European Biophysics Journal (2019) 48 (Suppl 1):S1–S264
https://doi.org/10.1007/s00249-019-01373-4

EBSA
Biophysics in Europe

JOINT 12th EBSA congress
10th ICBP – IUPAP congress

July 20-24, 2019, Madrid, Spain

This supplement was not sponsored by outside commercial interests. It was funded entirely by the publisher.

Acknowledgements

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JOINT 12th EBSA congress
10th ICBP – IUPAP congress
July 20-24, 2019, Madrid, Spain

Hosted by

The Spanish Biophysical Society (Sociedad de Biofísica de España, SBE)

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Welcome to the 12th European Biophysics Congress – 10th International Conference on Biological Physics

Dear colleagues and friends,

On behalf of the Spanish Society of Biophysics (SBE), the European Biophysics Societies Association (EBSA), and the Biological Physics Commission (C6) of the International Union of Pure and Applied Physics (IUPAP), it is our pleasure to welcome you to Madrid for the "12th European Biophysics Congress – 10th International Conference on Biological Physics 2019". After more than four years of organization and planning, in close interaction with national societies, the Congress chairs, and EBSA and C6-IUPAP Councils, we are delighted to offer you what we think is a spectacular scientific program, which combines the most advanced biophysics available today. Your participation will complete an outstanding level of talks, posters and scientific discussions, under the comfortable conditions of our congress venue, at the most modern congress infrastructure of the city of Madrid.

For the first time, the biennial EBSA Congress takes place in Madrid 2019 together with the triennial ICBP Conference organized by IUPAP, to have a Congress with a true worldwide coverage, where biologists, physicists, and bio- and physical-chemists will have an unprecedented opportunity to exchange their complementary views and building novel interdisciplinary collaborations. Scientists from 27 European countries are expected, plus a significant participation of biophysicists from a further 20 countries from Asia, Africa and North and South Americas.

The Scientific Committee of the Congress, together with the Councils of EBSA and IUPAP-C6, selected a fair collection of topics representative of modern biophysics and proposed an extraordinary selection of plenary speakers, including two recent Nobel Laureates. All EBSA member societies were also asked for suggestions of chairs and speakers for the different topics. Once chairs were appointed and confirmed, they explored extensively their domains of expertise to suggest additional speakers to complete their sessions. Chairs also selected additional oral contributions from over 800 abstracts received, which completed the scientific program that the Congress is now offering.

The Spanish Society of Biophysics (SBE, Sociedad de Biofísica de España) (www.sbe.es) is a very active and young society, established in 1986 with the main goal of favoring contact among biophysicists working in Spain and among these and the international Biophysics community. SBE has now almost 500 members, with a fair proportion of middle career scientists and students, and is strongly connected with other biophysical societies in Europe and North and South America.

Organizing a Congress today is still suffering from the short availability of funds for science derived from recent years of economic limitations, and a program like the one of EBSA-IUPAP Madrid 2019 would not be possible without the generosity of chairs and speakers, who accepted coming to Madrid with limited financial support. EBSA itself, also supports the congress to enable registration and other costs to be kept under control, and we also acknowledge complementary support from IUPAP. We are really thankful to all of them for their positive engagement and their commitment. We are also very grateful to our sponsors and exhibitors, and to the input of Grupo Pacífico, the company that has been assisting us with all the operational details, and in particular to Carine Saint-Rose, always available with a smile.

A large numbers of bursaries (60) were provided by EBSA to students, completed with a few bursaries for Spanish students awarded by the SBE. The Biophysical Society (USA) funded a fair number of prizes for the best poster communications, which will be managed by the new BPS Executive Officer, Jennifer Pesanaelli. We thank the members of the Organizing Committee, especially the fantastic help of Mercedes Echaide in most organizational topics along the four years of preparation of the Congress. Of particular relevance and support has been always the help and advice coming from Anthony Watts, a reference inspiring the best performance of EBSA and EBSA activities, including the Biophysics Summer School preceding the Congress and supported also by the International Union of Pure and Applied Biophysics (IUPAB). We thank Avanti for sponsoring again the prestigious Avanti/EBSA award for membrane

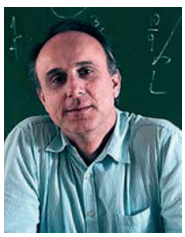
biophysics, and to Springer-Nature for the continuous support of these congresses – Springer-Nature publishes the European Biophysics Journal, the revenues of which support EBSA, and hence this congress.

Enjoying biophysics and enjoying the Congress should not prevent the attendants enjoying also the marvelous streets and museums of old Madrid downtown during the day as well as its splendid nightlife. Connecting attendance at the Congress with a few days of vacation in sunny and warm Spain will allow a full exploration of Spanish landscapes, culture and gastronomy, a must that millions of tourists look for every year. Don't miss Madrid "tapas", and do not forget to continue biophysical discussions around some "cañas" and "vinos" in the city's old quarter.

We look forward to an exciting time in Madrid, full of science, biophysics and Spanish culture!

Lastly, we hope to see you again at the 13th EBSA Congress in Vienna in 2021!

Jesús Pérez-Gil, Chair of the Organizing and Scientific Committees, President of SBE and Vice-president of EBSA



Juan Manuel R. Parrondo, Co-chair, IUPAP-C6 Council

A Welcome Message from the Commission on Biological Physics (C6) of the International Union of Pure and Applied Physics (IUPAP)

Dear colleagues,

Welcome to the 10th International Conference on Biological Physics 2019 that is held jointly with the 12th European Biophysics Congress. Our gathering in Madrid provides, for the first time, an opportunity for participation of scientists working in the full spectrum of this interdisciplinary field. By joining forces with the European Biophysical Societies Association (EBSA), we have been able to organize a conference that brings together internationally leading experts in various areas of biological physics.

IUPAP was founded in 1922 by a group of physicists led by William Bragg who wanted to establish a forum that would promote international cooperation among physicists, to counter the devastating effects of the 1st World War. The Union has been successful in helping to secure global agreements and safeguarding free movement of scientists and their science worldwide. IUPAP started with 13 member states and has grown into a global organization representing 56 member states today. The C6 Commission was formally established in 1990 on the initiative of Kai Siegbahn (the IUPAP president at the time) and the founding chair Hans Frauenfelder. By recognizing the achievements of Young Scientists from all countries, IUPAP has formed one of the most inclusive scientific organizations in the world.

I would like to extend to you my warmest greetings, and trust that you will find this unique event in Madrid a memorable experience.

Ramin Golestanian

Chair of IUPAP C6 Commission on Biological Physics

Welcome from the President of EBSA

EBSA biennial congresses are now becoming a major activity in the world of Biophysics, and this, the 12th congress, is no exception. With participants from over almost 40 countries registered, over 800 poster abstracts and around 140 invited talks, including (again) two Nobel Laureates, we are indeed looking forward to a spectacular congress. In line with EBSA tradition, we are delighted to be joining up with a local host Biophysical Society, in this case the Spanish Biophysical Society (SBE), one of the strongest in Europe. As with the 11th EBSA congress in Edinburgh which was jointly with the International Union of Pure and Applied Biophysics (IUPAB), this time in Madrid, we join with another international union, the biophysical physics section (C6) of the International Union of Pure and Applied Physics, (IUPAP). As always, we also very much appreciate the support of the US-based Biophysical Society for their generous support of bursaries and poster prizes and welcome to Madrid this year, the new Executive Officer, Jennifer Pesanaelli.

Bringing together all the ingredients for such a major congress, is no trivial task, and EBSA would like to thank Jesús Pérez-Gil and Juan Manuel R. Parrondo, and the members of the various committees, for driving through the organization of the congress, and ensuring that the scientific quality reflected in the congress, continues to be of such a high standard. Juggling geopolitical and gender balance with the programme of speakers, both invited, plenary and short oral talks, as well as maintaining the highest quality science, can be very daunting, but they have pulled off a very successful and balanced programme, covering a varied breadth of contemporary biophysics, a traditional characteristic of EBSA congresses. Additionally, since these congresses move throughout the 4-compass points in Europe (here we are in the west), each congress has its own local flavour reflecting local traditions and style – Madrid is a fun and cultured city, and we hope you will find time to appreciate its offerings.

The European Biophysical Societies' Association (EBSA) was formed in 1984 as a non-profit making organisation, with the objectives (www.ebsa.org) "*to advance and disseminate knowledge of the principles, recent developments and applications of biophysics, and to foster the exchange of scientific information among European biophysicists and biophysicists in general*".

It is composed of 31 Biophysical Societies in the European area, representing around 4000 – 5000 individuals who are national society members – these individuals do not pay a fee to EBSA, but their national societies pay a membership fee of just €2 per member, and the vast majority (95%) of the revenues of EBSA (a UK registered charity) come from the publishing revenues of the European Biophysics Journal, for which EBSA holds the copyright. EBSA also has an Associate member, the Iranian Biophysical Society, and has been instrumental in the formation of several new biophysical societies in Europe.

EBSA is associated with the international organizations International Union for Pure and Applied Biophysics (IUPAB) and Initiative for Science in Europe (ISE). EBSA maintains close links with Springer-Nature, the publishers of the European Biophysics Journal. The European Biophysics Journal, owned by EBSA, is free of charge for EBSA members.

Since the purpose of EBSA is to promote Biophysics in Europe, it supports, both financially and organizationally, the biennial congresses like this one – the next will be in Vienna in 2021. Also, EBSA offers sponsorships to organisers of meetings and schools that promote biophysics in Europe, as well as satellite meeting to the congress. Support can come in the form of grants to the organisers or bursaries to participants. EBSA also offers bursaries to individual young scientists to participate in the Biophysics Congress – 60 have been awarded this year. Furthermore, new initiatives have been generated, such as support for working visits or bursaries to attend scientific meetings.

EBSA is also proud to recognize the achievements of European biophysicists, by awarding two major prizes, namely the EBSA Young Investigator Award which this year goes to Pere Roca-Cusachs of the University of Barcelona (UB) and Institute for Bioengineering of Catalonia (IBEC) for his for contributions to the field of mechanobiology. The Avanti/EBSA award for membrane biophysics this year goes to Bruno Antonny, group leader at the Institut de Pharmacologie Moléculaire et Cellulaire, Valbonne-Sophia Antipolis, France, for his work on membrane biophysics. Both awards carry cash prizes from EBSA and generously from Avanti Polar Lipids Inc., respectively.

Finally, please do approach any of the committee members if you wish to discuss EBSA related matters, and discuss ways in which EBSA can fulfil its mandate more effectively. We also warmly welcome Presidents and Secretaries of the national societies to join us for the "Presidents' meeting" here in Madrid, and then also nominated Society voting members and observers to the General Meeting of EBSA.

EBSA – Biophysics for Europe – and the world.

Anthony Watts, President (2017 – 2019)

The EBSA Executive Committee at a committee meeting, Venice, January 2018. Members are: (from left to right) Mauro Dalla Serra, Trento, Italy; André Matagne, Liège, Belgium; José Carrescoa, Madrid, Spain; László Mátyus, Debrecen, Hungary; Jacqueline Cherfils, Cachan, France; Helmut Grubmüller, Göttingen, Germany; Anthony Watts, Oxford, England; Jesús Pérez-Gil, Madrid, Spain; John Seddon, London, England; Pavol Miskovsky, Kosice, Slovakia; Ilpo Vattulainen, Helsinki, Finland; Robert Gilbert, Oxford, England. Not present are: Pierre-Emmanuel Milhiet, Montpellier, France; Tony Wilkinson, York, England. New member from July 2019, Elena Pohl, Vienna, Austria.

Satellite Events

BIOLOGICAL AND BIO-INSPIRED MATERIALS: FROM RESPONSIVENESS TO ACTIVITY - 19 JULY, 2019- MADRID (UAM)
https://sites.google.com/view/bioreact2019
ORGANIZED BY: J.L. Aragones, L R. Arriaga, M. Velez and P. Tarazona

COMPLEX NETWORKS IN THE LIFE SCIENCES - JULY 25, 2019 ALCALÁ DE HENARES (MADRID, SPAIN)
https://complexnetworksebsa2019.weebly.com
twitter: @CompNetLife2019
ORGANIZED BY: Jacobo Aguirre, Saúl Ares

HIGH DENSITY DNA ARRAYS: MODELS, THEORIES AND MULTISCALE SIMULATIONS
https://www.cecam.org/workshop-1769.html

OUT-OF-EQUILIBRIUM SOFT MATTER IN COMPLEX MEDIA
https://www.cecam.org/workshop-1666.html

“PROTEO-LIPID NANOSTRUCTURES: FROM DOMAINS TO DEVICES” 25-27 JULY, 2019, Bilbao, Spain
http://prolin2019.com
ORGANIZED BY: Vadim Frolov (Chair), Anna Shnyrova, David Rodriguez-Larrea, Aitor Hierro and Sergi Padilla-Parra.

Congresses of the

INTERNATIONAL UNION FOR PURE AND APPLIED BIOPHYSICS

1st IUPAB CONGRESS, 1961 STOCKHOLM, SWEDEN
2nd IUPAB CONGRESS, 1966 VIENNA, AUSTRIA
3rd IUPAB CONGRESS, 1969 CAMBRIDGE, MASS., U.S.A
4th IUPAB CONGRESS, 1972 MOSCOW, U.S.S.R
5th IUPAB CONGRESS, 1975 COPENHAGEN, DENMARK
6th IUPAB CONGRESS, 1978 KYOTO, JAPAN
7th IUPAB CONGRESS, 1981 MEXICO CITY, MEXICO
8th IUPAB CONGRESS, 1984 BRISTOL, UNITED KINGDOM
9th IUPAB CONGRESS, 1987 JERUSALEM, ISRAEL
10th IUPAB CONGRESS, 1990 VANCOUVER, CANADA
11th IUPAB CONGRESS, 1993 BUDAPEST, HUNGARY
12th IUPAB CONGRESS, 1996, AMSTERDAM, THE NETHERLANDS
13th IUPAB CONGRESS, 1999, NEW DELHI, INDIA
14th IUPAB CONGRESS, 2002, BUENOS AIRES, ARGENTINA
15th IUPAB / 5th EBSA / SFB CONGRESS, 2005, MONTPELLIER, FRANCE
16th IUPAB CONGRESS, 2008, LONG BEACH, CALIFORNIA, USA
17th IUPAB CONGRESS, 2011, BEIJING, CHINA
18th IUPAB CONGRESS, 2014, BRISBANE, AUSTRALIA
19th IUPAB / 11th EBSA / CONGRESS, 2017, EDINBURGH, UNITED KINGDOM

—

20th IUPAB CONGRESS, 2020, Rio de Janeiro, Brazil
(see http://www.iupab.org/ for details)

Congresses of the

EUROPEAN BIOPHYSICAL SOCIETIES' ASSOCIATION

1st EUROPEAN BIOPHYSICS CONGRESS, 1971, BADEN, AUSTRIA
2nd CONGRESS, 1997, ORLEANS, FRANCE
3rd CONGRESS, 2000, MUNICH, GERMANY
4th CONGRESS, 2003, ALICANTE, SPAIN
5th EBSA / 15th IUPAB / SFB CONGRESS, 2005, MONTPELLIER, FRANCE
6th CONGRESS, 2007, LONDON, UNITED KINGDOM
7th CONGRESS, 2009, GENOA, ITALY
8th CONGRESS, 2011, BUDAPEST, HUNGARY
9th CONGRESS, 2013, LISBON, PORTUGAL
10th CONGRESS, 2015, DRESDEN, GERMANY
11th EBSA / 19th IUPAB / CONGRESS, 2017, EDINBURGH, UNITED KINGDOM
12th EBSA- 10th ICBP – IUPAP CONGRESS, July 20-24 2019, MADRID, SPAIN

—

13th EBSA: Vienna, Austria in the Summer of 2021
(see http://www.ebsa.org/ for details)

Recipients of EBSA Bursaries

Ábrahám, Ágnes	Hungary
Baranowski, Marek	Poland
Bialobrzewski, Michal	Poland
Bucataru, Cezara	Romania
Cano-Munoz, Mario	Spain
Cetin, Ebru	Turkey
Correa, Wilmar	Germany
Cullell-Dalmau,Marta	Spain
David, Melinda	Romania
Dietel, Lisa	Germany
Dragomir, Isabela	Romania
Dudás, Bálint	Hungary
Farcas, Alexandra	Romania
Ferreira, Rita	Portugal
Flores-Romero, Hector	Germany
Fuente, Diego	Spain
Galimzyanov, Timur	Russian Federation
Gehan, Pauline	France
Golovkova, Iaroslava	France
Gutiérrez-Rus, Luis	Spain
Herrera León, Claudia	France
Herrera León, Fabio	Finland
Ionescu, Sandra	United Kingdom
Kondrashov, Oleg	Russian Federation
Kozarski,Mateusz	Poland
KuzminaNatalia	Russian Federation
Liebe, Nils Laurin	Germany
Luckner, Madlen	Germany
Majaron, Hana	Slovenia
Martinez-Rojas, Vladimir	Italy
Marx, Lisa	Austria
Matwijczuk, Arkadiusz	Poland
Melero-Carrillo, Alejandro	United Kingdom
Misuraca, Loreto	France
Mlynarska-Cieslak, Agnieszka	Poland
Moga, Akanksha	Germany
Moreno-Pescador, Guillermo	Denmark
Nunes, Rafael	Portugal
Nuñez Viadero, Eider	Spain
Perzanowska, Olga	Poland
Pillai, Visakh	Ireland
Pinet, Louise	France
Pinto, Giulia	Italy
Robert, Charly	Belgium
Rodriguez Moraga, Nely	France
Roig-Merino, Sara	The Nederlands
Saeedimasine, Marzieh	Sweden
Silva, Ítala Cristine	Portugal
Talafová, Veronika	Slovakia
Tankovskaia, Svetlana	Russian Federation
Tetiana, Mukhina	France
Tosatto, Laura	Italy
Tymchenko, Ekaterina	Russian Federation
Uriati, Eleonora	Italy
Valenzuela-Gomez, Fernando	Spain
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Vignolini, Tiziano	Italy
Wang, Weiqiang	Spain
Zímová, Lucie	Czech Republic
Fonin, Alexander	Russian Federation

12th EBSA, 10th ICBP-IUPAP CONGRESS DETAILED PROGRAM

Saturday 20 July

17:30 WELCOME ADDRESS

18:00 **PLENARY LECTURE**
Harnessing Evolution to create new medicines
Sir Gregory Paul Winter

Sunday 21 July

09:00 **PLENARY LECTURE**
Eukaryotic organelles: deciphering their interdependency, structure and dynamics with new imaging technologies
Jennifer Lippincott-Schwartz, USA

10:00-12:45 **PROTEIN FOLDING**
Chairs: Ana Azuaga, Spain
Jacqueline Cherfils, France

Invited speakers:

10:00 Understanding alpha-synuclein amyloid self-assembly and its associated toxicity
Nunilo Cremades, Spain

10:30 Dodging the crisis of folding proteins with non-trivial topology - new challenges.
Joanna Sulkowska, Powland

11:00 – 11: 30 COFFEE BREAK

11:30 The structural determinants of the toxicity of misfolded protein oligomers associated with neurodegenerative diseases
Fabrizio Chiti, Italy

Short Talks:

12:00 Prion soft amyloid core driven self-assembly of globular proteins into bioactive nanofibrils
Weiqiang Wang, Spain

12:15 Unravelling the details of the conformational cycle of the Hsp90 chaperone
Katarzyna Tych, Germany

12:30 Strategically positioned slow codons support protein folding
Michal Perach, Israel

10:00-12:45 **DNA ARCHITECTURE AND GENE REGULATION**
Chairs: *Masaki Sasai, Japan*
Helmut Schiessel, Netherlands

Invited speakers:

10:00 DNA release from histones in nucleosome core particles: measurements and models
Lois Pollack, USA

10:30 Gene control with a “twist”: Exploring the dynamic chromatin landscape
Beat Fierz, Switzerland

11:00- 11:30 COFFEE BREAK
11:30 Chromosome organization in living human cells revealed by single nucleosome imaging
Kazuhiro Maeshima, Japan

Short Talks:
12:00 Non-specific action of polyamines leads to specific changes in structure and function of DNA
Takashi Nishio, Japan

12:15 Simulating the binding of Pioneer Transcription Factors to the nucleosome
Jan Huertas, Germany

12:30 Dependence of DNA Persistence Length on Ionic Strength and Ion Type
Catherine Tardin, France

10:00-12:45 **BIOPHYSICS OF CYTOSKELETON**
Chairs: *Marisela Velez, Spain*
Andreas Janshoff, Germany

Invited speakers:
10:00 Mind the gap: polyampholytes meet the neuronal skeleton
Roy Beck, Israel

10:30 Reconstitution of basic mitotic spindles in Cell-like confinement
Marleen Dogterom, the Netherlands

11:00 – 11:30 COFFEE BREAK

11:30 The spindle is a composite of two permeating polar gels
Jan Brugués, Germany

Short Talks:
12:00 Structure dissection of a contractile phage tail tube
Birgit Habenstein, France

12:15 Ultrafast tracking reveals the function of structural domains of single proteins
Kristýna Holanová, Czech Republic

12:30 Nucleotide and Osmolyte Induced Folding of FtsZ from Staphylococcus aureus
Sonia Huecas, Spain

10:00-12:45 **MECHANOBIOLOGY**
Chairs: *Malgorzata Lekka, Poland*
Pere Roca-Cusachs, Spain

Invited speakers:

10:00 Mechanically and biochemically induced pathways regulating integrin-dependent cell adhesion
Daniel Mueller, Switzerland

10:30 Molecular to cellular mechanics at short timescales
Felix Rico, France

11:00- 11:30 COFFEE BREAK

11:30 Mechanosensitive actomyosin network dynamics and migration plasticity in 3D environments
Verena Ruprecht, Spain

Short Talks:

12:00 Activation of human aortic valve interstitial cells by local stiffness in Calcific Aortic Valve Disease*Loredana Casalis, Italy*

12:15 Role of Rab8A and Caveolin-1 in the interplay between cell mechanotransduction and cholesterol trafficking
Giulio Fulgoni, Spain

12:30 The Mechanical Work of Vinculin Binding to Talin Regulates Vinculin Activation
Rafael Tapia Rojo, USA

12:45-14:30 LUNCH AND POSTER SESSION

14:30 **PLENARY LECTURE**
Shaping Membranes with Proteins and Cytoskeleton: Deciphering Mechanisms with Reconstituted Systems
Patricia Bassereau, France

15:15 **AVANTI PRIZE CONFERENCE**
Control of organelle dynamics by membrane curvature and lipid unsaturation
Bruno Antonny, France

16:00-16:30 **EBSA YOUNG INVESTIGATOR AWARD**
Sensing the matrix: transducing mechanical signals from integrins to the nucleus.
Pere Roca-Cusachs, Spain

16:30 – 17:00 COFFEE BREAK

17:00-19:15 **MEMBRANE STRUCTURE AND DYNAMICS**
Chairs: *Claudia Steinem, Germany*
Felix Goñi, Spain

Invited speakers:

17:00 Lessons Learned from Complex Mimics of Biological Membranes
Georg Pabst, Austria

17:30 Regulating the dynamic interactions between herpes simplex viruses and cell-surface glycosaminoglycans
Marta Bally, Sweden

18:00 IQGAP1 links phosphoinositide signaling to the cytoskeleton and promotes cytoskeletal reorganization
Arne Gericke, USA

Short Talks:

18:30 Adsorption kinetics of pulmonary surfactant complexes purified from bronchoalveolar lavages of porcine lungs and human amniotic fluid
José Carlos Castillo-Sánchez, Spain

18:45 Microfluidic platforms for the handling, manipulation, and analysis of model cells
Tom Robinson, Germany

19:00 Quantified Efficiency of Membrane Leakage Events Relates to Antimicrobial Selectivity
Maria Hoernke, Germany

17:00-19:15 **CELLULAR PROLIFERATION**
Chair: *Juan M. R, Parrondo, Spain*

Invited speakers:

17:00 Dynamics of bacterial swarms and biofilms
Knut Drescher, Germany

17:30 Biophysical Concepts Applied to Tumor Progression
Herbert Levine, USA

18:00 How molecular forces shape bacterial biofilms
Berenike Maier, Germany

Short Talks:

18:30 Cell-size regulation induces long-term oscillations in population growth rate
Farshid Jafarpour, USA

18:45 Molecular architecture of bacterial amyloids in Bacillus biofilms
Nadia El Mammeri, France

19:00 Microviscosity of bacterial biofilm matrix characterized by fluorescence correlation spectroscopy and single particle tracking
Valentin Dunsing, Germany

17:00-19:15 **NON-EQUILIBRIUM PHYSICS**
Chairs: *Udo Seifert, Germany*
Felix Ritort, Spain

Invited speakers:

17:00 How rotating ATP synthases can modulate membrane structure
Ivan Lopez-Montero, Spain

17:30 Quantifying and modelling active motion in biological systems
Timo Betz, Germany

18:00 Dynamics of nanoparticle and virus uptake at cell membranes
Ulrich Schwarz, Germany

Short Talks:

18:30 Non-Equilibrium Processes in Proteins Triggered by Light: Excited States Molecular Dynamics Perspective
Wieslaw Nowak, Poland

18:45 1/f noise in ion channels formed by the Classical Swine Fever Virus (CSFV) p7 protein
Antonio Alcaraz, Spain

19:00 Biophysical models of mRNA translation applied to ribosome profiling data
Juraj Szavits-Nossan, United Kingdom

17:00-19:15 **TRAFFICKING AND SIGNALLING**
Chairs: *Ana Garcia-Saez, Germany*
Carlo Manzo, Spain

Invited speakers:

17:00 Systematic superresolution analysis of endocytosis reveals an actin nucleation nano-template that drives efficient vesicle formation
Jonas Ries, Germany

17:30 Shedding new light on the nanoscale organization of GPCR signalling
Davide Calebiro, UK

18:00 Cryo-EM structural analysis of focal adhesion machinery
Naoko Mizuno, Germany

Short Talks:

18:30 TANGO1 Regulates Membrane Tension to Mediate Collagen Export from the Endoplasmic Reticulum
Felix Campelo, Spain

18:45 Facile membrane flow and tension equilibration at a presynaptic nerve terminal
Erdem Karatekin, USA

19:00 Allosteric regulation of small GTPases at the surface of membranes
Jacqueline Cherfils, France

Monday 22 July

09:00 **PLENARY LECTURE**
Overcoming the multiscale simulation challenge for biomolecular systems
Gregory Voth, USA

10:00-12:45 **BIOMOLECULAR SIMULATION I**
Chairs: *Claudio Soares, Portuga*
Ilpo Vattulainen, Finland

Invited speakers:

10:00 Deep learning for molecular biophysics
Frank Noe, Germany

10:30 Sculpting cell membranes by protein nanomachines
Andela Saric, UK

11:00 – 11:30 COFFEE BREAK

11:30 Pentameric ligand-gated ion channels: new crystal structures and MD simulations
Marc Delarue, France

Short Talks:

12:00 Automated cryo-EM structure refinement using correlation-driven molecular dynamics
Maxim Igaev, Germany

12:15 Aβ peptides and β-sheet breakers. A coarse grained molecular dynamics approach using GO-Martini

Aishwarya Dhar, Italy

12:30 Effect of pH on the influenza fusion peptide structure and activity: A constant-pH molecular dynamics approach
Diana Lousa, Portugal

10:00-12:45 **MACROMOLECULAR COMPLEXES**
Chairs: *Tony Wilkinson, UK*
Irene Diaz-Moreno, Spain

Invited speakers:

10:00 Recent insights into the peptide-loading complex machinery
Simon Trowitzch, Germany

10:30 LUBAC and linear ubiquitin chains: novel tools to study immune signalling
Katrin Rittinger, UK

11:00 – 11:30 COFFEE BREAK

11:30 Analysing cryoEM data quality in the post resolution revolution era: Validity criteria
José M. Carazo, Spain

Short Talks:

12:00 Histone tails in nucleosome: fuzzy interaction with DNA
Sevastyan Rabdano, Russia

12:15 New protein-protein interaction modulators for the therapeutic regulation of synapse dysfunction in neurodevelopmental disorders and neurodegeneration
Maria Jose Sanchez-Barrena, Spain

12:30 Structural characterisation of tissue-derived, disease-associated polymers of alpha-1-antitrypsin using conformation-selective antibodies and single-particle reconstructions from electron microscopy images
James Irving, UK

10:00-12:45 **EVOLUTIONARY DYNAMICS**
Chairs: *Susanna Manrubia, Spain*
Liedewij Laan, Netherlands

Invited speakers:

10:00 Eco-evolutionary dynamics during Escherichia coli colonization of the mouse gut
Isabel Gordo, Portugal

10:30 Evolutionary Biology of Cell Division: Integrating Quantitative Genetics and Cellular Biophysics
Daniel Needelman, USA

11:00- 11:30 COFFEE BREAK

11:30 Universal properties of genotype-phenotype maps
Sebastian Ahnert, UK

Short Talks:

12:00 ScarTrace: CRISPR/Cas9-mediated clonal tracing in zebrafish embryonic development and regeneration
Anna Alemany, Netherlands

12:15 Rare beneficial mutations cannot halt Muller's ratchet in spatial populations
Su-Chan Park, South Korea

12:30 Exploring phenotypic variability of bacteria using microfluidic cell traps
Ágnes Ábrahám, Hungary

10:00-12:45 **BIOPHYSICS OF MEMBRANE OXIDATION**
Chairs*: Manuel Prieto, Portugal*
Rosangela Itri, Brazil

Invited speakers:

10:00 Photosensitized oxidation of lipid membranes: yields and rates of chemical and physical changes
Mauricio da Silva, Brazil

10:30 Achievements, challenges and hopes in the biophysical analysis of oxidised (phospho)lipids
Ana Reis, Portugal

11:00-11:30 COFFEE BREAK

11:30 Oxysterols and Truncated Oxidized Phospholipids in Model Membranes
Martin Hof, Czech Republic

Short Talks:

12:00 Lipids as substrates and dynamic activators: pro-apoptotic lipid peroxidation at the mitochondrial membrane surface.
Patrick Van Der Wel, Netherlands

12:15 The effect of oxidised cholesterol on model red blood cell membranes
Paul Smith, UK

12:30 Lipid curvature modulates function of mitochondrial membrane proteins
Olga Jovanovic, Austria

12:45-15:00 LUNCH AND POSTER SESSION

13:00-15:00 MEETING OF EBSA Presidents

15:00 **PLENARY LECTURE**
Cryo-EM to visualize structure and dynamics of complex biological macromolecular assemblies
Eva Nogales, USA

15:45 **IUPAP Young Scientist Prize in Biological Physics 2019**
Bacterial collective behaviors
Knut Drescher, Germany

16:00 **IUPAP Young Scientist Prize in Biological Physics 2018**
Thermodynamics of biological active matter
Nikta Fakhari, USA

16:30-19:15 **PROTEIN STRUCTURE AND FUNCTION**
Chairs: *Mark Baldus, Netherlands*
Juan Hermoso, Spain

Invited speakers:

16:30 Unraveling the role of SepF in the early actinobacterial divisome
Pedro Alzari, France

17:00 Molecular Basis for the Extra-Mitochondrial Roles of Cytochrome C in Cell Life and Death
Miguel Angel de la Rosa, Spain

17:30- 18:00 COFFEE BREAK

18:00 Atomistic Simulation of Biomolecular Function: Ribosomal Translation and Ligand Binding Heterogeneity
Helmut Grubmueller, Germany

Short Talks:

18:30 Campylobacter jejuni Tlp3 dCache sensing domain specifically recognises hydrophobic amino acids

Mohammad Firoz Khan, Australia

18:45 Dynamics of intrinsically disordered and unfolded proteins: investigations using neutron spin-echo spectroscopy
Andreas Stadler, Germany

19:00 Side chain to main chain hydrogen bonds stabilize a polyglutamine helix in the activation domain of a transcription factor
Albert Escobedo, Spain

16:30-19:15 **GENE NETWORK DYNAMICS AND SIGNALING**
Chairs: Marta Ibañez, Spain
Jan Brugues, Germany

Invited speakers:

16:30 Stochastic Turing patterns in the development of a one-dimensional organism
Joel Stavans, Israel

17:00 Human time vs. Mouse time: in vitro segmentation clock as a model system
Miki Ebisuya, Spain

17:30- 18:00 COFFEE BREAK

18:00 Distinguishing dormant from dead by triggering life in spores
Hyun Youk, Netherlands

Short Talks:

18:30 A nonequilibrium phase transition theory for the formation of diverse homeostasis and the emergence of systematic aging in multicellular systems
Yuting Lou, Japan

18:45 Post-transcriptional regulation, noise and spatial transcript localization of small RNA-controlled genes in an Escherichia coli stress response network
Rinat Arbel-Goren, Israel

19:00 Modeling cytoneme guidance for Hedgehog signaling
Adrián Aguirre-Tamaral, Spain

16:30-19:15 **NEW FRONTIERS IN BIOIMAGING**
Chairs: *Silvia Caponi, Italy*
Christian Eggelin, Germany

Invited speakers:

16:30 Faster and gentler optical nanoscopy for brain cell imaging
Ilaria Testa, Sweden

17:00 The mesolens: a new instrument for 3d optical imaging of large biological specimens with sub-cellular resolution throughout
Gail McConnell, UK

17:30 – 18:00 COFFEE BREAK

18:00 Brillouin Microscopy for cell and tissue biomechanics
Giuliano Scarcelli, USA

Short Talks:

18:30 Wavefront shaping for low background, high resolution STED-FCS in three dimensions
Aurélien Barbotin, UK

18:45 High density single particle tracking reveals nano- and meso-scale dynamic organization of plasma membrane receptors in living cells
Nicolas Mateos Estevez, Spain

19:00 Photophysics and engineering of transgene labels for optoacoustics
Andre C. Stiel, Germany

16:30-19:15 **CELL MEMBRANE BIOPHYSICS** (CPL Symposium)
Chairs*: Edgard Koijmann, USA*
Gemma Fabries, Spain

Invited speakers:

16:30 Cholesterol modulation of nicotinic acetylcholine receptor single molecule and nanocluster trajectories
Francisco Barrantes, Argentina

17:00 Morphology of Membrane Contact Sites Contributes to the Regulation of the Phosphatidylinositol Cycle
Richard Epand, Canada

17:30 – 18: 00 COFFEE BREAK

18:00 Redox lipidomics deciphers phospholipid signals of programmed cell death
Valerian Kagan, USA

Short Talks:

18:30 Linking Structure, Stability and Function in the Anti-Measles Virus Action of Tocopherol-Derivatized Peptide Nanoparticles (Prize SBfP)
Tiago N. Figueira, Portugal

18:45 Antimicrobial Peptides Impair Bacteria Cell Structure Within Seconds
Enrico F. Semeraro, Austria

19:00 Elucidating the nanoscale architecture of the plasma membrane with super-resolution spectroscopy

Erdinc Sezgin, UK

19:15-20:15 NATIONAL ASSEMBLIES

Tuesday 23 July

09:00 **PLENARY LECTURE 6**
Titin folding powers muscle contraction
Julio Fernandez, USA

10:00-12:45 **MECHANISMS OF MEMBRANE PROTEINS**
Chairs: *Manuela Pereira, Portugal*
Antonio Ferrer, Spain

Invited speakers:

10:00 Factors affecting Ion Permeation through K^+-Channels
Carmen Domene, UK

10:30 The first human lacking functional TRPV1 channel: Implications on TRPV1 functions in nociception
Baruk Minke, Israel

11:00-11:30 COFFEE BREAK

11:30 Optoelectrical dynamics of BK channel activation
Teresa Giraldez, Spain

Short Talks:

12:00 Structural insight into TRPV5 channel function and modulation
Jenny Van Der Wijst, Netherlands

12:15 Biophysical insights into membrane fission mediated by ESCRT-III
Vasil Georgiev, Germany

12:30 Ion transport, interfacial effects and scaling behavior in protein channels
Vicente Aguilella, Spain

10:00-12:45 **SINGLE MOLECULE BIOPHYSICS**
Chairs: *Nuno Santos, Portugal*
David Rueda, UK

Invited speakers:

10:00 The mechanical stability of proteins regulates their translocation rate into the cell nucleus
Sergi Garcia-Manyes, UK

10:30 Mechanosensitive states of the giant muscle protein titin
Miklos Kellermayer, Hungary

11:00 – 11:30 COFFE BREAK

11:30 Single-molecule live-cell imaging of mRNA life cycle
María Carmo-Fonseca, UK

Short Talks:

12:00 ParB dynamics and the critical role of the C-terminal domain in DNA condensation unveiled by combined Magnetic Tweezers and TIRF Microscopy
Julene Madariaga-Marcos, Spain

12:15 A folding nucleus and minimal ATP binding domain of Hsp70 identified by single-molecule force spectroscopy
Gabriel Zoldak, Slovakia

12:30 Single-molecule dissection of the dihydrofolate reductase reaction revealed multiple conformers leading to a catalytic product release
Nicole Galenkamp, Netherlands

10:00-12:45 **BIOPHYSICS OF IMMUNE RESPONSE**
Chairs: *Jean Marie Ruysschaert, Belgium*
Mario Castro, Spain

Invited speakers:

10:00 Membrane Sensing and Remodelling during the immune synapse. A spatiotemporal adaptive tale of lipid packing dynamics and collective assembly
Jorge Bernardino de la Serna, Spain

10:30 Mechanosensing via immunoreceptors
Cheng Zhu, USA

11:00 – 11:30 COFFEE BREAK

11:30 The role of oligomeric scaffolds in inflammatory signal transduction through Toll-like receptors and MyD88
Nick Gay, UK

Short Talks:

12:00 Insights into the Molecular Mechanism of Lipids/Toll-Like Receptors Interaction
Malvina Pizzuto, Spain

12:15 Super-resolution microscopy analysis of molecular interactions between epithelial cells and tissue-resident T cells in mouse epidermis
Dmitry Ushakov, UK

12:30 Shape matters: Towards a molecular understanding of the innate immune response to microbial lipids

Andra Schromm, Germany

12:45-15:00 LUNCH AND POSTER SESSION

14:00-15:00 INFORMATION SESSION
The European Research Council – Funding for frontier research in Europe
Janne Salo, Research programme officer, European Research Council Executive Agency
Andela Šarić, London, Starting grantee
Cait MacPhee, Edinburgh, Advanced Grant panel chair 2018

15:00-16:15 **NEW AND NOTABLE**
Chairs*: Anthony Watts, UK*
Maria Garcia-Parajo, Spain

15:00 A molecular mechanism for transthyretin amyloidogenesis
Trevor Forsyth, France

15:25 Cryo-EM of Helical Polymers at Near Atomic Resolution Yields Surprises: From Microbial Nanowires to Indestructible Pili
Edward H. Egelman, USA

15:50 To spread or not to spread: the mechanosensitive dilemma of T cells
Kheya Senguptaa, France

16:15-19:00 **BIOMOLECULAR SIMULATION II**
Chairs: *Claudio Soares, Portugal*
Ilpo Vattulainen, Finland

Invited speakers:

16:15 Advances in Multiscale Simulation of DNA
Modesto Orozco, Spain

16:45 Nanoparticle-bio interactions: the role of interface composition, conformation and softness
Giulia Rossi, Italy

17:15 – 17: 45 COFFEE BREAK

17:45 Lipid regulation of structure, dynamics, energetics, and function of membrane proteins
Emad Tajkhorshid, USA

Short Talks:

18:15 The catalytic mechanism of MB-COMT: new drug design paradigm?
Alex Bunker, Finland

18:30 Combining theoretical and experimental approaches to understand the mechanism of antibiotic permeation: implications on the fight against bacterial resistance
Carla F. Sousa, Portugal

18:45 Exploring Conformational Transitions and Free Energy Profiles of Proton Coupled oligopeptide Transport
Mariana Batista, Brazil

16:15-19:00 **LIVE IMAGING AND OPTICAL MICROSCOPY**
Chair: *Sussane Fenz, Germany*

Invited speakers:

16:15 Faster and better: taking localisation microscopy into live cells
Susan Cox, UK

16:45 A Multi Messenger microscope to paint chromatin in cells
Alberto Diaspro, Italy

17:15 – 17: 45 COFFEE BREAK

17:45 Looking into the nursery of platelets: Imaging of the bone marrow in vivo, in vitro and in silico
Katrin Heinze, Germany

Short Talks:

18:15 Strong cytoskeleton activity on millisecond timescales upon particle binding revealed by ROCS microscopy
Felix Jünger, Germany

18:30 Multiplexed single-molecule fluorescence imaging by FRET-PAINT
Nina Deußner--elfmann, Germany

18:45 In vivo single-molecule imaging of DNA gyrase
Ji-Eun Lee, UK

16:15-19:00 **ACTIVE MATTER AND BIOLOGICAL SELF-ORGANIZATION**
Chairs: *Sriram Ramaswamy, India*
Ramin Golestanian, Germany

Invited speakers:

16:15 Active Polymers and Membranes
Gerhard Gompper, Germany

16:45 The microtubule/kinesin system: A versatile realization of an active nematic
Francesc Sagues, Spain

17:15 – 17: 45 COFFEE BREAK

17:45 Spontaneous tension cable generation and convergence-extension flows in the gastrulating chick embryo
Silke Henkes, UK

Short Talks:

18:15 Design principles for robust self-assembly of multiple biological structures from limited resources
Deb Sankar Banerjee, UK

18:30 Active phase separation in mixtures of chemically-interacting particles
Jaime Agudo-Canalejo, UK

18:45 Self-organization of ciliary beats in bronchial epithelium
Simon Gsell, France

16:15- 19:00 **IONIC LIQUIDS AND BIOMOLECULES**
Chairs: *Hans-Joachim Galla, Germany*
Pietro Ballone, Ireland

Invited speakers:

16:15 Ionic liquids in bio-systems: from model biomembranes to living cells
Antonio Benedetto, Ireland

16:45 Aqueous Ionic Liquids: Consequences for Biological Stabilities
Jens Smiatek, Germany

17:15 – 17: 45 COFFEE BREAK

17:45 Studying fundamental role of ionic interactions to connect structure and properties of ionic liquids with biological activity
Valentine Ananikov, Russia

Short Talks:

18:15 Probing the effect of a room temperature ionic liquid on self-assembled structure of phospholipid membranes
Saheli Mitra, India

18:30 The effect of imidazolium-based ionic liquids on human insulin aggregation/amyloid fibrillization

Vladimir Vanik, Slovakia

18:45 Effect of Ionic Liquids on the Mechanoelasticity of Biomembranes: from Model Phospholipid Bilayers to Living Cells
Pallavi Kumari, Ireland

19:00-20:00 EBSA GENERAL ASSEMBLY

Wednesday 24 July

09:00 **PLENARY LECTURE**
Nonequilibrium stresses and fluxes: compositional organization at the cell surface and organelle morphodynamics
Madan Rao, India

10:00-12:45 **LIPID AND LIPIDOME**
Chairs: *John Seddon, UK*
Francisco Monroy, Spain

Invited speakers:

10:00 Membrane remodeling by the amyloidogenic peptide IAPP: surface crowing relevance
Manuel Prieto, Portugal

10:30 How the globoside Gb3 partitions in phase-separated membranes
Claudia Steinem, Germany

11:00 – 11: 30 COFFEE BREAK

11:30 Curvature rigidity of asymmetric and differentially stressed membranes
Markus Deserno, USA

Short Talks:

12:00 Formation of membrane domains: insight from simulations on different scales
Andreas Heuer, Germany

12:15 Monte Carlo and molecular dynamics simulations to explain biomembrane meso-patterning by a composition-curvature coupling mechanism
Julie Cornet, France

12:30 Biophysical properties of 1-deoxyceramides in ordered and disordered bilayers
Felix M Goñi, Spain

10:00-12:45 **BIG DATA IN BIOPHYSICS**
Chairs: *Remy Monasson, France*
Alfonso Valencia, Spain

Invited speakers:

10:00 A systems approach to understanding the allosteric mechanism of pyruvate kinase M2
Franca Fraternali, UK

10:30 Data-driven models of protein sequence landscapes: inference, 3D structure prediction and protein design
Martin Weigt, France

11:00 – 11: 30 COFFEE BREAK

11:30 Combining imaging and gene expression data in mathematical models for immuno-oncology
Vera Pancaldi, France

Short Talks:

12:00 Real-time dynamic changes in serum metabolome during the anticancer treatment by means of NMR-based metabolomics
Lukasz Boguszewicz, Poland

12:15 Decrypting interaction fingerprints in protein molecular surfaces
Bruno Correia, Switzerland

12:30 Bayesian inference and machine learning approaches to determine protein copy number from localization microscopy
Carlo Manzo, Spain

10:00-12:45 **LIQUID-LIQUID PHASE SEPARATION IN BIOLOGICAL SYSTEMS**
Chairs*: Rumiana Dimova, Germany*
Mauro della Serra, Italy

Invited speakers:

10:00 Bacterial division protein phase-separated condensates: biochemical and functional consequences (Bruker SBE Prize)
German Rivas, Spain

10:30 Light controlled cytoplasmic transport to probe cellular organization
Mortiz Kreysing, Germany

11:00 – 11: 30 COFFEE BREAK

11:30 Multivalency of proteins in cellular phase separation
Samuel Safran, Israel

Short Talks:

12:00 General sol to gel transition of liquid-liquid phase separated protein under shear
Yi Shen, UK

12:15 Recapitulation of nucleocytoplasmic transport with phase-separation of engineered protein repeats
Sheung Chun Ng, Germany

12:30 Protein phase transition: from biology towards new dynamic protein materials
Paolo Arosio, Switzerland

10:00-12:45 **INSTRUCT-ERIC: INTEGRATING ACCESS TO BIOPHYSICS AND STRUCTURAL BIOLOGY IN EUROPE**
Chair: *Ray Owens, UK*

Invited speakers:

10:00 Structure and mechanism of bactericidal mammalian perforin-2, an ancient agent of innate immunity
Rob Gilbert, UK

10:30 NMR-based hybrid approaches for cellular structural biology
Marc Baldus, Netherlands

11:00 – 11: 30 COFFEE BREAK

11:30 Towards High Throughput cryoEM data acquisition and processing, assuring reproducibility
Jose-Maria Carazo, Spain

Short Talsk:

12:00 Access to structural biology and biophysics technologies through Instruct-ERIC
Naomi Gray, UK

12:15 Crystal structures of bacteriophage receptor binding proteins
Mark J. Van Raaij, Spain

12:30 The Cryo-EM Structure of a Non-toxic Greek Key Oligomer of Alpha Synuclein
Ritobrita Chakraborty, India

12:45-15:00 LUNCH AND POSTER SESSION

14:00-15:00 EBSA EC SECOND MEETING

15:00-17:45 **MOLECULAR MOTORS**
Chairs: *Christoph Schmidt, USA*
José Carrascosa, Spain

Invited speakers:

15:00 Cooperative Functions of Molecular Motor Assemblies
Shin'Ichi Ishiwata, Japan

15:30 A molecular hurdle race: How does DNA polymerase deal with SSBs stably bound to the template?
Borja Ibarra, Spain

16:00 – 16:30 COFFEE BREAK

16:30 A ratchet mechanism governs load adaptation by branched actin networks
Peter Bieling, Germany

Short Talks:

17:00 Kinesin-2 stepping reflects its heteromeric nature
Willi L. Stepp, Germany

17:15 Reverse stroke of cardiac myosin revealed by single molecule microscopy is essential for heart function
Yongtae Hwang, Japan

17:30 Artificial Assembly of the Bacterial Flagella Motor on DNA Scaffolds
Sophie Hertel, Australia

15:00-17:45 **TISSUE BIOPHYSICS AND MORPHOGENESIS**
Chairs*: Francoise Brochard-Wyart, France*
Stephan Grill, Germany

Invited speakers:

15:00 Forming, watching and stressing organoids
Pierre Nassoy, France

15:30 Physics of epithelial folds and flows
Guillaume Salbreux, UK

16:00 – 16:30 COFFEE BREAK

16:30 Bacterial colonies as active droplets - from single cells to continuum theory
Vasily Zaburdaev, Germany

Short Talks:

17:00 Biomimetic emulsions probe the mechanics of tissues
Lea-Laetitia Pontani, France

17:15 Mechano-transduction and coordination of epithelial cells during Drosophila morphogenesis
Matthias Häring, Germany

17:30 A hydraulic instability underlies oocyte size selection in C.elegans
Arghyadip Mukherjee, Germany

15:00-17:45 **EMERGING BREAKTHROUGH MOLECULAR-SCALE BIOPHYSICS METHODOLOGIES**
(ARBRE-MOBIEU COST Action)
Chairs: *Patrick England, France*
Isabel Alves, France

Invited speakers:

15:00 Adventures with dynamic and disordered systems
Perdita Barran, UK

15:30 Access to Atomic Resolution Structural Information of Homo-Repeats: The Huntingtin Case
Pau Bernado, France

16:00 – 16:30 COFFEE BREAK

16:30 Investigating the bacterial translocon at the single molecule level
Peter Hinterdorfer, Austria

Short Talks:

17:00	Correlative μ-Brillouin and μ-Raman spectroscopy: emerging tool for simultaneous mechanical and chemical analysis of cells and tissues *Silvia Caponi, Italy*
17:15	Revisiting the structure-function relationship with mass photometry *Philipp Kukura, UK*
17:30	Hierarchical micro- and nanostructured surface architectures for label-free spectroscopic and microscopic protein interrogation *Julia Flesch, Germany*
18:00	**CLOSING PLENARY LECTURE** MINFLUX Nanoscopy: Superresolution post Nobel *Stefan Hell, Germany*
19:00	CLOSING CEREMONY
21:00	CONGRESS DINNER

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Saturday 20th July

Plenary Lecture 1
O-001
Harnessing Evolution to create new medicines
Gregory Winter
Trinity College., Cambridge, UK
Abstract: Antibodies are produced naturally by the immune system in response to foreign proteins (or antigens) and help protect against infectious agents such as bacteria and viruses. In many senses, antibodies are nature's own pharmaceuticals. Generally, antibodies have a Y-shape, with two arms and stem. Like the Swiss Army knife, the antibody is a multi-functional tool. The tips of the antibody arms bind to the virus or bacterium; this alone may be sufficient to prevent it adhering to or infecting a cell. The antibody stem can also act as a flag to the immune system, and trigger other cells and molecules to attack the infectious organism.
Although in nature, antibodies have evolved to attack infectious agents and toxins, we have learned in the last thirty years how to redesign or evolve antibodies to attack non-infectious diseases such as cancer and immune disorders. Such engineered antibodies have transformed the treatment of these diseases, revolutionising the pharmaceutical industry and generating products worth more than $70bn per annum.
More recently we have applied the same methods to create small antibody mimics based on short peptides covalently attached to an organic core. We believe that these will have some advantages over antibodies, and are currently evaluating their potential in animal models and early human clinical trials.

Sunday 21st July

Plenary Lecture 3
O-003
Shaping Membranes with Proteins and Cytoskeleton: Deciphering Mechanisms with Reconstituted Systems
P. Bassereau.
Institut Curie.
Cell plasma membranes are highly deformable: they are strongly curved during cell migration upon the formation of cellular protrusions or during endo/exocytosis where small buds form and eventually detach. These membrane-shaping processes always require the interaction with proteins (for instance BAR-domain proteins with an intrinsically curved shape, or proteins that insert into the lipid bilayer) and in some cases with the actin cytoskeleton. Over the years, *in vitro* reconstituted membrane systems combined to theoretical models have been instrumental for understanding how proteins and cytoskeleton shape cellular membranes. I will present examples from my group that illustrate how with these model membranes we have deciphered some mechanisms underlying endocytosis as well as the generation of filopodia.

Monday 22nd July

Plenary Lecture 4
O-004
Overcoming the Multiscale Simulation Challenge for Biomolecular Systems
G. Voth.
The University of Chicago.
Department of Chemistry, James Franck Institute, and Institute for Biophysical Dynamics, The University of Chicago, Chicago, IL, USA
Advances in theoretical and computational methodology will be presented that are designed to simulate complex (biomolecular and other soft matter) systems across multiple length and time scales. The approach provides a systematic connection between all-atom molecular dynamics, coarse-grained modeling, and mesoscopic phenomena. At the heart of these concepts are methods for deriving coarse-grained (CG) models from molecular structures and their underlying atomic-scale interactions. This particular aspect of the work has strong connections to the procedure of renormalization, but in the context of CG models it is developed and implemented for more heterogeneous systems. An important new component of our work has also been the concept of the "ultra-coarse-grained" (UCG) model and its associated computational implementation. In the UCG approach, the CG sites or "beads" can have internal states, much like quantum mechanical states. These internal states help to self-consistently quantify a more complicated set of possible interactions within and between the CG sites, while still maintaining a high degree of coarse-graining in the modeling. The presence of the UCG site internal states greatly expands the possible range of systems amenable to accurate CG modeling, i.e., quite heterogeneous systems, including complex self-assembly processes involving large multi-protein complexes. Applications to experimentally important targets such as cytoskeleton actin filaments and HIV virions will be given.

Monday 22nd July

Plenary Lecture 5
O-005
Cryo-EM to visualize structure and dynamics of complex biological macromolecular assemblies
E. Nogales.
UC Berkeley.
Cryo-EM to visualize structure and dynamics of complex biological macromolecular assemblies
Eva Nogales, Avinash Patel and Basil Greber
UC Berkeley
Cryo-EM can be used to investigate complete and fully functional macromolecular complexes in different functional states, providing a richness of biological insight. We are using cryo-EM in the study of complex gene expression machinery acting at the transcription level.
TFIID and TFIIH are large protein complexes required for transcription initiation by eukaryotic RNA polymerase II (Pol II). A major effort in my lab during the last twenty years has been to describe the structure and dynamics of human TFIID, an assembly of over 1 MDa that binds to core promoter DNA and recruits the rest of the transcription initiation machinery. TFIID functions as a central hub in the recognition of the core promoter sequences and neighboring chromatin marks and loads the TATA-box binding protein onto the DNA. TFIIH, a complex of about 0.5 MDa, enables transcription bubble opening and promoter clearance by Pol II via its ATPase and kinase activities, respectively. Methodological advances in cryo-electron microscopy have led to recent breakthroughs in the characterization of the structures of both of these complexes. I will present these recent structural insights and discuss their implications for our understanding of eukaryotic transcription initiation.

Tuesday 23rd July

Plenary Lecture 6
O-006
Titin folding powers muscle contraction
Julio M Fernandez
Department of Biological Sciences
Columbia University
By combining springs with motors and chemical switches biological systems can achieve great scales of mechanical power amplification, for example when jumping or launching a projectile. However, the molecular mechanisms of how this is achieved remain unknown. The giant muscle protein titin, composed of hundreds of tandem Ig domains, is a complex elastic protein whose role in muscle function is still poorly understood. A crucial recent discovery that we have made is that titin domains do a surprisingly large amount of mechanical work when they fold against an opposing mechanical force. We have shown that the amount of mechanical work done by a folding titin Ig domain can be 2-3 times larger (~120 zJ) than that of the chemically powered motor myosin II; ~38 zJ. Titin molecules store mechanical energy by unfolding and extending under force. Elastic energy is stored this way by stretching caused by gravitational pulling during locomotion, inertia, chemical modifications, and ATP powered sources to name a few. Titin unfolding occurs at varying rates over a very wide range of forces above >8 pN. By contrast, most of the stored mechanical energy is delivered back only over a small range of forces where the folding probability increases from 0 to 1 (< 6 pN) and the folding protein does large amounts of mechanical work. Thus, protein folding/unfolding is likely to operate as a mechanical battery where different types of energy sources are stored, and then converted back into contractile power. Given that titin is now known to be the third filament of muscle, determining if protein folding can deliver work quickly enough to match the power output of the myosin motors, is a central question to be answered. The mechanical power output of protein folding is a novel concept and thus has never been studied before. Cryptic cysteine residues are common in the elastic I band region of titin where they can oxidize to form intra-domain disulfide bonds, limiting the extensibility of an unfolding Ig domain. Here we use magnetic tweezers force spectroscopy to study the folding dynamics of a disulphide bonded modular titin protein operating in the physiological range, with the ability to control the oxidation state of the protein in real time. We show that the midpoint folding probability of the parent Ig domain reversibly shifts up from 4.0 pN to 12.8 pN upon oxidation. In this force range, the folding contraction dominates the elastic recoil of the protein, delivering stepwise mechanical work which depends on the oxidation state in an all-or-none manner. For example, the output power of a folding contraction at 6 pN goes from 0 zW to 6,000 zW upon introduction of the disulfide bond. This large amount of power is delivered by folding at forces where single molecular motors are typically stalled. From our observations we predict that during muscle contraction, activation of myosin II motors by Ca++ leads to a drop in the force experienced by titin, triggering delivery of mechanical power by titin folding. Thus, it seems inevitable now that the three filaments of the muscle sarcomere act in concert to both store and deliver mechanical power, revolutionizing our understanding of the molecular mechanisms of muscle contraction.

Wednesday 24th July

Plenary Lecture 7
O-007
Nonequilibrium stresses and fluxes: composition organization at the cell surface and organelle morphodynamics
M. Rao.
Simons Centre for the Study of Living Machines
National Centre for Biological Sciences – TIFR
Bengaluru 560065, India
Abstract : I will discuss two levels of cellular organisation that are predominantly driven by nonequilibrium stresses and fluxes of material. (a) The physical organization and dynamics of many signalling molecules at the cell surface are locally controlled by their coupling to the thin cortical actomyosin layer, which actively drives local cell membrane composition and shape. In many cases, this organisation involves a collaboration with specific lipids on the cell surface. While this active organisation occurs constitutively, it gets locally amplified by external cues. These studies are revealing an elaborate mechanism of local composition control. (b) A defining feature of eukaryotic cells is the appearance of functional compartmentalisation in the form of membrane-bound organelles. Cellular compartments, such as the Endosomal or Golgi systems, are subject to stochastic trafficking that involves active fission and fusion of cargo vesicles. These exhibit stable structures driven far from equilibrium. I will discuss our recent attempts to understand the physical basis of nonequilibrium control of size, shape, spatial position and number of compartments, with special emphasis to the Golgi system.

Wednesday 24th July

Plenary Lecture 8
O-008
MINFLUX Nanoscopy: Superresolution post Nobel
Stefan W. Hell
Max Planck Institute for Biophysical Chemistry, Göttingen, GER
Max Planck Institute for Medical Research, Heidelberg, GER
The 2014 Nobel Prize in Chemistry was awarded “for the development of super-resolved fluorescence microscopy”. More than 125 years after Ernst Abbe’s definition of the supposedly insurmountable diffraction resolution limit, fluorescence “microscopes crossed the threshold”, as the Nobel poster put it. The result has been the breathtaking development of far-field optical super-resolution microscopy or, in short, ‘nanoscopy‘ as an entire field over the past years.
A fresh look at the foundations [1] shows that an in-depth description of the basic principles of nanoscopy spawns new powerful concepts such as MINFIELD [2], MINFLUX [3] and DyMIN [4]. Although they differ in some aspects, these concepts harness a local intensity minimum (of a doughnut or a standing wave) for determining the coordinate of the fluorophore(s) to be registered. Most strikingly, by using an intensity minimum of the excitation light to establish the fluorophore position, MINFLUX nanoscopy has obtained the ultimate (super)resolution: the size of a molecule [3]. The talk will highlight recent developments.
[1] Hell, S.W. Far-Field Optical Nanoscopy. **Science** 316, 1153-1158 (2007).
[2] Göttfert, F., Pleiner, T., Heine, J., Westphal, V., Görlich, D., Sahl, S.J., Hell, S.W.
Strong signal increase in STED fluorescence microscopy by imaging regions of subdiffraction extent. **PNAS** 114, 2125-2130 (2017).
[3] Balzarotti, F., Eilers, Y., Gwosch, K. C., Gynnå, A. H., Westphal, V., Stefani, F. D., Elf, J., Hell, S.W. Nanometer resolution imaging and tracking of fluorescent molecules with minimal photon fluxes. **Science** 355, 606-612 (2017).
[4] Heine, J., Reuss, M., Harke, B., D’Este, E., Sahl, S.J., Hell, S.W. Adaptive-illumination STED nanoscopy. **PNAS** 114, 9797-9802 (2017).

Sunday 21st July

PROTEIN FOLDING

O-009

Understanding alpha-synuclein amyloid self-assembly and its associated toxicity

Nunilo Cremades

Institute of Biocomputation and Physics of Complex Systems (BIFI), Joint Unit BIFI-Institute of Physical Chemistry "Rocasolano" (CSIC), University of Zaragoza, 50018 Zaragoza, Spain.

Many neurodegenerative disorders are characterised by the conformational change of normally soluble proteins or peptides into pathological conformers by a process of misfolding and self-assembly that leads to the formation of amyloid aggregates. Our recent studies on alpha-synuclein, the protein whose aggregation and deposition is associated with a number of neurodegenerative diseases collectively referred to as synucleinopathies, including Parkinson´s disease, support the idea that multiple aggregated species can be generated through diverse misfolding pathways during the process of amyloid aggregation of a given polypeptide, which possess different degrees of neuronal toxicity and infectivity [1,2]. We are elucidating some of the different mechanisms that lead to alpha-synuclein self-assembly and the formation of structurally different amyloid polymorphs, some of which could be involved in distinct pathologies and/or stages of the development of a particular disease, i.e. in the initial induction of neurotoxicity or in the propagation of pathology [3]. In addition, we have been able to trap and isolate some intermediate oligomeric species with significant biological relevance and determine the structural basis of their toxicity [4], which can help us to develop rational strategies based on amyloid toxicity for the early detection and treatment of synucleinopathies [5].

References

[1] Cremades N., *et al*. (2012). Direct observation of the interconversion of normal and toxic forms of alpha-synuclein. *Cell* 149, 1048-1059.

[2] Chen S.W., *et al*. (2015). Structural characterization of toxic oligomers that are kinetically trapped during alpha-synuclein fibril formation. *Proc. Natl. Acad. Sci USA*. 112, E1994-E2003.

[3] Cremades N. and Dobson C.M. (2018). The contribution of biophysics and structural studies of protein self-assembly to the design of therapeutic strategies for amyloid diseases. *Neurobiol. Dis*. 109, 178-190.

[4] Fusco G., *et al*. (2017). Structural basis of membrane disruption and cellular toxicity by alpha-synuclein oligomers. *Science* 358, 1440-1443.

[5] Perni M., *et al*. (2017). A natural product inhibits the initiation of alpha-synuclein aggregation and suppresses its toxicity. *Proc. Natl. Acad. Sci USA*. 114, E1009-E1017.

O-010

Dodging the crisis of folding proteins with non-trivial topology - new challenges

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University of Warsaw.

The mechanism of folding of deeply knotted proteins into their native structure is still not understood [1,2]. Current thinking about protein folding is dominated by the Anfinsen dogma stating that the structure of the folded proteins is uniquely dictated by the amino acid sequence of a given protein and that the folding is driven uniquely by the energy gained from interactions between amino acids that contact each other in the native structure of the protein. The role of ribosomes in protein folding was only seen as permitting the folding to progress from the N-terminal part of nascent protein chains. We propose here that ribosomes can participate actively in the folding of knotted proteins by actively threading nascent chains emerging from the ribosome exit channels through loops formed by a synthesized earlier portion of the same protein. Our simulations of folding of the protein with the deepest knot – Tp0624 [3] positively verify the proposed ribosome-driven active threading mechanism leading to the formation of deeply knotted proteins [4].

Suggested mechanism can be see here https://www.youtube.com/watch?v=wuUctgrQ0PA

[1] KnotProt: a database of proteins with knots and slipknots, M Jamroz, W Niemyska, EJ Rawdon, A Stasiak, KC Millett, P Sułkowski, JI Sulkowska, Nucleic Acids Res. (2014), 43: D306-D314.

[2] Dodging the crisis of folding proteins with knots, JI Sulkowska, P Sułkowski, JN Onuchic PNAS (2009) 106, 3119-3124

[3] Proteins' knotty problems, AI Jarmolinska, AP Perlinska, R Runkel, B Trefz, P Virnau, JI Sulkowska, Journal of Molecular Biology (2018) 431, 2

[4] Protein Knotting by Active Threading of Nascent Polypeptide Chain Exiting From the Ribosome Exit Channel P Dabrowski-Tumanski, M Piejko, S Niewieczerzal, S Stasiak, JI Sulkowska The Journal of Physical Chemistry B (2018) 122, 49, 11616-11625

O-011

The structural determinants of the toxicity of misfolded protein oligomers associated with neurodegenerative diseases

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The structural determinants of the toxicity of misfolded protein oligomers associated with neurodegenerative diseases

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Misfolded protein oligomers are thought to be the pathogenic species in protein deposition diseases. However, the morphological, structural and dynamic characteristics of these species causing cell toxicity are not entirely understood. We will show the characteristics of three pairs of toxic and nontoxic oligomers formed by $A\beta_{42}$, the peptide associated with Alzheimer's disease, α-synuclein, the protein associated with Parkinson's disease, and the model protein HypF-N.

Using a number of biophysical techniques, such as far-UV circular dichroism spectroscopy, Fourier-transform infrared spectroscopy, ANS, and ThT fluorescence assays, site-directed dye labelling, atomic force microscopy, etc. we will show the morphological characteristics, size, degree of solvent-exposed hydrophobic groups and secondary structure content of the six oligomeric species. In addition, using cell toxicity assays, such as the MTT reduction assay, intracellular Ca^{2+} and ROS measurements, calcein release measurements, the caspase-3 activity assay, etc. we will show the toxicity of the various species to cultured neuronal cells and primary neurons.

In each of the three pairs, the species found to be toxic have the same size, but a higher solvent-exposed hydrophobicity, relative to the biologically inert species. Using the model protein HypF-N, the toxic species can be converted into nontoxic ones by clustering them into larger assemblies in the presence of chaperones. By contrast, the toxicity of the oligomers was not found to be correlated with their β-sheet structure content.

The analysis of the three pairs of toxic and nontoxic oligomers of $A\beta_{42}$, α-synuclein and HypF-N indicate that (i) oligomer toxicity correlates positively with the surface hydrophobicity of the oligomers, (ii) negatively with their size and (iii) indicate that a β-sheet architecture in the oligomers is not a structural determinant of their toxicity, despite common assumptions.

O-012 (P-001)

Prion soft amyloid core driven self-assembly of globular proteins into bioactive nanofibrils

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Amyloids have been exploited to build up amazing bioactive materials. In most cases, short synthetic peptides constitute the functional components of such materials. The controlled assembly of globular proteins into active amyloid nanofibrils is still challenging, because the formation of amyloids implies a conformational conversion towards a β-sheet-rich structure, with a concomitant loss of the native fold and the inactivation of the protein. There is, however, a remarkable exception to this rule: the yeast prions. They are singular proteins able to switch between a soluble and an amyloid state. In both states, the structure of their globular domains remains essentially intact. The transit between these two conformations is encoded in prion domains (PrDs): long and disordered sequences to which the active globular domains are appended. PrDs are much larger than typical self-assembling peptides. This seriously limits their use for nanotechnological applications. We have recently shown that these domains contain soft amyloid cores (SACs) that suffice to nucleate their self-assembly reaction. Here we genetically fused a model SAC with different globular proteins. We demonstrate that this very short sequence act as minimalist PrDs, driving the selective and slow assembly of the initially soluble fusions into amyloid fibrils in which the globular proteins keep their native structure and display high activity. Overall, we provide here a novel, modular and straightforward strategy to build up active protein-based nanomaterials at a preparative scale.

O-013 (P-002)
Unravelling the details of the conformational cycle of the Hsp90 chaperone
K. Tych[1], M. Jahn[1], H. Girstmair[1], T. Hugel[2], J. Buchner[1], M. Rief[1].
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The molecular chaperone, heat shock protein 90 (Hsp90), is a large, dimeric ATP-driven molecular machine. It is essential in eukaryotes and is known to function as a part of many complexes. These complexes are involved, amongst other things, in the regulation of cell division and signalling [1]. As such, Hsp90 has emerged as an important target for the development of cancer therapeutics [2].
Despite the importance of this fascinating molecular machine, the exact mechanisms by which Hsp90 undergoes conformational changes, the roles of nucleotides, different domain orientations and how these influence the function of the chaperone in complex with its co-chaperones are mostly unknown.
Using a custom-built single molecule optical trapping experimental setup, we have so far elucidated the mechanism by which this large protein folds [3], characterised the role of its flexible charged linker region [4], performed a detailed comparison of Hsp90 orthologues [5] and uncovered the role of nucleotide binding in the stability of the dimeric interface of Hsp90 [6]. Current research efforts are building on these findings to study the effect of co-chaperones on the conformational cycle and single molecule mechanics of Hsp90.
[1] A. J. McClellan, et al. Cell 131, 121–135, (2007).
[2] J. S. Isaacs, et al. Cancer cell 3, 213–217, (2003).
[3] M. Jahn, et al. Proc. Nat. Acad. Sci. 113, 1232–1237, (2016).
[4] M. Jahn, et al. Proc. Nat. Acad. Sci. 111, 17881–17886, (2014).
[5] M. Jahn and K. Tych, et al. Structure 26(1), 96-105 (2018)
[6] K. Tych, et al. J. Phys. Chem. B 112(49), 11373-11380 (2018)

O-014 (P-003)
Strategically positioned slow codons support protein folding
M. Perach[1], Z. Zafrir[2], T. Tuller[2], O. Lewinson[3].
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The genetic code is redundant, where several alternative codons code for a single amino acid. Synonymous codons are translated at different rates by the ribosome, with some codons considered to be “fast-translating” and others to be “slow- translating”. Since many proteins fold co-translationally, synonymous codon usage, and the resulting translation speed, was suggested to influence nascent chain folding. A proposed underlying mechanism suggests that pausing between the translation of independently-folding units supports their correct folding. The importance of codon usage to protein folding and function was shown for a few individual cases, but a clear and general understanding of the phenomenon is still missing. In this work, we aim to identify conserved regions of slow codons that are important for correct protein folding and function.
To this end, we analyzed 1,115 orthologue protein groups from *E. coli* and *B. subtilis* to identify slow codon stretches that appear to be important for a specific 3-D protein fold. Based on this analysis, we selected several proteins for experimental validation. We generated a modified version of each gene in which the slow codon stretch was replaced with a synonymous fast one. We compared the modified version to the wild type one in a set of experiments checking expression, solubility and function. Preliminary results show that in several cases, especially in oligomeric proteins, strategically positioned slow codons are important for protein stability and function, suggesting that biased codon usage is a general mechanism that assists correct protein folding.

Sunday 21st July

DNA ARCHITECTURE AND GENE REGULATION

O-015
DNA release from histones in nucleosome core particles: measurements and models
L. Pollack.
Cornell University.
For efficient storage, DNA is tightly packaged in nucleosome core particles (NCPs). However, it must also be readily accessible for processing. My group is interested in understanding how DNA release from NCPs is affected by factors including histone variants, DNA sequence variation or the action of partner molecules, such as remodelers or chaperones. We apply new methods to detect the dynamic global structure(s) of the DNA in the presence of protein partners. Contrast variation small angle x-ray scattering is an ideal probe of the conformation of the nucleic acid component of a protein-nucleic acid complex and can be implemented in either static or time-resolved studies. Our first studies probed the equilibrium and time dependent conformations of DNA in NCPs as a function of increasing salt, which facilitates release. We applied an ensemble optimization method to identify plausible structural ensembles present under each different solution condition or at different times during a time-resolved release experiment. At first, our studies employed the tightly positioning Widom 601 DNA sequence and canonical histones. Recently this work has expanded to account for DNA sequence, histone variants, and the addition of chaperones, such as the Chd-1 remodeler.

O-016
Gene control with a “twist”: Exploring the dynamic chromatin landscape Gene control with a “twist”: Exploring the dynamic chromatin landscape
B. Fierz.
EPFL.
The dynamic organization of the eukaryotic genome into chromatin is integral to genome regulation. Chromatin structure and dynamics, modulated by histone post-translational modifications (PTMs) as well as architectural proteins, dictate DNA access for transcription factors and the gene expression machinery. While of key importance, the detailed mechanisms of DNA access regulation by chromatin dynamics are however still poorly understood.
We dissect chromatin signaling on the single-molecules scale, combining chemical biology approaches and mechanistic biophysics. We recently developed a single-molecule FRET (smFRET) approach to directly observe the dynamic architecture of chemically defined chromatin fibers. We find that local chromatin organization is based on tetranucleosome units, which undergo structural fluctuations on the micro- to millisecond timescale. These dynamics are regulated by histone PTMs and linked to function. Indeed, internal chromatin dynamics are exploited by transcription factors (TF) to invade chromatin structure. Employing single-molecule fluorescence imaging we could observe how a yeast pioneer TF, Rap1, binds its target promoter in compact chromatin. Importantly, we show that Rap1 binding opens chromatin fiber structure by inhibiting nucleosome-nucleosome contacts. Finally, we reveal that Rap1 collaborates with the chromatin remodeler RSC to shift promoter nucleosomes, paving the way to form long-lived bound states on now exposed DNA.
In summary, our results provide a mechanistic view of how a pioneer TF gains access and opens chromatin, thereby establishing an active promoter architecture and controlling gene expression.

O-017

Chromosome organization in living human cells revealed by single nucleosome imaging

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National Institute of Genetics.

Chromatin organization and dynamics play a critical role in gene transcription. However, how they interplay remains unclear. To approach this issue, the single-nucleosome imaging is a powerful technique because local chromatin motion reflects chromatin organization in living cells (1). Using the imaging technique, we investigated genome-wide chromatin behavior under various transcriptional conditions in living human cells. While transcription by RNA polymerase II (RNAPII) is generally thought to need more open and dynamic chromatin, surprisingly, we found that active RNAPII globally constrains chromatin movements. RNAPII inhibition or RNAPII rapid depletion by AID system released the chromatin constraints and increased chromatin dynamics. Perturbation experiments of P-TEFb clusters, which are associated with active RNAPII, had similar results. On the other hand, treatment of RNA polymerase I inhibitor or splicing inhibitor did not have significant effect on the chromatin dynamics. Furthermore, chromatin mobility also increased in resting G0 cells and UV-irradiated cells, which are transcriptionally less active. Our results demonstrated that chromatin is globally stabilized by loose connections through active RNAPII, which is compatible with models of classical transcription factories or liquid droplet formation of transcription-related factors. Together with our computational modeling, we propose the existence of loose chromatin domain networks for various intra-/interchromosomal contacts via active RNAPII clusters/droplets (2).

References:

Nozaki, T. et al. (2017) Dynamic organization of chromatin domains revealed by super-resolution live-cell imaging. Molecular Cell. 67, 282-293.
Nagashima, R. et al. (2019) Single nucleosome imaging reveals loose genome chromatin networks via active RNA polymerase II. Journal of Cell Biology.
doi: 10.1083/jcb.201811090

O-018 (P-037)

Non-specific action of polyamines leads to specific changes in structure and function of DNA

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Polyamines are found in all living organisms, where they are involved in many cellular processes including cell growth and proliferation. The most commonly occurring natural polyamines are putrescine, spermidine and spermine, and they exhibit linear-chain skeleton. In addition to these common polyamines, hyperthermophiles require long and branched-chain polyamines for growth at high temperatures. Therefore, structural differences among polyamines are expected to play an important role in the mechanism of self-control of living state.

Here, we report the effect of various polyamines including linear- and branched-chain isomers on the higher-order structure of DNA based on the results of our experiments. From atomic force microscopic (AFM) measurements, it was found that the branched polyamine induces a mesh-like structure on DNA at room temperature. Interestingly, with increasing temperature up to 80°C, DNA molecules tend to unwind, and form multiple nano-loops with a diameter of 10–50 nm along the DNA strand. On the other hand, linear polyamines tend to form flower-like structure, suggesting the parallel alignment of DNA and does not cause nano-loop structure at higher temperatures.

We have also studied the effect of various polyamines on the activity of gene expression through *in vitro* luciferase assay. It was revealed that gene expression activity is completely suppressed accompanying the folding transition of DNA into a compact state. It was also found that the activity of gene expression is enhanced several times at the polyamine concentrations just below the critical concentration to induced the compaction of DNA.

Based on these experimental observations, we may argue the biological role of polyamines in relation to the structural change of genomic DNA molecules in living cellular environment.

T. Nishio, et al., Branched-Chain Polyamine Found in Hyperthermophiles Induces Unique Temperature-Dependent Structural Changes in Genome-Size DNA. *ChemPhysChem*, 19, 2299(2018).
Y. Kashiwagi, T. Nishio, et al., Repulsive/attractive interaction among compact DNA molecules as judged through laser trapping: Difference between linear- and branched-chain polyamines. *Colloid Polym Sci.* https://doi.org/10.1007/s00396-018-4435-3 1-11 (2018)

O-019 (P-038)

Simulating the binding of Pioneer Transcription Factors to the nucleosome

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Transcription factors are proteins that bind to DNA to regulate gene expression. In most cases, accessibility to DNA is a prerequisite for their function. However, in the nucleus the DNA is packed into chromatin, which is often inaccessible. The fundamental unit of chromatin is the nucleosome, in which 147 DNA basepairs are wrapped around a core of eight histone proteins.

Interestingly, a series of transcription factors, known as pioneers, are able to bind to closed chromatin states, recognizing their binding sites even in the presence of nucleosomes. They can help open chromatin, increase DNA accessibility, and support binding of other transcription factors. For example, Oct4, a master regulator of stem cell pluripotency, is able to bind native nucleosomes in a sequence specific manner.

To understand the nucleosome properties that are involved in the binding of Oct4, we performed all-atom simulations of three nucleosomes with different DNA sequences, in presence and absence of Oct4. By comparing three sequences with characteristic Oct4 binding profiles, we identified differences in dynamics and structural properties of the three nucleosomes, most of which are located in the regions known to be important for nucleosome unwrapping. We also characterized how the addition of Oct4 alters the dynamics of the nucleosomes, and which are the relevant nucleosome properties that explain the binding and behavior change. These findings help us understand the role of pioneer transcription factors in the binding ofclosed chromatin.

O-020 (P-039)

Dependence of DNA Persistence Length on Ionic Strength and Ion Type

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The rigidity of double-stranded DNA plays a major role in the structuring of the chromosome and thus in the expression of genes, as well as in nanotechnology where DNA is used as a building block. But how is this rigidity influenced by the presence of different types of ions? In this work, we responded both experimentally and theoretically to this question. Thanks to the massive parallelization of the single-molecule technique of Tethered Particle Motion (TPM), we measured the dependence of persistence length, reflecting the stiffness of the polymer DNA, over a wide range of ions and salt concentrations. We demonstrated a unique decay for monovalent or divalent metal ions perfectly described by recent theories, which take into account the non-linear electrostatic effects as well as the finite diameter of the DNA. Our findings, published in *Phys. Rev. Lett.* **122** 028102 (2019), make it possible to predict conformational changes of complex structures formed by DNA both *in vitro* and *in vivo*.

Sunday 21st July
BIOPHYSICS OF CYTOSKELETON

O-021
Mind the gap: polyampholytes meet the neuronal skeleton
R. Beck.
Tel Aviv University.
Mind the gap: polyampholytes meet the neuronal skeleton
Prof. Roy Beck, School of Physics and Astronomy, Tel-Aviv University
A key hurdle in modeling biological systems originates from the complex interactions between multiple components. Physical models and experiments often reduce the number of components aiming to address the fundamental mechanisms. Nevertheless, in most cases, the inherent heterogeneity is an essential ingredient in the biological context. Similar to the skeleton of bridges and towers, the neuronal skeleton contains well-aligned arrays of rigid beams, made out of proteins, which resist deformations. However, neurons also contain many spaghetti-like flexible charged proteins, which protrude from the rigid beams and are otherwise free to move in between them. These flexible components naturally do not resist deformations very strongly, which raises the following fundamental questions: what can nature gain by such architecture? Can a simplified physical model capture the complexity of disordered proteins interaction? In the talk, I will try to answer this and other related questions on the advantages of disordered polyampholyte proteins have in functional biological systems, and on how the delicate balance between electrostatics and entropy bridge the gap between physics and biology.[1–5]

[1] Kornreich, M. *et al.* Neurofilaments Function as Shock Absorbers: Compression Response Arising from Disordered Proteins. *Phys. Rev. Lett.* **117,** 148101 (2016).
[2] Laser-Azogui, A. *et al.* Neurofilament assembly and function during neuronal development. *Curr. Opin. Cell Biol.* **32,** 92 (2015).
[3] Kornreich, M. *et al.* Order and disorder in intermediate filament proteins. *FEBS Lett.* **589,** 2464 (2015).
[4] Malka-Gibor, E. *et al.* Phosphorylation-Induced Mechanical Regulation of Intrinsically Disordered Neurofilament Proteins. *Biophys. J.* **112,** 892 (2017).
[5] Kornreich, M. *et al.* Composite bottlebrush mechanics: α-internexin fine-tunes neurofilament network properties. *Soft Matter* **11,** 5839 (2015).

O-022
Reconstitution of basic mitotic spindles in cell-like confinement
M. Dogterom.
Delft University of Technology.
Reconstitution of basic mitotic spindles in cell-like confinement
Bipolar organization of the mitotic spindle is the result of forces generated by dynamic microtubules and associated proteins in interaction with chromosomes and the cell boundary. Biophysical experiments on isolated spindle components have provided important insights into the force-generating properties of different components, but a quantitative understanding of the force balance that results from their concerted action is lacking. Here we present an experimental platform based on water-in-oil emulsion droplets that allows for the bottom-up reconstitution of basic spindles. We find a typical metaphase organization, where two microtubule asters position symmetrically at moderate distance from the mid-zone, is readily obtained even in the absence of chromosomes. Consistent with simulations, we observe an intrinsic repulsive force between two asters that can be counterbalanced alternatively by cortical pulling forces, anti-parallel microtubule crosslinking, or adjustment of microtubule dynamics, emphasizing the robustness of the system. Adding motor proteins that slide anti-parallel microtubules apart drives the asters to maximum separation, as observed in cells during anaphase. Our platform offers a valuable complementary approach to *in vivo* experiments where essential mitotic components are typically removed, instead of added, one by one.

O-023
The spindle is a composite of two permeating polar gels
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During cell division, correct segregation of chromosomes depends on the ability of microtubules to self-organize into a bipolar spindle. Our current understanding of large spindle assembly is based on the interplay between spatial microtubule nucleation and microtubule transport. It has been recently shown that branching nucleation is the main mechanism by which microtubule nucleation occurs away from centrosomes. However, microtubule branching naturally leads to explosive waves of microtubule nucleation that rapidly travel away from initially created microtubules at a speed much faster than the microtubule flux velocity. This behavior should normally result in spindles with outward polarity (or inverted polarity) originating from the chromosomes, where microtubule nucleation is highest. Yet, spindles manage to robustly assemble bipolar spindles despite branching nucleation and slow microtubule flux. Here, we used experiment and theory to study how spindles from Xenopus laevis egg extracts acquire the proper microtubule organization despite the slow microtubule transport and branching nucleation. We found that microtubules in spindles self-organize into two mechanically distinct microtubule networks that undergo a gelation transition. This gelation allows the propagation of long-range extensile stress from the center of the spindle that push these two gels apart. This process transports microtubules independently of their local polarity environment, and thus local sorting. The emergence of two gelated microtubule networks that push against each other explains how microtubules can be sorted out into the proper bipolar structure in the presence of branching nucleation despite the slow microtubule transport.

O-024 (P-053)
Structure dissection of a contractile phage tail tube
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Bacteriophages are infectious nano-objects that inject their genetic material into the host via complex molecular machineries. To cross the biological barriers encompassing the extracellular space and the bacterial membrane, phages expose a tail, composed of several proteins, which executes numerous functions during infection. The particularity of the *Myoviridae* bacteriophages resides in the additional sheath, surrounding the inner tail tube. This contractile sheath pushes the inner tube across the host membrane acting therefore like a molecular syringe transferring the viral genome into the host cell. This complex machinery increases the infection efficiency in this type of phages, containing a higher amount of DNA content to transfer than other phage families.
We here elucidate the structural basis and the molecular mechanisms of a *Myoviridae* phage inner tail tube. Among them, the tail tube of bacteriophage Mu stands out because the molecular subunit protein of the tube assembly (gpM) fulfils its biological function with the minimal structural motif. We investigate the structural features of the Mu tail tube using a combination of solid-state NMR, liquid-state NMR and cryo-EM performed on in vitro assembled filaments. We show that the subunit protein gpM is partially folded in its monomeric state and adopts a globular beta-rich structure in a very rapid polymerisation process towards the native assembly. Our results suggest that the minimal construction motif in gpM conserves the structural features shared also by the *Caudovirales* phages. The apparent resemblance of these structures supports the idea that the 3D fold of the tail tube protein is conserved despite the poor sequence conservation.

O-025 (P-054)

Ultrafast tracking reveals the function of structural domains of single proteins

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Protein-protein interactions and their dynamics have been extensively studied mostly by fluorescence. Fluorescent labels are usually incorporated to discriminate biological processes under study from the complex background or visualize small molecules.

To overcome spatio-temporal limitations of fluorescence [1], we employ interferometric detection of scattering (iSCAT). iSCAT detects the light scattered on a protein molecule or a scattering label via its interference with a reference wave, e.g. light partially reflected at a glass coverslip. By these means, it is possible to image very small scattering labels [2] or even unlabeled proteins [3].

PRC1 (protein regulator of cytokinesis 1) belongs to the Ase1/MAP65/PRC1 family of microtubule-associated proteins (MAPs) and plays an important role in cytokinesis. These proteins serve as rigid connections between MTs and can interact with other proteins. Each structural domain of the PRC1 protein seems to play its role but details remain unclear [4].

We studied two different domains via a specific attachment of scattering labels. 3D maps of PRC1 on a single microtubule were measured and analyzed. We observe that each domain has a different behavior and we propose a new functional model of the interaction.

References

[1] S. Weisenburger and V. Sandoghdar, "Light microscopy: an ongoing contemporary revolution," *Contemp. Phys.*, vol. 56, no. 2, pp. 123–143, 2015.

[2] K. Holanová, M. Vala, and M. Piliarik, "Optical imaging and localization of prospective scattering labels smaller than a single protein," *Opt. Laser Technol.*, vol. 109, pp. 323–327, 2019.

[3] M. Piliarik and V. Sandoghdar, "Direct optical sensing of single unlabelled proteins and super-resolution imaging of their binding sites," *Nat. Commun.*, vol. 5, p. 4495, 2014.

[4] R. Subramanian *et al.*, "Insights into antiparallel microtubule crosslinking by PRC1, a conserved nonmotor microtubule binding protein," *Cell*, vol. 142, no. 3, pp. 433–443, 2010.

O-026 (P-055)

Nucleotide and Osmolyte Induced Folding of FtsZ from *Staphylococcus aureus*

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The essential bacterial cell division protein FtsZ uses GTP binding and hydrolysis to assemble into filaments that treadmill around the Z-ring, guiding septal wall synthesis and cell division. FtsZ is a structural homolog of tubulin and a target for discovering new antibiotics. We have found that purified FtsZ monomers from the pathogen *S. aureus* (SaFtsZ) require bound nucleotide to keep a folded structure in solution. Nucleotide-less apo-SaFtsZ is essentially unfolded, as assessed by NMR and CD, and crystallizes in a non-native structure. Addition of GTP (≥ 1mM) dramatically shifts the equilibrium towards the folded protein, rendering SaFtsZ active for assembly. Other mesophile FtsZs are also stabilized by nucleotide binding. Apo-SaFtsZ also folds in CD with glycerol, and other stabilizing osmolytes such as TMAO, ethylene glycol, betaine and proline, which enable high-affinity GTP binding (K_d 20 nM in 3.4 M glycerol, determined by ITC) similar to thermophile stable FtsZ. We have devised a competition assay to detect any molecules that bind overlapping the nucleotide site of SaFtsZ or EcFtsZ, employing glycerol-stabilized apo-FtsZs and the specific fluorescence anisotropy change of *mant*-GTP upon dissociation from the protein. This robust assay provides a basis for robotic screening for high affinity GTP-replacing ligands, which combined with phenotypic profiling, may yield the next generation of FtsZ-targeting antibacterial inhibitors. Finally, by solving several SaFtsZ crystal structures, we identified a cavity behind the nucleotide-binding pocket that harbors distinct compounds, opening the way for designing extended inhibitors.

Sunday 21st July

MECHANOBIOLOGY

O-027

Mechanically and biochemically induced pathways regulating integrin-dependent cell adhesion

D. Mueller.

Nico Strohmeyer, Mitasha Bharadwaj, Patrizia Spoerri, Reinhard Fässler and Daniel J. Müller

Integrin-mediated sensing of the biochemical and biophysical properties of the extracellular matrix (ECM) allows cells to control adhesion and signaling. Currently, the understanding emerges on how different integrins binding to the same ECM ligand cooperate during initiating adhesion and how cells sense and respond to mechanical cues sensed during adhesion initiation. First, we will report how fibroblasts during initiating adhesion respond to mechanical load by strengthening integrin-mediated adhesion to fibronectin in a biphasic manner. In moderate load regime, fibronectin-engaged α5β1 integrins sense mechanical load via catch bonds and immediately trigger signaling cascades. This signaling activates additional integrins to bind fibronectin and to strengthen cell adhesion. If the mechanical load applied to fibronectin-bound α5β1 integrins exceeds a threshold, the bond properties of the individual integrins change and fibroblasts fail to activate additional integrins. This unique response to mechanical load regulates integrin-mediated adhesion much before visible adhesion clusters assemble. Additionally, we will report that αVβ3 integrins first outcompete α5β1 integrins for ligand binding and later induce signaling cascades that increases α5β1 integrin clustering. Finally, we will discuss how G-protein coupled receptors regulate the conformational equilibrium of α5β1 integrins and thereby regulate the adhesion formation of fibroblasts to fibronectin. The regulation of α5β1 integrins by GPCRs translates into changes in the spreading and migratory behavior of fibroblasts. The discussed results highlight how fibronectin-binding integrins coordinate adhesion initiation of fibroblasts to fibronectin via diverse signaling pathways and how these can be used to control cellular attachment

O-028

Molecular to cellular mechanics at short timescales

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The mechanical properties of living cells provide structural stability and mechanical flexibility, crucial for their function. Thus, molecular understanding of the mechanics of the cell is relevant to understand biological function. Atomic force microscopy (AFM) [1]) is a unique technology that combines nanometric-imaging capabilities with piconewton force resolution. While conventional AFM has been extensively used to probe cell mechanics, its time resolution is limited to a few milliseconds [2]. We have recently adapted high-speed AFM (HS-AFM) [3] to probe the mechanics of individual cytoskeletal proteins and adhesion complexes at microsecond time resolution, providing a more detailed description of the unfolding mechanism [4–6]. We have also adapted HS-AFM to probe the viscoelastic properties of living cells [7]. Here, I will first introduce HS-AFM for force measurements on single cytoskeleton molecules. I will then show that probing cell mechanics at the shortest time scales (~10 μs) provides a description of the response of the ultimate components of the cytoskeleton. At high frequencies, cells exhibit rich viscoelastic responses that reflect the state of the cytoskeleton filaments. Compared to benign cancer cells, malignant cells revealed a remarkably different scaling law at short timescales compatible with a more tensed cytoskeleton. Microrheology over a wide dynamic range provides mechanistic understanding of cell mechanics and a univocal fingerprint, applicable to diagnosis or prognosis of disease. Finally, I will discuss the application of HS-AFM to determine other mechanical behavior of living cells, such as poroelasticity, with microsecond time resolution.

References

[1] G. Binnig, C. F. Quate, and C. Gerber, Phys. Rev. Lett. **56**, 930 (1986).

[2] M. Radmacher, R. W. Tilmann, and H. E. Gaub, Biophys. J. **64**, 735 (1993).

[3] T. Ando, N. Kodera, E. Takai, D. Maruyama, K. Saito, and A. Toda, Proceedings of the National Academy of Sciences **98**, 12468 (2001).

[4] F. Rico, L. Gonzalez, I. Casuso, M. Puig-Vidal, and S. Scheuring, Science **342**, 741 (2013).

[5] F. Rico, A. Russek, L. González, H. Grubmüller, and S. Scheuring, PNAS **116**, 6594 (2019).

[6] H. Takahashi, F. Rico, C. Chipot, and S. Scheuring, ACS Nano **12**, 2719 (2018).

[7] A. Rigato, A. Miyagi, S. Scheuring, and F. Rico, Nat Phys **13**, 771 (2017).

O-029

Mechanosensitive actomyosin network dynamics and migration plasticity in 3D environments

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Abstract:
Cells constantly experience mechanical forces within their tissue microenvironment that influence cytoskeletal organisation, cellular dynamics and migration plasticity. We were recently able to show that mechanical forces in confined environments trigger a rapid switch to amoeboid cell migration (Ruprecht et al., Cell 2015). Amoeboid cell transformation depends on the activation of myosin II motor proteins and stochastic fluctuations in cortical contractility that lead to spontaneous cellular symmetry breaking and fast amoeboid motility. Still, mechanisms regulating actin network dynamics and cortical contractility levels by physical parameters of the tissue microenvironment remain largely elusive. Using a combination of controlled 3D biomimetic ex vivo assays with superresolution imaging and live-cell in vivo imaging, we study mechanosensitive mechanisms regulating actomyosin network architecture and cellular dynamics in embryonic progenitor stem cells. We show that mechanical forces and tissue stress in 3D environments regulate the setpoint of actomyosin network contractility through the mechanosensitive activation of myosin II motor proteins, thereby modulating cellular biomechanics, polarization and migration competence via the physical tissue environment. We further present evidence that the nucleus is a core element of high-sensitivity force sensing and mechanotransduction in soft and low-adhesive embryonic tissues. The regulation of cellular biomechanics by the 3D physical tissue environment enables a fast and reversible cellular response to shape changes and mechanical loads and confers mechanical robustness and cellular plasticity to tissue stress conditions.

O-030 (P-071)

Activation of human aortic valve interstitial cells by local stiffness in Calcific Aortic Valve Disease

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Differentiation of valve interstitial cells (VICs) into pro-calcific cells is one of the central events in calcific aortic valve (AoV) disease (CAVD). A contribution of mechanical factors to such disease has been recently hypothesized due to the cyclic load that valve tissue withstands during cardiac cycle and the susceptibility of VICs to mechanical forces supporting their role as tissue 'mechanosensors'. Here, we investigated the role of mechanical compliance on activation of VICs obtained from patients with valve stenosis and valve insufficiency. We employed a 2D culture system onto polyacrylamide gels with atomic force microscopy (AFM) controlled elastic modulus to establish correlations between nuclear translocation of mechanically-activated transcription factor YAP and substrate mechanical compliance. We found that high stiffness levels determined YAP nuclear translocation with a different dynamics in cells from the two pathologic settings, thus revealing a pathology-specific VICs response to mechanical cues. In stenotic VICs, YAP nuclear translocation was associated to stiffness-dependent formation of stress fibers, adhesion complexes, and loading of **α**SMA onto F-Actin cytoskeleton. AFM force mapping performed along radial sections of human calcific valve leaflets identified, finally, areas with high and low levels of rigidity within a similar range to those controlling YAP nuclear translocation in vitro. Since VICs juxtaposed to these areas exhibited nuclear localized YAP, we conclude that subtle variations in matrix stiffness are involved in mechanosensing-dependent VICs activation and pathological differentiation in CAVD.

O-031 (P-072)

Role of Rab8A and Caveolin-1 in the interplay between cell mechanotransduction and cholesterol trafficking

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Rab8A is a small GTPase involved in the regulation of intracellular traffic and cell shape. Preliminary data from our lab and others showed that Rab8A and Caveolin-1 participate of complementary traffic pathways. Given the key role of Caveolin-1 in mechano-transduction, we hypothesized that Rab8A activity and localization might be sensitive to changes in membrane tension. Using cell stretching and osmotic shock experiments, we demonstrate that Rab8A is recruited to the plasma membrane upon changes in membrane tension. Moreover, PM rupture induced by laser ablation promoted Rab8A recruitment to the damaged site. Since Rab8A is involved in cholesterol efflux, we are exploring its function in lipid homeostasis, and the potential relevance of such function for mechanoadaptation. Preliminary data obtained from CRISPr/Cas9-edited Rab8AKO cells suggest that feedback systems regulating cholesterol homeostasis require Rab8A. Rab8AKO cells exhibit a blunted response to cholesterol deprivation (i.e. reduced induction of SREBP2 activation and of the upregulation of the mevalonate synthesis pathway). Further supporting an important role for Rab8A in the regulation of cholesterol trafficking, Rab8 over-expression *per se* normalizes cholesterol levels at the endosomal compartment in Cav1KO cells. Our observations support a model whereby Rab8 plays a key role in both mechano-transduction pathways and cholesterol homeostasis. We propose that Rab8A is a candidate regulator coupling mechanoadaptation to cholesterol homeostasis through mechanisms intersecting with Cav1 function.

O-032 (P-073)

The Mechanical Work of Vinculin Binding to Talin Regulates Vinculin Activation

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Talin is a multidomain protein that bridges the extracellular matrix with the active cellular cytoskeleton in focal adhesions; hence, talin is a molecular adaptor that works as a force sensor to allow the cell to communicate mechanically with its environment. Talin is a promiscuous protein, and a multitude of molecular partners are known to bind talin at different stages of the mechanosensitive signaling pathways. Among them, vinculin stands particularly out, given that it binds to at least 11 of the talin helices to recruit the actin filaments, and that all of these sites are cryptic; talin must be mechanically unfolded in order for vinculin to bind. Therefore, the interaction between talin and vinculin requires of force spectroscopy techniques to be studied. Previous work has demonstrated that vinculin locks talin in an unfolded state, and that this state can be reversed if the force is increased, suggesting a feedback regulatory mechanism in this mechanosensing pathway. However, the origin of this effect is unknown, and the force range under which the talin-vinculin complex is stable has not been accurately measured. Here, we focus on the R3 domain of talin, and use our magnetic tweezers force spectrometer to directly observe the binding of vinculin to talin over a large range of forces and timescales. Our experiments demonstrate that vinculin binding is always preceded by a ~3 nm contraction of the unfolded talin polypeptide, which, interestingly, does not occur steeply, but rather with a slow ~500 millisecond-long time-scale. This observation implies that vinculin binding to talin does mechanical work, which might be the cause for the inhibitory effect at high forces. We measure the probability of vinculin binding in the range from 4 to 50 pN, and determine that binding occurs optimally between 8.5 and 15 pN. Our results are accurately represented by a simple analytical theory that combines the unfolding dynamics of talin, and the mechanical work necessary to contract a stretched polymer, which establishes the physical grounds for the formation of this complex. We propose this to be a universal mechanism for ligand binding to substrates extended under force.

Sunday 21st July

MEMBRANE STRUCTURE AND DYNAMICS

O-033

Lessons Learned from Complex Mimics of Biological Membranes

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LESSONS LEARNED FROM COMPLEX MIMICS OF BIOLOGICAL MEMBRANES

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Lipid-only mimics of biological membranes serve as valuable platforms for studying the functional role of membrane lipids under chemically well-defined conditions. Of recent, we have focused on complex mimics of mammalian and bacterial plasma membranes with either lateral or transbilayer inhomogeneities. In particular, we have developed protocols for fabricating and analyzing asymmetric lipid vesicles, which are sufficiently stable and which are amenable for biophysical studies using diverse techniques. We have specialized on small-angle X-ray/neutron scattering combined with complimentary techniques to address leaflet specific structure and transbilayer coupling mechanisms. Complementary, we are currently developing tools for reliable estimates for intrinsic lipid curvatures, which are known to play a pivotal role in coupling to protein function. I will present recent research highlights resulting from these efforts and discuss some applications with a specific focus on antimicrobial peptides and their synergistic activity.

O-034

Regulating the dynamic interactions between herpes simplex viruses and cell-surface glycosaminoglycans

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Virus entry is a complex dynamic multistep process requiring a series of fine-tuned events mediating virus diffusion through the glycocalyx, its attachment to the cell membrane and lateral diffusion to the point of entry. A number of enveloped viruses, including herpes simplex viruses (HSV) attach to susceptible host cells via interaction between their glycoproteins and cell-surface glycosaminoglycans (GAGs).

In our work, we study the molecular and physical mechanisms modulating HSV binding, diffusion and release from cell-surface glycosaminoglycans. Using single virus tracking in combination with either in vitro minimal models of the cell surface or live cell microscopy, we gain insights into the modulatory function of protein glycosylation (the presence of mucin-like regions on viral glycoproteins) and interrogate the role of GAG sulfation in the process. We show that mucin-like regions found on the glycoproteins of HSV-1 and HSV-2 play an important role in modulating the interaction, an observation further supported by cell experiments. We further show that the diffusion of virions on the surface depends on the type of GAGs and their degree of sulfation. Taken together, our research contributes to a better understanding of the mechanisms underlying the interaction between a virus and the surface of its host. Such insights will without doubt facilitate the design of more efficient antiviral drugs or vaccines.

1. Altgarde, N., et al., *Mucin-like Region of Herpes Simplex Virus Type 1 Attachment Protein Glycoprotein C (gC) Modulates the Virus-Glycosaminoglycan Interaction.* Journal of Biological Chemistry , 2015.
2. Peerboom, N., et al., *Binding Kinetics and Lateral Mobility of HSV-1 on End-Grafted Sulfated Glycosaminoglycans.* Biophysical Journal, 2017.
3. Delguste, M., Peerbom, N. et al. *The mucin-like region of gC-1 regulates the binding strength and mobility of herpes simplex virus type 1 during initial attachment to glycosaminoglycans*. ACS Chemical Biology, 2019.
4. Peerboom, N., et al. Cell Membrane Derived Platform To Study Virus Binding Kinetics and Diffusion with Single Particle Sensitivity. ACS Infectious diseases, 2018.

O-035

IQGAP1 links phosphoinositide signaling to the cytoskeleton and promotes cytoskeleta reorganization

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IQGAP1 is a multi-domain protein that acts as a scaffold for multiple signaling pathways, including the PI3K signaling pathway. IQGAP1 has been suggested to generate the lipid messenger $PI(3,4,5)P_3$ by scaffolding the phosphoinositide kinases, PIPKIs and PI3K that generate $PI(4,5)P_2$ from phosphatidylinositol via PI(4)P to be selectively used by PI3K for $PI(3,4,5)P_3$ generation. The dynamics of the assembly of these proteins on IQGAP1 in intact, living cells is unknown. Fluorescence lifetime imaging microscopy (FLIM) in combination with Fluorescence Resonance Energy Transfer is a technique uniquely suited to probe the dynamic assembly of proteins on the scaffolding protein since it provides information about the interaction of proteins (FRET) along with spatiotemporal information. In this presentation we focus on investigating the sequence of binding of PI3K pathway components to IQGAP1 and we investigate the potential IQGAP1 mediated cross-talk between the PI3K and cdc42 signaling pathways. We report on the change in association between components of the $PI(3,4,5)P_3$ signaling system in real time in live cells under basal and stimulated conditions. We show that IQGAP1 integrates $PI(3,4,5)P_3$ mediated signaling with cell migration through direct physical interaction between PIPKIγ and PI3K, and the cytoskeletal proteins talin and Cdc42. Our work shows that IQGAP1- provides a scaffold that allows different signaling pathways to converge to regulate cytoskeletal rearrangements in response to extracellular stimuli.

O-036 (P-099)

Adsorption kinetics of pulmonary surfactant complexes purified from bronchoalveolar lavages of porcine lungs and human amniotic fluid

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Surface tension forces are overwhelmed by lung surfactant complexes at the alveolar air-liquid interface. Specifically, type-II pneumocytes are the cells involved in synthesizing and assembling surfactant into dehydrated and highly packed multilamellar structures called lamellar bodies. Upon stimulation, alveolar type II cells secrete lamellar bodies to the alveolar air-liquid interface where they replace pulmonary surfactant spent during respiratory cycling. Owing to compression-expansion respiratory cycles, a wealth of membrane structures, which are thought to be related to surfactant metabolism, may be observed in alveolar spaces. In this context, lamellar bodies have been demonstrated to exhibit outstanding adsorption properties since they keep the molecular determinants defining surfactant activity. Nevertheless, the molecular mechanism of lamellar bodies adsorption to the interface is not well-understood yet. Additionally, lung surfactant preparations used for both research and clinical applications are routinely purified from bronchoalveolar lavages of animal lungs, thus they are mainly composed by already used pulmonary surfactant complexes in which lamellar bodies are a minority component. Alternatively, a pulmonary surfactant purified from human amniotic fluid has been recently demonstrated to keep structural features of a freshly secreted surfactant.

In the present work, we compare interfacial adsorption kinetics of a surfactant purified from porcine lungs with those of a surface-active preparation obtained from human amniotic fluid using a Wilhelmy surface balance. In addition, Langmuir interfacial films were transferred onto glass coverslips and observed under epifluorescence microscopy. We observed meaningful differences between the behaviour of both surfactants that may be related to their contrasting structural features.

O-037 (P-100)

Microfluidic platforms for the handling, manipulation, and analysis of model cells

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Biological cells in their natural environment experience a variety of external forces such as fluidic shear stress, osmotic pressures, and mechanical loads. How the cell membrane itself responses to such forces is of great interest. Synthetic membranes such as giant unilamellar vesicles (GUVs) offer a reduced cell model, whereby individual components can be isolated and studied without interference from cellular complexity (Robinson 2019). However, being able to handle and apply forces to these delicate objects in a controllable manner is non-trivial. Therefore we present several microfluidic methods to capture, analyse and apply a variety of forces to GUVs. First we present novel methods for the capture and isolation of vesicles for membrane pore analysis (Robinson 2013; Yandralli & Robinson 2019). Next we discuss a device that contains micro-patterned electrodes which allow the application of electric fields and observations of subsequent membrane fusion (Robinson 2014). While membrane proteins are a crucial part of the cellular response to external stimuli, lipid rafts are thought to play an important role in the spatial organization of membrane proteins. To this end, we produce vesicles with membrane domains to model them and explore their behaviour in response to external forces. We use a valve-based system to apply precise fluidic shear stresses to vesicles (Sturzenegger, Robinson 2016). The final device comprises an integrated micro-stamp which can mechanically compress GUVs to study the effects that deformation has on the membrane and domains (Robinson 2019 *submitted*).

Yandrapalli, N., and Robinson, T. (2019). Ultra-high capacity microfluidic trapping of giant vesicles for high-throughput membrane studies. *Lab Chip* 19, 626–633.
Sturzenegger, F., Robinson, T., et al. (2016). Membranes under shear stress: visualization of non-equilibrium domain patterns and domain fusion in a microfluidic device. *Soft Matter* 12, 5072–5076.
Robinson, T., et al. (2014). Controllable electrofusion of lipid vesicles: initiation and analysis of reactions within biomimetic containers. *Lab Chip*, 2852–2859.
Robinson, T., et al. (2013). Microfluidic trapping of giant unilamellar vesicles to study transport through a membrane pore. *Biomicrofluidics* 7, 44105.
Robinson, T. (accepted 2019). Microfluidic handling and analysis of giant vesicles for use as artificial cells. *Advanced Biosystems*.

O-038 (P-101)

Quantified Efficiency of Membrane Leakage Events Relates to Antimicrobial Selectivity

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In many therapeutic strategies like killing pathogenic microbes by antimicrobial peptides, drug delivery, endosomal escape, and in disease-related processes such as apoptosis, the membrane is permeabilized.

Our concept for quantifying the efficiency of individual membrane leakage events replaces less stringent descriptions of dye leakage. The concept is applicable to many types of leakage events including thinning, defects, (toroidal) pores, or channels.

We measure release and fluorescence lifetime of a self-quenching dye. Apart from the dose-response, our analysis also quantifies the efficiency of individual leakage events. Additionally, cumulative leakage kinetics can indicate certain membrane permeabilization mechanisms. For example, applying our concept to three series of antimicrobial peptide analogues shows how the leakage mechanism and leakage efficiency of a given compound change with lipid composition. Thus, lipids play an important role for the selectivity of membrane permeabilization.

I will also point out aspects to consider when comparing leakage in vesicle of various sizes or cells, the possible occurrence of more than one type of leakage event, and artefacts from vesicle aggregation or fusion.

The concept for the quantitative description of leakage behaviour and understanding of leakage mechanisms aids the design and improvement of membrane-active antimicrobials.

Sunday 21st July

CELLULAR PROLIFERATION

O-039

Dynamics of bacterial swarms and biofilms

K. Drescher.

In nature, bacteria often engage in a range of collective behaviors, which coordinate activities of communities. In this presentation, I will demonstrate how two bacterial behaviors, swarming and biofilm formation, are unified by physical interactions, chemical signaling, and dynamical transitions. I will show how these collective behaviors arise from cell-cell interactions, and the physiological state of individual cells. Furthermore, I will introduce new experimental methods for investigating bacterial collective behaviors.

O-040

Biophysical Concepts Applied to Tumor Progression

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In recent years many physicists have begun working on aspects of cancer research. These aspects include the growth of the primary tumor, the transition to metastasis, the emergence of drug resistance to targeted therapies, and most recently the interaction of the tumor with the immune system. This talk will survey some of these recent developments from a perspective first elucidated in our opinion piece on how insights from bacterial colony growth can provide a conceptual framework for modeling tumor progression (see Ben-Jacob, Eshel, Donald S. Coffey, and Herbert Levine. "Bacterial survival strategies suggest rethinking cancer cooperativity." *Trends in microbiology* 20.9 (2012): 403-410). Remarkably, many of these models have found significant applicability and this will be illustrated by comparisons to recent experimental data on metastasis obtained in the cancer biology community.

O-041

How molecular forces shape bacterial biofilms

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Communities of bacterial cells can live together embedded within a slime-like molecular matrix as a biofilm. This allows the bacteria to hide from external stresses. A single bacterium can replicate itself and develop into a biofilm, and over time the bacterial cells in specific regions of the biofilm will start to interact with their neighbors in different ways. Very little is known about the link between physical interaction forces and the structure of colonies and biofilms. In my talk I will discuss how mechanical interactions between bacteria govern the structure and dynamics of bacterial biofilms. We have generated a molecular toolbox that allows tuning the interaction forces systematically. Using this toolbox, we address the question how differential interaction forces govern cell sorting, biofilm structures, and shape relaxations. Interestingly, moderate changes in attractive interactions between pairs of bacteria induce dramatic changes in the materials properties of bacterial colonies. Currently, we are evaluating how biofilms might benefit from the structural diversity that develops due to differential interactions.

O-042 (P-141)

Cell-size regulation induces long-term oscillations in population growth rate

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There are negative correlations between the generation time of a bacterial cell and those of its descendants. If a cell grows for a longer time than expected, its daughter cells (and subsequent descendants) will be larger at birth and have to compensate for their sizes by dividing slightly earlier than expected. This process is known as cell-size regulation. In this talk, I discuss the effect of these correlations on the dynamics of population growth of microorganisms. I show that any non-zero correlation that is due to cell-size regulation can induce long-term oscillations in the population growth rate. The population only reaches its steady state due to the often-neglected variability in the growth rates of individual cells. The relaxation time scale of the population to its steady state is determined from the distribution of single-cell growth rates and is surprisingly independent of the details of both the division process and the cell-size regulation. I propose an experimental method to measure single-cell growth variability by observing how long it takes for the population to reach its steady state, a measurement that is significantly easier and less biased than single-cell measurements.

O-043 (P-142)

Molecular architecture of bacterial amyloids in Bacillus biofilms

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The formation of biofilms provides structural and adaptive bacterial response to the environment. In *Bacillus* species, the biofilm extracellular matrix is composed of exopolysaccharides, hydrophobins and several functional amyloid proteins.

We report the molecular architecture of *Bacillus subtilis* and pathogenic *Bacillus cereus* functional amyloids, using multi-scale approaches such as solid-state NMR, electron microscopy, X-ray diffraction, DLS, ATR-FTIR and immune-gold labeling. Solid-state NMR data reveal that the major amyloid component TasA in its fibrillar amyloid form contain β-sheet and α-helical secondary structure, suggesting a highly non-typical amyloid architecture, and species variability between *B. subtilis* and *B. cereus*. Proteinase K digestion experiments indicate the amyloid moiety is approximately ~100 amino-acids long, and subsequent solid-state NMR and FTIR signatures for *Bacillus subtilis* and *Bacillus cereus* TasA filaments highlight a conserved rigid amyloid core albeit with substantial differences in structural polymorphism and secondary structure composition. Structural analysis and cross-seeding data on the accessory protein TapA in *B. subtilis* and its counterpart CalY in *B. cereus* reveal a catalyzing effect between the functional amyloid proteins and a common structural architecture, suggesting a co-assembly in the context of biofilm formation.

Our findings highlight non-typical amyloid behavior of these bacterial functional amyloids, underlining structural variations between biofilms even in closely related bacterial species.

O-044 (P-143)

Microviscosity of bacterial biofilm matrix characterized by fluorescence correlation spectroscopy and single particle tracking

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Bacterial biofilms are surface-adherent communities of bacteria surrounded by an extracellular polymeric substance (EPS) consisting of secreted polysaccharides and other biomolecules. In healthcare settings bacterial biofilms represent a severe threat, causing chronic infections and contamination of medical devices. To remove biofilms, multiple strategies have been developed, e.g. treatment with antibiotics or bacteriophages, which are advantageous to specifically target bacterial species. In this context, it remains unclear to which extent the EPS matrix imposes a physical barrier to the transport of bacteriophages through the biofilm. To address this question, we have reconstituted the EPS matrix of the bacterium *Pantoea stewartii*, responsible for a severe disease of corn plants, and investigated the diffusion properties of fluorescent particles using fluorescence correlation spectroscopy and single particle tracking. This approach allows to study the EPS spatial organization under various physico-chemical conditions. We show that small probes diffuse freely in the EPS with diffusion coefficients similar to those measured in water. In contrast, large probes are drastically slowed down, showing anomalous subdiffusion. The degree of confinement increases with EPS concentration. Tracking single fluorescently labeled bacteriophages at physiological concentrations, we observe a population of strongly confined particles, showing distinct subdiffusive dynamics with anti-correlation of successive steps. To overcome the physical barrier imposed by the EPS, bacteriophages are equipped with EPS degrading enzymes. By treating the EPS with purified bacteriophage enzymes, we show that upon EPS degradation strongly confined diffusion rapidly turns to free diffusion. Thus, our approach allows the investigation of dynamic changes of the biofilm microviscosity and demonstrates that the EPS matrix imposes a probe size dependent diffusion barrier under physiological conditions. Our data suggests that the ability to degrade the EPS provides a key for bacteriophages to evade trapping in the biofilm.

Sunday 21st July

NON-EQUILIBRIUM PHYSICS

O-045

Quantifying and modelling active motion in biological systems

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Living biological systems are continuously reorganizing their structure to perform their function. The mechanical activity plays here an important role, as the constant generation of forces drives fluctuations as well as controlled motion of intracellular particles, membranes and cells. From a physical point of view, this active motion drives the system far away from thermodynamic equilibrium, which can be measured as a violation of equilibrium quantities such as the fluctuation dissipation theorem.

Quantifying the out-of-equilibrium components provides the possibility to model the active molecular processes. We measure the energy and the forces actively applied on membranes [1] as well as cellular granules [2] and model these with an active Langevin approach. By comparing the predictions of forces and mechanics with the measurement of the fluctuations and viscoelastic properties we can extract molecular parameters from mesoscopic measurements. This gives timescales and chemical reaction parameters such as forces, binding states and velocities of the underlying proteins using a simple average measurement of the active motion.

Active collective cell migration is furthermore studied in the early development of the zebrafish embryo. Here we can show that a wetting approach combined with a constant active pulling force can explain the time evolution of the contact angle between embryo and yolk [3].

References

[1] H. Turlier et al *Nature Physics* **12**, 513-519 (2016)

[2] M. Almonacid et al, *Nature Cell Biology* **17**, 470-479 (2015)

[3] B. Wallmeyer et al *Biophysical Journal* in press

O-046

Dynamics of nanoparticle and virus uptake at cell membranes

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Receptor-mediated uptake of nanoparticles and virus into cells requires that the energy of adhesion overcomes the deformation energy of the cell membrane. It is well known that this competition leads to a minimal particle size of around 50 nm and an optimal particle size of around 100 nm, which is a typical size for viruses. However, it is much less understood which role is played by the shapes of both the particle and the free membrane. Moreover, not much is known about the role of stochastic processes in these small systems. We have developed a comprehensive theoretical treatment that allows us to address all of these questions in one common framework. By considering the shape equations of the free membrane, we first show that its elastic energy can be neglected for tense and loose membranes. For the intermediate but biologically relevant regime, it can be approximated by a phenomenological expression that is similar to but different from a line tension. We then derive deterministic dynamical equations for particle uptake and show that in general, cylindrical particles are taken up faster than spherical ones. We finally map the deterministic equations onto one-step master equations and show that for large particles, now spheres can be taken up faster than cylinders due to stochastic effects. Our results demonstrate the importance of non-equilibrium physics for an essential biological process.

O-047

How rotating ATP synthases can modulate membrane structure

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Systems that convert energy into rotational motion are of particular interest due to their exotic out-of-equilibrium behavior. Specifically, the rotatory movement of microparticles can lead to collective effects with a major impact on the self-organization properties on the embedding media [1]. These effects have been reported theoretically and demonstrated at the microscale. In nature, the membrane protein F_1F_o-ATP synthase (ATP synthase) is the most important biological motor with rotational movement. ATP synthase plays a key role in creating more than 90% of the biochemical energy ATP in cells. The ATP synthase is composed of two linked multi-subunit complexes: the soluble catalytic domain F_1 and the membrane-spanning rotor F_0. An electrochemical gradient of protons across F_0 causes the central shaft to spin rapidly within the head inducing the synthesis of ATP in F_1 [2]. The molecular motor is reversible and an excess of ATP provokes a rotation in the opposite direction driven by a two-state Brownian ratchet mechanism [3]. I show here how the protein activity of ATP synthase can significantly alter the properties of the surrounding lipid membrane [4] and I present preliminary results that support the action of rotating ATP synthases in the out of equilibrium process of membrane structure [5].

1. van Zuiden, B. C., Paulose, J., Irvine, W. T. M., Bartolo, D. & Vitelli, V. Spatiotemporal order and emergent edge currents in active spinner materials. Proceedings of the National Academy of Sciences of the United States of America 113, 12919-12924 (2016).

2. Noji, H., Yasuda, R., Yoshida, M. & Kinosita, K. Direct observation of the rotation of F-1-ATPase. Nature 386, 299-302 (1997).

3. Kulish, O., Wright, A.D., & Terentjev, E.M. F1 rotary motor of ATP synthase is driven by the torsionally-asymmetric drive shaft. Scientific Reports. 6, 28180 (2016)

4. Almendro-Vedia, V. G. et al. Nonequilibrium fluctuations of lipid membranes by the rotating motor protein F1F0-ATP synthase. Proceedings of the National Academy of Sciences of the United States of America 114, 11291-11296 (2017).

5. Lenz, P., Joanny, J. F., Julicher, F. & Prost, J. Membranes with rotating motors. Physical review letters 91 (2003).

O-048 (P-153)

Non-Equilibrium Processes in Proteins Triggered by Light: Excited States Molecular Dynamics Perspective

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Classical molecular dynamics (MD) simulations of proteins are mainstream tools in biophysics. However, simulations of protein systems in which light absorption is taken into account are far from trivial. Many methods have been proposed so far [1]rydzewski nowak chapter], but there is no consensus on how to monitor rare events triggered by light absorption. Here, we present our results of protein structural rearrangements that are induced by absorbing photons in the chromophore parts of proteins. We adopt a simplified approach of switching the dynamics between electronic states, named the Landau-Zener model. We model fluorescent probes dynamics (Prodan, Aldan) embedded in apomyoglobin, the photodissociation of nitric oxide from a neuroglobin mutant [2], and structural changes induced by the cis-trans transition in proteins involved in the control of glucose level: EPAC2 and KATP Kir6.2/SUR1 channel [3,4]. We extract and indicate possible nano-mechanical events stimulated by light on a nanosecond time scale. Data show that systematic structural light-triggered effects in these proteins happen and that such motions may play a functional role. Our results indicate that developing light-controlled drugs related to diabetes treatment should be possible.

This work is supported by National Science Centre, Poland grants no.2016/23/B/ST4/01770, 2016/20/T/ST3/00488 and partly by ICNT NCU.

[1] J. Rydzewski, W. Nowak ,"Molecular Dynamics Simulations of Large Systems in Electronic Excited States", in Handbook of Computational Chemistry, Springer, 1917-1941, 2016.
[2] J. Rydzewski, W. Nowak, Photoinduced Transport in an H64Q Neuroglobin Antidote for Carbon Monoxide Poisoning, J. Chem. Phys., 148(11), 2018, 115101,
[3] Broichhagen J, Schonberger M, Cork SC et al. Optical control of insulin release using a photoswitchable sulfonylurea. Nat Commun 2014; 5:5116. doi: 10.1038/ncomms6116.
[4] K. Walczewska-Szewc, W. Nowak, Spacial models of malfunctioned protein complexes help to elucidate signal transduction critical for insulin release. Biosystems. 2019 Mar;177:48-55. doi: 10.1016/j.biosystems.2018.11.001.

O-049 (P-154)

1/f noise in ion channels formed by the Classical Swine Fever Virus (CSFV) p7 protein

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Permeabilization of the endomembrane system by viroporins is instrumental in the progression of host-cell infection by many viral pathogens. Previous studies showed that CSFV viroporin p7 assembles into channels of nanometric dimensions. Here we analyze the power spectrum of current traces. We focus on the low-frequency range where the power spectrum shows typically $1/f^{\alpha}$ behavior with $0.5< \alpha < 2$ (the so called "pink noise"). We investigate several lipid mixtures to show that lipid composition determines not only the absolute value of current fluctuations but also the α parameter in the power spectrum of the current traces. Our findings give support to previous studies hypothesizing that p7 viroporin forms channels with at least two different types of pore architecture. Overall, our observations suggest that pink noise is caused by conductance fluctuations governed by equilibrium processes, in particular the conformational dynamics involving the proteins and lipids that assemble together to form proteolipidic pores.

O-050 (P-155)

Biophysical models of mRNA translation applied to ribosome profiling data

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Protein synthesis is regulated at many different levels including transcription of DNA into mRNA and translation of mRNA into protein. There is an accumulated evidence that synonymous codon choice affects mRNA translation, particularly the rate of protein production and the quality of the protein produced [1]. Dynamical details of this intricate process have recently become available thanks to rapid advances in DNA sequencing and single-cell imaging. However, existing biophysical models of mRNA translation have had limited success so far interpreting the new data because of our lack of mathematical tools to describe nonequilibrium systems [2]. I will present a newly developed method for solving the most commonly used models of mRNA translation, the ones that are based on the totally asymmetric simple exclusion process. The method assumes that translation initiation is rate-limiting for protein synthesis and predicts the rate of translation and ribosome density from translation elongation rates of individual codons. I will demonstrate how to use the method for analyzing ribosome profiling data and discuss what it teaches us about codon optimization.

[1] Brule CE, Grayhack EJ. 2017. Synonymous codons: choose wisely for expression. Trends Genet 33:283-297.
[2] Zur H, Tuller T. 2016. Predictive biophysical modeling and understanding of the dynamics of mRNA translation and its evolution. Nucleic Acids Res 44:9031-9049.
[3] Szavits-Nossan J, Cinadrini L, Romano MC. 2018. Deciphering mRNA Sequence Determinants of Protein Production Rate. Phys Rev Lett 120:128101.

Sunday 21st July
TRAFFICKING AND SIGNALLING

O-051
Systematic superresolution analysis of endocytosis reveals an actin nucleation nano-template that drives efficient vesicle formation
Markus Mund, Jan van der Beek, Joran Deschamps Serge Dmitrieff, Jooske Monster, Andrea Picco, François Nédélec, Marko Kaksonen, Jonas Ries
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Clathrin-mediated endocytosis is an essential cellular function of all eukaryotes. It relies on a self-assembled macromolecular machine of over 50 different proteins in tens to hundreds of copies that mediate vesicle formation. How so many proteins can be organized to produce endocytic vesicles with high precision and efficiency is not understood. To address this gap, we developed high-throughput superresolution microscopy to reconstruct the nanoscale structural organization of 23 endocytic proteins from over 100,000 endocytic sites in yeast. This allowed us to visualize where individual proteins are localized within the machinery throughout the endocytic process.
By combining superresolution imaging, live-cell microscopy and Brownian dynamics simulations, we aim to identify the architectural features that allow the endocytic machinery to create vesicles with high efficiency and robustness. We found that actin filament nucleation is pre-patterned by a nucleation nanotemplate, which directly links molecular organization to the mechanics of endocytosis, and might represent a general design principle for directional force generation in other membrane remodeling processes such as during cell migration and division.
I will present first results on a dynamic reconstruction of the yeast endocytic machinery from thousands of images of fixed structures.
Mund et al. "Systematic analysis of the molecular architecture of endocytosis reveals a nanoscale actin nucleation template that drives efficient vesicle formation," *Cell*, (2018).

O-052
Shedding new light on the nanoscale organization of GPCR signalling
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My group investigates the basic mechanisms of G protein-coupled receptor (GPCR) signalling and their alterations in disease, which we study using innovative microscopy methods such as fluorescence resonance energy transfer (FRET) and single-molecule microscopy. Using these methods, we have demonstrated that GPCRs do not only signal via cyclic AMP at the cell surface but also at intracellular sites (Calebiro et al., PLoS Biology 2009). We have shown that this is required for the biological effects of hormones like TSH and LH. Moreover, we have shown that this occurs via retrograde trafficking of the internalized receptors to the trans-Golgi network (TGN), where they induce local cAMP signalling (Godbole et al., Nat Commun 2017). In parallel, we have developed an innovative single-molecule microscopy approach to investigate receptor interactions on the plasma membrane with unprecedented spatiotemporal resolution. Using this approach, we could demonstrate that GPCRs from transient complexes that differ considerably in size and location among receptors (Calebiro et al., PNAS 2013). Very recently, we have succeeded for the first time in directly visualizing individual receptors and G proteins as they interact and signal in living cells (Sungkaworn et al., Nature 2017). This has revealed "hot spots" for G protein signalling at the plasma membrane, which we hypothesize confer speed and specificity to GPCR signalling. Altogether, the most recent findings by our and other groups suggest that GPCR signalling is highly organized in time and space, which likely plays a key role in determining signal specificity downstream of this important family of membrane receptors. These findings also have major implications for drug discovery, as they might provide a new basis to precisely modulate GPCR activity, and, thus, develop innovative drugs with improved efficacy and less side effects.

O-053
Cryo-EM structural analysis of focal adhesion machinery
N. Mizuno.
Focal Adhesion (FA) is an intracellular protein machinery essential for cell migration, signaling and immune functions. Talin is a 270 kDa cytoplasmic adaptor protein that connects integrin receptors on the cell surface to the actin cytoskeleton within FA, thus, playing a key role in the assembly of FA. Upon its activation, talin can stretch up to ~80 nm within FA, acting as an activator of integrin, a recruitment platform for several signaling molecules, and finally a cross-linker of actin to the membrane. It is necessary for the tethering action of cells to the extracellular matrix (ECM). In cells, talin is highly expressed with a concentration of up to 25 μM. While activated talin is essential for the FA formation, it is also critical to be properly switched off during the resting state. The auto-inhibited state of talin has been long proposed and structural information of short talin fragments has been reported. However, the molecular organization and the functional relevance of the auto-inhibition has been a mystery. It has been challenging to obtain preparative amounts of talin and to control its conformational state. This is due to the complex and flexible domain organization of talin with 16 domains, sub-devided into a 50 kDa head and a 220 kDa rod with 13 helical bundles and a putative dimerization domain.
In order to understand the mechanistic features of talin's auto-inhibition, we characterized talin from structural (cryo-EM) and dynamic (biophysical) aspects. We recombinantly prepared a series of talin fragments including full-length. We further successfully obtained a cryo-EM structure of full-length talin in its autoinhibited state, showing a charge-based arrangement of the rod domains folding into a compact globular structure covered by the head domain. The dynamic analysis of talin revealed a molecular opening of talin, leading to a drastic change of the conformation from a 15-nm globular, autoinhibited to a 80-nm active string-like formation, which can be reversed. This conformational switch explains the dynamic behavior of talin and its long-range effects during cell migration and signaling.

O-054 (P-164)
TANGO1 Regulates Membrane Tension to Mediate Collagen Export from the Endoplasmic Reticulum
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Collagens are the main component of the extracellular matrix, a proteinaceous network that provides the structural integrity necessary for multicellularity. Collagens are bulky secretory proteins, which are *de novo* synthesized into the endoplasmic reticulum (ER), from where they are exported to the Golgi complex before being secreted. Despite their fundamental importance, the molecular and biophysical mechanisms of how collagens are exported from the ER still remain poorly understood. An ER-resident transmembrane protein, TANGO1, is required for the export of collagens by modulating and physically connecting the cytosolic COPII membrane-remodeling machinery to the collagens in the ER lumen. We recently monitored by super-resolution nanoscopy the organization of TANGO1 at collagen export sites, showing that TANGO1 assembles into rings around COPII proteins. How are these TANGO1 rings formed? How do they mediate collagen export from the ER? Here, we present a biophysical model in which TANGO1 forms a linear filament that wraps around COPII lattices to stabilize the neck of a growing carrier. Our model predicts how the different physical interactions of TANGO1 proteins regulate the size and shape of the rings. Moreover, our results indicate that the growth of a collagen-containing export intermediate may be driven by a local reduction of the ER membrane tension, which can be ascribed to the TANGO1-dependent tethering and fusion of ER-Golgi intermediate compartment (ERGIC) membranes to the sites of collagen export. Altogether, our results show that TANGO1 can induce the formation of transport intermediates by regulating ER membrane tension and thus controlling collagen export from the ER.

O-055 (P-165)
Facile membrane flow and tension equilibration at a presynaptic nerve terminal
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A crucial factor regulating synaptic transmission by neurons and hormone release by neuroendocrine cells is membrane tension, σ. Increased σ promotes fusion pore dilation leading to increased cargo release and strongly inhibits endocytosis, while decreased σ leads to transient pore opening resulting in incomplete cargo release and facilitates endocytosis. It has been proposed that a reduction in σ due to exocytic membrane addition to the presynaptic terminal surface could provide a mechanism that couples exo- and endocytosis. Such a coupling implies membrane flow from an exocytic site to an endocytic one, but such flows have never been demonstrated, and so the relationship between synaptic vesicle (SV) and σ dynamics is unknown. This is mainly due to challenges in the measurement of activity-dependent tension changes at nerve terminals, which are typically too small to allow direct access for tension measurements. We have overcome these challenges by using the giant synaptic terminal (~10 μm) of the goldfish retinal bipolar neuron. Such terminals are ideal for studying properties of presynaptic membranes, as each is filled with ~500,000 SVs, and respond to stimulation in a graded manner, increasing terminal area by up to ~15 % upon depolarization due to rapid exocytosis, followed by slower recovery due to endocytosis.
To measure σ, we use optical tweezers to manipulate a 1-3 μm bead, which is briefly brought into contact with a synaptic terminal, then pulled away to create a thin membrane tether between the bead and the terminal. The tether force reports σ. In addition, we manipulate membrane tethers to probe membrane flows and membrane-cortex drag.
We observe activity-dependent changes in σ consistent with exo- and endocytosis, as well as changes in tether force resulting directly from synapse depolarization– a mechano-electric effect. Unexpectedly, we also found that membrane tethers can be dragged across the terminal with little resistance, in sharp contrast to other cell membranes where cytoskeleton-PM interactions regulate σ and severely limit membrane flows. We conclude that exceptionally facile membrane flow and tension equilibration at the presynaptic plasma membrane is tuned for rapid turnover of synaptic vesicles, thus playing a key role in neurotransmission.

O-056 (P-166)
Allosteric regulation of small GTPases at the surface of membranes
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Lipidated small GTPases regulate most aspects of “cell logistics” including signaling, membrane traffic, cell shape and motility, and they are associated with myriad severe diseases. To function, they assemble multiprotein complexes at the surface of membranes to propagate actions in the cell, but an integrated understanding of their interactions with the lipid bilayer is still lacking. I will describe recent research in which we combined X-ray crystallography, SAXS, molecular dynamics, HDX-MS and fluorescence kinetics to understand the inner workings of small GTPases and their regulators on membranes, and how it led to new concepts in drug discovery.
References:
Aizel et al., Integrated conformational and lipid-sensing regulation of endosomal ArfGEF BRAG2. PLoS Biol. (2013) 11:e1001652.
Karandur et al. Multiple interactions between an Arf/GEF complex and charged lipids determine activation kinetics on the membrane. PNAS (2017), 114:11416-11421.
Nawrotek et al. PH-domain-binding inhibitors of nucleotide exchange factor BRAG2 disrupt Arf GTPase signaling. Nat. Chem. Biol. (2019) 15:358-366.

Monday 22nd July
BIOMOLECULAR SIMULATION I

O-057
Deep learning for molecular biophysics
Prof. Dr. Frank Noe
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We present new methods that exploit the power of deep learning to address fundamental problems of molecular biophysics. Firstly, VAMPnets exploit a variational approach for Markov processes in order to learn Markov state models (MSMs) using neural networks. We demonstrate that VAMPnets can automatically learn high-quality MSMs of protein kinetics and how MSMs can drive simulations in order to sample processes well beyond the seconds timescale, including protein-protein association and dissociation. Secondly, we demonstrate the use of deep learning in learning physically valid coarse-grained potentials from all-atom simulations, and the application of this approach to learn effective few-bead models of proteins that faithfully model the missing all-atom solvent.

O-058
Sculpting cell membranes by protein nanomachines
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Cellular membranes require constant remodeling to allow cells to maintain homeostasis, to grow and to divide. This involves protein machines that can sense mechanical forces and physically sculpt membranes in both directions, toward and away from the cytoplasm. Such machines are typically composed of a large number of membrane-bound proteins organised into filaments and other functional assemblies. Due to their multiscale nature, the formation and function of these assemblies are challenging to resolve with current experimental techniques and are too complex for detailed molecular simulations. Coarse-grained computer simulations, rooted in statistical mechanics and soft-matter physics, can be of great value in determining how functional protein nanostructures operate. Here we will discuss cooperative mechanosensing of membrane channels, and membrane remodelling by ESCRT-III filaments, studied using minimal coarse-grained models. We will provide quantitative predictions for cooperative action of bacterial mechanosensitive channels, from molecular to cellular scales. We will propose how geometrical transitions of ESCRT-III filaments can remodel and sever membranes of various shapes and topologies, creating membrane tubes, buds, and dividing tubular and spherical membranes. Beyond their biological context, our findings can also guide the design of artificial structures that mechanically sculpt cells and sense mechanical forces at the nanoscale.

O-059

Pentameric Ligand-gated Ion Channels: New Crystal Structures and MD simulations

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Pentameric Ligand-gated Ion Channels (pLGICs) are allosteric membrane receptors that mediate rapid chemo-electrical signal transduction in animals through the binding of neurotransmitters (agonists). Their functional response to agonists is regulated by a variety of molecules of pharmacological interest, such as benzodiazepines, alcohols, general anesthetics and barbiturates. pLGICs also exist in prokaryotes and display structure-function relationships remarkably similar to eukaryotic ones.

In the past, we have been able to solve the structures of both open and closed forms of a proton-gated ion channel GLIC, from the cyanobacteria *Gloeobacter violaceus*. Despite recent progress (H. Hu et al., PNAS, 2018, 115 :12172-81), its detailed mechanism is hard to decribe comprehensively, due to the large number of proton binding sites in the receptor.

We recently solved the crystal structure of another pLGIC from a bacterial symbiont of a large tubeworm living in deep sea (*T. jerichonana*) that can be open at basic pH in the presence of analogues of amino-acids (H. Hu et al., PNAS 2018, 115:3959-68). Surpisingly, its pore is widely open, while its extra-cellular domain contains a closed constriction ring.

We have used extensive MD simulations to probe the stability of this open state in a native lipidic environment and model its gating. This new structure sheds new light of the functional state of the closed form of ELIC, another receptor from the plant pathogen *E. chrystantemi*.

In addition, bacterial pLGIC are often fused to other domains, thus displaying a greater diversity in their architecture than their eukaryotic counterparts. Nothing is known at present on the higher architecture of these larger receptors of the pLGIC family.

We will present here the crystal structures at atomic resolution of two different conformations of DeCLIC, a pLGIC from a deltaproteobacterium *Desulfofustis* that contains two additional amino-terminal domains (NTD1 and NTD2), directly fused to the conventional ligand binding domain (LBD). Both NTD have a jelly-roll topology (H. Hu et al., submitted).

The structure obtained with Ca^{2+} shows a closed transmembrane pore. In contrast, the one obtained in the absence of Ca^{2+} displays a wide-open pore. The NTDs establish tight interactions with themselves and with the LBD. In the wide-open pore conformation, because of the global compaction and twisting motions of the NTDs and LBDs, the NTD2 modulates the conformation of the classical agonist binding site at the interface of subunits.

This pair of new structures also illuminates the remodeling of known allosteric sites in the LBD during the gating transition and provides a framework that allows to interpret a large body of experimental and structural data in other members of the pLGIC family.

O-060 (P-453)

Automated cryo-EM structure refinement using correlation-driven molecular dynamics

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We present a correlation-driven molecular dynamics (CDMD) method for automated refinement of atomistic models into cryo-electron microscopy (cryo-EM) maps at resolutions ranging from near-atomic to subnanometer. It utilizes a chemically accurate force field and thermodynamic sampling to improve the real-space correlation between the modeled structure and the cryo-EM map. Our framework employs a gradual increase in resolution and map-model agreement as well as simulated annealing, and allows fully automated refinement without manual intervention or any additional rotamer- and backbone-specific restraints. Using multiple challenging systems covering a wide range of map resolutions, system sizes, starting model geometries and distances from the target state, we assess the quality of generated models in terms of both model accuracy and potential of overfitting. To provide an objective comparison, we apply several well-established methods across all examples and demonstrate that CDMD performs best in most cases.

O-061 (P-454)

Aβ peptides and β-sheet breakers. A coarse grained molecular dynamics approach using GO-Martini

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The problem of protein misfolding is at the origin of a class of pathologies called protein conformational disorders (PCD) to which all neuro-degenerative diseases belong. PCD's are characterized by the misfolding of proteins that grow in aggregates of fibrillar shape. Among them, Alzheimer Disease (AD) is one of the most studied for its high impact on the modern society. The plaques present in the brain of AD patients show deposition of fibril made of amyloid β (Aβ) peptides [1]. The process that leads to misfolding, aggregation and amyloid plaques formation is not yet fully elucidated. It seems, however, that the "trigger" of the process is an abnormal switch of the peptide secondary structure leading to β-sheet formation.

Several factors are known to affect Aβ aggregation processes. An important role seems to be played by metal ions that have been observed to be quite abundant in fibrils [2-4]. Recently, the observation that short synthetic peptides, called β-sheet breaker (BSB's), are able to directly interact with Aβ, precluding (or disfavouring) amyloid polymerisation. This finding has stimulated a lot of work in the direction of trying to understand the molecular mechanism by which BSB's are able to slow down or even prevent Aβ aggregation and fibrillation [5].

In this presentation we show how one can get a good understanding of the role that BSBs play in the aggregation process of Aβ peptides by means of *coarse-grained molecular dynamics* simulations based on the Martini force-field. Since the secondary structure switching is a crucial event for the successive aggregation process, we have extended the standard Martini approach to incorporate GO-Martini algorithm [6] that allows to properly model structural switches and study the secondary structure dynamical evolution of Aβ peptides in the presence of BSBs.

1. LC Serpell, *BBA,* 1502(1):16-30, 2000
2. E De Santis et al., ***JPCB*, 119, 15813-15820,** 2015
3. S Morante, GC Rossi, *Advance Alz Res,2:100-147,* 2014
4. F Stellato et al., *Biophys Chem, 229, 110-114,* 2017
5. V Minicozzi et al., *JBC*, 289, 11242-11252, 2014
6. AB Poma et al., *J Chem Theory Comput*, 13(3):1366-1374, 2017

O-062 (P-455)

Effect of pH on the influenza fusion peptide structure and activity: A constant-pH molecular dynamics approach

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Influenza pandemics are serious health threats of our time, in view of the limited treatments available. Research on the molecular mechanisms of infection by the influenza virus (IV) is needed to find new therapeutic targets. Inactivating the fusion of the viral and host membranes is considered a promising strategy, but this process is poorly understood at the molecular level.

The IV fusion process is promoted by the protein hemagglutinin (HA). The IV is uptaken by endocytosis and the low pH of the late endosome triggers a large conformational change of HA that initiates fusion. HA contains a region that is essential for this process: the fusion peptide (FP), which binds to the host membrane and promotes fusion. Interestingly, this peptide is able to induce fusion of lipid vesicles, even in the absence of the rest of the protein, making it a privileged model to study fusion.

In the last years, our group has studied the molecular determinants of the FP activity, showing that this peptide can adopt two different conformations in the membrane, which have different impacts on the membrane properties. Our work also shed light into the mechanisms by which the peptide perturbs the membrane, which include promoting lipid disorder and lipid-tail protrusion.

Given that fusion takes place when the virus is exposed to the low pH of the endosome, we are currently investigating the effect of pH on the influenza FP structure and membrane-interacting properties, by using an in-house developed constant-pH molecular dynamics method to shed light into this question. Our results show that lower pH stabilizes a deeper insertion of the peptide in the membrane and strengthens the interaction of the peptide with the lipids, which results in a higher fusogenic activity. By combining the simulation results with experimental studies performed by our collaborators, this study provides a detailed molecular characterization of the effect of pH on the influenza FP, which can be useful for the design of novel therapies against this devastating pathogen.

Monday 22nd July
MACROMOLECULAR COMPLEXES

O-063
Recent insights into the peptide-loading complex machinery
S. Trowitzsch.
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Abstract
Identifying and eliminating infected or malignantly transformed cells are fundamental tasks of the adaptive immune system. For immune surveillance, the cell's metastable proteome is displayed as short peptides on major histocompatibility complex class I (MHC I) molecules to cytotoxic T-lymphocytes. Our knowledge about the track from the proteome to the presentation of peptides has greatly expanded, leading to a quite comprehensive understanding of the antigen-processing pathway, which comprises many transient and dynamic macromolecular machineries. I will report on the mechanisms of antigen translocation, chaperoning, and editing, as well as on quality control mechanisms for peptide-MHC I complexes that are key for the understanding of autoimmune diseases. Based on an integrative approach, we have elucidated the contribution of individual proteins to the architecture of the MHC I peptide-loading complex (PLC) and other MHC I editing complexes. Consequences of viral immune evasion strategies will be discussed. The data provide a framework for a mechanistic understanding of quality control steps during antigen selection and unveil the molecular details underlying the onset of an adaptive immune response.

O-064
LUBAC and linear ubiquitin chains: novel tools to study immune signalling
Katrin Rittinger
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Linear ubiquitin chains linked through Met1 are important players of immune and inflammatory signalling and apoptotic cell death. They are generated by a multi-subunit E3 ligase complex called linear ubiquitin assembly complex (LUBAC) that is thus far the only E3 ligase capable of forming linear ubiquitin chains. The complex consists of three subunits, HOIP, HOIL-1L and SHARPIN, with the HOIP subunit providing the E3 ligase activity of the complex. While the HOIL-1L and SHARPIN subunits are clearly required for the overall activity of the complex, their precise contribution to the observed biological functions of LUBAC remains unclear. I will discuss recent work on the design of novel tools to study LUBAC function and interfere with activity in a cellular context.

O-065
Analysing cryoEM data quality in the post resolution revolution era: Validity criteria
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CNB-CSIC (Spanish National center for Biotechnology).
Cryo Electron Microscopy (cryoEM) is possibly the fastest growing area in Structural Biology. Many specimens are amenable to their study by Electron Microscopy, and a logical question inside and outside of the cryoEM field is to ask for information about the quality and possible "peculiarities" of this new boom of data. Logically, we are all interested in validity criteria. In this talk I will start reviewing current practices in cryoEM to help addressing this issue, to then introduce a new quality/validation criterion that has the capacity to work only on the cryoEM maps and to derive an impressive amount of new quantitative information about map quality. We refer to the new Directional Local Resolution

O-066 (P-185)
Histone tails in nucleosome: fuzzy interaction with DNA
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New evidence from NMR spectroscopy suggests that histone tails remain highly dynamic even in the condensed state of chromatin. To probe the dynamic behavior of H4 histone N-terminal tail in greater detail, we prepared a sample of mononucleosome containing ^{15}N,^{13}C-labeled H4 histone. The HSQC spectrum of this sample features observable signals from the first fifteen residues in H4; half of these signals have been successfully assigned and used for site-specific ^{15}N relaxation measurements. The experimentally obtained chemical shifts and relaxation rates paint the picture of moderately mobile H4 tail with random-coil-like conformational properties. We have also recorded a μs-long MD trajectory of mononucleosome in the explicit TIP4P-D solvent, which has been designed specifically for (partially) disordered protein systems. This trajectory successfully reproduced the experimentally measured chemical shifts and relaxation rate constants. According to the MD data, the positively charged H4 tail hovers over the negatively charged ds-DNA, making transient contacts with both DNA backbone and major/minor grooves. This type of behavior, underpinned by electrostatic attraction and characterized by substantial mobility of H4 tail relative to the DNA chain, can be classified as "fuzzy interaction". The research was supported by RSF grant 15-14-20038 (modeling component) and NIH grant GM118664 (experimental component).

O-067 (P-186)
New protein-protein interaction modulators for the therapeutic regulation of synapse dysfunction in neurodevelopmental disorders and neurodegeneration
A. Mansilla[1], A. Chaves-Sanjuan[2], C. Roca[3], A. Canal-Martin[3], M. Daniel-Mozo[2], L. Martinez-Gonzalez[3], L. Infantes[2], A. Ferrus[4], A. Martínez[3], R. Perez-Fernandez[3], N. Campillo[3], M.J. Sanchez-Barrena[2].
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The protein complex formed by the Ca^{2+} sensor neuronal calcium sensor 1 (NCS-1) and the guanine exchange factor protein Ric8a co-regulates synapse number and probability of neurotransmitter release, emerging as a potential therapeutic target for diseases affecting synapses [1]. In neurodevelopmental disorders, such as Fragile X syndrome (FXS) or Autism, neurons show an abnormally high synapse number. On the contrary, in neurodegeneration, such as Alzheimer´s, Huntington´s or Parkinson´s diseases, patients show a low synapse number. In the recent years, we have been investigating the structural basis of the NCS-1/Ric8a interaction and found out that the formation of this complex is essential to increase synapse number [1,2]. Therefore, an inhibition of the NCS-1/Ric8a complex would constitute a potential strategy to regulate synapse function in FXS and related disorders. Conversely, the stabilization of this protein-protein interaction could be key to regulate synapses in neurodegeneration. With this aim, virtual screenings and dynamic combinatorial chemistry approaches have been used to find out regulatory molecules of this protein-protein interaction. Further, a multidisciplinary approach including, biochemical, biophysical, crystallographic, cellular and *in vivo* studies have been performed to demonstrate the activity of the compounds, their therapeutic potential and molecular mechanism of action [3,4].
[1] Romero-Pozuelo, J. *et al.* (2014) *Journal of Cell Science* **127**, 4246-4259.
[2] Baños-Mateos, S. *et al.* (2014) *Acta Crystallographica* A**70**, 530-534.
[3] Mansilla, A. et al. (2017) *PNAS* **114(6)**, E999-E1008.
[4] Canal-Martín, A. et al. (2019) *Under review*.

O-068 (P-187)

Structural characterisation of tissue-derived, disease-associated polymers of alpha-1-antitrypsin using conformation-selective antibodies and single-particle reconstructions from electron microscopy images

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α1-Antitrypsin is an abundant plasma inhibitor of neutrophil elastase, expressed at high levels by hepatocytes, and one of the causative agents of a class of conformational diseases termed serpinopathies. In its active state, α1-antitrypsin is in a kinetically stable, but thermodynamically unstable, configuration, rendering it susceptible to inappropriate conformational change. In individuals homozygous for the Z (E342K) mutation, α_1-antitrypsin accumulates in the liver as dense intracellular deposits, leading to a reduced level in circulation. These deposits are the consequence of an 'ordered aggregation' that yields linear, unbranched protein chains, termed polymers, that are both extremely stable and functionally inactive. The circulating deficiency results in a protease-antiprotease imbalance in the lung, predisposing affected individuals to emphysema and COPD, whilst the hepatic accumulation can lead to liver disease, including cirrhosis and hepatocellular carcinoma.

Our aim is to define the molecular details of the polymerisation pathway, in which α_1-antitrypsin passes through different conformational states as it transitions from the active monomer via one or more structural intermediates to a hyperstable polymeric form. Different models have been proposed for the terminal structure adopted by the pathological polymer; these are are largely based on characterisation of polymers produced under conditions mechanistically or biologically distinct from those existing in vivo, and as such their relevance to the pathological context has not been established. To probe the structural and energetic aspects of the polymerisation pathway, we have generated a molecular toolkit of conformation-specific monoclonal antibodies (mAbs), and mapped their epitopes. We have utilised these mAbs and applied single-particle reconstruction techniques to negative stain and cryo-EM images of polymers extracted from patient explant liver tissue. The resulting maps, in conjunction with molecular modelling, have allowed us to critically evaluate the proposed mechanisms of polymer formation.

Monday 22nd July

EVOLUTIONARY DYNAMICS

O-069

Eco-evolutionary dynamics during Escherichia coli colonization of the mouse gut

I. Gordo.

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ABSTRACT: Bacterial laboratory experiments where evolution is followed in real time have allowed us to test theoretical predictions on microbial adaptation and to unravel how pervasive the spread of beneficial mutations can be when they face novel environments.

Much less is known about bacterial real time evolution in more natural environments, such as that comprising the gut microbiota. The pace and pattern of evolutionary change during the life of a health mammal is currently unknown. We have been following the emergence of new strains in commensal E. coli when it colonizes the gut of laboratory mice (in vivo experimental evolution).

These semi-controlled experiments have revealed that rapid evolutionary change occurs, which is marked bystrong effect mutations, evolution of mutator clones and high rates of horizontal gene transfer.

O-070

Evolutionary Biology of Cell Division: Integrating Quantitative Genetics and Cellular Biophysics

Daniel Needleman

Gordon McKay Professor of Applied Physics and Professor of Molecular and Cellular Biology

Harvard University

We are using a combination of biophysics, quantitative genetics (i.e. QTL mapping), and comparative approaches to study spindle elongation and positioning in C. elegans and other nematodes. This work has lead to a quantitative understanding of how cortical pulling forces can stably position the spindle (and asters), and revealed the genetic basis of natural variations in spindle behaviors among C. elegans wild isolates. Taken together, our work provides a mechanistic explanation for the patterns of within species variations in the spindle, and it's changes over ~100 million years of evolution across different nematode species.

O-071

Universal properties of genotype-phenotype maps

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Over the past three decades the genotype-phenotype (GP) maps of several simple biological model systems have been studied in great detail. These model systems include RNA secondary structure and the HP model of protein folding. Because these systems are relatively tractable it is possible to study large-scale properties of the GP map, such as the distribution of genotypes per phenotype, the robustness and evolvability of phenotypes, and the accessibility of phenotypes in a given number of mutational steps. In recent years the measurement of these properties has been extended to further model systems, such as the Polyomino GP map of biological self-assembly, among others. In all of these maps similar relationships between phenotypic evolvability, robustness, and frequency are observed, such as a positive correlation between phenotypic evolvability and robustness, a negative correlation between genotypic evolvability and robustness and a linear scaling of phenotypic robustness with the logarithm of phenotype frequency. These properties therefore appear to potentially be universal to GP maps. Here we address possible reasons for this universality and introduce several highly simplified, abstract GP maps that nevertheless succeed in reproducing the same properties. These simplified maps allow us to identify possible underlying causes for the universal properties of GP map structure, which we find to be (a) the partition of sequence regions into constrained and unconstrained parts, and (b) non-local interdependence of sequence positions with regard to their constraints. Since biological sequences display these characteristics in almost every biological context it is likely that the same GP map properties we observe in abstract model systems also hold for much more complex and biologically realistic phenotypes.

O-072 (P-213)

ScarTrace: CRISPR/Cas9-mediated clonal tracing in zebrafish embryonic development and regeneration

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Embryonic development is a crucial period in the life of multicellular organisms when limited sets of embryonic progenitors produce all cells in the adult body. Determining which fate these progenitors acquire in adult tissues requires simultaneously measuring clonal history and cell identity at single-cell resolution, which has been a major challenge. In this talk, I will present ScarTrace, a single-cell sequencing strategy that allows to simultaneously quantify clonal history and cell type for thousands of cells obtained from different organs of the adult zebrafish. Using ScarTrace we show that a small set of multipotent embryonic progenitors generates all hematopoietic cells in the kidney marrow. ScarTrace also reveals that epidermal and mesenchymal cells in the caudal fin arise from the same progenitors, and osteoblast-restricted precursors can produce mesenchymal cells during regeneration. Remarkably, we identify a subsetof immune cells in the fin with an epidermal and mesenchymal clonal origin, while a very similar cell type detected in the brain (microglia) shares clonality with the hematopoietic system. This suggests the existence of organ-dependent mechanisms in the role of immunity in tissue repair and maintenance in zebrafish. Similar approaches will have major applications in other experimental systems, in which matching the embryonic clonal origin to the adult cell type will ultimately allow the reconstruction of how the adult body is built from a single cell. Because ScarTrace provides a glimpse of the cellular past, it will be interesting to explore how this history is predictive of the current epigenetic and expression state.

O-073 (P-214)

Rare beneficial mutations cannot halt Muller's ratchet in spatial populations

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Muller's ratchet describes the irreversible accumulation of deleterious mutations in asexual populations. In well-mixed populations the speed of fitness decline is exponentially small in the population size, and any positive rate of beneficial mutations is sufficient to reverse the ratchet in large populations. The behavior is fundamentally different in populations with spatial structure, because the speed of the ratchet remains nonzero in the infinite size limit when the deleterious mutation rate exceeds a critical value. Based on the relation between the spatial ratchet and directed percolation, we develop a scaling theory incorporating both deleterious and beneficial mutations. The theory is verified by extensive simulations in one and two dimensions.

O-074 (P-215)

Exploring phenotypic variability of bacteria using microfluidic cell traps

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HAS BRC, Szeged, Hungary.

In changing environments cell-to-cell variation of phenotypic characteristics and behaviour plays a key role in the survival, adaptation and evolution of bacterial populations. Therefore, single cell level observations are fundamental in order to understand the development and functioning of bacterial populations and communities.

We are developing microfluidic devices consisting single cell traps for bacteria that are used to study long-term growth of bacterial cells and their responses to various environmental factors. We use *Escherichia coli* and *Pseudomonas aeruginosa* cells to study cell to cell variability in quorum sensing, growth, division, cell size, and response to nutrient stress and antibiotics.

In a modified mother machine we are able to trap and observe the descendants of single bacterial cells through many generations. This enables us to correlate phenotypic and behavioural variability to cell relatedness and ageing.

Monday 22nd July

BIOPHYSICS OF MEMBRANE OXIDATION (Sponsored by IUPAB)

O-075

Photosensitized oxidation of lipid membranes: yields and rates of chemical and physical changes

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Photosensitized oxidations occur when photosensitizers absorb light and transform the energy of light photons into chemical potential to allow oxidation of biological targets. Damages in membranes (cytoplasm and/or organelle) are of particular importance since they are key elements in terms of biological structure of cells and organelles and also are fundamental in triggering cell signaling responses. We aim to unravel the role of these mechanisms in membrane oxidation and correlate the physical changes observed in membranes with the chemical modifications induced in the lipids. Changes in phospholipids are initiated as a consequence of the formation of free radicals and singlet oxygen. The first reaction usually involves the "ene" reaction between the lipid and triplet oxygen (formed from PS triplet reaction with molecular oxygen) leading to the formation of hydroperoxide (LOOH), causing important membrane changes such as the increase in the area per lipid, reduction in the membrane stretching module and the reorganization of domains. However, even in the presence of 100% of lipid hydroperoxides, membrane leakage does not occur. Irreversible damage starts with the abstraction of a hydrogen atom from an unsaturated fatty acid (LH), leading to the formation of a carbon-centered lipid radical (L •) that suffers the addition of an oxygen molecule forming peroxyl radical (LOO •). The propagation phase comprises the initiation of a new oxidation chain by the peroxyl radical (LOO •) and the decomposition of the lipid hydroperoxides into other intermediate radicals. In light-induced reactions, formation of alkoxides is catalyzed by direct contact reactions between the triplet photosensitizer, the lipid double bond and the lipid hydroperoxide, leading to chain breakage by β-scission. This process leads to the formation of lipid truncated aldehydes, which are the molecules responsible for starting of the leakage process. We are confident that this information will allow the development of more efficient photosensitizers for photomedicine as well as better strategies for sun protection.

O-076

Achievements, challenges and hopes in the biophysical analysis of oxidised (phospho)lipids

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Lipid peroxidation products are becoming increasingly recognized as bioactive mediators in signalling events in inflammation, immunity and infection. Phospholipids as components of lipid bilayers are one of the main targets of radical species and prone to oxidative modification. The randomness of radical-based reactions generates a myriad of primary and secondary phospholipid oxidation products (OxPL) that has been confirmed by mass spectrometry conducted on model membranes. The oxidative modification of one single phospholipid results in dozens of OxPL. On the other hand, the panel of OxPL described for fluids and cells is narrow and the structural homogeneity found *ex vivo* contrasts with the structural heterogeneity reported *in vitro*. Moreover, quantitative analyses of OxPL in fluids and tissues have shown the presence of OxPL in sub-micromolar concentrations and a residual OxPL/PL ratio (<1%).

To date, the reported biophysical changes of OxPL to lipid membranes including the increase of hydration layer, reversal of oxidized chain into the aqueous medium with increasing water permeability, with decrease of bilayer thickness and even pore formation result from work with individual OxPL, which do not reflect the structural diversity formed. Moreover, the amounts of OxPL used in model membranes as high as 50% are unlikely to occur in cell membranes. At the moment, due to experimental, instrumental and technological challenges little is known about the spatial distribution of OxPL at the surface of cells. Nevertheless, the biophysical changes reported using high OxPL amounts may find relevance in other scenarios namely in macromolecular aggregates with very low volumes (lipoproteins) with co-localization of OxPL in nanoscale domains ("patches") as predicted by the "*lipid whisker model*".

The challenges underlying the *ex vivo* analysis of OxPL and the influence of these lipid microenvironments in the permeability, absorption and delivery of polyphenols transported by lipoproteins will be discussed.

O-077

Oxysterols and Truncated Oxidized Phospholipids in Model Membranes

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Products of lipid and cholesterol oxidation are commonly, although in limited quantities, present in our bodies both under physiological and pathological conditions. While their recognition by proteins triggers many signaling pathways, their presence can also have severe effects on the physical properties of lipid membranes. In the lecture we will summarize our experimental and computational research on the behavior of truncated oxidized lipids and oxysterols in model lipid membranes. [1-6]

1-P. Jurkiewicz, et al., *Biochimica Et Biophysica Acta-Biomembranes 2012, (1818) 2338*
2-M. Stefl, et al., *Biochimica Et Biophysica Acta-Biomembranes 2014, (1838)1769*
3-P. Parkkila, et al., *Biochimica Et Biophysica Acta-Biomembranes 2015, (1848) 167*
4-W. Kulig, et al., *Free Radic. Biol. Med 2015, (84)30*
5-W. Kulig, et al., *J. Phys. Chem. Lett. 2018, (9) 1118*
6-I. Vinklarek et al., *submitted*

O-078 (P-220)

Lipids as substrates and dynamic activators: pro-apoptotic lipid peroxidation at the mitochondrial membrane surface.

M. Li[1], A. Mandal[1], V. Tyurin[1], M. Delucia[1], J. Ahn[1], V. Kagan[1], P. Van Der Wel[2].
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Oxidative chemical modifications of lipids change their biophysical properties and thus the behavior and structure of the surrounding membrane. An important recent realization is that the modified lipids are also employed by the cell as crucial quality control signals. The oxidation of mitochondrial lipids is an important trigger of apoptosis, in line with the common observation that pathogenic mitochondrial dysfunction generates excessive reactive oxygen species (ROS). Remarkably, it has been shown that cells do not rely merely on *incidental* oxidative events, but actually facilitate the *pro-active peroxidation of specific mitochondrial lipids* with the help the mitochondrial heme-containing protein cytochrome c.

We dissect the molecular interactions that mediate the formation and activation of the protein-lipid complex behind this pro-apoptotic peroxidation reaction [1]. By solid-state NMR, we probe the interactions between the protein and the lipid bilayer surface, interactions with solvent molecules, and the conformation and dynamics of the membrane-bound protein. Crucially, we also measure the lipid-specific peroxidase activity under these sample conditions via mass spectrometry-based lipidomics, finding a recapitulation of the type of pro-apoptotic cardiolipin peroxidation seen *in vivo*.

We observe the structural and dynamic changes affecting the protein as it associates with the negatively charged bilayer surface. While attaining a lipid-dependent activation of cardiolipin peroxidase activity, the native fold is largely preserved. This is apparent from spectral signatures of the membrane-bound protein, which are clearly distinct from those of denatured cytochrome c. Instead, specific spectral changes indicate localized dynamics that respond to the membrane fluidity. These lipid-associated dynamic changes in the protein rationalize the peroxidase activation in absence of a large-scale unfolding reaction. Upon protein-induced clustering into lipid nanodomains, the cardiolipin lipids themselves act not only as the preferred substrate but also as *dynamic regulators* in this lethal engagement between proteins and lipids at the mitochondrial inner membrane.

[1] M. Li, A. Mandal, V.A. Tyurin, M. DeLucia, J. Ahn, V.E. Kagan, & P.C.A. van der Wel (2019) Structure, **in press**, DOI: https://doi.org/10.1016/j.str.2019.02.007

O-079 (P-221)

The effect of oxidised cholesterol on model red blood cell membranes

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The human erythrocyte is responsible for delivering oxygen to all the body's tissues. To do so effectively, a red blood cell must be able to deform its 8 µm, biconcave plasma membrane to flow through blood vessels as small as 3 µm in diameter. As such, decreased deformability of a red blood cell can significantly reduce its ability to oxygenate tissues. Under oxidative stress, the red blood cell membrane becomes enriched with oxysterols, which increase membrane stiffness, decrease cell deformability and reduce the ability of the cell to transmit signals. A particularly cytotoxic oxysterol, 7-ketocholesterol, is associated with many pathologies such as type 2 diabetes, cardiovascular disease and numerous neurodegenerative diseases. It has previously been found that high levels of 7-ketocholesterol leads to more disordered and less condense lipid membranes. In a erythrocyte, a disordered inner leaflet will interact more strongly with the cytoskeleton, which leads to a stiffer, less deformable cell. We have used a series of all-atom and coarse-grain molecular dynamics simulations to probe the effects of 7-ketocholesterol on model red blood cell membranes. In doing so, we have gained a mechanistic understanding of how 7-ketocholesterol effects the order, dynamical, structural and mechanical properties of the model red blood cell membranes.

O-080 (P-222)

Lipid curvature modulates function of mitochondrial membrane proteins

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Lipid curvature plays an important role in the function of membrane proteins. Oxidative stress and formation of reactive oxygen species (ROS) in mitochondria result in significant modification of membrane lipids: (i) lysolipids are formed due to activation of mitochondrial phospholipase A2 (mt-PLA2), (ii) reactive aldehydes 4-hydroxy-2-nonenal (HNE) and 4-oxo-2-nonenal (ONE) covalently modify phosphatidylethanolamine (PE) headgroup forming HNE-PE, ONE-PE – adducts[1]. We focused on the effect of native and modified membrane lipids with different spontaneous curvatures on the activity of mitochondrial uncoupling protein 1 (UCP1) and ATP/ADP carrier (ANT). By combining measurements of total membrane conductance[2] and lipid bilayer bending moduli we revealed that (i) lysophosphatidylcholines with positive curvature, such as MPC and OPC, decrease bending modulus in neat lipid bilayers and increase protonophoric activity of UCP1 and ANT, (ii) negatively curved PE does not influence protein activity and (iii) PE modified by reactive aldehydes (HNE-PE, ONE-PE – adducts) acts similar to lipids with a positive spontaneous curvature. Molecular dynamics simulations revealed that modified PEs and lysolipids alter lateral pressure profile in lipid bilayer membrane in the same direction and range. Molecular mechanism described in this work brings new perspective in understanding (i) the transport function of mitochondrial membrane proteins and (ii) processes associated with mitochondrial dynamic, including fusion and fission, during oxidative stress.

REFERENCES

1.Jovanovic, O. *et al.* The molecular mechanism behind reactive aldehyde action on transmembrane translocations of proton and potassium ions. *Free Radic Biol Med* **89**, 1067-1076 (2015).
2.Beck, V. *et al.* A new automated technique for the reconstitution of hydrophobic proteins into planar bilayer membranes. Studies of human recombinant uncoupling protein 1. *Biochim Biophys Acta* **1757**, 474-479 (2006).

Monday 22nd July

PROTEIN STRUCTURE AND FUNCTION

O-081

Unraveling the role of SepF in the early actinobacterial divisome

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One of the most ancient and diverse prokaryotic phyla, Actinobacteria lack several of the components that are essential in bacterial model systems such as Escherichia coli for the formation of the divisome, the protein machine responsible for cytokinesis. Divisome assembly at the cell division site depends on the initial placement of tubulin-like FtsZ into a structure called the Z-ring. Here we will present our structural and functional characterization of actinobacterial SepF, a crucial factor for Z-ring formation and early divisome assembly, using an integrative approach going from structural biochemistry and biophysics to bacterial genetics and cellular imaging.

O-082

Molecular Basis for the Extra-Mitochondrial Roles of Cytochrome c in Cell Life and Death

M.A. De La Rosa[1], M.A. De La Rosa[2], K. González-Arzola[2], A. Guerra-Castellano[2], F. Rivero-Rodríguez[2], A. Velázquez-Cruz[2], G. Pérez-Mejías[2], M.A. Casado-Combreras[2], I. Rodríguez-González[2], A. Díaz-Quintana[2], I. Díaz-Moreno[2].
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O-083

Atomistic Simulation of Biomolecular Function: Ribosomal Translation and Ligand Binding Heterogeneity

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Ribosomes are highly complex biological nanomachines which operate at many length and time scales. We combined single molecule, x-ray crystallographic, and cryo-EM data with atomistic simulations to elucidate how tRNA translocation, the action of antibiotics, and frameshifting work at the molecular level. We describe a new combined allosteric mechanism for erythromycin-induced translational stalling of the antibiotics sensor peptide ErmB, as well as a free energy model that can explain and predict frameshifting efficiencies. Using streptavidin/biotin as a model system with super-strong affinity, we show that the underlying free energy landscape which governs ligand binding and unbinding can be extracted from combined atomic force microscopy (AFM) and force probe simulation data, which covers loading rates of 11 orders of magnitude.

[1] Arenz S, Bock LV, ... , Grubmüller H, Vaiana AC, Wilson DN. Nature Comm. 7, 12026 (2016)
[2] Fischer N, ..., Stark H. Nature 540 (7631), 80-85 (2016)
[3] Bock LV, Blau C, Vaiana AC, Grubmüller H. Nucl. Acids Res. 43 (14), 6747–6760 (2015)
[4] Bock LV, ..., Grubmüller H. Nature Struct. Molec. Biol. 20 (12), 1390-1396 (2013)
[5] Bock LV, Kolar MH, Grubmüller H. Curr. Opin. Struct. Biol. 49, 27–35 (2018)
[6] Huter P et al. Mol. Cell 68 (3), 515–527 (2017)
[7] Rico F, Russek A, Gonzalez L, Grubmüller H, Scheuring S. PNAS, 116 (14) 6594-6601 (2019)

O-084 (P-223)

***Campylobacter jejuni* Tlp3 dCache sensing domain specifically recognises hydrophobic amino acids**

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In *Campylobacter jejuni*, chemotaxis and motility have been identified as important virulence factors that are required for host colonisation and invasion. The chemotaxis process involves recognition of chemical cues by the ligand binding domain (LBD) of chemoreceptors also known as methyl-accepting chemotactic proteins. Recently, we determined the crystal structure of *C. jejuni* Tlp3-LBD chemoreceptor in complex with attractant isoleucine, revealing this receptor belongs to the dCache_1 family of sensing modules. In this work, we performed a high-throughput screening of potential ligands and identified additional small molecules that directly interact with Tlp3-LBD. All of the new ligands (leucine, valine, α-amino-N-valeric acid, 2-Amino-3,4-dimethylpentanoic acid, 2-Amino-3-methylhexanoic acid, amino-3,3-dimethylpentanoic acid, alanine and phenylalanine) are hydrophobic amino acids chemically and structurally similar to isoleucine. Analysis of the crystal structures of Tlp3-LBD in complex with these ligands showed that like isoleucine, they bind to the membrane-distal subdomain of the dCache Tlp3 sensing module. The Tlp3-LBD residues that interact with the main chain of isoleucine, leucine, valine, α-amino-N-valeric acid, 2-Amino-3,4-dimethylpentanoic acid, 2-Amino-3-methylhexanoic acid, amino-3,3-dimethylpentanoic acid, alanine and phenylalanine are located at equivalent positions in all complex structures, whilst residues that interact with the side chain move to accommodate the different amino acid ligands up to the length of 5 carbon chain, beyond this length the side chain is flipped from inside of the pocket towards the β3β4 loop as in the case of 2-Amino-3-methylhexanoic acid. In addition, analysis of the structure activity relationship (SAR) reveals that isoleucine possesses the most favored structure to interact with Tlp3-LBD and hence, has highest binding affinity.

O-085 (P-224)

DYNAMICS OF INTRINSICALLY DISORDERED AND UNFOLDED PROTEINS: INVESTIGATIONS USING NEUTRON SPIN-ECHO SPECTROSCOPY

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A characteristic property of intrinsically disordered and unfolded proteins is their high molecular flexibility, which enables the exploration of a large conformational space. We present neutron scattering experiments on the structure and dynamics of the intrinsically disordered myelin basic protein (MBP) and the chemically denatured bovine serum albumin (BSA) in solution (1,2,3). Small-angle neutron scattering (SANS) experiments allowed us to gain information of structural aspects of MBP and denatured BSA as response to denaturant conditions. Using neutron spin-echo (NSE) experiments, we were able to investigate collective motions of the protein chain up to several hundred nanoseconds on the nanometre length-scale. NSE results showed a high flexibility of the unfolded proteins. Internal motions of the intrinsically disordered MBP and denatured BSA were described using normal mode analysis and concepts derived from polymer theory. The contribution of residue-solvent friction was accounted for using the Zimm model including internal friction. Motions of MBP are well described by collective normal modes, while dynamics of denatured BSA show polymer-like dynamics. Disulphide bonds forming loops of amino acids of the peptide backbone have a major impact on internal dynamics of denatured BSA. We see directly in a molecular picture that topological restrictions due to disulfide bridges in denatured BSA create confinement effects: Long-wavelength Zimm modes are strongly reduced in amplitude due to loops formed by disulfide bridges.

Dynamics in folded native BSA was measured by NSE as reference. The observed internal dynamic process in native BSA can be attributed to opening and closing motion of the BSA dimer. An intermolecular disulphide bridge between the BSA monomers can form a covalent cross-link establishing a molecular hinge in dimeric BSA in solution. The effect of that hinge on the observed motion of BSA in the dimer is discussed in terms of normal modes in a molecular picture.

References

[1] Stadler et al. J. Am. Chem. Soc., 2014, 136 (19), pp 6987–6994
[2] Ameseder et al. J. Phys. Chem. Lett, 2018, 9, 2469−2473
[3] Ameseder et al. Phys Chem Chem Phys, 2018, 20 (7), 5128-5139

O-086 (P-225)

Side chain to main chain hydrogen bonds stabilize a polyglutamine helix in the activation domain of a transcription factor

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Poly-glutamine (polyQ) tract expansions have been linked to nine human neurodegenerative diseases. Their biological role has been suggested to involve their organization into secondary structure elements depending on their protein sequence context[1,2,3]. For the particular case of the Androgen Receptor (AR) – linked to Kennedy's disease - we recently reported that the Leu-rich segment N-terminal to the polyQ tract acts as a helix-inducing sequence that propagates helicity into the tract itself[1]. We have collected CD, NMR and molecular simulations data on a set of recombinant, isotopically enriched peptides representing increasingly longer AR polyQ tracts up to the lengths found in the average human population (16-25 Gln residues, depending on ethnicity)[4]. Experimental data shows that the helicity of the sequence positively correlates with tract length, and that a rotameric selection affects the sidechains of the initial glutamine residues in the tract upon helicity gain. In turn, chemical shift-reweighted, MD and QM/MM simulation-derived conformational ensembles unveil that helix stabilization is achieved through bifurcate hydrogen bonds involving both the backbone and glutamine sidechains, resulting in a non-canonical helical arrangement and providing a rationale for the sidechain rotameric selection observed by NMR. Leucine to alanine mutations N-terminal to the polyQ tract result in helix destabilization, despite of Ala being intrinsically more efficient at helix propagation. We propose that Leu residues generate a hydrophobic shielding for Gln side-chain-involving hydrogen bonds, providing energetic stabilization by preventing water competition[5]. Such effect can also be observed in the polyQ tract of huntingtin, which has also been reported to populate a helical ensemble[3]. Thus, our observations provide a mechanistic basis for the link that exists between polyQ tract length and transcriptional activity in AR[6] and, more generally, between tract length and aggregation via helical oligomeric intermediates in polyQ diseases.

1. Eftekharzadeh, B. *et al.* **Biophys. J**. *(*2016) Jun 7; *110(11)*: 2361-6.
2. Warner, JB. *et al*. **JACS** (2017) *139*; 14456-15569.
3. Baias, M. *et al.* **JACS** (2017) *139*; 1168-1176.
4. Weng *et al.* **Scientific Rep.** (2017), Jan 16; *7:*40554.
5. Gao, J. *et al.* **Nat. Struct. Mol. Biol.** (2009) *16;* 684-690.
6. Beilin, J. *et al.* **J. Mol. Endocrinol.** (2000) *25*; 85-96.

Monday 22nd July

GENE NETWORK DYNAMICS AND SIGNALLING

O-087

Stochastic Turing patterns in the development of a one-dimensional organism

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Cells having the same genetic information can behave very differently, due to inevitable stochastic fluctuations in gene expression, known as noise. How do cells in multicellular organisms achieve high precision in their developmental fate in the presence of noise, in order to reap the benefits of division of labor? We address this fundamental question from Systems Biology and Statistical Physics perspectives, with Anabaena cyanobacterial filaments as a model system, one of the earliest examples of multicellular organisms in Nature. These filaments can form one-dimensional, nearly-regular patterns of cells of two types. The developmental program uses tightly regulated, non-linear processes that include activation, inhibition, and transport, in order to create spatial and temporal patterns of gene expression that we can follow in real time, at the level of individual cells. We study cellular decisions, properties of the genetic network behind pattern formation, and establish the spatial extent to which gene expression is correlated along filaments. Motivated by our experimental results, I will show that pattern formation in Anabaena can be described theoretically by a minimal, three-component model that exhibits a deterministic, diffusion-driven Turing instability in a small region of parameter space. Furthermore, I will discuss how noise can enhance considerably the robustness of the developmental program, by promoting the formation of stochastic patterns in regions of parameter space for which deterministic patterns do not form, suggesting a novel, much more robust mechanism for pattern formation in this and other systems.

O-088

Human time vs. Mouse time: in vitro segmentation clock as a model system

M. Ebisuya Matsuda.
Miki Ebisuya (EMBL Barcelona)

Different species have different tempos of development: larger animals tend to grow more slowly than smaller animals. My group has been trying to understand the molecular basis of this interspecies difference in developmental time, using the segmentation clock as a model system. The segmentation clock is the oscillatory gene expressions that regulate the timing of somite formation from presomitic mesoderm (PSM) during embryogenesis. We have recently succeeded in inducing PSM from both human iPS cells and mouse ES cells, detecting the oscillation and traveling wave of segmentation clock in vitro. Interestingly, the oscillation period of human segmentation clock was 5-6 hours while that of mouse was 2-3 hours. Taking advantage of our in vitro system and simple mathematical models, we have been comparing the genome sequences and molecular processes of the segmentation clock between human and mouse to explain the interspecies difference in the oscillation period.

O-089

Distinguishing dormant from dead by triggering life in spores

Y. Hyun.
Delft University of Technology.

The fact that dormant cells show no outward signs of life makes it challenging to distinguish them from dead cells. By tuning and measuring the expression of a reporter gene within dormant yeast spores, we quantified the spores' ability to express a generic gene. We show that this ability varies greatly between genetically identical spores and is a predictor of (1) how much nutrient is necessary to revive it and (2) how long it can remain viable in the absence of nutrients. With this investigation, we address why some spores would germinate but not others, when spores die.

O-090 (P-294)

A nonequilibrium phase transition theory for the formation of diverse homeostasis and the emergence of systematic aging in multicellular systems

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The breakdown of homeostasis in tissues is fatal to living beings. Numerous factors across multiple scales from subcellular to extracellular levels could account for the deregulation of homeostasis. Here we present a prototypical multicellular homeostasis model in the form of a 2D stochastic cellular automaton with three cellular states, proliferative, dead and quiescent, of which the state-updating rules based on fundamental cell biology regarding self-replication, apoptosis and cell cycle arrest. This simple model can reproduce the formation of diverse homeostatic patterns with distinct morphologies, turnover rates and lifespans without considering genetic, metabolic or other exogenous variations. Besides, through mean-field analysis and Monte-Carlo simulations, those homeostatic states are found to be classified into extinctive, proliferative and degenerative phases, whereas healthy multicellular organizations (characterized by a large proportion of quiescent cells with few proliferative cells) could evolve from proliferative to degenerative phases over long time, undergoing a systematic aging. From statistical analyses, this aging process is found to be deeply related to the phase transitions into an absorbing state in some nonequilibrium physical systems featuring generalized epidemic process. A theory in the language of nonequilibrium statistical physics is thence proposed to attribute the collapse of homeostasis (at the multicellular level) to an isomorphism between a generalized epidemic process and the fundamental nature of biology regarding cell proliferation, death and cell cycle arrest.

O-091 (P-295)

Post-transcriptional regulation, noise and spatial transcript localization of small RNA-controlled genes in an *Escherichia coli* stress response network

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Post-transcriptional regulatory processes may change transcript levels and affect cell-to-cell variability or noise. We study small-RNA regulation to elucidate its effects on noise in the iron homeostasis network of Escherichia coli. In this network, the small-RNA RyhB undergoes stoichiometric degradation with the transcripts of several target genes, as well as upregulates the translation of other target genes, in response to iron stress. Using single-molecule fluorescence in situ hybridization (smFISH), we measured transcript numbers of RyhB-regulated genes in individual cells, as a function of iron deprivation. We observed a monotonic increase of noise with iron stress, but no evidence of theoretically predicted, enhanced stoichiometric fluctuations in transcript numbers, nor of bistable behavior in transcript distributions. Direct detection of RyhB in individual cells shows that its noise is much smaller than that of these two targets, when RyhB production is significant. A generalized stochastic, two-state model of bursty transcription that neglects RyhB fluctuations describes quantitatively the dependence of noise and transcript distributions on iron deprivation, enabling extraction of in vivo parameters such as RyhB-mediated transcript degradation rates. The transcripts' threshold-linear behavior indicates that the effective in vivo interaction strength between RyhB and its two target transcripts is comparable. Visualization of various transcripts by smFISH and super-resolution microscopy indicates that their subcellular localization is not significantly affected by iron stress. The results do not support predictions of a theoretical model that claims that excluded volume effects favor transcript localization at the cellular poles.

O-092 (P-296)

Modeling cytoneme guidance for Hedgehog signaling.

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Cell-cell communication is crucial during the development of an organism and is mediated through signal molecules called morphogens, which are distributed in a morphogenetic field in graded form, activating different target genes in a concentration-dependent manner. Classical models assume that the gradient distribution of those signaling is through a diffusion mechanism. However, new experimental results challenge this idea since most of the morphogens cannot diffuse freely through the extracellular matrix (ECM) due to their biochemical properties. A new mechanism for morphogen transport has been proposed that solves this issue and is based on the idea of distribution of the signal molecules through filopodia-like structures, also called signaling filopodia or cytonemes. These actin-based structures seem to play a critical role in the process of cell communication in several biological systems during development. Cytonemes protrude from the cell membranes of both producing and receiving cells and extend through the ECM to reach distant cells to deliver and collect the signaling molecules. Therefore, knowing the mechanism that control their dynamics, elongation, polarization and orientation is key to understand the correct signaling pattern. We are studying those cytonemes implicated in the Hedgehog (Hh) signaling pathway in *Drosophila* epithelia. We have analyzed in this system the possible role of certain adhesion and ECM proteins of this pathway in the cytoneme guiding process. We have used a set of genetic tools to modify the local levels of those proteins and then quantify biophysical parameters, such as length and orientation of cytonemes, under these conditions. Due to the non-linear dynamics of the process, we have developed a mathematical model using a novel theoretical approach where cytoneme guiding can be explained as an emergent biophysical property of potentials generated by concentration-dependent sources of several proteins.

Monday 22nd July
NEW FRONTIERS IN BIOIMAGING

O-093
Faster and gentler optical nanoscopy for brain cell imaging
Ilaria Testa
The formidable ability of fluorescent nanoscopy to image features closer than half the wavelength of light often comes at the expense of time and increased dose of energy for recording. We developed a gentle fluorescent nanoscope based on the reversible switch of fluorescent proteins, capable of resolving cellular structures within the whole cell at spatial resolutions down to between 40–60 nm using minimal light intensities (50-500 W/cm2). Our approach, named MoNaLISA for molecular nanoscale long term imaging with sectioning ability, is based on thousands of focal spots that switch and read-out the fluorescence signal emitted by reversible switchable fluorescent proteins. MoNaLISA illumination scheme happen in three steps and it is highly parallelized enabling acquisition time in the order of the 0.2–2Hz for large fields of view (50μm). The spatially modulated illuminations in ON-switching and read-out lead to images with tenfold enhanced contrast and intrinsic optical sectioning. MoNaLISA imaging is extendable to the whole range of reversible switchable fluorescent proteins without compromising image contrast. We demonstrate the general use of MoNaLISA in living cells and tissues such as human fibroblast, hippocampal neurons, colonies of mouse embryonic stem cells and organotypic slice culture.

O-094
The Mesolens: a new instrument for 3D optical imaging of large biological specimens with sub-cellular resolution throughout
G. Mcconnell.
University of Strathclyde.
For more than a century, the design of low-magnification microscope objectives has been guided by the angular acuity of the human eye (approximately 1 second of arc). At x4 magnification, this requires a numerical aperture no greater than 0.1 or 0.2, which can be achieved cheaply and easily by simple optical designs. With the advent of confocal and multiphoton microscopy, however, it became apparent that the poor axial resolution of more than 30 microns with low magnification objectives was intolerable for these 3D methods.
To overcome this, we have developed a new and complex objective with a magnification of 4x and an NA of just less than 0.5 which we call the Mesolens. We specified this lens for mammalian embryology, and have shown that it can image every cell of a 6mm-long embryo 3mm thick with sub-cellular resolution if the tissue is cleared appropriately. A by-product of the high NA is that the optical throughput is approximately 20x greater than a conventional 4x objective. The pupil size of the lens is so great that it cannot be used with a conventional microscope frame, so we have built the imaging system around the lens, and use either a sensor-shifting camera for widefield imaging or point-scanning fluorescence detection to create images. We have applied the Mesolens to a range of biomedical specimens: I will present data from current applications and describe our plans to further develop the lens.

O-095
Brillouin microscopy for cell and tissue biomechanics
G. Scarcelli.
UMD
The ability to measure the mechanical properties of biological tissue and biomaterials *in vivo* would have a significant impact in many areas of biomedical research and clinical application. At the cellular scale, the biomechanical component of the interaction between cells and their local microenvironment has emerged as a crucial regulator of cellular function. However, current instruments to assess cell mechanics, including atomic force microscopy and microrheology, require contact or are limited to the analysis of few points at random locations. As a result, it is difficult to map cellular mechanical properties when cells are not physically accessible such as in microfluidics chips or in 3D microenvironments.
Brillouin microscopy is an intriguing solution to the non-invasive measurement of material mechanical properties without contact. In Brillouin light scattering, the interaction of light with intrinsic mechanical vibrations of material, allows to read out mechanical information optically via the spectral analysis of the scattered light. Brillouin spectroscopy has long been employed as a non-contact method for mechanical testing, but its poor temporal resolution limited the analysis to few points per sample. In the past years, by developing a spectrometer with several orders of magnitude improved throughput and extinction, we created a Brillouin imaging technology, where label-free contrast is provided by the local longitudinal elastic modulus[1, 2], or viscosity and density [3] .
In this talk we will discuss the recent progress in terms of sensitivity and measurement speed as well as the applications in ocular tissue [4], developmental biology[5] and cell biomechanics [6].
References
[1] G. Scarcelli et al. *Nature Photonics,* 2, 39 (2008).
[2] G. Scarcelli *et al. Nature Methods,* 12, 1132 (2015).
[3] A. Fiore et al., *Physical Review Letters, (*2019).
[4] G. Scarcelli et al. *JAMA ophthalmology,* 133, 480 (2015).
[5] R. Raghunathan *et al. Journal of Biomedical Optics,* 22, 086013 (2017).
[6] J. T. Zhang et al., *Lab on a Chip,* 17, 663 (2017).

O-096 (P-297)
Wavefront shaping for low backgound, high resolution STED-FCS in three dimensions
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Fluorescence correlation spectroscopy (FCS) is a powerful tool to study molecular diffusion. However, the spatial resolution of FCS experiments is restrained by the diffraction limit and many diffusion processes happening at the nanoscale cannot be resolved by conventional FCS. To overcome this limitation, FCS can be used together with stimulated emission depletion (STED) microscopy, and this STED-FCS approach has already found multiple applications, particularly when studying the cellular plasma membrane. STED-FCS has however rarely been used to study 3-dimensional diffusion, mainly because with the most widely used STED depletion pattern, a ring-shaped focus (“doughnut”, 2D STED), the non-depleted out-of-focus volumes contribute to high background noise and deteriorate the effective spatial resolution. A solution to this problem consists in using a bottle-shaped pattern as the depletion beam (3D STED); but this pattern suffers from an exacerbated sensitivity to optical aberrations.
We present here an adaptive optics method that corrects aberrations affecting 3D STED-FCS experiments. Using a spatial light modulator (SLM) as a wavefront shaping device in the depletion path of a STED microscope, we corrected aberrations affecting STED-FCS measurements in the cytoplasm of living cells, leading to increased resolution, improved signal quality, and larger accessible depth range for measurements.

O-097 (P-298)

High density single particle tracking reveals nano- and meso-scale dynamic organization of plasma membrane receptors in living cells

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Transmembrane adhesion receptors at the cell surface, such as CD44, are often equipped with modules to interact with the extracellular matrix (ECM) and the intracellular cytoskeletal machinery. CD44 has been recently shown to compartmentalize the membrane into domains by acting as membrane pickets, facilitating the function of signalling receptors. While spatial organization and diffusion studies of membrane receptors are usually conducted separately, here we combine observations of the organization and diffusion of CD44 using high spatial and temporal resolution imaging on living cells to reveal a hierarchical organization of the receptor. We used high-density single particle tracking (HD-SPT) to generate cartography maps, with nanometre localization accuracy, of regions explored by the receptor as it diffuses in the cell membrane. Using this approach, we found that CD44 dynamically organizes along a meshwork-like pattern at the mesoscale. Interestingly, these patterns are enriched by actin-dependent CD44 nanoclusters. We characterized the properties of these clusters in terms of size and time evolution for different CD44 mutants and reconstructed an in-silico meshwork to further quantify to strength of the interaction of CD44 with the underlying network. Overall, our methodology provides valuable insight into the hierarchical organization of CD44 at the cell surface and should be equally applicable to the study of other membrane receptors.

O-098 (P-299)

Photophysics and engineering of transgene labels for optoacoustics

J.P. Fuenzalida-Werner[1], K. Mishra[1], I. Weidenfeld[1], A. Chmyrov[1], T. Drepper[2], V. Ntziachristos[1], A.C. Stiel[1].

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Photo- or Optoacoustic (OA) imaging combines optical contrast with ultrasound resolution enabling high-resolution real time *in vivo* imaging well-beyond the 1 mm penetration depth typical of purely optical methods. While OA already successfully employs endogenous contrast like blood or lipids to inform on tumor states, vascularization, inflammation or metabolic processes, targeted labels similar to those used in fluorescence imaging are few. This scarcity, particularly of labels that can be expressed as transgenes, limits the full exploitation of OA in life science applications. Since both modalities rely on optical absorption, OA labels can be developed from fluorescent ones, but their photophysical characteristics have to be engineered specifically for OA.

Our group studies the photophysics of OA signal generation from major classes of chromophore-bearing proteins (GFP-like, Bacteriophytochromes and Phycobiliproteins) using OA, absorption and fluorescence spectroscopy under ns-pulsed laser excitation as in OA imaging [1]. We use the insights from these analyses to engineer novel OA labels through screening- and structure-based rational design [2]. A focus of our group is on photo-controllable labels to overcome the abundant signal from natural absorbers during OA imaging *in vivo* [1,3-4]. In parallel, we explore ways to develop molecular sensors for OA imaging (e.g. Ca^{2+}-responsive) as well as cell-based sensors, e.g. our recent work in tracking macrophage activity within tumors using changes in the spectra of bacteriochlorophyll [5].

Our long-term goal is to provide a varied toolbox of OA labels and sensors that will allow researchers to exploit the penetration depth and non-invasiveness of this powerful imaging technique for their basic and preclinical studies of neural signaling, metabolism, cell physiology and tumor biology in entire organisms. Our conference contribution will introduce to this novel area of label development and its underlying photophysics.

Further reading:

Vetschera, *Analytical Chemistry*, 2018
Fuenzalida-Werner, *JSB*, 2018
Stiel, *Optics Letter*, 2015
Deán-Ben XL, *Optics Letters*, 2015
Peters, *Nat. Comm.*, 2019

Monday 22nd July

CELL MEMBRANE BIOPHYSICS Sponsorized by CPL, Elsevier

O-099

Morphology of Membrane Contact Sites Contributes to the Regulation of the Phosphatidylinositol Cycle

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The phosphatidylinositol cycle (PI-cycle) plays an important role in a number of signal transduction pathways and it is the principle pathway for the synthesis of phosphatidylinositol (PI) and its phosphorylated derivatives . In primary mammalian cells the major fraction of PI is a single molecular species containing 18:0 and 20:4 acyl chains on the sn-1 and sn-2 positions, respectively. This acyl chain enrichment is accounted for in part by the substrate acyl chain specificity of enzymes of the PI-cycle, in addition to acyl chain remodeling enzymes. Another unusual property of the PI-cycle is that some steps of the cycle occur in the plasma membrane (PM), while other steps occur in the endoplasmic reticulum (ER). This requires that lipid is transferred between the ER and PM in both directions. One example of this is the transfer of diacylglycerol (DAG) and/or phosphatidic acid (PA) from the PM to the ER. DAG is produced in the PI-cycle by the action of phospholipase C on PI(4,5)P2 that occurs in the PM. DAG can be converted to PA by the epsilon isoform of the enzyme diacylglycerol kinase (DGKε). The PA that is produced is used in the PI-cycle in the ER for the synthesis of CDP-DAG. There is evidence that DGKε is present in both the PM and the ER and that it is also present at PM-ER contact sites. DGKε acts on a lipid substrate, DAG, and therefore requires a membrane in which to act. Recently it has been shown that the enzyme´s activity and substrate acyl chain specificity is very sensitive to the physical properties of the membrane in which it acts. In stable planar bilayers DGKε has lower activity and lower acyl chain specificity. Furthermore it is not sensitive to the intrinsic curvature properties of the constituent lipids, as long as a bilayer arrangement is maintained. However, DGKε activity and substrate acyl chain specificity is remarkably increased by a change in membrane morphology. Specifically it has been shown that membranes with negative Gaussian curvature (bicontinuous cubic phase or membrane fusion intermediates) have the required morphology to activate the enzyme and endow it with acyl chain specificity. DGKε is thus the first example of an enzyme whose properties are modulated by membrane shape but not by membrane intrinsic curvature. It is proposed that DGKε acts as a bridge for the hierarchic coupling of membrane physical properties and lipid composition to synergistically regulate membrane signaling events.

O-100

Cholesterol modulation of nicotinic acetylcholine receptor single molecule and nanocluster trajectories

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Translational motion of neurotransmitter receptors is an important factor for determining receptor number and stability at the synapse and hence, synaptic efficacy. We combine single-molecule STORM superresolution localization microscopy of nicotinic acetylcholine receptor (nAChR) with single-particle tracking, mean-squared displacement, turning angle, ergodicity, and clustering analyses to characterize the diffusional properties of individual molecules and their collective behavior in living cells. nAChR diffusion is highly heterogeneous: a mix of anomalous subdiffusive, Brownian and superdiffusive. Cholesterol-depleted trajectories exhibit weak ergodicity breaking. At the single-track level, the free walks of an individual trajectory are transiently interrupted by confinement sojourns in small nanodomains (~50 nm radius), with millisecond-long lifetimes. Nanocluster assembly occurs in bursts lasting for seconds, separated by periods of cluster disassembly. The two dynamic processes occur in similar spatial scales, but in different time frames and with different cholesterol sensitivities. The ms-long confinement sojourns and the s-long reversible nanoclustering affect all trajectories and determine the resulting macroscopic motional regime and the breadth of the heterogeneity in the ensemble population. The emerging picture is that the nAChR operates in a complex variety of motional regimes, including anomalous diffusion, and that these are subject to cholesterol modulation at the cell surface.

O-102 (P-317)

Linking Structure, Stability and Function in the Anti-Measles Virus Action of Tocopherol-Derivatized Peptide Nanoparticles

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Amyloids have been exploited to build up amazing bioactive materials. In most cases, short synthetic peptides constitute the functional components of such materials. The controlled assembly of globular proteins into active amyloid nanofibrils is still challenging, because the formation of amyloids implies a conformational conversion towards a β-sheet-rich structure, with a concomitant loss of the native fold and the inactivation of the protein. There is, however, a remarkable exception to this rule: the yeast prions. They are singular proteins able to switch between a soluble and an amyloid state. In both states, the structure of their globular domains remains essentially intact. The transit between these two conformations is encoded in prion domains (PrDs): long and disordered sequences to which the active globular domains are appended. PrDs are much larger than typical self-assembling peptides. This seriously limits their use for nanotechnological applications. We have recently shown that these domains contain soft amyloid cores (SACs) that suffice to nucleate their self-assembly reaction. Here we genetically fused a model SAC with different globular proteins. We demonstrate that this very short sequence act as minimalist PrDs, driving the selective and slow assembly of the initially soluble fusions into amyloid fibrils in which the globular proteins keep their native structure and display high activity. Overall, we provide here a novel, modular and straightforward strategy to build up active protein-based nanomaterials at a preparative scale.

O-103 (P-318)

Antimicrobial Peptides Impair Bacteria Cell Structure Within Seconds

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Novel antibiotics based on membrane active antimicrobial peptides (AMPs) are promising candidates for defending the spread of diseases caused by multi-resistant pathogenic bacteria [1]. Notwithstanding the number of works that explore the relationship between AMP activity and membrane architecture [2,3], the dynamics and full mechanism that lead to cell death are currently not clear. This prompted us to investigate the cinematographic effects of human lactoferricin derivatives on live *E. coli* using high-resolution (ultra) small-angle X-ray scattering (USAXS/SAXS) measurements and complementary techniques.

In order to discriminate scattering signals originating from peptides and bacteria, SAXS measurements of AMP suspensions were firstly analysed by means of colloidal and polymer models. Secondly, dependencies of AMP Minimum-Inhibitory-Concentration on bacterial number density were modelled via a phenomenological Hill equation in order to extract effective association constants and the minimum number of AMPs required for inhibiting cell growth. Finally, time-resolved USAXS/SAXS combined with stopped-flow mixing allowed us to probe AMP activity on the submicron to nanometre length (cell body and cell envelope, respectively) and time scales using a recently reported multi-scale model [4]. First results suggest that part of the cell function is lost due to a general disruption of the cell wall, involving a decrease of correlations between inner and outer membranes, as well as a loss of cytoplasm. These key events of killing occur just within a few seconds after exposure to AMPs, i.e. much faster than anticipated from previous reports.

[1] K. Lohner, editor, *Development of novel antimicrobial agents: Emerging strategies.* Horzon Scientific Press, Norfolk, UK (2001).
[2] K. Lohner, E. Sevcsik and G. Pabst, *Liposome-based biomembrane mimetic systems: implications for lipid-peptide interactions*. In *Advances in Planar Lipid and Liposomes* (Vol. 6). A. Leitmannova-liu, editor. Elsevier, Amsterdam, pp. 103-137 (2008).
[3] D. Zweytick et al., *J. Biol. Chem.* **286**(24), 21266 (2011).
[4] E. F. Semeraro et al., *IUCrJ* **4**(6), 751 (2017).

O-104 (P-319)

Elucidating the nanoscale architecture of the plasma membrane with super-resolution spectroscopy

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Cellular homeostasis is usually maintained via complex signalling mechanisms. Although these signalling events involve a multitude of signal transduction molecules, they are usually triggered by interactions between a ligand and a receptor on the cell membrane. The investigation of spatiotemporal interactions between signalling components in the membrane has been hampered by the complex structure of the cell membrane: signalling in the plasma membrane involve not only the core protein components, but also membrane lipids, the actin cytoskeleton and the glycocalyx. Moreover, early phases of signalling occur at very fast temporal (milliseconds) and small spatial (nanometre) scales, which further hampers thorough elucidation of these processes. Therefore, studies aiming at a complete understanding of signalling require both advanced imaging techniques with high spatiotemporal resolution and well-defined reconstituted systems that can pinpoint the role of each functional component. Here, I will explain how we utilize super-resolution STED microscopy combined with fluorescence correlation spectroscopy (STED-FCS) to access the diffusion characteristics of fluorescently labelled lipids and proteins in the live cell plasma membrane. Our data showed that nanoscale mobility of lipids and proteins in the plasma membrane is highly heterogeneous and this heterogeneity gives invaluable information on the molecular bioactivity. We also developed simplified biomimetic of cell membrane to clarify the principles underlying cell signalling at the molecular level and to dissect the essential drivers of these processes.

Tuesday 23rd July

MECHANISMS OF MEMBRANE PROTEINS

O-105

Factors affecting Ion Permeation through K^+-Channels

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The energetic barriers associated with ion translocation across the selectivity filter of K^+-channels have been computed in various studies, leading to the proposal of two alternate mechanisms of conduction, involving or neglecting the presence of water molecules in between the permeating ions. The effect of membrane composition, specifically anionic lipids, and the protonation states of key protein residues are assessed, serving as contemporary aspects of this work. The information gained brings to light several novel prospects concerning conduction of potassium ions.

O-106

Optoelectrical dynamics of BK channel activation

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In neurons, sites of Ca^{2+} influx and Ca^{2+} sensors are located within 20-50 nm, in subcellular "Ca^{2+} nanodomains". Such tight coupling is crucial for the functional properties of synapses and neuronal excitability. Two key players act together in nanodomains, coupling Ca^{2+} signal to membrane potential: the voltage-dependent Ca^{2+} channels (Cav) and the large conductance Ca^{2+} and voltage-gated K^+ channels (BK, hslo or KCa1.1). BK channels are high conductance potassium channels characterized by synergistic activation by intracellular Ca^{2+} and membrane depolarization, but the complex molecular mechanism underlying channel function is not adequately understood. Numerous studies have identified the Ca^{2+} binding sites within an intracellular structure known as the gating ring. However, many questions remain about the complex structural rearrangements involved in coupling Ca^{2+} binding to channel gating. Information about the pore region, voltage sensing domain or isolated intracellular domains has been obtained separately using electrophysiology, biochemistry and crystallography. In our laboratory we use a combination of genetics, biochemistry, electrophysiology and spectroscopy, which we correlate with protein structural analysis, to investigate the real time structural dynamics underlying the molecular coupling of Ca^{2+}, voltage and activation of BK channels in the membrane environment, its regulation by accessory subunits and channel effectors. BK subcellular localization and role in Ca^{2+} neuronal nanodomains make these channels perfect candidates as reporters of local changes in [Ca^{2+}] restricted to specific subcellular regions close to the neuronal membrane. We have created fluorescent variants of the channel that report BK activity induced by Ca^{2+} binding, or Ca^{2+} binding and voltage. In addition to the above-mentioned structure-function studies, we aim to optimize and deploy these novel optoelectrical reporters to study physiologically relevant Ca^{2+}-induced processes both in cellular and animal models. Overall, optically-active BK channels with spectrally-separate photoactivation and FRET modules offer many possibilities for the study of physiologically relevant processes involving ion channel activation in mammalian cells.

O-107

The first human lacking functional TRPV1 channel: Implications on TRPV1 functions in nociception

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# Contributed equally to this work

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Many body functions, in particular, noxious heat sensation and inflammatory pain have been attributed to activation of the TRPV1 channel. Nevertheless, the functional roles of the human TRPV1 (hTRPV1) channel remain poorly define. We performed whole-exome sequencing analysis in a patient who eats "hot" chili peppers without any signs of taste aversion, blotching, or tears. This patient was found to carry a novel homozygote mutation in the hTRPV1 channel. He also revealed no sensitivity to capsaicin, elevated noxious heat tolerance and reduced sensitivity to noxious heat. Surprisingly, he showed unusually high sensitivity to noxious cold, but had normal sensitivity to mechanical stimuli and to induction of inflammation by histamine. Other sensory modalities, immune response, blood sugar and general health were normal. *In vitro* Ca^{2+} imaging, electrophysiological, biochemical and messenger RNA assays clearly showed elimination of the hTRPV1 functions without eliminating the TRPV1 protein. Our results show that hTRPV1 is a determinant of noxious heat detection, but surprisingly other properties of TRPV1 knock-out mice have not been observed in human.

O-108 (P-347)

Structural insight into TRPV5 channel function and modulation

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TRPV5 (transient receptor potential vanilloid) is a uniquely calcium-selective TRP ion channel that plays a crucial role in the maintenance of calcium homeostasis. TRPV5 and its close homologue TRPV6 do not exhibit thermosensitivity or ligand-dependent activation, unlike other TRPV channels. They are constitutively opened at physiological membrane potentials. Both are tightly regulated by calcium and the calcium-sensing protein calmodulin (CaM). However, little was known on CaM binding and stoichiometry, or how it arranges TRPV5/6 channel inactivation. Full length and truncated TRPV5 proteins, expressed in human embryonic kidney (HEK293) cells, were affinity-purified and reconstituted into lipid nanodiscs (disc-shaped membrane mimics) or detergent micelles. After assessing the reconstitution efficiency with size-exclusion chromatography, SDS-PAGE and negative stain EM, pure TRPV5 protein fractions were pooled, concentrated and used for cryo-EM analysis. Data collection took place on a Titan Krios electron microscope, operated at 300kV. Data analysis was carried out in CryoSPARC and RELION software. We report high resolution cryo-EM structures of full length and truncated TRPV5 in lipid nanodiscs, a TRPV5 W583A mutant structure, and a complex structure of TRPV5 with CaM. Overall, TRPV5 closely resembles previously reported TRPV channel structures but we highlight some new features, which include an extended S1-S2 linker that forms tight interactions with the upper pore region, as well as an essential tryptophan residue (W583) at the bottom pore. While the W583A mutation does not affect the conformation at the upper pore, the lower pore region is clearly open for ion permeation. Our TRPV5-CaM complex structure demonstrates interaction of CaM with specific carboxy-terminal regions of TRPV5. Interestingly, residue K115 of CaM inserts deeply into the lower pore surrounded by W583, thereby blocking the pore. Furthermore, 3D classification suggests a flexible stoichiometry of 1:1 or 2:1 CaM binding to TRPV5. Our structures highlight interesting channel features divergent from the thermosensitive TRPV channels and extend our understanding from what is currently known for TRPV5 and its homolog TRPV6. Most notable, we provide insight into TRPV5 channel gating and propose a model for CaM-dependent channel regulation.

O-109 (P-348)

Biophysical insights into membrane fission mediated by ESCRT-III

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The endosomal sorting complex required for transport (ESCRT) engages in processes of membrane remodelling and fission, such as formation of multivesicular bodies, plasma membrane repair, neuron pruning, virus budding and autophagy as reviewed in [1]. The ESCRT machinery contains more than 15 proteins organized in four sub-complexes (ESCRT-0, ESCRT-I, ESCRT-II and ESCRT-III), among which the ESCRT-III (composed by Vps2, Vps20, Vps24 and Vps32) is highly conserved across the eukaryotic lineage and mediates the processes required for membrane deformation and fission [2]. Membrane models such as giant unilamellar vesicles (GUVs) [3] can be employed to unravel the ESCRT action in vitro, given the complexity and the number of proteins involved. It has been recently shown that solely Vps20, Vps32 and Vps24 from the phagocytic parasite *E. histolytica* are required to generate intraluminal vesicles (ILVs) in GUVs [4]. However, the current models do not provide a complete picture of the biophysical mechanisms by which the ESCRT-III components reshape the membrane. Moreover, the role of the membrane material properties in tuning the ESCRT-III activity is unrevealed. In this study, we observed for first time the consecutive action of the ESCRT-III proteins on a single-vesicle level, combining GUVs and microfluidics. We characterized several mechanisms involved in the membrane remodelling by the ESCRT-III complex and the regulation of the protein activity. Namely, (i) increase in the membrane tension results in distortion of the ESCRT-III scaffold in the intraluminal buds; (ii) the ESCRT-III proteins influence both the membrane stiffness and the spontaneous curvature, and thus control the size of the ILVs; (iii) a membrane fluid-fluid phase separation was induced in the presence of the ESCRT-III machinery, whereby the ILVs formed from the liquid-ordered phase.

[1] L. Christ et al., Trends Biochem. Sci., 2017, vol. 42, p. 42

[2] J Schöneber et al., Nature Rev., 2017, vol. 18, p. 5

[3] R. Dimova, Annu. Rev. Biophys., 2019, DOI: 10.1146/annurev-biophys-052118-115342.

[4] Y Avalos-Padilla et al., Front. Cell. Infect. Microbiol., 2018, vol.8,p. 53

O-110 (P-349)

Ion transport, interfacial effects and scaling behavior in protein channels

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Many protein channels have in common the importance of electrostatic interactions between the permeating ions and the nanochannel. Since ion transport occurs under confinement conditions, interfacial effects such as access resistance (AR) may play a significant role. We measure AR in a large ion channel, the bacterial porin OmpF, by means of single channel conductance measurements in electrolyte solutions containing varying concentrations of high molecular weight PEG, sterically excluded from the pore. We found that AR might reach up to 80% of the total channel conductance in diluted solutions, where electrophysiological recordings register essentially the AR of the system and depend marginally on the pore characteristics. On the other hand, charged polar groups of the lipid may have a strong influence on the electric potential and the ionic concentration near the membrane-solution interface. Charged residues within the protein located near the pore mouth can also play a role, although to a lesser extent than AR and membrane surface charges. These three factors are obviously coupled and are strongly dependent on the channel aperture size, 3D structure and channel-lipid assembling. Comparison of experiments performed in charged and neutral planar membranes shows that lipid surface charges modify the ion distribution and determine the value of AR, indicating that lipid molecules are more than passive scaffolds even in the case of large transmembrane proteins. These findings are relevant to the fact that ionic conductance in membrane channels exhibits a power-law dependence on electrolyte concentration ($G \sim c^{\alpha}$). We critically evaluate the predictive power of scaling exponents by analyzing conductance measurements in four biological channels with contrasting architectures. We show that scaling behavior depends on several interconnected effects whose contributions change with concentration so that the use of oversimplified models missing critical factors could be misleading. In fact, the presence of interfacial effects could give rise to an apparent universal scaling that hides the channel distinctive features. We complement our study with 3D structure-based Poisson−Nernst−Planck calculations, giving results in line with experiments and validating scaling arguments.

Tuesday 23rd July

SINGLE MOLECULE BIOPHYSICS

O-111

The mechanical stability of proteins regulates their translocation rate into the cell nucleus

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The translocation of mechanosensitive transcription factors (TFs) across the nuclear envelope is a crucial step in cellular mechanotransduction. Yet the molecular mechanisms by which mechanical cues control the nuclear shuttling dynamics of TFs through the nuclear pore complex (NPC) to activate gene expression are poorly understood. Here, we show that the nuclear import rate of myocardin-related transcription factor A (MRTFA) — a protein that regulates cytoskeletal dynamics via the activation of the TF serum response factor (SRF) — inversely correlates with the protein's nanomechanical stability and does not relate to its thermodynamic stability. Tagging MRTFA with mechanically-stable proteins results in the downregulation of SRF-mediated gene expression and subsequent slowing down of cell migration. We conclude that the mechanical unfolding of proteins regulates their nuclear translocation rate through the NPC and highlight the role of the NPC as a selective mechanosensor able to discriminate forces as low as ~10 pN. The modulation of the mechanical stability of TFs may represent a new, general strategy for the control of gene expression.

O-112

Mechanosensitive states of the giant muscle protein titin

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Titin, a multi-domain filamentous protein is thought to act as a sensor of stress and strain in the muscle sarcomere. The exact mechanisms of this mechanosensing function are not fully understood. To gain an insight into the mechanosensitive structural states of titin, we have manipulated single molecules with high-resolution optical tweezers and imaged structural states of extended molecules with atomic force microscopy. Discrete, stepwise transitions can be resolved in titin during stretch at forces as low as 5 pN. Numerous mechanisms and molecular regions contribute to a pattern of transitions which is sensitive to mechanical history. Globular domains are selected for unfolding according to a spatial gradient of mechanical stability, which acts as a safety mechanim against sarcomeric structural disintegration. A C-terminally located region corresponding to the titin kinase unfolds systematically under overstretching forces, suggesting that this domain may be mechanically activated in intrasarcomeric conditions. Mechanically unfolded weak domains may dynamically reorganize towards the molten-globule and the folded state, thereby generating force that aids saromere contraction. Altogether, titin displays a complex pattern of history-dependent, force-driven structural transitions which, by dynamically exposing ligand-binding sites, may set the stage for the sensing of the sarcomere's mechanical status.

O-113

Single-molecule live-cell imaging of mRNA life cycle

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Expression of genetic information in eukaryotes involves a series of interconnected processes that ultimately determine the quality and amount of proteins in the cell. Many individual steps in gene expression are kinetically coupled, but tools are lacking to determine how temporal relationships between chemical reactions contribute to the output of the final gene product. Previous studies have imaged RNAs in living cells by genetically inserting the binding sites for bacteriophage coat proteins in the RNA of interest. However, multiple nascent RNAs were simultaneously detected at the site of transcription, necessitating a modelling approach to infer kinetic information. To circumvent these significant limitations and potential problems in data interpretation, we developed a strategy that permits direct tracking of single nascent pre-mRNA molecules in live cells. We are using this approach to study kinetics of the different steps of mRNA life cycle.

O-114 (P-397)

ParB dynamics and the critical role of the C-terminal domain in DNA condensation unveiled by combined Magnetic Tweezers and TIRF Microscopy

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ParB is a central component of partition systems responsible for the faithful segregation of chromosomes and low-copy number plasmids in bacteria. *Bacillus subtilis* ParB forms multimeric networks involving non-specific DNA binding leading to DNA condensation. In previous work [1], it was found that the C-terminal domain (CTD) of ParB is essential for the formation of those higher-order structures, and that an excess of the free CTD impeded DNA condensation or promoted decondensation of pre-assembled networks. However, interpretation of the molecular basis for this phenomenon was complicated by the inability to uncouple protein binding from DNA condensation or to correlate both measurements in parallel. Here, we have combined lateral magnetic tweezers with total internal reflection fluorescence microscopy to simultaneously control the restrictive force against condensation and to visualize ParB protein binding by fluorescence. At non-permissive forces for condensation, ParB binds non-specifically and highly dynamically to DNA, whereas at low forces ParB condenses DNA as shown previously [2]. Our new approach allowed direct tests of different models to explain CTD-dependent condensation inhibition. We conclude that the free CTD blocks the formation of ParB networks by heterodimerization with full length ParB which remains bound to the DNA. This strongly supports a model in which the CTD acts as a key bridging interface between distal DNA binding loci within ParB networks [3].

[1] Fisher, G.L.M., *et al.*, Elife, 2017. 6: e28086
[2] Taylor, J.A., *et al.*, NAR, 2015. 43(2): p. 719-31
[3] Madariaga-Marcos, J., *et al.*, Elife, 2019 (*accepted*)

O-115 (P-398)

A folding nucleus and minimal ATP binding domain of Hsp70 identified by single-molecule force spectroscopy

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The folding of large proteins can be a very complex process; many large proteins critically rely on the aid of molecular chaperones while some equally large proteins fold spontaneously. Along the folding pathways, partially folded intermediate states are frequently populated and can accelerate or even decelerate efficient folding process. The structures of these intermediates are generally unknown because they are often very short-lived. In our recent work [1], single-molecule force measurements were used to scrutinize the hierarchy of intermediate states along the folding pathway of the nucleotide binding domain (NBD) of Escherichia coli Hsp70 DnaK. DnaK-NBD is a member of the sugar kinase superfamily that includes Hsp70s and the cytoskeletal protein actin. Using optical tweezers, a stable nucleotide-binding competent en route folding intermediate comprising lobe II residues (183-383) was identified as a checkpoint for productive folding. We have obtained a structural snapshot of this folding intermediate that shows native-like conformation. To assess the fundamental role of folded lobe II for efficient folding, we turned our attention to yeast mitochondrial NBD, which does not fold without a dedicated chaperone. After replacing the yeast lobe II residues with stable E. coli lobe II, the obtained chimeric protein showed native-like ATPase activity and robust folding into the native state, even in the absence of chaperone. In summary, lobe II is a stable nucleotide-binding competent folding nucleus that is the key to time-efficient folding and possibly resembles a common ancestor domain.

[1] Bauer D, Meinhold S, Jakob RP, Stigler J, Merkel U, Maier T, Rief M, Žoldák G. A folding nucleus and minimal ATP binding domain of Hsp70 identified by single-molecule force spectroscopy. Proc Natl Acad Sci U S A. 2018 May 1;115(18):4666-4671.

O-116 (P-399)

Single-molecule dissection of the dihydrofolate reductase reaction revealed multiple conformers leading to a catalytic product release

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It is generally accepted that enzymes stabilise the transition-state of a catalysed reaction. Here using a nanopore as a single-molecule nano-reactor we provide experimental evidence for a more sophisticated narrative. The sampling of hundreds of consecutive reactions from single enzymes revealed that dihydrofolate reductase (DHFR) populates frequent non-productive transition-state conformations and undergoes second-long catalytic pauses. We also found that the free-energy landscape of the enzyme is sculpted with multiple ground-state conformers with different affinity for substrate, cofactor and product that undergo hierarchical changes during the catalytic cycle. As in a two-stroke engine, the chemical step provides the power stroke to switch the enzyme to the product-bound conformer, promoting the simultaneous release of the oxidised cofactor. The subsequent binding of a reduced cofactor to the vacated site provides the free energy for the recovery stroke, which induce the allosteric release of the product and resets the initial configuration. This catalytic remodeling of the affinity landscape of DHFR is likely to be a general feature for complex enzymatic reaction where the release of the products must be facilitated.

Tuesday 23rd July

BIOPHYSICS OF THE IMMUNE RESPONSE

O-118

Mechanosensing via immunoreceptors

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The immune response is orchestrated by a variety of immune cells, the function of which then is determined by the collective signals from different immunoreceptors. Recent studies have highlighted the presence of mechanical force on these receptor–ligand pairs and its important role in regulating antigen recognition/discrimination and function. In this perspective, we use the T cell receptor as an example to review the current understanding of the mechanosensing properties of immunoreceptors. We discuss the types of forces that immunoreceptors may encounter, the effects on ligand recognition, conformational changes and mechanosensing mechanisms, as well as the consequences in downstream signal transduction and function.

O-119

The role of oligomeric scaffolds in inflammatory signal transduction though Toll-like receptors and MyD88

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Toll like receptors (TLRs) are pivotal in triggering the innate immune response to pathogen infection. Ligand binding to leucine rich repeats on the receptor induces the dimerization of both receptor ectodomain and the cytosolic Toll/interleukin-1 receptor (TIR) domain, providing a nucleation signal for assembly of an oligomeric scaffold, the Myddosome, leading to inflammatory signalling. Myeloid differentiation primary response 88 (MyD88) is required by all TLRs except TLR3 and signalling is thought to proceed via a mechanism that involves the stepwise, sequential assembly of individual components. Recent studies in our lab find that the death domains of human MyD88 spontaneously and reversibly associate to form helical filaments in vitro. A 3.1 Å cryo-EM structure reveals that the architecture of the filament is identical to that of the MyD88-IRAK4-IRAK2 heterooligomeric Myddosome. Additionally, the death domain of IRAK4 interacts with the filaments to reconstitute the non-stoichiometric 6:4 MyD88-IRAK4 complex. Single molecule analysis in vivo shows that in the resting state MyD88 is present as short oligomers or monomers and that interaction with the activated receptor relieves autoinhibition allowing the rapid assembly of the closed Myddosome complex. Together, these data suggest that the MyD88 scaffold may be preformed or rapidly assembled on receptor activation and TIR engagement.

O-120 (P-437)

Insights into the Molecular Mechanism of Lipids/Toll-Like Receptors Interaction

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Toll-like Receptors (TLRs) are the main protagonists of the innate immune system. Among them TLR2 and TLR4/MD2 recognize lipid patterns located in bacterial membrane and alert the immune system of pathogen invasion through stimulating secretion of pro-inflammatory cytokines. We showed that TLR4 and 2 are able to recognize also synthetic lipids, used as nucleic acid nanocarriers, and the mitochondrial lipid cardiolipin.

In silico analysis coupled with in vitro and in vivo experiments brought us to understand the molecular parameters for which a lipid would be inert or recognized by TLR2 or TLR4/MD2. More specifically, we demonstrated that short saturated di-acyl cationic lipids lipopolyamines (LPAs) activate TLR2 and 4, whereas longer saturated cationic lipids activate only TLR2(Lonez et al., 2015 CMLS; Pizzuto et al., 2017, 2018 J Control Release). Moreover the tetra-acyl mitochondrial lipid cardiolipin (CL) acts as a TLR4/MD2 agonist or antagonist depending on its unsaturation degree (Pizzuto et al. 2019 CMLS).

Although we found that all CL docked to the hydrophobic cavity of MD2, unfortunately, molecular docking failed to predict physiologically meaningful conformations or the pharmacology of CLs, revealing the limitations of such an approach with this family of molecules.

By contrast, docking of di-acyl LPAs in TLR2/TLR1 and TLR2/TLR6 was able to predict their activity, suggesting potential TLR2 binding modes reminiscent of bacterial lipopeptide sensing (natural TLR2/1/6 ligands). The saturated or mono-unsaturated LPAs share the ability of burying their lipid chains in the hydrophobic cavity of TLR2 and, in some cases, TLR1, at the vicinity of the dimerization interface. The cationic headgroups form multiple hydrogen bonds, thus crosslinking TLRs into functional complexes. According to our in silico analysis and in vitro data, TLR2 activation could no longer be achieved if both LPA chains were unsaturated. (Pizzuto et al., 2017 J Control Release)

We therefore recommend the use of unsaturated C18 chains for the synthesis of inert transfection agents or TLR4 antagonists. On the other hand, we recommend saturated LPAs in vaccine formulation as their immunostimulatory activity coupled to their carrier properties conferred good adjuvant properties as demonstrated by our in vivo studies(Pizzuto et al., 2018 J Control Release).

O-121 (P-438)

Super-resolution microscopy analysis of molecular interactions between epithelial cells and tissue-resident T cells in mouse epidermis

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Epithelial tissues, such as skin, gut or lung, are the first line of animal defence against external factors. Mammal epidermis is populated by a large number of immune-cells. In mouse these include immune monitoring γδ T cells, also known as dendritic epidermal T cells (DETC), which form a dense network in parallel to the network of Langerhans cells. Previously, a transmembrane-protein Skint1 expressed by keratinocytes was suggested to play a pivotal role in molecular interactions between T cells and surrounding epithelial cells. Here we applied a super-resolution microscopy approach to investigate the role of Skint1 in defining tissue homeostasis and cell functionality. Using Structured Illumination Microscopy, we were able to visualize Skint1 clustering in vicinity of T cell receptor (TCR) clusters at dendrite contact points of DETC, suggesting their direct interaction in tissue. The clustering of Skint1 on epithelial cell surface was further characterised in Skint1 mutant, Skint1 knockout, Skint1 transgenic and γδ TCR knockout mouse lines. The role of dimerization between Skint1 and Skint2 molecules was investigated by analysing Skint1 in Skint2 knockout line and by quantitating Skint2 clustering in Skint1 knockout and transgenic lines. Moreover, we applied two-colour Single Molecule Localization Microscopy approach revealing Skint1-TCR interactions in tissue at 15 nm isotropic resolution. These super-resolution imaging data provide a direct support for Skint family protein -mediated immune sensing in epithelial tissues.

O-122 (P-439)

Shape matters: Towards a molecular understanding of the innate immune response to microbial lipids

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Bacterial membrane components such as lipopolysaccharides (LPS) and lipopeptides are highly effective activators of the innate immune response and trigger inflammation and antimicrobial defense mechanisms by activation of Toll-like receptors (TLR). Dysregulation of this immune response can lead to severe diseases like pneumonia, acute respiratory distress syndrome, and sepsis. We characterize the structural and physical prerequisites for the inflammatory activity of bacterial lipids to elucidate how lipids are recognized by the immune system as danger signals. To this aim we investigate natural and reconstituted membrane systems and use biophysical methods such as fluorescence and infrared spectroscopy to characterize membrane properties. Small angle X-ray diffraction (SAXS) at the electron synchrotron PETRA III at DESY is employed to determine the membrane organization in solution. We have analyzed a variety of isolated natural bacterial lipids, synthetic analogs, and synthetic microbial-lipid-mimetics. These studies reveal that the three-dimensional organization of lipid aggregates correlates with their ability to activate or antagonize cell activation. Accordingly, only cubic inverted aggregate structures exhibit high inflammatory activity, whereas cylindrical molecules, forming lamellar aggregate structures, exhibit low or no activity. Interestingly, we found several examples showing that this behaviour is not correlated with a specific degree of acylation, but depends on the respective packing of the acyl chains in the hydrophobic moiety. Thus, inflammation inducing structures can be adopted by di- and triacyl-as well as highly acylated hexa-acyl lipids of different molecular origin.

Comparing reconstituted lipid aggregates from purified lipopolysaccharide (LPS) of Gram-negative bacteria with purified outer membrane vesicles (OMV) isolated from bacterial cultures we can demonstrate that the interaction mechanisms of both membrane systems with the host cell are quite different. Whereas LPS requires protein mediated transport and activates cell surface receptors, LPS as part of OMVs can enter the host cell by membrane interaction without transporter and activates intracellular signalling pathways. Our data suggest that physico-chemical characteristics represent a key to an understanding of the biological activity of bacterial lipids and determine the molecular mode of action of activators and inhibitors. This knowledge is essential for the further development of lipid-based drugs as adjuvants and therapeutics.

Tuesday 23rd July

NEW AND NOTABLE

O-124

Cryo-EM of Protein Polymers Yields Many Surprises

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Large amounts of protein in eukaryotic, bacterial and archaeal cells is often found in the form of helical polymers. Viruses infecting these cells can also be helical. We have been using cryo-EM to study the structure and function of many of these polymers. Since the introduction of direct electron detectors about six years ago, there has been a "resolution revolution" where near-atomic levels of resolution can now almost routinely be achieved for many macromolecular complexes. While some of these complexes can, in principle, be crystallized, cryo-EM has emerged as the method of choice for structural studies of such complexes as it does not require crystallization, uses far less sample, and is much faster. But for helical polymers most can never be crystallized and cryo-EM is not only the preferred method but the only method available for reaching near-atomic resolution. I will describe our most recent applications of cryo-EM to a range of systems, from viruses that infect organisms living in nearly boiling acid (1-3), to an archaeal pilus that is nearly indestructible (4), to "microbial nanowires" that conduct electrons (5). All of these studies provide not only new understanding of biology and evolution, but yield insights into novel structures that can have applications to drug delivery, biomedical engineering and nanotechnology.

1. Liu Y, Osinski T, Wang F, Krupovic M, Schouten S, Kasson P, Prangishvili D, & Egelman EH (2018) Structural conservation in a membrane-enveloped filamentous virus infecting a hyperthermophilic acidophile. *Nature communications* 9(1):3360.
2. Kasson P, DiMaio F, Yu X, Lucas-Staat S, Krupovic M, Schouten S, Prangishvili D, & Egelman EH (2017) Model for a novel membrane envelope in a filamentous hyperthermophilic virus. *eLife* 6:doi:10.7554/eLife.26268.
3. DiMaio F, Yu X, Rensen E, Krupovic M, Prangishvili D, & Egelman EH (2015) A Virus that Infects a Hyperthermophile Encapsidates A-Form DNA. *Science* 348:914-917.
4. Wang F, Cirvkaite-Krupovic, V., Kreutzberger, M., Su, Z., de Oliveira, G., Sherman, N., DiMaio, F., Wall, J., Prangishvili, D., Krupovic, M., Egelman, E.H. (2019) An extensively glycosylated archaeal pilus survives extreme conditions. *Nature Microbiology* in press.
5. Wang F, *et al.* (2019) Structure of Microbial Nanowires Reveals Stacked Hemes that Transport Electrons over Micrometers. *Cell* 177(2):361-369.e310.

Tuesday 23rd July
BIOMOLECULAR SIMULATION II

O-126
Advances in Multiscale Simulation of DNA.
M. Orozco
DNA is not only the key molecule of life and one of the most promising biotechnological entities, but a paradigm of a multiscale system whose study implies the representation of Å-scale details in a meter long polymer. This 10^{10} scale problem forces the need to use multi-physics approaches where different levels of theoretical treatments are combined from high- level QM approaches to rough mesoscopic modeling approaches. I will summarize in my talk recent advances done in Barcelona in the development of new approaches for the multiscale representation of DNA.

O-127
Nanoparticle-bio interactions: the role of interface composition, conformation and softness
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The design of ligand-protected metal nanoparticles (NP) with biomedical applications relies on the understanding, at the molecular level, of their solubility in the biological environment and of their interactions with different biological systems. Here we focus on gold NPs, functionalized by an organic ligand shell, in water and in contact with serum proteins and lipid membranes. We use molecular dynamics and enhanced sampling techniques, at atomistic and coarse-grained resolution, to characterize the molecular mechanisms and the thermodynamics of NP-NP aggregation, NP-protein and NP-lipid interactions.
Depending on the composition of the ligand shell, NPs can form different aggregates in water, including planar, linear or porous 3D aggregates. Once interacting with a biological target, such as a lipid membrane or a serum protein, the NP aggregation state, surface composition (e.g. charge and/or hydrophobicity) and conformation (e.g. folded vs extended, ordered or disordered) concur to dictate the thermodynamics and the kinetics of the interaction. We will show examples in which a) the NP charge state affects the kinetics of interaction with a zwitterionic membrane; b) the NP surface patterning affects the kinetics and thermodynamics of interaction with a zwitterionic membrane and c) the hydrophobicity and conformation of the NP ligand shell determines the strength of interaction of the NP with a serum protein.

O-128
Lipid Regulation of Structure, Dynamics, Energetics, and Function of Membrane Proteins
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Membrane proteins have evolved to function optimally while embedded within the heterogeneous and dynamically changing environment of biological membranes. As a matter of fact, the structure and dynamics, and thus the function of membrane proteins are largely affected by the membrane. Given their complex lipid composition, wide range of shape and charge variation, and diverse mechanical properties, biological membranes can modulate the function of membrane proteins through a number of molecular mechanisms. It is now well accepted that lipid-protein interactions are central to the modulation of fundamental cellular processes, including signaling and transport across the biological membranes. While the importance of lipid-protein interactions is widely recognized, characterization of lipid-protein interactions and the lipid-modulated protein dynamics is often challenging, if not impossible, with currently available experimental techniques. Application of molecular dynamics simulations opens up the possibilities for obtaining atomic-level details as well as the associated free energies of such interactions. In this talk, I will provide recent examples of computational studies in which the role of lipid bilayers and their constituents on a number of transport proteins has been successfully characterize at an atomic resolution. These studies, often complemented by experimental validation, offer invaluable insights on the functional mechanisms of physiologically important membrane proteins and how lipids can control and regulate energy landscapes associated with their function

O-129 (P-456)
The catalytic mechanism of MB-COMT: new drug design paradigm?
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We used computational molecular modelling in synergistic combination with a wide range of experimental methodologies, to study a pharmaceutically important enzyme: catechol-O-methyltransferase (COMT) [1]. The enzyme has both water soluble (S-COMT) and membrane bound (MB-COMT) isoforms; MB-COMT is a drug target in the treatment of Parkinson's disease: it is desirable to selective target this isoform. This should also be possible since the substrate profiles of S-COMT and MB-COMT differ, in spite of the fact that they possess identical catalytic domains [2]. While our study culminates in the determination of the catalytic mechanism of MB-COMT [3] that differentiates it from S-COMT, and opens the door to the possibility of selective targeting, this presentation describes a ten-year arc of research that preceded this publication.
We hypothesized that the mechanism of substrate differentiation was the interaction with the membrane of both potential inhibitors or substrates and the catalytic domain of MB-COMT; we then proceeded to determine the catalytic mechanism specific to MB-COMT. We ascertained that (1) substrates with a preferred affinity for MB-COMT over S-COMT had an orientation in the membrane conducive to catalysis from the membrane surface and (2) binding of COMT to its cofactor ADOMET induced a conformational change that causes the catalytic surface of the protein to adhere to the membrane surface, where substrates and Mg^{2+} ions, required for catalysis, are found. Through bioinformatics analysis we found evidence of this mechanism in other proteins, including several existing drug targets.
References
[1] P. T. Männistö and S. Kaakkola, Pharmacol. Rev., **51**, 593–628, (1999).
[2] R. G. Robinson *et al.* ACS Chem. Neurosci., **3**, 129–140, (2012).
[3] A. Magarkar *et al.* Chem. Commun., **54**, 3440, (2018).

O-130 (P-457)

Combining theoretical and experimental approaches to understand the mechanism of antibiotic permeation: implications on the fight against bacterial resistance

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Bacterial resistance is a critical public health threat whose importance has been increasing over the years. Fluoroquinolones (FQ) are a group of antibacterial drugs that are classified as "highest priority critically important antimicrobials" by the World Health Organization. The extensive use of FQs led to an exponential increase in bacterial resistance to these drugs. Known mechanisms of bacterial resistance to FQs, often encompass a reduction of the antibiotics' permeation through the Omp porin channels present at the bacterial cell membrane. Since FQs have intracellular targets, the study of their permeation is crucial to comprehend bacterial resistance and develop alternative drugs.

With the perspective of finding alternatives to the use of FQs, copper complexes of FQs have been studied, as they are expected to have an increase permeation through the membrane. In the present study the translocation of Ciprofloxacin (Cpx) and its copper ternary complex (CuCpxPhen) are compared. Model membranes mimicking the bacterial membrane in the presence or absence of the OmpF porin were used. Spectroscopic methods were employed to determine drugs' partition coefficient to the model membranes and drugs' binding constant to the porin. To further explore structural and dynamical aspects of the translocation process, a theoretical approach was considered, using molecular dynamics simulations. Using umbrella sampling we were able to describe the thermodynamics of the translocation process for each pathway. The results show that CuCpxPhen has a greater partition in lipid membranes, with theoretical results pointing for a greater ability of this complex to cross the lipid membrane, as it shows lower energy barriers at bilayer's centre. Additionally, and despite experimental results showing similar binding constants for Cpx and CuCpxPhen with OmpF, theoretical results show a more favourable energy profile for Cpx translocation across this channel. Overall, our results indicate that CuCpxPhen's permeation in the bacterial membrane should be porin independent. Copper complexes are then expected to be good candidates to bypass the bacterial resistance related with reduction of antibiotic permeation by porin mutation. The findings of this work are important to proceed with the study of CuFQPhen complexes as an alternative to free FQs in resistant bacteria.

O-131 (P-458)

Exploring Conformational Transitions and Free Energy Profiles of Proton Coupled oligopeptide Transporters

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Proteins involved in peptide uptake and transport belong to the proton-coupled oligopeptide transporter (POT) family. Crystal structures of POT family members reveal a common fold consisting of two domains of six transmembrane α helices that come together to form a "V" shaped transporter with a central substrate binding site. Proton coupled oligopeptide transporters operate through an alternate access mechanism, where the membrane transporter undergo global conformational changes, alternating between inward-facing (IF), outward-facing (OF) and occluded (OC) states. Conformational transitions are promoted by proton and ligand binding, however, due to the absence of crystallographic models of outward-open state, the role of H^+ and ligands are still incomplete. To provide a comprehensive picture of the POT conformational equilibrium, conventional and enhanced sampling molecular dynamics simulations of $PepT_{st}$ in the presence or absence of ligand and protonation were performed. Free energy profiles of the conformational variability of $PepT_{st}$ were obtained from microseconds of adaptive biasing force (ABF) simulations. Our results reveal that both, proton and ligand, change significantly the conformational free energy landscape. In the absence of ligand and protonation, only transitions involving IF and OC states are allowed. After protonation, the wider free energy well for E300 protonated $PepT_{st}$ indicates a greater conformational variability relative to the apo system, and OF conformations became accessible. For the E300 Holo-$PepT_{st}$, the presence of a second free energy minimum suggests that OF conformations are not only accessible, but also, stable. The differences in the free energy profiles demonstrate that transitions toward outward facing conformation occur only after protonation and, probably, this should be the first step in the mechanism of peptide transport. Our extensive ABF simulations provide a fully atomic description of all states of the transport process, offering a model for the alternating access mechanism and how protonation and ligand binding control the conformational changes.

Tuesday 23rd July

LIVE IMAGING AND OPTICAL MICROSCOPY

O-132

A Multi Messenger microscope to paint chromatin in cells.

A. Diaspro.

A Multi messenger microscope designed within liquid tunable microscopy approach - LIQUITOPY - is presented. The possibility of integrating different light-matter interactions for imaging is the starting point for the design and realization of a LIQUITOPY architecture (Won R (2018) NatPhot; Diaspro A et al. (2018) BJ). It aims integrating simultaneous acquisition mechanisms coupled with supervised machine learning to foster a brand new way of imaging. It is liquid because it operates with a blend of mechanisms of contrast and tunable in terms of spatial and temporal resolution. It aims to contribute to elucidate an open universal question in biology about the way chromatin organization in the nucleus rules the compaction and function of the human genome in the interphase of cells and mitotic chromosomes. As label free approach the multi messenger microscope will integrate the possibility of performing Mueller matrix microscopy specially oriented to detect changes in helical structures within the cell.

O-133

Looking into the nursery of platelets: Imaging of the bone marrow *in vivo*, *in vitro* and *in silico*

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In mammals, platelets are produced by megakaryocytes (MKs) that are predominantly residing in the bone marrow (BM). MKs originate from hematopoietic stem cells and are thought to migrate from an endosteal niche towards the vascular sinusoids during their maturation.

Light sheeet Fluorescence Microscopy (LSFM) is a powerful tool to study megakaryopoiesis, and has already widened our perspective on MKs in their 3D environment. Through LSFM imaging of MKs and the vasculature in the intact BM, we show that MKs can be found within the entire BM, without a bias towards bone-distant regions. We developed and compared different image processing pipelines and simulation scenarios for precise identification of MKs in 3D light-sheet fluorescence microscopy of uncut murine bones. By combining *in vivo* two-photon microscopy and in situ LSFM with computational simulations, we reveal surprisingly slow MK migration, limited intervascular space, and a vessel-biased MK pool. These data challenge the current thrombopoiesis model of MK migration and support a modified model, where MKs at sinusoids are replenished by sinusoidal precursors rather than cells from a distant periostic niche. If MKs themselves do not need to migrate to reach the vessel, therapies to increase MK numbers might be sufficient to raise platelet counts.

We supported our imaging approaches by realistic computational simulations. Typically, simulation studies use artificial meshes as templates to minimize the computational effort or due to the lack of experimental data. Here, we show that 3D cells and vessels derived from LSFM can dramatically improve simulations as those maximally mimic the physiological situation. Thus, imaging and simulations go hand in hand when segmented objects perfectly serve as biological templates for advanced simulations. Such reliable whole-bone analysis *in silico* identify MKs as biomechanical restraints for bone marrow cell mobilization and extravasation influencing for example neutrophil and HSC migration.

O-134

Faster and better: taking localisation microscopy into live cells

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Super-resolution microscopy is a powerful tool for imaging structures at a lengthscale of tens of nm, but its utility for live cell imaging is limited by the time it takes to acquire the data needed for an image. For localisation microscopy the acquisition time can be cut by more than two orders of magnitude by using advanced algorithms which can analyse dense data, trading off acquisition and processing time. Information can be traded for resolution: for example, the whole dataset can by modelled as arising from blinking and bleaching fluorophores (Bayesian analysis of Blinking and Bleaching), although at a high computational cost. However, all these approaches will come with a risk of artefacts, which can mean that the image does not resemble the underlying sample. We have recently developed Harr Wavelet Kernel (HAWK) analysis, a multi-timescale prefiltering technique which enables high density imaging without artefacts. The results of benchmarking with other techniques reveal that at high activation densities many analysis approaches may achieve high apparent precision (very sharp images), but poor accuracy (the images don't look like the sample). However, HAWK analysis produces images free from sharpening artefacts allowing accurate images to be rapidly taken. Furthermore, this property of HAWK can be used to identify artificial sharpening artefacts and assess the quality of localisation microscopy images.

O-135 (P-499)

Strong cytoskeleton activity on millisecond timescales upon particle binding revealed by ROCS microscopy

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Cells change their shape on the scale of seconds, cellular protrusions even on subsecond timescales enabling various responses to stimuli of approaching bacteria, viruses or pharmaceutical drugs. Typical response patterns are governed by a complex reorganization of the actin cortex, where single filaments and molecules act on a much smaller and faster timescale. These dynamics have remained mostly invisible due to a superposition of slow and fast motions, but also due to a lack of adequate imaging technology. Whereas fluorescence techniques require too long integration times, novel coherent techniques such as ROCS microscopy can achieve sufficiently high spatiotemporal resolution. ROCS uses rotating back-scattered laser light from cellular structures and generates a consistently high image contrast at 150nm resolution and frame rates of up to 100 Hz - without fluorescence or bleaching. Here, we present an extension of ROCS microscopy that exploits the principles of dynamic light scattering for precise localization, visualization and quantification of the cytoskeleton activity of mouse macrophages. The locally observed structural reorganization processes, encoded by dynamic speckle patterns, occur upon distinct mechanical stimuli, such as soft contacts with optically trapped beads. We find that a substantial amount of the near-membrane cytoskeleton activity takes place on millisecond timescales, which is much faster than reported ever before.

O-136 (P-500)

Multiplexed single-molecule fluorescence imaging by FRET-PAINT

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We combined DNA-PAINT imaging with single-molecule FRET read-out and demonstrate multiplexed detection with sub-diffraction optical resolution [1]. For this purpose, we designed pairs of short oligonucleotides labeled with donor and acceptor fluorophores for different FRET efficiencies, and integrated the design into the concept of DNA-PAINT. We demonstrate this FRET-PAINT approach by designing and imaging DNA origami, on which several target binding sites were spaced ~ 55 nm. We resolved the individual binding sites, and in addition determined the FRET efficiency for each site in single and mixed populations. The combination of FRET read-out and DNA-PAINT allows for multiplexed super-resolution imaging with low background, in conjunction with distance sensitive readout in the 1-10 nanometer range. We apply FRET-PAINT as a multiplexing imaging tool in combination with RNA-FISH.

[1] Deussner-Helfmann et al. (2018). Correlative Single-Molecule FRET and DNA-PAINT Imaging. Nano Letters *18*, 4626-4630.

O-137 (P-501)

In vivo single-molecule imaging of DNA gyrase

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DNA gyrase is a type II topoisomerase that performs a vital function in bacteria of introducing negative supercoils and relaxing positive supercoils generated by DNA replication and transcription. This essential role of DNA gyrase has resulted in the development of several antibiotics that kill the cell by specifically targeting it and interfering with its function. Although there have been extensive biochemical, structural and genetic data, we know little of how it operates in complex cellular environments. Here, we used genetic techniques to attach specific fluorescent proteins to the subunits of gyrase and to other parts of the cellular molecular machinery, which are involved in the activities of gyrase. We tracked gyraseA (GyrA) and gyraseB (GyrB) subunits in real time using high-speed single-molecule fluorescence microscopy in living *Escherichia coli* cells, allowing us to explore accurately where in the cell these molecules act to super-resolution precision and how many of them are involved in their cellular activities. We correlated the data on GyrA and GyrB to assess if both gyrase subunits are permanently coupled or if they assemble during catalytic engagement with DNA. We also studied how the activities respond to antibacterials, which target gyrase, to address how gyrase poisons can be tolerated by cells and lead to antibacterial resistance.

Our single-molecule approach with DNA gyrase using advanced light microscopy in living cells is allowing us to address fundamental questions concerning the role of ATP hydrolysis in general topoisomerase activity in order to perform its vital role of relaxing torsional stress in DNA.

Tuesday 23rd July

ACTIVE MATTER AND BIOLOGICAL SELF-ORGANIZATION

O-138

Active Polymers and Membranes

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Active matter exhibits a wealth of emerging non-equilibrium behaviors [1]. A paradigmatic example is the interior of cells, where active components, such as the cytoskeleton, are responsible for its structural organization and the dynamics of the various components. Of particular interest are the properties of polymers and filaments [2]. The intimate coupling of thermal and active noise, hydrodynamic interactions, and polymer conformations implies the emergence of novel structural and dynamical features.

Recent theoretical and simulation developments and results for the structural and dynamical properties of polymers and filaments exposed to activity will be reviewed. Different propulsion mechanisms are considered, such as chains of active Brownian particles [3], or filaments propelled along their contours [4,5,6]. This leads to interesting single-particle behavior, such as a softening of a semiexible filament of active Brownian particles at intermediate levels of activity [3], or a sperm-like beating motion of a filament pushing a load. At high polymer densities in two dimensions, collective dynamics characterized by active turbulence is observed [5]. Closed polymer rings (in two-dimensions) can be considered as a model of cell membranes. Here, active components lead to enhanced uctuations [7] and cell motility [8].

[1] J. Elgeti, R.G. Winkler, and G. Gompper, Rep. Prog. Phys. 78, 056601 (2015).
[2] R.G. Winkler, J. Elgeti, and G. Gompper, J. Phys. Soc. Japan 86, 101014 (2017).
[3] T. Eisenstecken, G. Gompper, and R.G. Winkler, J. Chem. Phys. 146, 154903 (2017).
[4] R.E. Isele-Holder, J. Elgeti, and G. Gompper, Soft Matter 11, 7181 (2015).
[5] O. Duman, R.E. Isele-Holder, J. Elgeti, and G. Gompper, Soft Matter 14, 4483 (2018).
[6] A. Ravichandran, O. Duman, M. Hoore, G. Saggiarato, G.A. Vliegenthart, T. Auth, and G. Gompper, eLife 8, e39694 (2019).
[7] S.M. Mousavi, G. Gompper, and R.G. Winkler, J. Chem. Phys. 150, 064913 (2019).
[8] C. Abaurrea Velasco, S.D. Ghahnaviyeh, H.N. Pishkenari, T. Auth, and G. Gompper, Soft Matter 13, 5865 (2017).

O-139

The microtubule/kinesin system: A versatile realization of an active nematic

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Microtubule-based systems are viewed as minimal in vitro reconstitutions of the cytoskeleton. They are made active by mixing micron-size microtubules with kinesin proteins fueled with ATP. When this material is two-dimensionally interfaced with oil, it conforms nematic textures pervaded by topological defects and active flows. As an Introduction, I will start by briefly reviewing the field of active systems, singularly those with a biophysical interest. In the central part of the talk I will present experimental results corresponding to different scenarios of the microtubule/kinesin active nematic system. First, I will introduce recent observations relative to the onset dynamics and full characterization of a turbulent-like regime, identifying the basic length scales involved in the instability mechanism [1,2,]. Later, a strategy of control of these active flows will be commented, based on patterning the viscous coupling of the active nematic at the oily interface [3]. Finally, I will refer to situations of active nematics droplets, dispersed in isotropic and anisotropic oils (liquid crystals) [4].

[1] B. Martinez et al., Nature Physics 15:362, 2019
[2] P. Guillamat et al. Nat. Comm. 8:564, 2017
[3] P. Guillamat et al., PNAS 114, 5498, 2016
[4] P. Guillamat et al., Sci. Adv. 4:eeao1470, 2018

O-140

Spontaneous tension cable generation and convergence-extension flows in the gastrulating chick embryo

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During gastrulation, and other development stages like germband extension, epithelial cell sheets spontaneously organise to exert contractile mechanical forces, resulting in convergence-extension flow. Current models assume different types of chemical signalling based pre-patterning of the junctions, leading to both tension and flow. In the chick embryo, in the absence of pre-patterning and in the presence of strong local mechanical disorder, contractile mechanical forces and flows still emerge. Here, we present the active junction model (AJM), a model of self-amplifying contractile cell sheets that posits a myosin-dependent junction contractility with a tension-dependent feedback loop. This active mechanics model leads to the spontaneous formation of tension chains with directionality in the presence of an applied stress. Spontaneous active flow results from the activated junctions through active T1 transitions, and convergence-extension flow results for appropriate boundary conditions. This active intercalation pattern is only productive under highly specific conditions, including a hierarchy of time scales, with slow myosin response, an intermediate viscoelastic time scale and fast elastic response.

O-141 (P-519)

Design principles for robust self-assembly of multiple biological structures from limited resources

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Controlling the size of sub-cellular structures is very important for proper execution of physiological functions of the cell. While it is well known that the sizes of intracellular structures (filaments, organelles, networks) scale with cell size, the underlying physical mechanisms for size control remains poorly understood. The limiting pool hypothesis- the assembly rate of macromolecular structures scales with the available amount of resources- provides a robust mechanism for size control of a single structure. However it fails to capture size control of multiple structures assembled from the same pool of resources. Examples include, assembling multiple protein structures from a limited pool of ribosomes, assembly of multiple organelles, multiple distinct cytoskeletal structures etc.

Here we present a physical model for active self-assembly and size regulation of 3-dimensional sub-cellular structures, which provides a mechanism for robust size regulation of multiple structures in the presence of stochasticity and competition for resources. Using this model we predict size regulation of multiple organelles, filaments and networks in quantitative agreement with experimental data. We also discuss how competition for resources between multiple networks can lead to cell polarization.

O-142 (P-520)

Active phase separation in mixtures of chemically-interacting particles

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Microorganisms and cells can chemotax in response to gradients of chemicals that they themselves produce or consume. The same behaviour has been recently observed at the nanoscale for individual enzymes, and can be mimicked in synthetic systems using catalytically-active phoretic colloids. Importantly, when many such particles are placed in solution, they interact with each other through their influence on the chemical's concentration field. We show that mixtures of chemically-interacting particles can undergo macroscopic phase separation, displaying a wealth of different configurations that are intimately related to the active, non-equilibrium character of the interactions. The fundamentally new class of phase separation behaviour arises generically from the chemical interactions, making our results directly applicable to self-organisation in heterogeneous populations of microorganisms and cells (e.g. quorum sensing and competition for nutrients in bacterial ecosystems, or cell-cell communication via chemokines); to aggregation of enzymes that participate in common catalytic pathways into a metabolon, which may be harnessed in the design of better synthetic pathways; or to the development of new active materials using catalytic colloids. Our results are also of relevance to fundamental studies of active phase separation phenomena, given that in our system the activity arises from the non-equilibrium nature of the interactions between particles that are otherwise non-motile, rather than from the intrinsic activity of self-propelling particles as commonly studied.

O-143 (P-521)

Self-organization of ciliary beats in bronchial epithelium

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In the lungs, the bronchial epithelium is covered by motile cilia whose coordinated beating drives the transport of mucus along the bronchial tree. This large-scale transport requires a global directional organization of ciliary beats. Yet the self-organization mechanisms leading to a collective dynamics of cilia remain to be explored.

In vitro experiments on reconstituted bronchial epithelium show that the mucus flow exhibits multi-scale swirly patterns during ciliogenesis. These patterns are closely connected to the underneath ciliary-beat organization. The nature of the fluid has a major impact on the spontaneous emergence of these patterns, as shown by the re-organization of the ciliary-beat orientations when replacing the mucus by a model fluid. This emphasizes the prominent role of the active response of cilia to their hydrodynamic interactions with mucus. A simple physical model of ciliary-beat organization is proposed to investigate the possible emergence of large-scale ciliary patterns due to hydrodynamic interactions. The mucus flow over the modeled epithelium is computed using numerical simulations. Depending on ciliary density and mucus properties, the model solutions can exhibit several ciliary patterns, including a swirly pattern similar to that observed experimentally and a fully aligned pattern that is optimal for mucus transport. This supports that the flow can carry the necessary information for large-scale ciliary-beat organization.

Tuesday 23rd July

IONIC LIQUIDS AND BIOMOLECULES

O-144

Ionic liquids in bio-systems: from model biomembranes to living cells

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Ionic liquids (IL) are a relatively new class of organic salts consisting of organic cations and organic or inorganic anions. Their joint ionic and organic nature gives them intriguing properties such as being liquid at ambient temperature and having low vapour pressures, and, for this reason, they have been investigated for basic research and for industrial applications. The first interest in their interaction with "bio" systems has been motivated by the need to assess their toxicity. Evidence of their specific interaction with bio-systems is the reason of the most recent interest fuelling the emerging field of "ILs and biomolecules". The research activity of the last few years [1-3] has shown, for example, that selected ILs are able to (i) diffuse into biomembranes and eventually disrupt them; (ii) stabilize proteins and enzymes, preserving their biochemical functions; (iii) either promote or prevent the aggregation of proteins into amyloids; (iv) extract, purify, and even preserve DNA; (v) dissolve polysaccharides and cellulose; and (vi) kill bacteria and cancer cells, leaving healthy eukaryotic cells almost unaffected. Research in these subjects aims at understanding the micro-scopic mechanisms behind the effects relevant in biomedicine, pharmacology and in bio-nanotechnology. In my invited talk I will present first an overview of the subject, then I will focus on specific cases currently under investigation in our Lab [4-6]. I will discuss how ILs, dissolved at low doses at the biomembrane-water interfaces, penetrate into biomembranes, changing their microscopic structures, dynamics and mechanoelastical properties. I will consider a variety of systems, from model phospholipids bilayers up to living cells. Magnetic ILs will also be considered. Our major approaches include neutron and light scattering, atomic force microscopy, optical tweezers and computer simulations as well as a variety of optical and macroscopic techniques.

Key-words: Ionic liquids; Organic salts

[1] Benedetto, Ballone *ACS Sustainable Chem Eng*. 2016; 4:392-412.
[2] Benedetto, *Biophys Rev*. 2017; 9:309-320.
[3] Benedetto, Galla *Biophys Rev*. 2018; 10:687-690.
[4] Benedetto, et al. *J Phys Chem B*. 2014; 118:12192−12206.
[5] Rotella, et al. *Biophys Rev*. 2018; 10:751-756.
[6] Benedetto, Ballone *Langmuir*. 2018; 34:9579-9597.

O-145

Aqueous Ionic Liquids: Consequences for Biological Stabilities

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Aqueous mixtures of ionic liquids (ILs) reveal a plethora of interesting effects. Of particular importance are structure-stabilizing or structure-destabilizing mechanisms on proteins and DNA, which crucially depend on the concentration and the nature of the ions in solution. In this talk, I will present an overview about our recent research in this direction [1,2]. By means of atomistic molecular dynamics simulations in combination with molecular theories of solution, one can show that most effects depend on preferential exclusion or preferential binding mechanisms [3-5]. The results of free energy calculations for short peptides in aqueous IL solutions reveal a good agreement with our theoretical approach. Furthermore, I will shed more light on the molecular differences between complex ions, simple ions and standard organic co-solutes with regard to their crucial influence on protein structures.

1. D. Diddens, V. Lesch, A. Heuer, J. Smiatek, Phys. Chem. Chem. Phys. 19, 20430 (2017)
2. V. Lesch, A. Heuer, C. Holm, J. Smiatek, Phys. Chem. Chem. Phys. 17, 26049 (2015)
3. J. Smiatek, J. Phys. Condens. Matter 29, 233001 (2017)
4. E. A. Oprzeska-Zingrebe, J. Smiatek, Biophys. Rev. 10, 809 (2014)
5. E. A. Oprzeska-Zingrebe, M. Kohagen, J. Kästner, J. Smiatek, Europ. Phys. J. Spec. Top. 227, 1665 (2019)

O-146

Studying fundamental role of ionic interactions to connect of structure and properties of ionic liquids with biological activity

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Ionic interactions in the liquid phase (water solutions and organic solvents) influence structure and properties of the system on different levels [1]. Molecular interactions of ions, clustering and structuring effects, formation of polar domains and mico-/nano-scale ordering play the key role in understanding biological activity of ionic species. Dynamic interactions with biomolecules add another level of complexity towards biological activity on living cells [2]. To reflect ongoing challenges, IOLIOMICS was emerged as a rapidly growing area of research dealing with the studies of ions in liquids (or liquid phases) and stipulated with fundamental differences of ionic interactions [2].

In the ongoing project interactions between ionic liquids, water and biomolecules are studied with a number of experimental methods and the results dealing with field-emission scanning electron microscopy of ionic liquid system [3], visualization of dynamic interactions in micro- and nano-scale systems [4], self-organization of water in ionic liquids [3,4], regulation of biological activity and comparison of different drug development platforms based on ionic liquids [5] will be presented and discussed.

References

[1] Egorova K. S., Ananikov V. P., *J. Mol. Liq.*, **2018**, 272, 271; doi: 10.1016/j.molliq.2018.09.025.

[2] Egorova K.S., Gordeev E.G., Ananikov V.P., *Chem. Rev.*, **2017**, 117, 7132; doi: 10.1021/acs.chemrev.6b00562.

[3] Kashin A.S., Galkin K.I., Khokhlova E.A., Ananikov V. P., *Angew. Chem. Int. Ed.*, **2016**, 55, 2161; doi: 10.1002/anie.201510090.

[4] Seitkalieva M. M., Kachala V. V., Egorova K. S., Ananikov V. P., *ACS Sustainable Chem. Eng.*, **2015**, 3, 357, doi: 10.1021/sc500770v.

[5] Egorova K. S., Seitkalieva M. M., Posvyatenko A. V., Khrustalev V. N., Ananikov V. P., *ACS Med. Chem. Lett.*, **2015**, 6, 1099, doi: 10.1021/acsmedchemlett.5b00258.

O-147 (P-551)

Probing the effect of a room temperature ionic liquid on self-assembled structure of phospholipid membrane

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A cellular membrane, which is mainly composed of phospholipids and proteins, is the outer layer of a cell that protects the inner components from the adverse effects of any foreign molecule. It also plays important roles in many physiological activities of cell including communication and endocytosis-exocytosis process. Any deviation from its structure, dynamics, and stability can influence the biochemical and physiochemical activities of the membrane. The interaction of inorganic salts is well known to generate such deviation and thereby affect the cell functionality.

Recently, there has been significant research interest in understanding the interaction of room temperature ionic liquids (ILs) with the cell membrane. An IL is a salt in the liquid state below 100°C. These molecules are non-explosive, non-flammable, and are having good electrical and thermal conductivities. They do not pollute air as they have low vapour pressure. Because of these properties, they have a large number of industrial applications. But some recent studies have revealed the lysing effect of ILs on environment friendly microorganisms living in soil and water.

The molecular mechanism of toxic activities of ILs is yet to understand. In this work, multilamellar vesicles (MLV) of phospholipids have been used to shed light on the effect of an IL on the structure of cellular membrane. The MLVs formed by zwitterionic lipid, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) are found to shrink as a consequence of interaction with an imidazolium-based IL, 1-decyl-3-methylimidazolium tetrafluoroborate ([DMIM][BF4]). The absorbed IL significantly modify the surface charge of the MLVs. While these observations indicate a strong membrane-IL interaction, synchrotron-based small angle x-ray diffraction (SAXD) measurements have provided a structural description of the interaction. SAXD and Fourier transform infrared spectroscopy studies have clearly revealed disordering effect of the IL on the conformational organization of lipid chains. The presence of negativily charged lipid 1,2-dipalmitoyl-*sn*-glycero-3-phospho-L-serine sodium salt (DPPS) in DPPC MLVs plays an important role in disordering the chains in membrane and inter-bilayer interaction.

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O-148 (P-552)

The effect of imidazolium-based ionic liquids on human insulin aggregation/amyloid fibrillization

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Proteins are one of the most important molecules in living organisms possessing immense amount of functions in their native state. Under certain conditions, native proteins could aggregate. These misfolded proteins may accumulate in a form of protein aggregates called amyloid fibrils. Amyloid fibrils are elongated unbranched fibers consisting of β-structures of separate monomers positioned perpendicular to the fibril axis and stacked strictly above each other. Amyloid aggregates are often correlated with diseases known as amyloidoses, including Alzheimer's, Parkinson's or insulin-derived amyloidosis. Moreover, amyloid fibrils have also been recently tested as novel biomaterials due to their specific properties such as high stability, strength, elasticity or resistance against degradation [1]. Solvent conditions play an important role in controlling the amyloid aggregation *in vitro*. Ionic liquids (ILs) are a new class of media with interesting properties and low melting-point (< 100°C), that may substitute the volatile organic solvents. They can be used neat or diluted in water, eventually in other solvents. ILs consists of large organic cations combined with various anions and can be designed to match desired properties for various applications.

In this work, we have studied the effect of ILs with 1-ethyl-3-methyl imidazolium (EMIM) cation and various anions (NO_3^-, BF_4^-, Cl^-, $CH_3CO_2^-$, HSO_4^-) on fibrillization kinetics and morphology of insulin fibrils using ThT fluorescence assay, calorimetry, CD and FTIR spectroscopy and AFM. We have found that the effect of ILs strongly depends on their concentration and the extent is not correlated to the position in Hofmeister series. At lower concentration of ILs, the acceleration of kinetics is observed, and polymorphic fibrils are formed. At higher concentration, amorphous aggregates are prevalent for all studied ILs. It is important to recognize and define the relations between physico-chemical properties of ILs and the kinetics of amyloid fibrillization or morphology of fibrils in order to expand our understanding of the amyloid fibrillization process.

[1] Assenza, S., et al., *Phys Rev Lett.* 113 (2014) 268103

O-149 (P-553)

Effect of Ionic Liquids on the Mechanoelasticity of Biomembranes: from Model Phospholipid Bilayers to Living Cells

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The interaction between ionic liquids (ILs) and biomolecules is an emerging area of research [1]. Among biological structures, model biomembranes such as phospholipid bilayers have been the first to be investigated in relation with ILs. Recently, by neutron reflectivity and computer simulations, it has been shown that ILs dispersed at low concentrations at water-biomembrane interfaces diffuse into biomembranes without disrupting their overall integrity [2,3]. Computer simulations [3] also suggested that the mechanoelasticity of biomembranes is affected by the presence of ILs. To study this experimentally, we recover to atomic force spectroscopy (AFS) by which we measure the mechanoelasticity of supported lipid bilayers interacting with water solutions of ILs. Different lipids and different ILs have been employed in these investigations. As a result, we found that different ILs change the mechanoelasticity of phospholipid bilayers in different ways. More precisely, the same ILs can make phospholipid bilayers either softer or stiffer depending on the lipid composition of the bilayer. We have also indications that this effect is IL dependent. In my contribution, I will present these experimental results, partially published recently [4], and show how the understanding of the microscopic mechanism behind this behaviour can be used to study the effect of ILs on living cells. This research holds the promise for new applications in bio-nanotechnology [5].

[1] A. Benedetto, P. Ballone (2016) Room temperature ionic liquids meet bio-molecules: a microscopic view of structure and dynamics. ACS Sustainable Chem Eng 4:392

[2] A. Benedetto, et al (2014) Structure and stability of phospholipid bilayers hydrated by a room-temperature ionic liquid/water solution: a neutron reflectometry study. J Phys Chem B 118:12192

[3] A. Benedetto, R.J. Bingham, P. Ballone (2015) Structure and dynamics of POPC bilayers in water solutions of room temperature ionic liquids. J Chem Phys B 142:124706

[4] C. Rotella, P. Kumari, B.J. Rodriguez, S.P. Jarvis, A. Benedetto (2018) Controlling the mechanoelasticity of model biomembranes with room-temperature ionic liquids. Biophys Rev 10:751

[5] A. Benedetto, P. Ballone (2018) Room-Temperature Ionic Liquids and Biomembranes: Setting the Stage for Applications in Pharmacology, Biomedicine, and Bionanotechnology. Langmuir 34(33):9579

Wednesday 24th July

LIPID AND LIPIDOME

O-150

Membrane remodeling by the amyloidogenic peptide IAPP: surface crowing relevance

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Membrane-catalyzed amyloid fibril formation of human islet amyloid polypeptide (hIAPP) or amylin has been implicated as a mechanism by which hIAPP exerts its toxicity in type 2 diabetes. On the other hand, the membrane-bound aggregates of the rat variant of IAPP (rIAPP) are unable to progress into fibrillar structures since this is a non-amyloidogenic peptide. Here, we combined steady-state and time-resolved fluorescence methods and microscopy techniques to perform a comparative study on how anionic lipid membranes control the self-assembly of these two peptides. Our studies confirmed that membrane-catalyzed fibrillation of hIAPP results in fibril growth into the solution. However, the membrane-mediated self-assembly of rIAPP is confined to the lipid bilayer. To evaluate the impact of unlabeled rIAPP/lipid concentration on the membrane surface coverage reached by the peptide, FRET-based measurements of membrane binding of a tracer amount of fluorescently-labeled Atto488-rIAPP were combined with fluorescence anisotropy measurements of its membrane-bound oligomerization state. We found that progressive membrane saturation with rIAPP correlated with its ability in reducing the average size of anionic liposomes. Furthermore, a time-resolved emission spectra (TRES) study of Laurdan revealed that membrane binding and oligomerization of rIAPP produced an increased rigidity and surface dehydration in the vicinity of the probe that ultimately might be responsible for rIAPP ability in remodeling the lipid membranes.

O-151

How the globoside Gb3 partitions in phase-separated membranes

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Glycosphingolipids serve as receptors for a number of bacterial toxins and viruses and are thus involved in a variety of physiological and pathophysiological processes. As an example, the Shiga toxin B subunit (STxB) being responsible for cell membrane attachment and trafficking of Shiga holotoxin, binds specifically to the glycosphingolipid Gb3. There is strong evidence that the fatty acid of Gb3 influences the binding behavior of STxB as well as the intracellular routing of the protein. To elucidate the impact of the fatty acid, different Gb3 species were reconstituted into lipid bilayers resembling the chemical composition of the outer leaflet of eukaryotic plasma membranes. Such lipid mixtures phase separate into liquid-ordered (lo) and liquid-disordered (ld) domains. To investigate the phase behavior of the different Gb3 molecules in these lipid mixtures and to understand their influence on STxB binding, three fundamentally different model membrane systems were applied, namely giant unilamellar vesicles (GUVs), supported lipid bilayers (SLBs) as well as pore-spanning membranes (PSMs). From fluorescence microscopy imaging of these membrane systems, we concluded that the fatty acid of Gb3 significantly influences the partition of the glycosphingolipid in phase-separated membranes before and after STxB binding. In addition, also the lateral organization of STxB on the membranes is influenced, which is discussed in the context of Shiga toxin induced invaginations and its internalization into the cell.

O-152

Curvature rigidity of asymmetric and differentially stressed membranes

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All lipid biomembranes are asymmetric: their two individual leaflets differ in their lipid composition. Such a state is only metastable, decaying over a time scale of (presumably) hours to days into a symmetric distribution via lipid flip-flop between the leaflets. Cells hence must put work into maintaining asymmetry as a nonequilibrium steady state. Recent experimental advances have succeeded in artificially creating such asymmetric membranes, and to study their properties before the asymmetry decays. Such experiments suggest that asymmetric membranes have quite unexpected properties. For instance, they have been found to be much stiffer than what one would predict based on the curvature elasticity of their individual leaflets. In this talk I will try to explain these findings, using a combination of theory and coarse-grained molecular dynamics simulations. A key part of the puzzle will be that asymmetric membranes are not fully characterized by their two leaflet compositions alone. One needs at least one more observable, the differential stress between leaflets, to fully characterize the state. I will argue that it is not the compositional asymmetry but the differential stress which is directly responsible for the experimentally observed stiffening. However, lipid asymmetry is an indirect cause for that stress, which can be understood by looking at the processes by which asymmetric membranes are experimentally created.

O-153 (P-556)

Formation of membrane domains: insight from simulations on different scales

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The formation of domains in lipid membranes is a key requisite for signal-processing in cells. In this contribution a profound understanding will be provided from a simulation perspective. Results will be presented for lipid mixtures with and without transmembrane domains for a variety of different lipid types.

First, extensive Molecular Dynamics (MD) simulations of lipid membrane mixtures are presented on the atomistic as well as the coarse-grained level (MARTINI) [1]. A close comparison of both levels of description involve the spatial properties of the domain formation (in case of mixtures of saturated and unsaturated lipids and cholesterol) and the time evolution of characteristic observables such as the chain order parameter, indicating the emergence of domain formation [2,3]. Among others, we check to which degree the coarse-grained representation matches the atomistic one from a thermodynamic perspective and get quantitative insight into the enthalpic and entropic change of the gel-to-liquid transition of a specific saturated lipid in good agreement with experiments. In this context, the individual enthalpic and entropic driving forces of the lipid mixtures are quantified [4]. Second, we can map the systems on a lattice model which is exclusively characterized by properties, inherited from the MD simulations (see [4,5] for first results). In this way, it is possible to reach length- and timescales which extend the typical MD scales by orders of magnitudes. Furthermore, due to the direct accessibility of enthalpic and entropic contributions a close thermodynamic understanding of the domain formation becomes accessible.

[1] H. J. Risselada and S. J. Marrink, Proc. Natl. Acad. Sci. U. S. A 105, 17367 (2008).
[2] D. Hakobyan and A. Heuer, J. Phys. Chem. B 117, 3841 (2013).
[3] D. Hakobyan and A. Heuer, PLoS One 9, e87369 (2014).
[4] D. Hakobyan and A. Heuer, J. Chem. Phys. **146**, 064305 (2017).
[5] R. Friedman et al., J. Membr. Biol. **251**, 609 (2018).

O-154 (P-557)

Monte Carlo and molecular dynamics simulations to explain biomembrane meso-patterning by a composition-curvature coupling mechanism

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Plasma membrane forms a selective barrier for the cell, yet its role goes far beyond a simple frontier. Indeed, it plays a crucial role in biological functions such as endo and exocytosis, cell communication or adhesion. It is now widely agreed that membrane lipid and protein spatial repartition is not homogeneous but that these components are organized into nanodomains. These domains have proven to be key players in the above-mentioned biological functions. Combining statistical physics analytical tools and numerical simulations, we propose a physical mechanism for this membrane organization in a simple model vesicle. At the mesoscale, we describe the membrane with a composition-curvature coupling mechanism. We use an elastic description of the membrane surface via a Helfrich Hamiltonian and study the species mixture with an Ising model, relevant in describing phase transition phenomena. We perform Monte Carlo simulations for different membrane parameters (temperature, composition, spontaneous curvature, surface tension) and study its equilibrium states. Depending on the range of parameters, we get systems either undergoing a macrophase separation, either mixing homogeneously, or featuring domains. We characterize the range of parameters leading to these phase modulations by drawing phase diagrams from the simulation results. We compare this phase diagram with the one previously obtained by analytical field-theoretic techniques. In the case of meso-patterning, different observables are computed such as correlation functions and domain size distributions to extract information about the emerging membrane domains, such as their typical shape, size or spacing. In order to propose a valid rationale for membrane structuring at a lower scale, we also perform coarse-grained molecular dynamics simulations (MARTINI) of lipid bilayers including curvature-generating components. To assess the validity of our results, we compare them to available analytical predictions and experimental data.

O-155 (P-558)

Biophysical properties of 1-deoxyceramides in ordered and disordered bilayers

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Membrane sphingolipids have attracted a considerable attention in the last decades due to the discovery of the sphingolipid signaling pathway and its importance in regulating important processes such as cell growth and apoptosis. Our views on the complexity and variety of this lipid class have been enriched with the recent discovery of a novel sphingolipid subclass, the (1-deoxy) sphingolipids that lack the 1-hydroxy group. These lipids differ in their properties from the canonical (or 1-hydroxy) sphingolipids and they are toxic when accumulated in cells, inducing neurodegeneration and other dysfunctions. (1-Deoxy)ceramides, (1-deoxy)dihydroceramides, and (1-deoxymethyl)dihydroceramides, the latter two containing a saturated sphingoid chain, have been studied in this work using differential scanning calorimetry and atomic force microscopy to evaluate their behavior in bilayers composed of mixtures of three or four lipids. When compared to canonical ceramides, C16:0 (1-deoxy)ceramides show a lower miscibility in mixtures of the kind pSM/Chol/XCer (54:23:23 mol ratio), where XCer is any 1-deoxyceramide, giving rise to the coexistence of a liquid-ordered phase and a gel phase. The latter resembles, in terms of thermotropic behavior and nanomechanical resistance, the gel phase of the pSM/Chol/pCer mixture [Busto et al., Biophys. J. 2014, 106, 621-630]. Differences are seen between the different C16:0 (1-deoxy)ceramides under study in terms of nanomechanical resistance, bilayer thickness and bilayer topography. When examined in a more fluid environment (an ER-mimicking model membrane composed of ePC:ePE:ePI, 1:1:1 mol ratio), segregated gel phases are still present. Interestingly, and probably related to segregated gel phases, (1-deoxy)ceramides preserve the capacity for membrane permeation, but their effects are significantly lower than those of canonical ceramides. Moreover, C24:1 (1-deoxy)ceramides show significantly lower membrane permeation capacity than their C16:0 counterparts. The above data may be relevant in the pathogenesis of certain sphingolipid-related diseases, including certain neuropathies, diabetes, and glycogen storage diseases.

Wednesday 24th July
BIG DATA IN BIOPHYSICS

O-156
A SYSTEMS APPROACH TO UNDERSTANDING THE ALLOSTERIC MECHANISM OF PYRUVATE KINASE M2.
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Allostery is a crucial phenomenon in biology, which enables cells to tune metabolic and signaling pathways in response to subtle chemical cues. Despite its importance we are unable to ascribe a quantifiable structural description to allosteric regulation in many cases, leaving it somewhat of a biophysical enigma. While allosteric sites hold great promise as therapeutic targets in recently described cases, little is known about how proteins propagate allosteric information between distal sites. To better understand the structural mechanisms behind protein allostery, we combine molecular dynamics (MD) simulations with experimental biophysical tools to identify allosteric pathways within key metabolic enzymes.
Initially, we investigated the allosteric mechanism of pyruvate kinase M2 (PKM2). A number of studies have revealed that PKM2 expression may be enriched in proliferating cells and that allosteric control of its activity is crucial for their growth and survival. Although PKM2 has been well characterised, the structural basis of how its activity is regulated remains poorly understood. To address this apparent gap in the literature, we use PKM2 as a proof-of-principle to demonstrate that an integrated systems approach can uncover dynamic and energetic events associated with the allosteric mechanism of enzymes. Using atomistic molecular dynamics simulations we find that a network of cooperative residues communicate structural information to the active site, upon binding of allosteric ligands, to stabilize either the active or the inactive conformation of the enzyme.

O-157
Data-driven models of protein sequence landscapes: inference, 3D structure prediction and protein design
Martin Weigt
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In the course of evolution, proteins undergo substantial changes in their amino-acid sequences, while conserving their three-dimensional fold and their biological functionality. Modern sequencing techniques provide us with increasingly large families of evolutionary related proteins. Such data can be used to infer statistical models of sequence variability. I will overview the surprising efficiency of pairwise models (Potts models / Markov random fields), which reaches from protein structure prediction to novel, data-driven approaches to protein design.

Cocco, S., Feinauer, C., Figliuzzi, M., Monasson, R., & Weigt, M. (2018). Inverse statistical physics of protein sequences: A key issues review. Reports on Progress in Physics, 81(3), 032601.

Figliuzzi, M., Barrat-Charlaix, P., & Weigt, M. (2017). How Pairwise Coevolutionary Models Capture the Collective Residue Variability in Proteins. Molecular Biology and Evolution, 35(4), 1018-1027.

O-158
Combining imaging and gene expression data in mathematical models for immuno-oncology
V. Pancaldi.
INSERM, Centre de Recherches en Cancerologie de Toulouse.

O-159 (P-587)
Real-time dynamic changes in serum metabolome during the anticancer treatment by means of NMR-based metabolomics
L. Boguszewicz, A. Bieleń, J. Mrochem-Kwarciak, A. Skorupa, M. Ciszek, A. Heyda, A. Wygoda, A. Kotylak, K. Składowski, M. Sokół.
Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice Branch, Gliwice, Poland.
Head and neck squamous cell carcinomas (HNSCC) are mainly located in larynx, pharynx and oral cavity, which play crucial roles in respiratory, nutritional, social and communicative functions. The standard organ preservation treatment method for HNSCC is sequential and/or concurrent radiotherapy (RT) and chemotherapy (CHT). However, it is associated with significant temporary or permanent toxic side effects in normal tissue and/or involved regions (Acute Radiation Sequelae, ARS). We aimed to investigate the real-time (during-treatment) changes in the serum metabolome and to correlate these changes with available patients' clinical data (ARS, laboratory blood tests, weight/BMI). 230 HNSCC patients were enrolled into the study. Patients were treated radically with RT and CHT-RT. CHT was realized as induction and/or concurrent treatment. Blood samples were collected weekly, starting from the day before the treatment and stopping within the week after the treatment completion, resulting in a total number of approximately 1900 samples. Patients were clinically monitored until the resolution of all ARS symptoms. Serum samples were analyzed using 1H-NMR spectroscopy followed by multivariate projection techniques (MPT) and batch analysis (BA). Significant metabolic alterations correlated with ARS escalation were successfully identified. Patients with significant treatment induced weight loss showed increased serum ketone bodies (KB) concentrations. The increase of KB preceded signaling from the clinical nutritional parameters (albumin, BMI) and may be used as a prognostic marker. Furthermore, significant changes in concentrations of inflammatory and energy involved metabolites were observed during the RT/CHRT treatment. The application of MPT and BA methods allows to track the trajectories of temporal (during-treatment) changes in the serum metabolome of HNSCC patients. Which, in turn, gives the opportunity to predict metabolic alterations and treatment response in new patients. Multivariate models trained and validated on big datasets may be useful in introducing personalized medicine to anticancer treatment.
The work has been funded by National Science Centre grant 2015/17/B/NZ5/01387.

O-160 (P-588)
Decrypting interaction fingerprints in protein molecular surfaces
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Interactions between proteins and other biomolecules are the basis of protein function in all biological processes. Many proteins bear functional signatures that can be effectively inferred from sequence and structure, but require the existence of sequence or structural homologues functionally annotated. A higher-level description of a protein structure is presented by its molecular surface, which models a protein as a continuous molecule where geometric and chemical features are presented, to some extent, independently of the underlying protein sequence. We hypothesized that molecular surfaces carry fingerprints that can be identified and enlighten their interaction with other biomolecules. Thus, we develop a computational algorithm to identify molecular surface interaction fingerprints (MaSIF). MaSIF exploits geometric deep learning tools to extract geometric and chemical features from protein surfaces. We used MaSIF to study three fundamental aspects related to biomolecular interactions: I) determining the binding specificity of enzymes, where we achieve a 74% accuracy on highly similar cofactors; II) predicting protein-protein interaction sites, where we outperform state-of-the-art site predictors with a ROC AUC of 0.85; III) large scale protein docking, a new paradigm based on surface fingerprints that outperforms other methods by a factor of 1000 in computational time, enabling multitarget docking campaigns. In summary, we present a novel approach - MaSIF – that leverages deep learning techniques to identify patterns in protein surfaces and from those infer important function-related properties. We anticipate that these emerging modeling techniques will be the next generation tools to improve our understanding of protein function and design.

O-161 (P-589)

Bayesian inference and machine learning approaches to determine protein copy number from localization microscopy

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Single-molecule localization microscopy has become an important tool for nanoscale imaging and a considerable effort has been devoted to quantifying protein copy number in super-resolution images. In localization microscopy, the imaging procedure results in the mapping of a labeled protein into a stochastic number of molecular localizations, randomly placed around the actual protein position. Therefore, the determination of the exact protein copy number is impaired by the stochasticity of the labeling and the complex photophysics of the fluorescent probes. High protein density and tight arrangement in oligomeric structures at the nanoscale further complicate this task. Consequently, even when using *ad hoc* calibration standards, the inverse problem of determining protein copy number and positions from the collected localizations is technically and computationally challenging. We present two methods to tackle this problem at different levels. The first consist in a brute force approach, based on a convolutional neural network architecture to create the most-likely protein spatial arrangement compatible with an experimental localization map. The neural network is trained on a calibration image dataset, based either on experiments or numerical simulations, thus offering the possibility of transfer learning. We discuss performance and limitations of the method at varying imaging parameters. While the former approach is directly applied to raw localization lists, other methods previously described involve the application of a segmentation algorithm to cluster nearby localizations and then the fit of the distribution of localization per cluster to infer the relative abundance of different oligomerization species. In these cases, the latter step is complicated by the lack of knowledge about the largest oligomeric structure and the increasing number of free parameters. To improve the fitting step, we describe a method for Bayesian model comparison and parameter inference via a nested sampling algorithm that robustly estimate the weight of each oligomeric population without overfitting. The method has a wide applicability and - in the presence of a proper calibration - can be also applied to STED, confocal and TIRF imaging.

We gratefully acknowledge the support of NVIDIA Corporation with the donation of the Titan Xp GPU used for this research.

Wednesday 24th July

LIQUID-LIQUID PHASE SEPARATION IN BIOLOGICAL SYSTEMS

O-162

EBSA-2019 lecture

Role of macromolecular phase separation and crowding in bacterial cell division: synthesis of minimal divisomes in the test tube

Role of macromolecular phase separation and crowding in bacterial cell division: self-organization and reactivity of FtsZ in cytomimetic media

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The interior of living cells consists of a variety of distinct microenvironments characterized by high total concentration, the immediate presence of membranes and cytoskeletal fibers, and heterogeneity in composition. Among the elements of intracellular complexity present in these media, macromolecular condensation resulting from biologically regulated liquid-liquid phase separation is emerging as a mechanism to organize intracellular biochemistry in eukaryotes, with broad implication for cell physiology and pathology. Intrinsically disordered proteins or those containing multivalent domains involved in macromolecular interactions are the ones more prone to form these condensates. Excluded volume effects due to natural crowding promote these phase separation processes. Bacteria do not have organelles, that generally argues for a relatively strong impact of processes connected to liquid phase separation to segregate or otherwise spatially organize molecules. Recent experimental pieces of evidence suggesting that phase-separated condensates also occur in bacterial systems will be summarized. In particular, our findings that the FtsZ protein, an essential element of the division machinery in most bacteria, forms these dynamic condensates in crowded cell-like conditions will be described. The potential implications of macromolecular phase separation and crowding to organize intracellular space in bacteria and to reconstructing minimal division machines from the bottom up in the absence of cells will be discussed.

O-163

Light controlled cytoplasmic transport to probe cellular organization

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Throughout the last decades, genetic perturbations massively advanced our molecular understanding of cell biological processes. At the same time however, the spatio-temporal organization of cells and developing embryos is widely believed to also depend on physical processes as well as on emergent mechanical properties of the cellular compartments. Here we demonstrate focused-light induced cytoplasmic-streaming (FLUCS). FLUCS uses light controlled thermoviscous expansion phenomena to induce well-defined flows in single cells and developing embryos. These non-invasive flows are localized, directed, highly dynamical, probe-free, and non-invasive. By controlling flows inside the cytoplasm of one-cell C. elegans embryos, we directly demonstrate the causal role of flows for the establishment of the head-to-tail body axis (aka PAR-polarization). Moreover, we show how FLUCS perturbations are suitable for an active micro-rheology of the cytoplasm and even within cell nuclei. Ultimately, we demonstrate how the underlying temperature stimuli can provide rich insights into biological phase-separation systems.

References:

Mittasch et al, Nature Cell Biology 20 (2018)

O-164

Multivalency of proteins in cellular phase separation

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Cellular phase separation of proteins results in a variety of membrane-less compartments which can serve as reaction centers, regions in which macromolecular assemblies can nucleate, or nuclear domains in which chromatin is concentrated. The heterogeneous chemical environment of the cell provides a variety of binding partners for multivalent proteins that can then form a relatively concentrated phase in coexistence with an aqueous-rich, dilute phase. Motivated by recent experiments, we have focused on the role of multivalency in modifying the usual theoretical concepts and predictions of phase separation. A minimal model of multivalent proteins that interact with divalent binding partners is analyzed to predict the phase diagram as a function of the protein/binding partner concentrations. The symmetry of the phase diagram is related to the protein valence and the concentration dependence is shown to scale exponentially with the binding energy. These predictions are compared with recent experiments by the Levy group at the Weizmann Institute. In the nucleus, multiconformational, multivalent proteins such as HP1 can be non-binding, bind to themselves and/or other proteins, and to chromatin, resulting in a rich phase diagram. The involvement of the macromolecular chromatin in the phase separation changes the properties (sizes, distribution, interfacial tension, dynamics) of the resulting phase-separated domains compared with the case of small molecule, liquid-liquid phase separation. Both simple models as well as computer simulations are compared with experiments by the Karpen group at Berkeley that show how HP1 phase separation results in (relatively concentrated) heterochromatin formation.

O-165 (P-597)

General sol to gel transition of liquid-liqud phase separated protein under shear

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It has been discovered that protein undergo reversible liquid-liquid phase separation (LLPS) forming membraneless compartments with condensed protein in living cells. This process is essential for information transportation and spatial organization. However, irreversible phase transition caused by protein aggregation or fibrillation results in neurological diseases, such as Amyotrophic lateral sclerosis (ALS) and Frontotemporal lobar degeneration (FTLD). Nonetheless, the aspects affecting this irreversible phase transition are not well understood. We have discovered, for several biological relevant proteins, such as FUS, Annexin A11 and Ded1, that shear stress triggers the sol-gel transition and can further transform condensed liquid protein droplets into solid fibres. The process is remarkably similar to the silk formation. In past studies, a few attempts have been made to generate fibres mimicking silk spinning but limited only to silk proteins. Our results have shown that proteins containing intrinsic disordered regions undergo LLPS, gelation and form solid fibres upon the response to the shear stress. To well study this phenomenon, microfluidic techniques have been applied to observe protein phase transition under controlled laminar flow. We characterized the change of structural conformation and mechanical properties of these materials of the different phases. All these discoveries are suggesting the possible mechanism of irreversible phase transition of liquid-liquid phase separated droplets in biological systems and opened a door to new sources of shear sensitive biomaterials.

O-166 (P-598)

Recapitulation of nucleocytoplasmic transport with phase-separation of engineered protein repeats

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Nuclear pore complexes (NPCs) in eukaryotes are equipped with a selective permeability barrier, which conducts transport between nucleus and cytoplasm. The barrier is composed of ~10 subtypes of FG domains, which are low-sequence-complexity, intrinsically disordered proteins (IDPs) containing numerous phenylalanine-glycine (FG) dipeptide motifs. The barrier allows passive diffusion of small molecules, but the diffusion of larger macromolecules (≥ 5nm) is restricted. Transport of the latter is mediated by binding to the shuttling Nuclear Transport Receptors (NTRs), like Importinβ. Such kind of "facilitated translocation" is achieved by the interactions between NTRs and FG domains.

We previously demonstrated that many FG domains, including the functionally important Nup98 FG domains, are "cohesive": they phase-separate spontaneously from aqueous solutions to form a hydrogel-like protein rich phase with remarkable NPC-like selectivity: it favours the fast partition of NTRs but excludes inert large proteins. We proposed that FG motifs within FG domain molecules interact with each other by polyvalent π-π/ hydrophobic interactions and these interactions lead to phase-separation. The phase can be locally and transiently disrupted by the binding of NTRs but not by inert molecules, thus allowing selective passage of NTRs and the associated cargos.

One challenge for systematic analyses of FG domain sequences is that they have very degenerated repeats. Here we attempted to engineer the simplest possible sequence of FG domain which still confers functions. Along this line, we discovered that both phase-separation propensity and selectivity remain unchanged if all xxFG motifs are converted to GLFG motifs; however, they are correlated with the density of FG motifs. We also compared different natural existing xxFG motifs systematically: GLFG and SLFG motifs are of similar cohesiveness. FSFG motifs, however, make the phase hyper-cohesive and NTRs fail to enter.

Finally, we designed an ultimately simplified FG domain comprising a perfectly repeated 12mer peptide. Remarkably, it also phase-separates to form a selective barrier. This dataset establishes that, although NPCs contain different FG domains with diverse repeat units, sequence heterogeneity is no fundamental requirement. Instead, the overall cohesive property of FG domains is essential. The simplified variants set the stage for structural/ atomic-level analyses.

O-167 (P-599)

Protein phase transition: from biology towards new dynamic protein materials

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It is now recognized that cells can form dynamic membraneless compartments by liquid-liquid phase separation (LLPS) of proteins and nucleic acids. Most of the proteins associated with this process contain low complexity domains (LCDs) or low complexity sequences (LCSs), which are intrinsically disordered domains enriched in specific amino acids. These sequences play a crucial role in tuning weak, attractive intermolecular interactions that compete with the entropic cost associated with de-mixing.

Here, we demonstrate the possibility to generate a new class of bio-inspired dynamic protein materials by conjugating low complexity domains to soluble globular regions. Specifically, we derived low complexity domains from a series of DEAD-box proteins strongly associated with the formation of processing bodies (P-bodies) in yeast. We show that these biologically derived molecular velcros enable the self-assembly of globular proteins into supramolecular architectures via a multistep process. This multistep pathway involves an initial liquid−liquid phase transition, which creates protein-rich droplets that mature into protein aggregates over time. These protein aggregates consist of permeable structures that maintain activity and release active soluble proteins. We further demonstrate that this feature, together with the dynamic state of the initial dense liquid phase, allows one to directly assemble different globular domains within the same architecture, thereby enabling the generation of both static multifunctional biomaterials and dynamic microscale bioreactors.

Faltova L., Küffner A. et al, "Multifunctional Protein Materials and Microreactors using Low Complexity Domains as Molecular Adhesives", ACS Nano, 2018, 12, 9991-9999

Wednesday 24th July

INSTRUCT-ERIC: INTEGRATING ACCESS TO BIOPHYSICS AND STRUCTURAL BIOLOGY IN EUROPE

O-168

Structure and mechanism of bactericidal mammalian perforin-2, an ancient agent of innate immunity

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Perforin-2 (MPEG1) is thought to enable the killing of invading microbes engulfed by macrophages and other phagocytes, forming pores in their membranes. Loss of Perforin-2 renders individual phagocytes and whole organisms significantly more susceptible to bacterial pathogens than their wild-types. I will describe the mechanism of Perforin-2 activation and activity determined using a combination atomic structures of pre-pore and pore assemblies from cryo-EM and X-ray crystallography, high-speed atomic-force microscopy, molecular dynamics simulations, and functional assays. Perforin-2 binds to negatively-charged lipid membranes such that its pore-forming domain points directly away from the membrane surface in the pre-pore state. Acidification triggers pore formation, via a 180° conformational change that results in the orientation of its membrane-inserting regions towards the target bilayer. This novel and unexpected mechanism prevents premature bactericidal attack, and may have played a key role in the evolution of all perforin-family proteins.

O-169

NMR-based hybrid approaches for cellular structural biology

M. Baldus

Increasing evidence suggests that the highly complex and dynamic environment of the cell interior and its physiochemical setting imposes critical control on cellular functions, which is hardly reproducible under in vitro conditions. Cellular NMR provides increasing possibilities to study molecular interactions and modifications that take place inside and at the periphery of cells at the atomic level. In our contribution, we outline the latest advancements in NMR methodology that enable such studies. In addition, applications will be shown of such methods to membrane proteins involved in cellular signaling [1] and molecular transport [2]. Combination of such approaches with modalities such as cryo-electron tomography (CET) and Fluorescence microscopy carries the potential to obtain structural insight into cell organization and function from the atomic to sub-micrometer scale [3].

[1] M. Kaplan, S. Narasimhan, C. de Heus, D. Mance, S. Van Doorn, K. Houben, D. Popov-Celeketic, R. Damman, E. A. Katrukha, P. Jain, W. J. C. Geerts, A. J. R. Heck, G. E. Folkers, L. C. Kapitein, S. Lemeer, P. M. P. V. B. E. Henegouwen and M. Baldus, *Cell*, 2016, **167**, 1241–1247.
[2] C. Pinto, D. Mance, T. Sinnige, M. Daniëls, M. Weingarth and M. Baldus, *Nature Communications*, 2018, **9**, 4135.
[3] L.A. Baker, T. Sinnige, P. Schellenberger, J. de Keyzer, C. Alistair Siebert, A. J.M. Driessen, M. Baldus, K. Grünewald, *Structure*, 2018, **26**, 161-170

O-170

Towards High Throughput cryoEM data acquisition and processing, assuring reproducibility

J.M. Carazo
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Electron Microscopy under cryogenic conditions (cryoEM) has reached in the last few years the capacity to solve the structure of large and complex systems at quasi atomic resolution (down to 2 A in a number of cases). However, to do so requires the processing of large amounts of data (Terabytes of information), which is done over several days of automated data acquisition using sophisticated and expensive equipment. It is imperative to assure high levels of data quality during the whole process, and for that a continuous analysis of the acquired data is imperative. We will show how we have achieved this in large resources like eBIC (Diamond) and the ERSF using our software Scipion, that also interfaces with local data bases and assure traceability and reproducibility in high demanding environments. Scipion has been developed as part of the Spanish contribution to the European Infraestructucture for Integrated Structural Biology, Instruct.

O-171

Access to structural biology and biophysics technologies through Instruct-ERIC

G. Naomi.
Instruct-ERIC.

Modern biophysics projects are complex and often require access to a variety of different state-of-the-art technologies, which are not always available to researchers. European Research Infrastructures are publicly funded, not-for-profit organisations which support scientists to implement their projects by providing access to cutting-edge technology platforms and expertise. Instruct-ERIC provides Open Access to world-class technology, expertise, and training in Structural Biology. Through the Instruct hub of world-leading research centres, users can access a catalogue of high-end techniques in sample preparation, biomolecular characterisation and 3D structural analysis including ultra high-field NMR, CryoEM and Xray crystallography. As well as providing access to a catalogue of advanced research tools, Instruct-ERIC offers training in emerging technologies, funded internships, and R&D funding for technology development. The Instruct-ERIC infrastructure is available to academic and industry researchers worldwide, and funded access is available to researchers from all member countries. The technology catalogue and access point can be found at https://instruct-eric.eu During this short session, Naomi Gray will introduce Instruct-ERIC and explain how European biophysics researchers can benefit from the services.

O-172 (P-621)

Crystal structures of bacteriophage receptor binding proteins

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Bacteriophages have specialised receptor binding proteins (RBPs) for initial, reversible, host cell wall recognition. Once a suitable host is found, the phage commits to infection by irreversible attachment via a secondary receptor interaction. The crystal structures of several of these receptor-binding proteins have been solved and have been shown to be mainly beta-structured, but structurally highly diverse and containing several new protein folds. Here we present structures of the receptor-binding proteins of the Escherichia coli phages T4, T5 and T7, and of Staphylococcus phages S24-1 and K. Bacteriophage receptor-recognising proteins may be used for bacterial detection, while modification by natural or experimental mutation of bacteriophage receptor-binding domains may allow retargeting of phages to alternative host bacteria. Their shape and stability may also allow their use in nano-technological applications.

O-173 (P-622)

The Cryo-EM Structure of a Non-toxic Greek Key Oligomer of Alpha Synuclein

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Aggregation of the intrinsically disordered protein alpha-Synuclein (α-Syn) into insoluble fibrils with a cross-β sheet amyloid structure plays a key role in the neuronal pathology of Parkinson's disease (PD). The fibrillation pathway of α-Syn encompasses a multitude of transient oligomeric forms differing in size, secondary structure, hydrophobic exposure, and their ability to inflict toxicity. According to a recent ssNMR study, an amyloid fibril of α-Syn contains the core residues of the protein arranged into in-register parallel β sheets with a unique C-terminal Greek key topology. We report that the physiologically available small molecule heme (hemin chloride) when added at sub-stoichiometric ratios to either monomeric or aggregated α-Syn, inhibits fibril formation by stabilizing a population of 'mace'-shaped oligomers. Using cryo-EM, we observed that these mace-oligomers consist of approximately four monomers, which complements previous reports of a physiologically stable tetramer. Incidentally, these heme-stabilized oligomers contain the Greek key topology and are essentially the smallest fundamental nuclei/ units that make up the ssNMR Greek key fibril model. This 'Greek key oligomer' fits well as a segment of the previously-described annular oligomers and appears to be its structural predecessor in the hierarchical pathway of fibril formation. This heme-stabilized oligomer also shares resemblances with the recently-determined cryo-EM structure of 'protofilament kernels.' However, these oligomers differ from the conventional Greek key model due to a 'distortion' in the Greek key architecture at the C termini of the protein molecules. We propose that this distortion prevents further appending of the twisted units into annular oligomers as well as protofilaments. Furthermore, heme inhibits fibrillation by binding to a crucial histidine (His50) residue located in the inter-protofilament preNAC interface, thus interfering with a salt bridge formation with a Glu57 residue located in the opposite protofilament, thereby weakening the inter-protofilament steric zipper integrity. When compared with the untreated fibril-forming on-pathway oligomers, the heme-treated 'distorted Greek key oligomers' showed lesser liposome permeation and toxicity to neuroblastoma cells.

Wednesday 24th July
MOLECULAR MOTORS

O-174
Cooperative Functions of Molecular Motor Assemblies
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I will present two topics in the present talk: First, on spontaneous oscillatory contraction (SPOC) observed in sarcomeres of skinned contractile system of striated (skeletal and cardiac) muscles at intermediate activation conditions (e.g., Ca-SPOC that occurs at pCa~6.0), in which a self-controlled distance between actin and myosin II motors in the myofilament lattice plays an essential role [1-6]. Second, on a contractile actin ring spontaneously formed at the equatorial plane in a spherical water-in-oil droplet (an artificial cell model), where the contractile force is produced by an assembly of actin filaments with myosin II motors [6, 7].
The conditions common to these two phenomena are the spatial constraint and its self-regulation: That is, the nano- to micro-scopic spatial constraint, i.e., the thick (myosin) and thin (actin) filament lattice (the lattice constant of about 20 nm) for the former and the spherical water-in-oil droplet (the radius of about 10 um) for the latter. Myosin II motors are not processive, which is in contrast to processive motors such as myosin V, a non-muscle molecular motors, so that myosin II does not function as a single molecule but works only as an assembly. I will stress that the spatial constraint is essential for the cooperative functions of non-processive myosin II motors (i.e., stochastic nano-machines) not only in muscle but also in non-muscle cells.
References: [1] Ishiwata, S. *et al.* 2010. *HFSP J* 4, 100-3. [2] Ishiwata, S. *et al.* 2011. *Prog. Biophys. Mol. Biol.* 105, 187-98. [3] Sato, K. ··· Ishiwata, S. 2011. *Prog. Biophys. Mol. Biol.* 105, 199-207. [4] Sato, K. ··· Ishiwata, S. 2013. *Phys. Rev. Lett.* 111, 108104. [5] Nakagome, K. ··· Ishiwata, S. 2016. *Biophys. Physicobiol.* 13, 217-26. [6] Ishiwata, S. *et al.* 2017. *Mol. Cryst. Liq. Cryst.* 647, 127-50. [7] Miyazaki, M. ··· Ishiwata, S. 2015. *Nat. Cell Biol.* 17, 480-9.

O-175
A molecular hurdle race: How does DNA polymerase deal with SSBs stably bound to the template?
B. Ibarra[1], F. Cerron[1], J. Jarillo[2], J. Morin[1], L.S. Kaguni[3], F.J. Cao[2], B. Ibarra[1].
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Single-stranded DNA binding (SSB) proteins provide a platform that allows replicative DNA polymerases to process efficiently the lagging single-stranded DNA (ssDNA) template generated during DNA replication. Multimeric SSBs bind with great affinity to ssDNA in different binding modes, which may have differente functions. To date, several questions remain unanswered about how apparently competing functions of polymerase and SSB are coordinated during synthesis of the lagging strand. For example: Which is the SSB binding mode to ssDNA relevant for DNA replication? What is the mechanism used by replicative DNA polymerases to dislodge the long-lived SSB-DNA complexes in a manner that does not compromise the advance of the replication fork? What is the nature of the stimulatory effect of SSB on the polymerase activity?
We have addressed these questions by interrogating at the single molecule level the human mitochondrial DNA replisome. Using optical tweezers, we measured: *i*) the structure and energetics of individual mitochondrial SSB-DNA complexes and *ii*) the real-time replication kinetics of the coordinated activity of the mitochondrial SSB and DNA polymerase in a single-molecule assay mimicking lagging strand synthesis under different mechanical tensions. Our data revealed that: *i*) the gradual generation of ssDNA during replication plays a key role in modulating the binding mode of the SSB and *ii*) strong, specific polymerase-SSB interactions are required for the lagging strand polymerase to dislodge SSB from the template without compromising its instantaneous replication rate.

O-176
A ratchet mechanism governs load adaptation by branched actin networks.
P. Bieling.
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Branched actin networks are self-assembling motors that move membranes and support a variety of essential cell biological processes. Load forces dictate branched network architecture and mechanics, but the molecular mechanisms that govern force adaptation are not well understood. We performed simultaneous AFM cantilever loading and multi-color TIRF imaging on reconstituted branched actin networks to measure the force dependence of all steps in network assembly. We find that force increases network density by shifting the steady-state balance between nucleation and capping. Unexpectedly, we find that loading inhibits nucleation due to negative feedback from growing barbed ends. Consistent with a Brownian Ratchet mechanism, force exponentially slows capping, creating a disbalance between filament nucleation and capping that increases network density. Remarkably, the elongation and capping rates exhibit nearly identical force-responses, because capping protein increments filament length similarly as an incoming actin protomer. This match in force dependence of capping and polymerization results in robustness of filament length with mechanical loading. Our work reveals the biochemical mechanisms that allow branched actin network to stiffen and become mechanically more efficient in response to hindering forces.

O-177 (P-623)
Kinesin-2 stepping reflects its heteromeric nature
W.L. Stepp, Z. Ökten.
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The functional significance of heteromerization in kinesin-2 motors remains largely unknown. Having coevolved with cilia, this motor is a prime example for environmental adaptation. Performing dual-color superresolution microscopy (dcFIONA), we followed the two different heads of the KLP11/20 motor from *C. elegans* walking on microtubules. Observing the stepping of both heads for the first time in a kinesin, we show that the heads have distinct stepping behaviors. Looking for the cause of this irregularity, our data unexpectedly points towards the C-terminal tails of the motor. We observe a potential allosteric interaction, that holds the promise to deliver key insights into the specialization of kinesin-2 for IFT.

O-178 (P-624)
Reverse stroke of cardiac myosin revealed by single molecule microscopy is essential for heart function
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In order to elucidate the molecular mechanism of how dynamics of cardiac myosins contribute to heart function, we measured forces of synthetic β-cardiac myosin filaments using optical tweezers and revealed stepwise displacements of actin filaments driven by myosins under a wide range of loads. The stepping ratio, which is the ratio of the numbers of forward steps relative to backward steps, under unloaded conditions decreased with increasing ATP concentrations. Compared with skeletal myosin, the stepping ratio of cardiac myosin is much lower than that of skeletal myosin, indicating cardiac myosin shows frequent backward steps. Meanwhile, the peak forces generated by cardiac myofilaments with ~15 interacting molecules were 1.5-2 times higher than those observed in skeletal myofilaments with nearly the same number of interacting molecules. Based on these findings, we developed a simulation model to understand which molecular properties critically affect on stepping behaviors and force outputs in cardiac myofilaments. The simulation suggested that reverse stroke in ADP states is a key feature to cause frequent backward steps at higher ATP concentrations, resulting lower stepping ratio. Moreover, switching between two ADP states associated with the alternate execution of power and reverse strokes keeps many myosins populated in force-generating states, enhancing the duty ratio and force outputs. Therefore, we further investigated whether single cardiac myosin can execute the power and reverse strokes in ADP state under a variety of loading conditions. When single cardiac myosins interacting with single actin filaments were stretched by optical tweezers, beads' positions were occasionally switched between two discrete levels for high loads, implying the load-dependent execution of power and reverse strokes. To know physiological meaning of reverse stroke, we simulated dynamics of myosins in sarcomere and found that the reverse stroke plays a crucial role in reducing the rate of ATP consumption during isometric contraction. Also, we implemented such molecular properties into a whole heart simulator and found that the reverse stroke is a unique future of cardiac myosin and essential for maintaining high systolic blood pressure and a rapid relaxation of diastolic blood pressure.

O-179 (P-625)
Artificial Assembly of the Bacterial Flagella Motor on DNA Scaffolds
S. Hertel, C.W. Liew, S. Xu, A. Tuckwell, L. Lee.
UNSW, Sydney, Australia.
The bacterial flagella motor (BFM) is an 11 MDa protein complex, composed of ~13 different component proteins, which powers the rotation of the bacterial flagella filament. Generation of torque and rotational switching of the BFM are mediated in the rotor by the proteins FliG, FliM and FliN. These proteins assemble into ring-shaped oligomers to form a multi-layered cytosolic ring called C-ring. The assembly of these proteins is controlled by inter- and intra-molecular interactions. In solution, FliG as well as the complex of FliM/N exist predominantly as monomers. However, after binding to a structural template these proteins can self-assemble into the ring-shaped polymers observed in the BFM. This study aims to control C-ring assembly using DNA nanostructures as an artificial template to functionally and structurally characterize interactions in the C-ring *ex vivo*. Rationally-designed DNA templates were used to immobilise an arbitrary number of protein molecules in a spatial configuration similar to that in a functional motor *in vivo*. This was achieved via the hybridisation of a single stranded DNA that was covalently attached to the protein. The use of DNA templates allowed us to measure the kinetics of intra- and intersubunit interactions in a C-ring assembly for the first time with surface plasmon resonance (SPR). Furthermore we characterized rationally designed point mutations and truncations of FliG using small-angle X-ray scattering (SAXS) and showed that these new protein constructs exhibit different conformations in solution compared to the wild-type protein. Additionally, SPR measurments with these constructs on DNA templates revealed the influence of these structural changes on the kinetics of the protein assembly, which allowed us to develop a model for self-assembly of the C-ring. This study demonstrates the use of synthetic DNA templates to probe molecular mechanisms underlying the self-assembly of the BFM and other complex protein machinery from their component parts.

Wednesday 24th July
TISSUE BIOPHYSICS AND MORPHOGENESIS

O-180
Forming, watching and stressing organoids
P. Nassoy
LP2N, UMR5298 CNRS/IOGS/Univ. Bordeaux, Talence, France
We present a simple microfluidic method based on the encapsulation and growth of cells inside permeable, elastic, hollow micro-spheres or micro-tubes. We will present and discuss how this approach allows us understand the biomechanical regulation of tumor progression and cell escape. We also describe applications to tissue bioengineering that exploit self-assembly of cell mixtures or that rely on the production of stem cells-derived organoids for cell therapy.

O-181
Physics of epithelial folds and flows
G. Salbreux
The Francis Crick Institute.
The shape of a biological tissue is determined by mechanical stresses acting within the tissue cells. During embryonic morphogenesis, forces generated in the actomyosin cytoskeleton in the cell of epithelia result in cell deformation, cell rearrangements, and 3D bending of the epithelium. To understand tissue morphogenesis, force generation at the cellular scale must be related to flows and deformation occurring at the tissue scale. Here I will discuss how this relation can be captured by a 3D vertex model or by a continuum theory of active surfaces to understand epithelial fold formation. I will also discuss how planar tissue flows are related to cell shape changes and cell division, and show how the growth of the Drosophila histoblast nests can be decomposed into basic cellular events.

O-182
Bacterial colonies as active droplets - from single cells to continuum theory
Hui-Shun Kuan[1,2], Frank Jülicher[3], and Vasily Zaburdaev[1,2,*]
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Cellular aggregates are common in many biological settings, ranging from bacterial biofilms to organoids and tumors. The dynamics of these systems is intrinsically non-equilibrium and driven by active processes in individual cells. Bacterial colonies are often considered as prototypical model systems for multicellularity. Many bacterial species use long flexible and retractile filaments called type IV pili to attach to surfaces and interact with other cells. Cycles of growth, attachment, retraction and detachment of the pili is the central active force generation mechanism that mediates cell motility and colony formation. Using *Neisseria gonorrhoeae* bacteria as an example, we go all the way from describing the motility of individual bacterial cells to the physics of colony formation in theory, numerical simulations and experiment. In this talk, we will focus on our latest development of a continuum theory of bacterial aggregates driven by attractive pili-mediated forces with excluded volume interactions. This approach allows us to study the process of aggregate formation as an active phase separation phenomenon, understand the physics of the coalescence between two aggregates, and explicitly describe the viscoelastic behavior of this multicellular system.

O-183 (P-632)

Biomimetic emulsions probe the mechanics of tissues

I. Golovkova, E. Wandersman, A. Prevost, L.L. Pontani.
Laboratoire Jean Perrin - UMR 8237 - CNRS / Sorbonne Université, Paris, France.

We study tissue-mimetic systems to understand the physical basis of collective remodeling in biological tissues. In particular, we seek to understand how the interplay between adhesion and forces controls the emergence of tissue architecture during morphogenesis. Indeed, during morphogenesis the homogeneous cell aggregate is subjected to large movements that give rise to the highly organized 3D structures found in the embryo. Using a bottom-up approach would thus allow to identify the minimal ingredients necessary to reproduce such collective processes by isolating the passive mechanical pathways of self-assembly in adhesive synthetic tissues. In particular, we use biomimetic emulsions that were shown to reproduce the minimal mechanical and adhesive properties of cells in biological tissues. These emulsions are stabilized with a monolayer of phospholipids that reproduce the fluidity of the cell membrane, and can be functionalized with adhesive proteins to mimic cell-cell adhesion in tissues. We then study the mechanical behavior of these emulsions under mechanical perturbations. In particular, we impose a global compression of the emulsions by flowing them in microfluidic constrictions with controlled geometries. Image analysis allows us to distinguish between two types of behavior in the emulsion: (1) the droplets can keep their respective positions in the packing and only be elastically deformed by the perturbation; (2) the droplets can adapt to the perturbation by rearranging positions with their neighbors, thus exhibiting an irreversible plastic response. In order to correlate these observations with *in vivo* measurements we also use emulsions as biocompatible force probes inside developing embryos. Since the droplets exhibit mechanical properties that are close to those of tissues they are visibly deformed by cellular forces. This technique is useful to map out forces in the tissue and shed light on mechanical processes at stake during development. Altogether, those findings will reveal the underlying regulations of adhesion and mechanical properties that take place in cells during the remodeling of tissues, and will more generally shed light on the physical processes at stake during embryonic development.

O-184 (P-633)

Mechano-transduction and coordination of epithelial cells during Drosophila morphogenesis

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Epithelial cells are capable of sensing and reacting to the forces and movements of their neighbors. These forces are multicellular forces at the tissue level and are transmitted by multi-protein complexes at the cell-cell adhesion sites. We hypothesize that mechano-sensitive ion channels could behave as molecular switches and respond to change in force at the cell-cell junction. The resulting biochemical signaling could be crucial for the maintenance of morphology and coordination in epithelial cells. We investigate the coordination of cells by fully quantifying the dynamics of the Amnioserosa tissue in Drosophila using a novel high-throughput image analysis pipeline based on deep neural networks. This method allows near-complete segmentation, yielding feasible analysis of a large ensemble of embryos. Inspired by graph theory, we decompose cell-cell interactions into three distinct coupling types. With this approach, the epithelium can be represented by a planar graph of cell couplings whereby cells are interpreted as vertices and junctions between cells as edges. We compare wild type embryos and mutants with impaired ion-channel functionality (TMC^{Gal4}) and weakened adhesion complexes (xit), revealing significant differences in e.g. composition of coupling types and spatial distributions. In contrast to the wild type, we find tension in those mutants to be anisotropically distributed, indicating that local cell-cell coordination through mechano-sensing is essential for the function of an epithelium as force-generating tissue.

O-185 (P-634)

A hydraulic instability underlies oocyte size selection in *C.elegans*

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The process of making an oocyte starting from a germline tissue is a fundamental cellular process that also demonstrates remarkable mechanical as well as hydrodynamic phenomena across organisms. In various cases it involves heterogenous growth of germcells within a collective structure leading to elimination of a significant proportion of germcells, this eludes to fundamental questions of dynamic size regulation and mechanical symmetry breaking. The roundworm C. elegans has a tubular syncytial (tissue architecture with connected cytoplasm) germline, which achieves germ-cell growth by hydrodynamic flows that range across 400 microns. We unravel the physical basis of oogenesis and cell elimination by combining cellular mechanics and hydrodynamics. We develop a novel theoretical framework that couples mechanics of a syncytial tissue with active fluid dynamics. By quantitative analysis and theoretical modeling, we discover that germcells actively generate long-range hydrodynamic flows along the germline, while also locally maintaining their homogenous size. The coupling of cell mechanics and hydrodynamic fields lead to active pressure-tuning, which yields a hydraulic instability setting a critical size for the germ-cells in the absence of active sources. This mechanism ensures selection and growth of germcells beyond a critical size at the expense of smaller cells and is independent of the apoptotic machinery.

Wednesday 24th July

EMERGING BREAKTHROUGH MOLECULAR-SCALE BIOPHYSICS METHODOLOGIES

O-186

Adventures with Dynamic and Disordered Systems and Joy

P. Barran
The University of Manchester.

In the last ten years direct infusion mass spectrometry (MS) coupled with electrospray ionisation (ESI) has been extensively applied to identify proteins and elucidate stoichiometry of protein complexes, often without the need for labels. Because desolvated species are affected by solvent conditions such as pH, buffer strength and concentration, ESI-MS is an appropriate method by which to consider the range of conformational states that proteins may occupy including natively folded, disordered, denatured and amyloid. Rotationally averaged collision cross sections of the ionized forms of proteins, provided by the combination of mass spectrometry and ion mobility (IM-MS), are also instructive in exploring conformational landscapes in the absence of solvent. The mass and conformer selected ions can also be subjected to dissociation (CAD, ECD, SID, UVPD) which can delineate the structure further. The use of hydrogen deuterium exchange coupled with MS as well as comparison to SAXs and other biophysical data can help to prove that gas phase results are relevant to solution. This presentation will present recent results from our group in this area. Sometimes working in one area can lead science in a radically different direction, and so it came to pass. The latter part on the talk will concentrate on how we have developed the first chemical test for Parkinson's Disease and how Joy Milne helped us to do that.

O-187

Access to Atomic Resolution Structural Information of Homor-Repeats: The Huntingtin Case

Annika Urbanek[1], Anna Morató[1], Matija Popovic[1], Alejandro Estaña[1,2], Frédéric Allemand[1], Carlos Elena-Real[1], Aurélie Fournet[1], Anabel Jiménez[3], Carlos Cativiela[3], Stephane Delbecq[4], Juan Cortés[2], Nathalie Sibille[1], Pau Bernadó[1]

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Homorepeats (HRs), protein fragments composed by the same amino repeated multiple times, are very common in eukaryotes and are involved in key biological processes and multiple pathologies. HRs are enriched in particular biophysical properties that enables them to perform very specialized functions but that can also trigger disease. Despite their interesting properties, the high-resolution structural characterization of HRs has been impaired due to their inherent flexibility and polymeric nature, which give poorly dispersed NMR spectra. Huntingtin (Htt), the causative agent of Huntington's disease (HD), is the prototypical example of a HR hosting protein. Htt has a poly-Glutamine tract of variable length that becomes toxic when the number of consecutive glutamines exceeds 35 (pathological threshold). Moreover, Htt contains two Poly-Proline tracts with 11 and 10 consecutive prolines. The aim of our study is to decipher the structural perturbations exerted by the extension of Poly-Glutamine tract beyond the pathological threshold, and the role that flanking regions, including the Poly-Proline tracts, have in the pathology.

To overcome challenges posed by HRs, we have developed a chemical biology strategy to isotopically label individual glutamines and prolines within HRs by combining nonsense tRNA suppression and cell-free protein synthesis. Our method disentangles the spectroscopic complexity of the HR and has enabled the NMR investigation of two huntingtin exon1 versions with 16 and 46 consecutive glutamines. In addition, the application to poly-Proline has allowed us to precisely explore the proline *cis/trans* isomerization in these HR regions. Implications of these observations to understand the structural bases of HDs, and the future perspectives of the site-specific isotopic labelling will be discussed.

O-188

Investigating the Bacterial Translocon at the Single Molecule Level

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Many membrane and secretory proteins are translocated across the endoplasmic reticulum membrane or the bacterial/archaeal plasma membrane through a conserved channel, the Sec61 or the SecYEG complex, respectively. In post-translational translocation the SecYEG channel associates with the cytoplasmic motor protein SecA to deliver secretory proteins to the periplasm. In this study we mimicked the translocation process by AFM. A sandwich structure was established that consisted of a mica-supported lipid bilayer containing biotinylated lipids, followed by a 2-D streptavidin crystal. On top SecYEG proteoliposomes were fused, forming periodically suspended membranes [1]. We covalently coupled the precursor form of outer membrane protein A (ProOmpA) to AFM cantilevers and performed simultaneous topographic and recognition (TREC) imaging in order to map functional SecYEG sites on the surface with nanometer accuracy. Dynamic force spectroscopy experiments on the targeted SecYEG molecules allowed us to extract information on interaction forces, energy landscapes and kinetic rate-constants of the bond formed between ProOmpA and SecYEG. ProOmpA constructs lacking the signal peptide were unable to interact with SecYEG, indicating that the signal sequence of the preprotein triggers the opening of the SecYEG channel. With the addition of SecA and ATP into solution we investigated the forces and dynamics of the fully assembled translocon. Our single molecule approach provides the unique possibility to validate the major assumptions of the two suggested translocation models: the "push and slide" [2] and the Brownian ratchet [3] mechanism.

[1] A. Karner, et al., *Nature Nanotechnology* **2017**, *12*, 260-266.
[2] B. W. Bauer, et al., *Cell* **2014**, *157*, 1416-1429.
[3] W. J. Allen, et al., *eLife* **2016**, *5*.

O-189 (P-643)

Correlative µ-Brillouin and µ-Raman spectroscopy: emerging tool for simultaneous mechanical and chemical analysis of cells and tissues

S. Caponi[1], M. Mattarelli[2], S. Mattana[2], L. Urbanelli[3], K. Sagini[3], C. Emiliani[3], M. Dalla Serra[4], D. Fioretto[2].
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In biological tissues and cells, chemical and mechanical properties are strictly correlated: their balance ensures the correct biological functionality. Here we present an innovative label-free microscopy able to jointly characterize the viscoelastic properties and the biochemical composition of biological materials with sub-micrometric resolution [1,2]. Employing Raman and Brillouin spectroscopies, this breakthrough methodology is able to analyse an exceptionally wide spectral range accessing from molecular to collective vibrational dynamics. We report relevant biophysical cases starting from the single living cells analysis to the investigation of ex-vivo tissues to demonstrate the potentiality of this emerging method. In some proof-of-principle experiments, the ability to characterize subcellular compartments and to distinguish cell status has been successfully tested [1] as well as, on ex-vivo tissues, the link between morphological structures and biomechanics has been evidenced. The results demonstrate the wide applicability of the technique.

1. S. Mattana, et. al. Light: Science & Applications 7, 17139 (2018).
2. F. Scarponi et al. Physical Review X 7, 17139; (2017).

O-190 (P-644)

Revisiting the structure-function relationship with mass photometry

P. Kukura

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The cellular processes underpinning life are orchestrated by proteins and the interactions they make with themselves and other biomolecules. A range of techniques has been developed to characterise these associations, but structural and dynamic heterogeneity remain a fundamental challenge. I will show that mass photometry based on interferometric scattering microscopy can mass-image single biomolecules in solution with nanometre precision and mass accuracy comparable to native mass spectrometry in the gas phase. Thereby, we can resolve oligomeric distributions at high dynamic range, detect small-molecule binding, and mass-measure polypeptides, glyco- and lipoproteins. These capabilities enable us to quantify the molecular mechanisms of processes as diverse as homo- and hetero-oligomeric protein assembly, amyloidogenic protein aggregation and actin polymerisation [1]. Our results illustrate how single molecule mass imaging provides access to protein dynamics and interactions and introduces a third, light-based approach to measuring mass in addition to the historical mechanical and spectrometric methodologies. This ability to investigate biomolecules in their native state with high mass accuracy and resolution provides critical, complementary information to static structural techniques in the context of protein function and regulation.

1. Young G, Hundt N, Cole D, Fineberg A, Andrecka J, Tyler A, Olerinyova A, Ansari A, Marklund EG, Collier MP, Chandler SA, Tkachenko O, Allen J, Crispin M, Billington N, Takagi Y, Sellers JR, Eichmann C, Selenko P, Frey L, Riek R, Galpin MR, Struwe WB, Benesch JLP, Kukura P. Quantitative mass imaging of single biological macromolecules. *Science* **2018** 360: 423-327

O-191 (P-645)

Hierarchical micro- and nanostructured surface architectures for label-free spectroscopic and microscopic protein interrogation

J. Flesch[1], M. Bettenhausen[2], M. Kazmierczak[3], O.E. Psathaki[1], W. Klesse[3], G. Capellini[3], T. Schroeder[4], B. Witzigmann[2], C. You[1], J. Piehler[1].

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The ambition to probe proteins in their most native state creates a high demand for label-free protein interrogation techniques. These techniques are currently limited by their lack of specificity and therefore time-consuming protein purification is needed. We here aimed to develop surface architectures that enable specific interrogation of protein-protein interactions and conformational organization by label-free surface-enhanced spectroscopy and microscopy without the need for protein purification. To ensure structural integrity and full functionality of immobilized proteins, we tailored surface biofunctionalizations for site-specific protein capturing into micro- and nanostructured sensor surfaces in *vitro*, from crude cell lysates and in live cells. Two label-free sensing approaches were explored: hierarchical Silicon micropillar (SiMP) arrays with nanoscale roughness combined with orthogonal surface chemistry were used for high density *in situ* capturing of soluble and membrane proteins from cells cultured on-chip. SiMP arrays serve as IR-resonators allowing for resonant sensing of captured proteins through a strong IR field enhancement. An augmented specificity can be achieved by tuning their resonance frequencys to protein absorption bands. Protein interrogation was carried out with a FTIR spectrometer which enables optimal probing of IR-active secondary and tertiary protein structures by sensing shifts of SiMP IR resonances upon protein deposition. As a second approach gold nanoparticles (AuNP) immobilized onto a glass surface were employed for localized surface plasmon resonance (LSPR) detection. Surface functionalization of immobilized AuNP with tris-(nitrilotiacetic acid) or HaloTag-Ligand yielded site-specific reversible and irreversible capturing of His or HaloTag fusion proteins that allowed real-time monitoring of protein-protein interactions by LSPR reflectance spectroscopy. By micropatterning and integrating of AuNP into SiMP arrays we aim to achieve a strong and spatially confined electromagnetic field enhancement which allows for highly sensitive interrogation of ligand receptor interactions at the plasma membrane of living cells.

S80

Sunday 21st July

PROTEIN FOLDING AND ASSEMBLY

P-001 (O-012)

Prion soft amyloid core driven self-assembly of globular proteins into bioactive nanofibrils

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Amyloids have been exploited to build up amazing bioactive materials. In most cases, short synthetic peptides constitute the functional components of such materials. The controlled assembly of globular proteins into active amyloid nanofibrils is still challenging, because the formation of amyloids implies a conformational conversion towards a β-sheet-rich structure, with a concomitant loss of the native fold and the inactivation of the protein. There is, however, a remarkable exception to this rule: the yeast prions. They are singular proteins able to switch between a soluble and an amyloid state. In both states, the structure of their globular domains remains essentially intact. The transit between these two conformations is encoded in prion domains (PrDs): long and disordered sequences to which the active globular domains are appended. PrDs are much larger than typical self-assembling peptides. This seriously limits their use for nanotechnological applications. We have recently shown that these domains contain soft amyloid cores (SACs) that suffice to nucleate their self-assembly reaction. Here we genetically fused a model SAC with different globular proteins. We demonstrate that this very short sequence act as minimalist PrDs, driving the selective and slow assembly of the initially soluble fusions into amyloid fibrils in which the globular proteins keep their native structure and display high activity. Overall, we provide here a novel, modular and straightforward strategy to build up active protein-based nanomaterials at a preparative scale.

P-002 (O-013)

Unravelling the details of the conformational cycle of the Hsp90 chaperone

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The molecular chaperone, heat shock protein 90 (Hsp90), is a large, dimeric ATP-driven molecular machine. It is essential in eukaryotes and is known to function as a part of many complexes. These complexes are involved, amongst other things, in the regulation of cell division and signalling [1]. As such, Hsp90 has emerged as an important target for the development of cancer therapeutics [2].

Despite the importance of this fascinating molecular machine, the exact mechanisms by which Hsp90 undergoes conformational changes, the roles of nucleotides, different domain orientations and how these influence the function of the chaperone in complex with its co-chaperones are mostly unknown.

Using a custom-built single molecule optical trapping experimental setup, we have so far elucidated the mechanism by which this large protein folds [3], characterised the role of its flexible charged linker region [4], performed a detailed comparison of Hsp90 orthologues [5] and uncovered the role of nucleotide binding in the stability of the dimeric interface of Hsp90 [6]. Current research efforts are building on these findings to study the effect of co-chaperones on the conformational cycle and single molecule mechanics of Hsp90.

[1] A. J. McClellan, et al. Cell 131, 121–135, (2007).
[2] J. S. Isaacs, et al. Cancer cell 3, 213–217, (2003).
[3] M. Jahn, et al. Proc. Nat. Acad. Sci. 113, 1232–1237, (2016).
[4] M. Jahn, et al. Proc. Nat. Acad. Sci. 111, 17881–17886, (2014).
[5] M. Jahn and K. Tych, et al. Structure 26(1), 96-105 (2018)
[6] K. Tych, et al. J. Phys. Chem. B 112(49), 11373-11380 (2018)

P-003 (O-014)

Strategically positioned slow codons support protein folding

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The genetic code is redundant, where several alternative codons code for a single amino acid. Synonymous codons are translated at different rates by the ribosome, with some codons considered to be "fast-translating" and others to be "slow-translating". Since many proteins fold co-translationally, synonymous codon usage, and the resulting translation speed, was suggested to influence nascent chain folding. A proposed underlying mechanism suggests that pausing between the translation of independently-folding units supports their correct folding. The importance of codon usage to protein folding and function was shown for a few individual cases, but a clear and general understanding of the phenomenon is still missing. In this work, we aim to identify conserved regions of slow codons that are important for correct protein folding and function.

To this end, we analyzed 1,115 orthologue protein groups from *E. coli* and *B. subtilis* to identify slow codon stretches that appear to be important for a specific 3-D protein fold. Based on this analysis, we selected several proteins for experimental validation. We generated a modified version of each gene in which the slow codon stretch was replaced with a synonymous fast one. We compared the modified version to the wild type one in a set of experiments checking expression, solubility and function. Preliminary results show that in several cases, especially in oligomeric proteins, strategically positioned slow codons are important for protein stability and function, suggesting that biased codon usage is a general mechanism that assists correct protein folding.

P-004

Sensitivity analysis of kinetics variability in amyloids and heterogeneous nucleation events

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In vitro kinetic assays of amyloid formation represent a central tool in many areas of biotechnological and biomedical sciences, including drug discovery against a variety of neurodegenerative disorders. The self-assembly of soluble peptides and proteins into insoluble amyloid aggregates follows macroscopic kinetic profiles that are commonly characterized by a certain degree of variability. This variability challenges the investigation of the molecular determinants of the aggregation process, and its molecular origin remains largely elusive. By analyzing the aggregation profiles of four different amyloidogenic proteins, we show that the variability of the kinetic traces is linearly proportional to the duration of the aggregation process. By applying a sensitivity analysis, we demonstrate that this behaviour arises from an initial fixed perturbation of one or more of the kinetic parameters of the aggregation network, and does not involve intrinsic stochasticity or amplification of perturbations during the aggregation process. Moreover, our analysis shows that the sensitivity of the amyloid aggregation network with respect to the initial monomer concentration is higher than for all the other kinetic parameters involved.

One of the possible microscopic sources of variability is surface-induced heterogeneous nucleation, which is difficult to understand and control and yet is ubiquitously observed in a variety of biophysical contexts. Here we develop a platform, based on polymeric nanoparticles, which provides a highly controlled surface-mediated driving force for aggregation. This high-throughput and flexible assay represents a convenient system to perform *in vitro* kinetic assays and investigate the fundamental physics underlying surface-induced protein self-assembly. In particular, we demonstrate the potential of this strategy by unraveling the synergistic effect of hydrophobic surfaces and mechanical agitation on the aggregation of human insulin under physiological conditions. We show that while hydrophobic surfaces specifically and dramatically increase primary heterogeneous nucleation events, mechanical agitation accelerates the formation of amyloid fibrils by favoring mass transport and by further amplifying the number of fibrils through fragmentation events. These results suggest that the inhibition of surface-induced heterogeneous nucleation should be considered a primary target to suppress amyloid self-assembly, and explain why in many systems the simultaneous presence of specific surfaces and hydrodynamic flow enhances protein aggregation.

P-005

The efficacy of designed anti-measles virus peptides depends on the stability of self-assembled clusters.

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The resurgence of several infectious diseases, like measles, has driven the search for new chemotherapeutics to prevent and treat viral infections. Self-assembling antiviral peptides are a promising class of entry inhibitors capable of meeting this need. Fusion inhibitory peptides derived from the heptad repeat of the C-terminal (HRC) of the measles fusion protein, dimerized and conjugated with lipophilic groups, were found to be efficacious against measles virus. The structures of the self-assembled nanoparticles formed by these peptides modulated their activity. Based on the analysis of a L454W mutation in the fusion protein of a naturally occurring measles viral isolate, HRC peptides bearing the tryptophan residue at position 454 (HRC-L454W) were synthesized with the goal of improving membrane anchoring and manipulating self-assembly. Monomeric and dimeric peptides, whether conjugated or not to a single lipophilic group, reduced infection *in vivo*. Bis-conjugation with lipophilic groups, in contrast, abrogated activity.

Based on the physicochemical properties of self-assembly and membrane insertion kinetics of the HRC-L454W peptides we show that bis-conjugation increases the stability and order of the inner core of the spontaneously self-assembled nanoparticles, resulting in their compaction. The presence of the tryptophan residue also increases steric hindrance effects in the nanoparticle of the dimeric peptides, contributing to inter-peptide cluster meshing, but the same level of compaction is not achieved. We propose that the highly ordered packing and stability of molecular clusters forming the inner core of self-assembled nanoparticles prevent efficient dissociation of the peptides *in vivo*, hindering their release and therefore eliminating their antiviral efficacy.

P-006

Exploring novel concepts of α-synuclein amyloid aggregation with a combination of bulk and single-particle spectroscopy

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α-synuclein is the principal amyloidogenic protein causing Parkinson's Disease (PD) in more than 5 million people worldwide. During disease conditions α-synuclein transitions from its native, intrinsically disordered (IDP) conformation to a neurotoxic, degradation-resistant β-amyloid aggregated form (1). Over the past years a number of long fibrillar aggregates have been structurally characterised by a number of techniques (2, 3). However, there is growing evidence suggesting that multiple aggregated species named polymorphs, some of them with independent aggregation mechanisms, might also occur and even coexist during the pathological process. This, together with the subtlety of the initial conformational changes that might be responsible for defining the aggregation pathways of α-synuclein, greatly increases the difficulty to study and therefore understand the disease in depth as well as the action mechanism of inhibitors and chaperons and thus asks for new tools to be developed. In our lab, we are developing new assays and tools based on a combination of bulk and single-particle techniques such as pyrene fluorescence, Fluorescence Cross-Correlation and FRET spectroscopy together with Electron Paramagnetic Resonance to gain new insights in all scales of the amyloid aggregation of α-synuclein: from observing the very first conformational changes that drive aggregation to being able to identify individual, distinct polymorphs that coexist in a complex mixture of aggregates thereby creating a PD fingerprint. Besides, we are applying this combined experimental strategies to the deeper understanding of the inhibition mechanisms of different molecules on α-synuclein amyloid aggregation.

1. Chiti F, Dobson CM (2006). Protein misfolding, functional amyloid, and human disease. *Annu. Rev. Biochem.* 75:333–366.

2. Stefani M, Dobson CM (2003). Protein aggregation and aggregate toxicity: new insights into protein folding, misfolding diseases and biological evolution. *J. Mol. Med. (Berl)*. 81(11):678-99.

3. Cremades N, *et al.* (2012), Direct observation of the interconversion of normal and toxic forms of alpha-synuclein. *Cell* 149(5):1048-59

P-007

MEOTA-BTZ derivatives inhibit amyloid aggregation of lysozyme in linker-length dependent manner

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The formation and accumulation of amyloid aggregates are the accompanying phenomena of amyloidoses, which include Alzheimer's and Parkinson's diseases or non-neuropathic lysozyme systemic amyloidosis. Amyloidoses are currently untreatable and the incidence of Alzheimer's disease, the most common form of dementia, reached 50 million patients worldwide in 2018. Furthermore, this number is expected to grow by almost 10 million per year. Nowadays, an approach to the treatment of these diseases is mostly symptomatic and aimed at temporary improvement in the quality of life.

One of the very promising approaches allowing to solve the problem of amyloidoses is inhibition of amyloid aggregation of poly/peptides or removal of insoluble amyloid fibrils from the affected tissues. According to studies, small molecules have a great potential to interfere with amyloid fibrillation of poly/peptides. The three suggested features of compounds able to inhibit amyloid aggregation are: the presence of aromatic rings, the position of side groups on the aromatic rings and the length and flexibility of the linker between functional groups. In our study, we focused on small organic heterodimers, namely tacrine-benzothiazole (MEOTA-BTZ) molecules which consist of 7-methoxytacrine and 2-aminobenzothiazole functional groups linked by an alkylamine linker.

Using *in vitro* and *in silico* methods, we investigated the ability of the studied compounds to significantly inhibit the amyloid aggregation of hen egg white lysozyme (HEWL). The effect of compounds was quantified using thioflavin T (ThT) fluorescence assay. The inhibitory effect was observed in the case of all compounds with IC_{50} values in the micromolar range; however, the effectivity of compounds differed based on the length of the linker. Measured data correlate with images from atomic force microscopy. The most effective derivative contains the longest, eight carbon long linker. These observations were supported by *in silico* calculations suggesting the direct dependence between the binding free energy of the derivatives and the length of the linker. MEOTA-BTZ derivatives have convincingly demonstrated their ability to inhibit the amyloid aggregation of HEWL at tolerable cytotoxicity level.

P-008

Understanding the Biophysics of Protein-Surface Interactions

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Despite the importance of protein-surface interactions in both biology and biotechnology, our understanding of how and why they occur is still limited: What are the thermodynamic consequences of the interaction of proteins with surfaces? Why do proteins generally remain folded and functional on biological surfaces and cell membranes, but often unfold, adhere to, and inactivate on artificial surfaces? To respond to these questions we need to understand the biophysical origins of protein-surface interactions, but unfortunately the scarcity of experimental measurements of the thermodynamics of such interactions has precluded its quantitative analysis. In response, we have developed an approach to measure the free energy of protein-surface interactions, which we have employed here to explore the extent to which attachment to a specific, macroscopic surface alters the thermodynamic stability of protein L. We have achieved so by modifying protein L with the redox reporter methylene blue and then covalently tethering its N-terminus to a gold electrode passivated with a hydroxyl-terminated alkanethiol monolayer. Unfolding of the surface-attached protein alters the ease with which the methylene blue transfers electrons to the surface, therefore allowing to monitor the unfolding by electrochemical techniques to extract protein stability. Comparing the thus obtained stability of the surface-attached protein to that of the same protein in bulk solution we find that surface-attachment stabilizes the protein due to excluded volume effects that restrict the conformational entropy of the unfolded state. We have also explored the role of macromolecular crowding, solvent composition, and electrostatics, to find that these effects are markedly different for surface-interacting proteins compared to bulk solution. We believe that our studies refine our understanding of the biophysics underlying protein-surface interactions, which may in turn improve the design of protein-surface pairs for protein-incorporating biotechnologies, such as protein-based sensors, or improve our understanding of biological processes occurring at cellular interfaces.

P-009

Comparison between Abeta-(1–40) and Abeta-(1–42) in terms of membrane interactions and their cross interactions

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Amyloid formation by amyloid β-protein (Aβ) is a pathological hallmark of Alzheimer's disease. Accumulating evidence has suggested that the binding of Aβ to neuronal membranes plays an important role in the aggregation of Aβ. We have elucidated that Aβ-(1–40) forms toxic tape-like fibrils composed of a single layer of mixed 2-residue-shifted antiparallel and in-resister parallel β-sheets on membranes containing GM1 clusters [1, 2]. In this study, Aβ-(1–40) and Aβ-(1–42), the latter being more amyloidogenic and toxic, were compared in terms of membrane interactions. Both proteins exhibited similar affinities for GM1-containing lipid bilayers [3]. They formed preamyloid oligomers of similar size (~15mer) and eventually toxic fibrils on neuronal cells, although a 10 times higher concentration was needed for Aβ-(1–40) [3, 4]. In their coexistence, both types of Aβ formed preamyloids independently, whereas they formed mixed fibrils. An aggregation model in compatible with these observations will be proposed.

[1] Matsuzaki, K., Acc. Chem. Res. 47, 2397 (2014).
[2] Okada, Y. et al., ACS Chem. Neurosci. 10, 563 (2019).
[3] Ogawa, M. et al., J. Neurochem. 116, 851 (2011).
[4] Itoh, N. et al., ChemBioChem, 19,430 (2018)

P-010

Computational design of a nine-and eight-bladed beta-propeller

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Beta-propellers are pseudo-symmetrical proteins composed of a repeating motif consisting of four anti-parallel beta-strands called a blade. These blades are arranged around a central axis and vary in number between four and nine. Nine bladed propellers however seem to be rare as currently the eukaryotic translation initiation factor 2A and homologs are the only known examples. A computational approach developed to design the first symmetrical beta-propeller, the Pizza protein, was used to design a nine-bladed symmetrical beta-propeller. Its structure was validated by X-ray crystallography. The protein consists of nine identical repeats of 42 residues, adopts the classical beta-propeller fold and is very thermostable. The structure of this designed proteins is different from the currently known nine-bladed propellers found in nature and has more in common with eight-propellers. Proteins carrying two to ten identical blades were also expressed, purified and crystalized. While ten repeating units assembled into the nine bladed structure, the protein with only eight repeats folded into an eight bladed propeller. This fold is almost as thermostable as the nine-bladed variant, indicating that a sequence can fold into different thermostable structures depending on the number of times it is repeated. These proteins named Kiku, are great model proteins to investigate the evolution of beta propellers to understand how the number of blades may have changed over time.

P-011

Polysorbate 20 – insights into micelle formation

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Polysorbate 20 is one of the most widely used detergents in liquid pharmaceutical formulations. It has been found to stabilize biologicals in their functional state and it serves to solubilize insoluble active ingredients.

For all these applications, it is important to be aware of the complex association behavior, which differs fundamentally from the usual concept of one characteristic type of micelle forming above the CMC. This is a result of the commercial product polysorbate 20 representing a very diverse mixture of polyoxyethylene ethers and esterified fatty acids with individual CMC values ranging over several orders of magnitude. As a consequence, PS 20 micelles change their composition and subsequent thermodynamic and structural properties quite markedly as a function of overall concentration.

Isothermal titration calorimetry (ITC) and dynamic light scattering (DLS) show that some kind of micelles form in a dispersion of PS 20 already at concentrations well below 1 mM. Increasing concentrations cause an additional partitioning of higher-CMC components into these "primary" micelles, causing substantial changes in micelle properties. For example, micelles forming at 10 mM are smaller and exhibit a significantly more endothermic micellization enthalpy compared to those formed at 1 mM.

P-012

Lysozyme amyloid fibrils are destroyed by amino acids functionalized magnetic nanoparticles

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The clearance of amyloid fibrils represents an attractive strategy to develop therapies for amyloid-related diseases. Magnetic nanoparticles (MNPs) have drawn a lot of attention in biomedical fields due to their unique properties as small size, large surface area, and relative non-toxicity. We have synthesized and characterized the physical and chemical properties of MNPs functionalized with different amino acids (AA), namely with lysine (Lys), glycine (Gly) or tryptophan (Trp). Using ThT assay and atomic force microscopy was determined that the AA-MNPs significantly destroy lysozyme amyloid fibrils. There is a correlation between AA adsorbed layer thickness and anti-amyloid properties of AA-MNPs. The best anti-amyloid properties were found out for the Trp-MNPs with the largest hydrodynamic diameter that can be explained by the increase in a layer thickness of AA or/and by the presence of Trp aromatic rings at the Trp-MNPs surface. The obtained results suggest that AA-MNPs represent potent agents for the treatment of diseases associated with amyloid aggregation of proteins. The cytotoxicity of AA-MNPs was verified as well. It was found that studied AA-MNPs are non-toxic to human SHSY5Y cells. (This work was supported by the project of VEGA grants 2/0145/17, 2/0033/19, and 2/0030/18, APVV-14-0120 and APVV-14-0932, SAS-MOST JRP 2015/5 and MVTS COST 083/14 action BM1405).

P-013

Silk: A natural example of a sticky entangled polymer

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The Bombyx Mori silk worm produces natural silk from an "aquamelt" of randomly coiled proteins, which upon applying elongational flow and extraction of water rapidly undergoes a transition to a solid fibre with outstanding mechanical properties. This process is remarkably more efficient than the spinning of synthetic polymers in terms of both energetic costs and solvent recycling, but remains poorly understood. We show, using quantitative modelling of the linear viscoelastic response of the silk feedstock, that this aquamelt may be viewed as a supramolecular polymer netwerk, where the protein is topologically entangled and uses salt bridges as reversible crosslinks. Our findings provide a viable starting point to physically understand the "flow-induced self-assembly" of silk fibres.

P-014

The ratio of spectral signals is not a reliable parameter for evaluation of protein transitions

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Monitoring the spectral properties of proteins, particularly the fluorescence of intrinsic tryptophan residues, is a popular method often used in the analysis of unfolding transitions (induced by temperature, chemical denaturant, and pH) in proteins. The tryptophan fluorescence provides several suitable parameters, such as steady-state fluorescence intensity, apparent quantum yield, mean fluorescence lifetime, position of emission maximum that are often utilized for the observation and subsequent analysis of the conformational/unfolding transitions of proteins. It has been previously pointed out that the critical condition to use of any of these signals is whether the signal is proportional to the population of macrostates. In fact, out of the commonly used fluorescence signals, this criterion is fulfilled only for the fluorescence emission intensity measured at different excitation and emission wavelengths. Other, above mentioned, fluorescence signals have to be analyzed in more complex way, e.g. they have to be corrected by weighting both the fraction of states and by the fluorescence quantum yield of each state and/or as it has been proved useful to analyze these signals in combination. The fluorescence intensities ratio at different wavelengths (usually at 330 nm and 350 nm) is popular parameter for the evaluation of thermal transitions due to empirical observation that such signal has better quality regarding signal/noise ratio and more pronounce shape of the transition. We demonstrate that the ratio of fluorescence emission at two different wavelengths, which are used in thermodynamic analysis of conformational transitions of proteins can lead to the incorrect determination of thermodynamic parameters characterizing unfolding transitions in proteins (e.g., melting temperature), and therefore needs to be corrected.

P-015

Co-Aggregation of Ganglioside-Containing Membranes and α-Synuclein

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Parkinson's disease (PD) is accompanied by the formation of Lewy bodies, whose main components are fibrils of the protein α-synuclein. These aggregates include membrane lipids, while little is known about the process of lipid/α-synuclein co-aggregation and its implications for fibril and Lewy body formation. Ganglioside lipids are abundant in neuronal membranes and have been found to accelerate fibril formation [1] and to accumulate in PD brains.

We address the effects of ganglioside-phospholipid (GM3-POPC) model membranes on the dynamics (regarding the order parameter and the correlation time of C-H bond reorientation) and structure of co-aggregates with α-synuclein using polarization-transfer ^{13}C solid-state NMR [2], wide-angle x-ray scattering (WAXS) and cryogenic electron microscopy. ^{31}P NMR powder patterns were recorded to monitor the lipid phase behavior and the spinning sidebands at 1250 Hz MAS were used to fit the chemical shift anisotropy before and after co-aggregation.

It was found that both lipids are co-assembled with α-synuclein with reduced headgroup and acyl chain mobility, and that the mobility of α-synuclein is modulated by the lipid-to-protein ratio and membrane composition. As the ganglioside content of membranes was increased the mobility of alanine residues is affected, implying a modulation of the fibril core which could impact the fibrillation propensity. These effects of co-aggregation on α-synuclein fibril mobility are currently being further investigated by us and raises questions on the specific lipid-protein interactions and the organization of lipids inside co-aggregates.

References:

[1] Grey, M.; Dunning, C. J.; Gaspar, R.; Grey, C.; Brundin, P.; Sparr, E.; Linse, S. *J. Biol. Chem.* 290, **2015**, 2969-2982.

[2] Nowacka, A.; Mohr, P. C.; Norrman, J.; Martin, R. W.; Topgaard, D. *Langmuir*, 26(*22*), **2010**, 16848-16856.

P-016

Multi-variable study on the *in vitro* reassembly of virus-like particles derived from the minute virus of mice

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Elucidating the physical principles regarding virus assembly might support the development of new therapies against viral diseases and the emergence of new nanobiotechnological strategies based on viral like particles (VLPs). The detailed description of the assembly of simplest icosahedral viruses has been hampered by the difficulties in observing populated intermediates experimentally. Recently, we described the assembly-disassembly pathways of one of the structurally simplest virus, the minute virus of mice (MVM), and demonstrated the reversibility of the assembly-disassembly process in vitro. Reassembled capsids are a promising nanobiotechnological tool but the efficiency of the reassembly process might be still low for large scale industrial purposes therefore, it is crucial to establish the conditions that maximize the efficiency of the reassembly process. Moreover, improving the reassembly efficiency could provide further insights into the biophysical aspects of this reaction and their relationship with virus biology. In this work we have studied the effects of pH, ionic strength, protein concentration, temperature and macromolecular crowding conditions on the reassembly efficiency of VLPs derived from MVM. Slightly acidic conditions (pH=6.5), relatively high ionic strength (0.5 M NaCl) and (under certain conditions) or crowding agents increased the reassembly efficiency. On the other hand, reassembly efficiency (though not absolute yields) was reduced at higher capsid protein concentrations.

P-017

Analysis of protein folding using persistent hoomology

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The process of protein folding has been attracted interests of many scientists. Recent improvement of computer simulation enables us to simulate this process. However, the results of these simulations are complicated, and we need further computation to extract the essential part of the folding process. Several methods for the analysis of protein folding have been proposed.

In this study, we propose another method to analyze protein folding simulation. Our method is based on persistent homology (PH), an emerging method for topological data analysis. In this approach, we put balls with radius r at the positions of atoms, and investigate the creation and destruction of "loops" when we gradually change r from 0 to infinity. Our method is composed of three steps. First, we list up all "loops" using PH. Second, we evaluate the contribution from "edges" to the formation of loops. Third, we reduce the dimension using non-negative matrix factorization.

We applied this method to the MD simulation of chignolin. Using the method described above, we reduced the dynamics of this system into two-dimensional space. Our result shows that chignolin has three metastable states: folded, misfolded and unfolded state. Folded state has loops that include edge between Tyr2 and Trp9, while misfolded state has loops with edges between Tyr2 and Thr8. The dynamics in reduced space shows that there are two stable fixed points and one saddle point, which correspond to folded, misfolded, and transient state, respectively. Our method gives intuitive insights on the protein folding process of this molecule.

P-018

Understanding the multiplicity of amyloid polymorphs and self-assembly pathways of α-Synuclein in Parkinson's disease

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α-Synuclein (α-Syn) is a presynaptic intrinsically disordered neuronal protein whose misfolding and aggregation in the form of amyloid fibrils is the hallmark of different neurodegenerative disorders known as synucleinopathies, including Parkinson's disease, the second most common neurodegenerative disease in the world. Increasing experimental evidences suggest that α-Syn can aggregate in multiple fibrillar polymorphs displaying different toxicities and degrees of infectivity, suggesting a potential link between the structure and pathology of different types of α-Syn fibrillar polymorphs. With the aim of understanding the different mechanisms that lead to the variety of α-Syn fibril polymorphs, we have characterised the amyloid pathways and amyloid polymorphs of α-Syn under a variety of experimental conditions including in vitro cytomimetic conditions. We have found conditions that lead to both heterogeneous and homogeneous nucleation that yield to drastically different amyloid structural polymorphs that differ at all the possible levels of structural variability, from their secondary structural content and peptide orientation to the quaternary organization of the constituent protofilaments. Interestingly, all the possible amyloid pathways that we have found can be grouped in two main types of general self-assembly mechanisms for α-Syn. Our findings are greatly contributing to understand the complex conformational landscape of α-Syn aggregation, the different ability of each polymorphs to induce neuronal toxicity and infectivity; and can be of significant help in guiding the rational design of therapeutic tools selectively targeting the formation of distinct structural types of α-Syn fibril polymorphs.

P-019

Structural dissection of amyloid fibrils of TDP-43 and its C-terminal fragments TDP-35 and TDP-16

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The TAR DNA binding protein of 43 kDa (TDP-43) is observed as the main component in amyotrophic lateral sclerosis (ALS) and frontotemporal lobar dementia (FTLD) cytoplasmic inclusions (1). TDP-43 consists of a well-folded N-terminal domain (NTD), two RNA recognition motif domains (RRM1 and RRM2) and an intrinsically disordered C-terminal domain. The prion-like C-terminal domain possesses most of the pathologically relevant mutations and plays a critical role in the spontaneous aggregation of TDP-43 and associated proteinopathy (2, 3). For a detailed structural analysis of the amyloid-forming C-terminal region, we have analyzed the full-length TDP-43, two C-terminal fragments (TDP-35 and TDP-16) and a C-terminal truncated fragment (TDP-43 ΔGaroS2) in their fibrillar state. Although the different protein constructs exhibit similar fibril morphology and a typical cross-β signature by X-ray diffraction, solid-state NMR indicates that TDP-43 and TDP-35 share the same polymorphic molecular structure, while TDP-16 encompasses a well-ordered amyloid core. We identified several residues in the so-called C-terminal GaroS2 region that participate in the rigid core of TDP-16 fibrils, underlining its importance during the aggregation process. Our findings demonstrate that C-terminal fragments can adopt a different molecular conformation in isolation or in the context of the full-length assembly, suggesting that the N-terminal domain play a significant role in the TDP-43 amyloid transition (4).

References 1. Neumann, M. et al. Science 314, 130–3 (2006). 2. Johnson, B. S. et al. J. Biol. Chem. 284, 20329–20339 (2009). 3. Pesiridis, G. et al. Hum. Mol. Genet. 18, (2009). 4. Shenoy et al., manuscript submitted

P-020

8-Anilinonaphthalene-1-sulfonic acid as a potential novel HIV-1 gp41 fusion inhibitor.

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During HIV-1 infection, the envelope subunit gp41 mediates fusion between viral and host cell membranes. This process is driven by gp41 folding into a highly-stable trimer of helical hairpins formed by the interaction between N-terminal (NHR) and C-terminal (CHR) heptad-repeat regions. In this interaction a hydrophobic pocket on the NHR surface reveals to be of high importance and its occupancy interferes with the NHR-CHR interaction of gp41. Accordingly, small-molecules binding the hydrophobic pocket constitute promising therapeutics against HIV-1 infection.

In our previous work, we rationally designed and engineered single-chain protein constructs that accurately mimic the NHR coiled-coil surface. These three-helix protein constructs (named covNHR) show high structural stability and solubility, bind the CHR region of gp41 with high affinity and possess nanomolar *in vitro* inhibitory activity for a variety of HIV-1 strains.

8-Anilinonaphthalene-1-sulfonic acid (ANS) is a fluorescent probe that binds to solvent –exposed hydrophobic patches in proteins and is widely used to characterize exposed hydrophobicity and partial unfolding or misfolding in proteins. Here, we investigate whether ANS can interact with our covNHR protein used as structural mimetic of the gp41 NHR region. Binding of ANS to our covNHR protein (covNHR-VQ) was measured by Near-UV Circular Dicroism and Fluorescence. Isothermal Titration Calorimetry (ITC) experiments indicate direct binding between ANS and covNHR-VQ with a 1:1 stoichiometry and a K_D of ~5 μM. The mode of binding was assessed using molecular docking using the X-ray structure of covNHR-VQ and the results show that ANS binds within the NHR hydrophobic pocket also revealing that protein-ligand interactions share common characteristics with known small-molecule inhibitors. Molecular docking results were validated using 100 ns Molecular Dynamics simulations that show the stability of the docking pose through the entire simulation time.

These results indicate that ANS is capable of binding to the NHR hydrophobic pocket with moderate affinity and therefore, ANS and its derivatives constitute promising lead compounds to inhibit the NHR-CHR interaction. ANS displacement, as a fluorescent probe, can also serve as tool for High Troughput Screening (HTS) using covNHR-VQ as structural mimic of gp41.

P-021

Rational oligomerization control for a designed metamorphic protein with *de novo* toroidal hexameric rings

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The old idea of one protein/one function has been challenged in the last two decades by many examples of proteins with more than one active conformation and/or function. Amongst them, moonlighting, metamorphic or highly flexible one state proteins can be included, with a growing significance in the last few years. In particular, metamorphic proteins show at least two alternative conformations with different structure and, possibly, different function, showing an equilibrium exchange between these conformations dependent on the environmental conditions.

Using a careful design we have created a metamorphic protein from a simple two state protein, chymotrypsin inhibitor 2 from barley seeds, by introducing 10 mutations along the sequence. The new protein version is able to specifically oligomerize into hexameric and dodecameric toroidal rings after experiencing a conformational change, expected for metamorphic proteins, related with the opening of the C-terminal beta strand. The structure and the oligomerization tendency have been tested by a battery of techniques, confirming the existence of a necessary intermediate conformational switch between the monomer and the oligomer.

In this scenario the oligomerization control can be performed by modulating both the monomer/switch and the switch/oligomer processes using external cues, as can be temperature or protein concentration, or mutational strategies. Following this last option, we have designed a set of mutants following three different strategies to increase oligomerization tendency: by facilitating the C-terminal opening, by stabilizing the interactions between monomers in the oligomer and by introducing metal binding sites in the oligomer. Here we present the effect and differences of these three strategies on the stabilization of the oligomer and the possibility to combine them in order to obtain thermophilic complexes.

P-022

Bexarotene does not directly clear Aβ plaques but influences Aβ processing: a possible mechanism

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It has been showed that anti-cancer drug bexarotene can remarkably destroy amyloid beta (Aβ) plaques in mouse models suggesting therapeutic potential for the Alzheimer's disease. However, the effect of bexarotene on clearance of plaques has not been seen in some mouse models. Therefore, an interesting question emerges whether bexarotene can destroy Aβ plaques by direct interaction with them or by preventing the production of Aβ peptides.

The results obtained in *in vitro* experiments suggest that bexarotene slows down the process of $Aβ_{42}$ fibrils formation but it does not affect the amount of formed fibrils. We also did not observe the ability of bexarotene to degrade $Aβ_{42}$ fibrils. We explored the possibility of inhibiting Aβ production via binding of bexarotene to β-secretase which can cleavage Aβ peptides from the amyloid precursor protein. Obtained fluorescence intensities for the whole concentration range of bexarotene were similar to the fluorescence intensities of BACE-1 product suggesting that bexarotene has no inhibitory effect on β-secretase activity. Therefore, we assume that bexarotene weakly binds to $Aβ_{42}$ peptide, $Aβ_{42}$ amyloid fibrils and β-secretase suggesting that bexarotene-induced clearance of amyloid plaques is probably not through direct interaction with Aβ or inhibition of Aβ production. Using *in silico* simulation we have also shown that bexarotene can tightly bind to peroxisome proliferator-activated receptor γ (PPAR-γ) and to retinoid X receptors (RXRs) with the inhibition constant in the sub-nanomolar range suggesting the other pathway of APOE4 overexpression via activating PPAR-γ or both receptors leading to enhancement of the Aβ clearance mechanisms.

This work was supported by the research grants from the Slovak Grant Agency VEGA 2/0145/17, 2/0176/14, MVTS COST 083/14-NGP-NET Action BM1405 and Narodowe Centrum Nauki in Poland (grant No 2015/19/B/ST4/02721).

P-023

Molecular insights into the structural effects of pathogenic CFTR mutations and the action of a pharmacological corrector

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To drive the rational design of cystic fibrosis (CF) therapies, it is important to elucidate how mutational defects in the cystic fibrosis transmembrane conductance regulator (CFTR) lead to its impairment, and how pharmacological compounds interact with CFTR. Here, we investigate structural effects of the patient-derived CF-phenotypic mutations V232D and E217G located in CFTR's membrane-spanning domain 1. For this purpose, we employ a helical hairpin construct derived from CFTR's transmembrane (TM) helices 3 and 4 (TM3/4) and their intervening loop. Single-molecule FRET is used to probe the folding status of reconstituted hairpins in lipid bilayers. While wild type (WT) TM3/4 folds efficiently in bilayers of various thicknesses, the V232D hairpin resides predominantly in an open conformation suggesting that hairpin insertion and folding are perturbed upon nonpolar-to-polar mutation, which could cause the maturation defect in V232D CFTR. In contrast, the E217G hairpin exhibits an altered adaptive packing behavior stemming from an additional GXXXG helix–helix interaction motif created in E217G TM3/4. This implies that misfolding and functional deficiency of E217G CFTR arises from an impaired conformational adaptability of CFTR's TM helical segments. Remarkably, addition of the small molecule drug Lumacaftor (VX-809) restores a compact WT-like fold in V232D TM3/4 and causes helix stabilization of E217G TM3/4 and WT TM3/4. Thus, we find that Lumacaftor has a general mode of action likely connected to its membrane-destabilizing properties, through which it efficiently improves maturation of various CFTR mutants.

P-024

Exploring Structural Characterization of the Human Golgi Matrix Protein GRASP55 in Solution

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In mammalian cells, the Golgi apparatus is a central hub for intracellular trafficking, sorting and post-translational modifications of proteins and lipids. The Golgi reassembly and stacking proteins (GRASPs) play a pivotal role in the biogenesis of Golgi stacking. However, the structural details of human GRASPs are still elusive. In this context, we have explored the biophysical properties of the human full-length GRASP55 in solution. Sequence-based analyses and circular dichroism spectroscopy suggest that the GRASP55 presents multiple intrinsically disordered sites although keeping considerable contents of the secondary structure. Size exclusion chromatography coupled with multi-angle light scattering studies show the GRASP55 forms a monomer in solution. Differential scanning calorimetry analysis displays two endothermic transitions for GRASP55, indicating the existence of an intermediate state prior to unfolding. The thioflavin T and ANS fluorescence show the GRASP55 can form protein aggregates/fibrils at the intermediate state. Transmission electron microscopy and fluorescence lifetime imaging microscopy proved that GRASP55 forms aggregates at the intermediate state. We will discuss the significance of these results to the proper function of human GRASP55 in both the conventional and unconventional secretory pathway.

P-025

Molecular characterization of tunable microscale protein-based biomaterials

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Protein aggregates have been related to many diseases, but recently, there has been a grown interest on using them as biomaterials due to their organic composition and their stability. Specific attention was focused from literature to different types of amyloid aggregates: ordered structures stabilised by a regular pattern of H-bonds. Among all, protein particulates are amyloid superstructures that are formed at a pH near the isoelectric point of the protein they are made of. They have a perfect spherical shape and a size ranging between hundreds of nanometres to a few micrometres, and up to now they weren't related to any disease.

We present an experimental study showing that maturation controls the molecular properties of alphalactalbumin (ALA) particulates. The kinetics of ALA particulates formation is studied at high temperature; at different time points, after massive aggregation is occurred, we select different key points representative of different maturation stages that were characterised with a combination of spectroscopy and microscopy methods.

Fluorescence Lifetime Imaging Microscopy (FLIM) on Thioflavin T stained particulates reveals different surface properties depending on the maturation stages, which relate with differences at secondary structure level as monitored by FTIR. We found that maturation varies the structure resistance of these microsized spheres to stresses like high pressure or dramatic pH changes, this being related to fundamental forces (intra and inter-protein interactions e.g. electrostatic, hydrophobic, H-bonds) stabilising the microscopic structure. Near maturated particulates are disassembled unlike far maturated ones. Far maturation stages result not to be toxic to cells possibly due to their unreactive surface and high stability. Moreover, it is evident that the size of particulates can be readily controlled by initial protein concentration.

A global view of experimental data clearly proves the possibility of growing spherical micro-sized aggregates with different surface properties, stability and size using the same protein without any chemical modification but simply by varying the incubation time and the concentration of protein prior incubation.

P-026

The influence of Hofmeister series ions on the process of self-assembly of the recombinant spider silk protein eADF4(C16)

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Spider silk represents a perspective material with high mechanical strength and, at the same time, is biocompatible. One of the best understood spider silk is dragline silk. Spider silk has a unique balance of strength and elacticity. Protein eADF4(C16) presents an engineered spider silk variant based on the sequence of the core domain of the natural dragline silk protein ADF4 of *Araneus diadematus*. Previously, eADF4(C16) has been shown to self-assemble into cross-β-fibrils, but also sub-micrometer particles. [1] The main aim of this work was to analyse the *in vitro* assembly kinetics of eADF4(C16) fibrils. Measurements of the turbidity and the ThT fluorescence were also used for studying of ions influence on the formation of the fibrils. Obtained results show that kinetics of eADF4(C16) fibrillization clearly depend on position of anions in the Hofmeister series as well as on the salt concentrations. We also discuss the ions relevance to the assembly of spider silk proteins *in vivo* and compare it with the assembly formation *in vitro*.

[1] Humenik, M.; Smith, A. M.; Arndt, S.; Scheibel, T. *Journal of Structural Biology,* **2015**, *191*, 130-138.

P-027

Anisotropic Protein-Protein Interactions

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Non-covalent forces between molecules in solution mediate protein-protein interactions and it is increasingly clear, that anisotropy in the interaction potential between proteins is important in driving their assembly. Anisotropy in protein-protein interactions are due to the chemical heterogeneity of the surface of the protein arising from the different amino acid side chains. This can have some unexpected and dramatic effects on protein behaviour, where small modifications on their surface can change solution behaviour significantly [1, 2]. Protein phase diagrams can be used to quantify this phenomenon since some phase transitions (e.g. fluid-crystal equilibrium and liquid-liquid phase separation) are sensitive to even to single amino-acid modifications.

Here we will present further work, using Human γD-Crystallin (HGD) as a model indicating how small modifications introduced to the protein surface by mutagenesis can impact on protein behaviour. We use a combination of protein phase diagrams and static and dynamic light scattering measurements to investigate these effects.

[1] James, S., Quinn, M.K., McManus, J.J., *Phys.Chem.Chem.Phys*. (2015), **17**, 5413-5420.
[2] Quinn, M.K., Gnan, N., James, S., Ninarello, A., Sciortino F., Zaccarelli, E., McManus, J.J., *Phys.Chem.Chem.Phys*. (2015), **17**, 31177-31187.

P-028

Effect of natural and synthetic small molecules on aggregation of globular proteins

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Amyloid diseases are characterized by the formation and deposition of amyloid aggregates inside or outside the cell. Amyloid-associated human diseases include Alzheimer's disease, Parkinson's disease, prion diseases and type II diabetes. Currently, these diseases are incurable; thus, comprehensive search for novel inhibitors is required to develop treatment strategies. Several types of potential inhibitors among them nanoparticles, short peptides, antibodies and small organic molecules have been studied so far but in spite of intense research, the mechanism of inhibition is poorly understood. However, several drug molecules are proven to be effective against amyloid fibrillization process and have potential to either prevent the aggregate formation or to some extent reverse the process of protein aggregation.

Herein we opted to test several small compounds with different structure, either extracted from herbs or obtained from multi-step synthesis, for anti-amyloid properties towards globular proteins. Hen egg white (HEW) lysozyme and human insulin were picked as model systems. The ability of extracts from traditional Chinese herbs (DB series) and synthetized tacrine – coumarin derivatives (SH series) to inhibit amyloid fibrils formation of globular proteins were studied using Thioflavin T fluorescence assay, atomic force microscopy and docking methods. The inhibitory activities were quantified through IC_{50} values.

The obtained data suggest that inhibitory effect of compounds on lysozyme or insulin fibrillization depends on their composition, not origin as each of three studied series contained compound with weak, mild or strong inhibitory activity. Based on the results we can suggest that important factor is the planarity of the molecule. Extracts from herbs, DB series, were non planar and possess only weak inhibitory activity with IC_{50} values 100-times higher than concentration of protein. On the other hand, the most potent tacrine-coumarin derivatives influenced the protein aggregation at the stoichiometric concentration (IC_{50} ~ 19-100 μM). Functional groups and types and length of linker also play a key role in inhibiting activity of compounds.

Our results provide useful information about structure and composition for design of novel and potential lead compounds for a treatment of amyloid-related diseases.

P-029

Molecular dynamics simulations of amyloid-β fragments at hydrophilic/hydrophobic interface

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Amyloids are insoluble and misfolded fibrous protein aggregates and associated with more than 40 serious human diseases. For example, amyloid-β (Aβ) fibrils are known to be associated with the Alzheimer's disease. We have performed molecular (MD) dynamics simulations to study dynamical ordering of amyloid fibril as follows: (1) Hamiltonian replica-permutation simulations of aggregation of a few Aβ fragments, Aβ(29-42) peptides [1, 2]. (2) Conformational difference of an Aβ peptide at hydrophobic/hydrophilic interface and in bulk water [3]. (3) Conformational difference of the Aβ amyloid fibril at the odd end and even end [4]. (4) Disruption of the Aβ amyloid fibril by supersonic wave [5]. (5) Rapid QM/MM approach to reveal metal effect at the initial stage of the Aβ peptide aggregation [6]. In this presentation, we will show our recent MD simulation results of aggregation of Aβ(16-22) peptides. We performed NVT MD simulations of 100 Aβ(16-22) peptides in explicit water solvents. We observed intermolecular β-sheet structures in aggregated conformations of the Aβ(16-22) peptides. We will also discuss the effects of the water solvent.

References

1. S. G. Itoh and H. Okumura, *J. Phys. Chem. B* **120**, 6555-6561 (2016).
2. S. G. Itoh and H. Okumura, *J. Phys. Chem. B* **118**, 11428-11436 (2014).
3. S. G. Itoh, M. Yagi-Utsumi, K. Kato, and H. Okumura, *J. Phys. Chem. B* **123**, 160-169 (2019).
4. H. Okumura and S. G. Itoh, *Sci. Rep.* **6**, 38422 (9 pages) (2016).
5. H. Okumura and S. G. Itoh, *J. Am. Chem. Soc.* **136**, 10549-10552 (2014).
6. H. Nishizawa and H. Okumura, *J. Comput. Chem.* **37**, 2701-2711 (2016).

P-030

Studying virus evolution mechanisms using resurrected ancestral proviral proteins.

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We have provided experimental evidence that a back-to-the-ancestor replacement on a proviral factor may bypass virus spreading by avoiding recruitment of the modern protein. In particular, we have shown that resurrected ancestral thioredoxins confer resistance for bacteriophage T7 propagation in *Escherichia coli* (Delgado et al., 2017, Cell Reports 19, 1247–1256).

Unpublished results based on virus evolution experiments show that a small population of the virus can solve the challenge posed by the ancestral thioredoxins and, furthermore, several rounds of infection leads to a promiscuous, generalist virus able to recruit both the modern and the ancestral thioredoxins with similar efficiencies. We are currently in the process of identifying the particular molecular mechanism underlying these results.

P-031

Modular protein-gold nanocluster hybrids as models for protein structure control.

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Modular protein-gold nanocluster hybrids as models for protein structure control.

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The control of the structure of proteins is a promising feature to develop smart protein-based materials. Protein-based engineered materials combine the large diversity of protein structures and the functionalities that can be added when conjugating proteins with functional nanoelements. The spatial organization of these functional-protein hybrids can be controlled using a bottom-up approach and self-assembly principles. In particular, we use consensus tetratricopeptide repeats (CTPR proteins) since their modularity allows facile design and combination.

In this work we present the design of photoswitchable systems in which the protein structure and the functionality of nanoelements are coupled, meaning that a change in the structure induces a change in the functionality. The main aim of this design is to control the functionality of the conjugated nanoelements *via* structural changes using light as switch. We apply a stepwise strategy: first to control the folding dynamics of the protein using molecular photoswitches (structural part), and second to synthetize fluorescent protein-gold nanoclusters (AuNCs) hybrids (functional part). The use of photoisomerizable molecules allows controlling the secondary structure of the protein and consequently changing the local environment of the gold nanocluster and therefore their photoluminescence properties, which can be used as reporter of the protein structural changes. This technology will be the basis for the development of novel sensors.

P-032

Mapping the Diversity of Protein Aggregates

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Deposits of protein aggregates, often appearing as fibrils, are associated with the onset of pathologies as Alzheimer´s and Parkinson´s diseases. Equally important is the impact that protein aggregates may have on the quality of a protein drug product. Finally yet importantly, protein aggregates have unique structural, physico-chemical and mechanical properties, making them appealing bio-inspired materials for applications. Either one looks at protein aggregation in the context of diseases, drug development or biomaterials, understanding how protein-protein (PPIs) and protein-solvent interactions (PSIs) determine self-assembly kinetics and morphology of the aggregates is a *conditio sine qua non* to unravel the mechanisms ruling the self-assembly reaction. In our group, we have reported the possibility for a large group of proteins to form a variety of protein aggregates, not limited to amyloid fibrils [1]. I will present our approach based on advanced fluorescence microscopy, small angle X-ray scattering and spectroscopy and aimed at identifying the key PPIs and PSIs responsible for such variability in structures and morphologies [2-6]. We use surfactants, salts, alcohols in bulk and microfluidic setups to finely tune the interactions between proteins and control the self-assembly process. Our results show that subtle changes in the PPIs and PSIs do not only affect the kinetics, but they may also have a dramatic effect on the microscopic structures, mechanical properties and stability of the final species. Our findings provide a scenario in which a pool of heterogeneous structures can be generated as a result of interconnected aggregation pathways. This aspect is of key relevance for a deeper understanding of the generalized protein energy landscape as well as for protein drug development and the design of protein materials.

References

[1] Vetri V. and Foderà V. **2015 FEBS Letters,** 589, 2448–2463
[2] Vetri, V. et al. **2018 J Phys Chem B**, 122, 3101-3112.
[3] Skamris T. et al. **2019 Sci. Rep.**, 9, 1733
[4] Foderà V. et al. **2014**, **J Phys Chem Lett**, 5, 3254–3258.
[5] Foderà V. et al. **2013 Phys Rev Lett**, 111, 108105.
[6] Foderà V. et al. **2012 J Phys Chem Lett,** 3, 2803-2807.

P-033

Membrane domains modulate Aβ$_{1-42}$ oligomer interactions with Supported Lipid Bilayers: an Atomic Force Microscopy investigation

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Alzheimer's disease is a devastating pathology with an increasing number of affected individuals following the rise of the life expectancy. Amyloid peptide Abeta$_{1-42}$ has been identified as one the main culprits of the disease. The peptide has been shown to create major effects on lipid membranes, including membrane solubilization. Even though many discrepancies remain, the membrane composition has been identified as a factor that plays a pivotal role in regulating peptide/membrane interactions. Cholesterol and ganglioside GM1, two lipids suspected to create lipid domains in membranes, are proposed to play a crucial role in the promotion of these interactions. Liquid-liquid phase separations, associated with lipid rafts, were also shown to favour peptide-induced membrane damages. We aimed at revealing the effects of lipid segregation on the membrane-perturbing ability of Abeta$_{1-42}$ and an oligomeric mutant (oG37C), a peptide that presents many common features with the suspected toxic intermediates involved in the neurodegeneration process. Atomic Force Microscopy (AFM) was used to characterize and compare these peptide effects on supported lipid bilayers with 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) as the main component. Abeta$_{1-42}$ interaction with pure POPC bilayers appeared to be poor, while oG37C accumulated on membranes, targeting disordered lipid areas. The presence of cholesterol or GM1 (10 mol%) in model membranes greatly favoured the association of both peptides to the supported lipid bilayers, leading to important membrane solubilization. In lipid systems showing liquid-liquid phase separations (1,2-dioleoyl-sn-glycero-3-phosphocholine/1,2-dipalmitoyl-sn-glycero-3-phosphocholine/cholesterol (DOPC/DPPC/cholesterol), and DOPC/Brain Sphingomyelin/cholesterol ternary mixtures), Abeta$_{1-42}$ and oG37C exclusively aggregated on liquid-disordered-phase domains, creating large deposits and even extracting lipids in the case of DOPC/BSM/cholesterol bilayers. The AFM results clearly establish that the presence of domains favour the interaction of Abeta$_{1-42}$ and oG37C with membranes.

P-034

Infrared Nanospectroscopy to probe the interaction of toxic Aβ1-42 variant oligomers with membrane

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Toxicity of Aβ peptides involved in Alzheimer's disease is linked to the interaction of intermediate species with membranes.[1] In the present study we investigated the interaction of Aβ1–42 (WT) and toxic oligomers; (oG37C) with the different model membranes using an innovative technique coined nanoscale infrared spectroscopy (Nano-IR). Nano-IR can not only produce high resolution images as classical atomic force microscopy but also gives access to the local IR absorption spectra through the vibrations of the sample that can be detected by the AFM tip. In our case, it makes it possible to access the secondary structure of the tested area at the nanoscale. The WT fiber adopts mostly a parallel β-sheet secondary structure (1631 cm−1) mixed with a β-turn structure (1662 cm−1). The oG37C is mostly structured in anti-parallel β-sheets characterized by the bands at 1625 cm−1 and 1689 cm−1. Membrane models are used to investigate the role of different lipids in their interactions with Aβ peptides. The model membranes are composed of 1-Palmitoyl-2-oleoylphosphatidylcholine (PC), sphingomyelin (SM) and cholesterol (Chol). Cholesterol is an essential component of cell membranes and plays important role on the development, proliferation, differentiation and maintenance of neuronal tissues and cells. This work clearly brings to light that the presence of cholesterol in membranes is favorable to the interaction with Aβ peptides in oligomers or aggregates. [2]

References

[1] S. Henry, H. Vignaud, C. Bobo, M. Decossas, O. Lambert, I.D. Alves, C. Cullin, S. Lecomte, Biomacromolécules, 2015 16, 944-950.
[2] S. Henry, B. Bercu, C. Bobo, M. Molinari, S. Lecomte, Nanoscale, 2018, 10, 936–940.

P-035

Peptide assemblies: some of the rules of this complex puzzle

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In nature, evolution as fine-tuned effective and often amazingly efficient strategies for building functional assemblies such as biological membranes, virus capsids, or cytoskeletons. The intra- and intermolecular interactions that tie together these macromolecular and supramolecular architectures are almost all noncovalent. Only the combination of those multiple weak interactions allows stability of the architectures, but also their responsiveness and dynamics since they may fluctuate, rearrange, reassemble when exposed to the proper stimuli. Both stability and dynamics of such architectures is most often highly cooperative, rendering quantification of individual contributions particularly challenging since they come into play simultaneously. In this context, in the group we study the self-assembly structures and mechanism of natural systems such as virus capsid but also synthetic ones such as oligopeptide. The seminar will focus on the mechanisms of peptide self-assembly and of the structures of these assemblies. The studies of the self-assembly properties of small peptides (8 to 14 amino-acids) allow precise understanding and characterization of the physical and physico-chemical rules guiding the self-assembly of these molecules.

P-036

Disruption of monomeric alpha-synuclein long-range interactions alters its misfolding propensity

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Disruption of monomeric alpha-synuclein long-range interactions alters its misfolding propensity

The aggregation of alpha-synuclein (aSyn) occurs as part of the pathology of Parkinson's disease (PD). However, several questions remain unanswered regarding the physiological and pathophysiological role of aSyn, and the molecular mechanism of the disease is far from understood. As aSyn visits different conformations in space, some of the structural conformers are expected to be more toxic and thus implicated in the disease mechanism. By studying the system in vitro, we aim to detect the most toxic species and the environmental conditions that induce their formation, to aid in designing a treatment for the disease. We focus on the effect of the familial point mutations A30P, A53T, E46K, A53E, H50Q, and G51D on aSyn aggregation, as well as the association between aSyn and calcium, a divalent ion of great biological importance. The aggregation kinetics of WT aSyn and the familial mutants upon calcium addition were studied with Thioflavin –T (ThT) assays. As previously shown, the aggregation kinetics of WT aSyn were enhanced in high calcium concentrations. However, the familial mutants displayed very varied aggregation kinetics upon the calcium stimulus. The difference in response to calcium addition between WT aSyn and the mutants is of great potential interest for the disease mechanism. For that reason, the study of the sub-molecular dynamics of the protein variants by Hydrogen-Deuterium Exchange Mass Spectrometry (HDX-MS) at the first steps of the aggregation process was employed to help elucidate further the molecular mechanism of aggregation. We attribute this difference in aggregation upon the calcium stimulus to be due to subtle differences in monomer conformation at the start of aggregation, as shown by HDX-MS. Moreover, by employing Atomic Force Microscopy (AFM), we show that the fibrils formed from different mutants and under different conditions display different fibril morphology.

Sunday 21st July

DNA ARCHITECTURE AND GENE REGULATION

P-037 (O-018)

Non-specific action of polyamines leads to specific changes in structure and function of DNA

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Polyamines are found in all living organisms, where they are involved in many cellular processes including cell growth and proliferation. The most commonly occurring natural polyamines are putrescine, spermidine and spermine, and they exhibit linear-chain skeleton. In addition to these common polyamines, hyperthermophiles require long and branched-chain polyamines for growth at high temperatures. Therefore, structural differences among polyamines are expected to play an important role in the mechanism of self-control of living state.

Here, we report the effect of various polyamines including linear- and branched-chain isomers on the higher-order structure of DNA based on the results of our experiments. From atomic force microscopic (AFM) measurements, it was found that the branched polyamine induces a mesh-like structure on DNA at room temperature. Interestingly, with increasing temperature up to 80°C, DNA molecules tend to unwind, and form multiple nano-loops with a diameter of 10–50 nm along the DNA strand. On the other hand, linear polyamines tend to form flower-like structure, suggesting the parallel alignment of DNA and does not cause nano-loop structure at higher temperatures.

We have also studied the effect of various polyamines on the activity of gene expression through *in vitro* luciferase assay. It was revealed that gene expression activity is completely suppressed accompanying the folding transition of DNA into a compact state. It was also found that the activity of gene expression is enhanced several times at the polyamine concentrations just below the critical concentration to induced the compaction of DNA.

Based on these experimental observations, we may argue the biological role of polyamines in relation to the structural change of genomic DNA molecules in living cellular environment.

Refs.

1) T. Nishio, et al., Branched-Chain Polyamine Found in Hyperthermophiles Induces Unique Temperature-Dependent Structural Changes in Genome-Size DNA. *ChemPhysChem*, 19, 2299(2018).
2) Y. Kashiwagi, T. Nishio, et al., Repulsive/attractive interaction among compact DNA molecules as judged through laser trapping: Difference between linear- and branched-chain polyamines. *Colloid Polym Sci.* https://doi.org/10.1007/s00396-018-4435-3 1-11 (2018)

P-038 (O-019)

Simulating the binding of Pioneer Transcription Factors to the nucleosome

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Transcription factors are proteins that bind to DNA to regulate gene expression. In most cases, accessibility to DNA is a prerequisite for their function. However, in the nucleus the DNA is packed into chromatin, which is often inaccessible. The fundamental unit of chromatin is the nucleosome, in which 147 DNA basepairs are wrapped around a core of eight histone proteins.

Interestingly, a series of transcription factors, known as pioneers, are able to bind to closed chromatin states, recognizing their binding sites even in the presence of nucleosomes. They can help open chromatin, increase DNA accessibility, and support binding of other transcription factors. For example, Oct4, a master regulator of stem cell pluripotency, is able to bind native nucleosomes in a sequence specific manner.

To understand the nucleosome properties that are involved in the binding of Oct4, we performed all-atom simulations of three nucleosomes with different DNA sequences, in presence and absence of Oct4. By comparing three sequences with characteristic Oct4 binding profiles, we identified differences in dynamics and structural properties of the three nucleosomes, most of which are located in the regions known to be important for nucleosome unwrapping. We also characterized how the addition of Oct4 alters the dynamics of the nucleosomes, and which are the relevant nucleosome properties that explain the binding and behavior change. These findings help us understand the role of pioneer transcription factors in the binding ofclosed chromatin.

P-039 (O-020)

Dependence of DNA Persistence Length on Ionic Strength and Ion Type

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The rigidity of double-stranded DNA plays a major role in the structuring of the chromosome and thus in the expression of genes, as well as in nanotechnology where DNA is used as a building block. But how is this rigidity influenced by the presence of different types of ions? In this work, we responded both experimentally and theoretically to this question. Thanks to the massive parallelization of the single-molecule technique of Tethered Particle Motion (TPM), we measured the dependence of persistence length, reflecting the stiffness of the polymer DNA, over a wide range of ions and salt concentrations. We demonstrated a unique decay for monovalent or divalent metal ions perfectly described by recent theories, which take into account the non-linear electrostatic effects as well as the finite diameter of the DNA. Our findings, published in *Phys. Rev. Lett.* **122** 028102 (2019), make it possible to predict conformational changes of complex structures formed by DNA both *in vitro* and *in vivo*.

P-040

Dynamic response of the bacterial chromosome locus induced by the gene expression of membrane proteins

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Mediated by the transcription and translation machinery, the DNA segments of expressed genes can be spatially reorganized. Especially in bacterial cells, these interactions have been suggested to majorly determine the chromosome organization and segregation. Transertion, as a hypothetic DNA-protein interaction, which involves the simultaneous expression and insertion of membrane proteins, has been proposed to provide the tethers between the chromosome and cell membrane and drive the segregation of the replicated chromosome pairs. In this study, to verify the hypothesis of transertion, the motion behavior of the local chromosomal segments around the gene of the target membrane protein, i.e., lactose permease, has been observed at single gene locus level. Two-color self-regulated fluorescent TetR-*tetO* and ParB-*parC2* systems were applied to visualize the spatial distributions and the trajectories of the locus pair around the lactose operon. From the static and dynamic analyses on the motions, including the mechanical response and the reorganization of the local chromosomal segments have been quantitatively indicated. Also, the differences between the wild-type operon and the mutant (Δ*lacY*::*cfp*) also provide the evidence to differentiate the effects from the expressions of the cytoplasmic and the membrane protein genes. The results support the hypothesis of transertion phenomenon in the expressions of membrane proteins.

P-041

Molecular dynamics insights into KstR transcriptional regulator function

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The TetR family of transcriptional regulators is widespread in bacteria. They regulate biological processes such as multidrug resistance, biofilm formation, biosynthesis of antibiotics, catabolic pathways, nitrogen fixation, and stress responses amongst others, and as such are often important for pathogenicity. KstR and KstR2 are two TetR family repressors that regulate cholesterol metabolism in *Mycobacterium tuberculosis* and other actinomycetes. KstR is essential for pathogenesis in *M. tuberculosis* and is a candidate for drug development.

Molecular dynamics simulations have provided insight into which regions of KstR change conformation, and the extent of conformational deviation that can occur between different states of KstR. This demonstrated distinct conformational paths between apo KstR, DNA-bound KstR, and ligand-bound KstR. Here, we use molecular dynamics simulations and dynamical network analysis to identify the communication pathways that propagate the allosteric changes that occur in KstR upon ligand and DNA binding. This mechanistic insight will be useful for the design of drugs to selectively inhibit KstR.

P-042

Understanding the role of proto-oncogene Kras G-quadruplexes: Structural and ligand binding characterization.

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Kras is one of the most mutated proto-oncogene and codes for a 23.3 kDa (ras) protein associated in different steps that lead to propagation of cancer cells. Kras oncogene is involved in different types of cancer especially in PDAC pancreatic and lung cancers. PDAC is one of the main causes of death in western countries, due to its aggressive nature and resistance to conventional chemotherapy. More than 90 % of human PDACs harbor oncogenic KRAS mutations such as the constitutively active mutant KRAS (G12D) required in all stages of PDAC: initiation, progression and metastasis. In the promotor region of Kras gene, there is a G-rich quadruplex-forming sequence (G4) important for gene regulation, notably by a protein hnRNP A1. When the action of hnRNP A1 is abolished KRAS expression is severely diminished. As the inactivation of mutant KRAS results in the reversion of the carcinogenic process, targeting oncogenic KRAS is imperative for a successful strategy against PDCA. Our structural and binding results propose the exploitation of a newly discovered route not investigated before by directly targeting the unusual G4 structures of DNA in the promotor region of the gene that encodes for KRAS (G12D).

P-043

Investigation of KRAS promoter region G-quadruplexes and interaction studies with hnRNP A1 protein

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KRAS gene codes for a highly mutated GTPase protein acting as a « switch » between an active and an inactive state, a mechanism found to be important in processes such as cell replication and proliferation. When dysregulated those processes are in the origin of cancer. KRAS mutations are particularly implicated in lungs (30%), colorectal (44%) and pancreatic (97%) cancers. Despite the fact that those mutations are well known, KRAS is still an undruggable target. That is why new strategies emerged some years ago by directly targeting the KRAS promoter region and especially some specific structures called G-quadruplexes (G4). It was shown that G4 within KRAS promoter region can bind transcription related proteins and disturb transcription process acting as a block mechanism when transcription machinery is reading the genetic sequence. Stabilization or destruction of these structures, using small chemical ligands for example, could become a new area of therapy. This project is focused on a 32 residues sequence (KRAS32R) which can form G4 and also corresponds to the minimal interaction domain of transcription proteins such as MAZ or hnRNP1. This last protein is capable of binding to KRAS32R G-quadruplexes and unfolding favouring the transcription of KRAS. Due to G4 polymorphism we found that the sequence can adopt two different conformations in equilibrium. We studied the interaction between KRAS32R conformations and UP1 a protein derived from hnRNP A1 containing two RRM (RNA Recognition Motifs) domains. We also probed small chemical ligands from different families in order to prevent UP1 binding to KRAS32R conformations.

With several biophysical methods we found some ligand which can interact with KRAS32R conformers and act as competitors in UP1 binding. By NMR methods we want to determine specific interaction between G4 and ligands aiming to optimized the best ligands to work as pro-drugs in cancer therapy.

P-044

Interplay between nucleophosmin, the DNA repair protein APE1 and DNA: regulation of base excision repair?

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Nucleophosmin (NPM1) is an abundant nucleolar protein in charge of several functions related to cell growth and homeostasis, such as ribosome assembly, response to stress and DNA damage repair. NPM1 is a multidomain oligomer, where a pentameric core is connected to small, C-terminal globular domains through long, flexible linkers. To perform its multiple activities, NPM1 is able to continuously shuttle between cytoplasm, nucleoplasm and nucleolus. Upon occurrence of DNA double strand breaks, a phosphorylated form of NPM1 has been shown to be recruited to lesion foci. Similarly, we have observed that oxidative damage elicits NPM1 phosphorylation, nucleolar release and enrichment on particular chromatin regions. Moreover, NPM1 has been reported to interact with apurynic apyrimidinic endonuclease 1 (APE1), a key enzyme in the Base Excision Repair (BER) pathway, suggesting that NPM1 might locate, together with APE1, in repair platforms on the chromatin and participate in that route. Herein, we have focused on the interactions between NPM1, APE1, and abasic DNA, to understand how they could be related to BER regulation. We have shown, based on native electrophoresis and isothermal titration calorimetry (ITC), the formation of stable complexes between the two proteins, and dissected the involvement of NPM1 domains in the interaction. Our data indicate that NPM1 C-terminal domain is dispensable for APE1 recognition; however, the isolated core domain is not enough for stable binding. On the other hand, based on circular dichroism thermal scans, we have observed that DNA binding conformationally stabilizes APE1. Fluorescence anisotropy binding assays reveal that NPM1 is able to compete with a secondary, lower affinity, APE1 binding site on DNA. Based on our results, we propose that NPM1 might cooperate in the BER process favouring the specific binding of APE1 to abasic DNA, and its discharge from the incised, product DNA, which could explain NPM1 stimulation of APE1 incision activity. NPM1 regulation of APE1 repair activity represents a novel therapeutic target that could be exploited for chemosensibilization / radiosensibilization.

P-045

Novel conformers of the G·C DNA base pair and their mutual interconversions *via* the proton transfer: A quantum-mechanical study

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Since the discovery of the spatial structure of the DNA by Watson and Crick, researchers were limited only by the canonical A·T and G·C Watson-Crick DNA pairs with canonical geometry, involving nucleotide bases in the main tautomeric form. However, consideration of this issue should be more comprehensive in order to take into account different biologically-important conformers of these pairs.

In this study for the first time we have revealed novel conformers of the G*·C* DNA base pairs (asterisks denote rare tautomers), in particular, reverse Löwdin G*·C*(rWC), Hoogsteen (H) G*′·C*(H) and reverse Hoogsteen G*′·C*(rH) base pairs.

We found out that they are formerd through the tautomerization processes via the proton transfer (PT) between the bases along the intermolecular H-bonds:

- G*·C*(rWC)↔G+·C-(rWC) (Gibbs free energy of activation 4.38 kcal·mol-1)↔G·C*O2(rWC) (3.64 kcal·mol-1) and G*·C*(rWC)↔G+·C-(rWC)↔G*N2·C(rWC) (9.27 kcal·mol-1) reactions occur via the two-stage sequential PT via the dynamically-unstable zwitterion-like G+·C-(rWC) intermediate, and transition states (TSs), one of which is joint;
- G*·C*(rWC)↔G*′N2·C(rwWC) (27.43 kcal·mol-1), G*′·C*(H)↔G*N7·C(H) (4.01 kcal·mol-1) and G*′·C*(rH)↔G*′N7·C(rH) (25.30 kcal·mol-1) reactions occur through the one-stage double PT.

Also, we have analysed in details the evolution of the main physico-chemical parameters along the intrinsic reaction coordinate by applying the unique author's methodology.

All these data are useful for the better understanding of the conformational and tautomeric variability of the classical G*·C* DNA base pairs.

P-046

Exploring the conformational ensemble of DNA Helix-Junction-Helix construct by MD simulations and FRET

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Many complex nucleic acid structures contain rigid base-paired regions connected by flexible linkers. Helix-Junction-Helix (HJH) DNA constructs are ideal systems to study the tertiary folding of these structures. Experimental studies show that the conformational ensembles sampled by these constructs show differences depending on the counter ion valence and its concentration. However, atomic detail necessary to understand these differences, as well as nucleic acid folding, in general, is lacking. Here, we combine fluorescence resonance energy transfer (FRET) measurements and all-atom molecular dynamics simulations to study the interplay between DNA conformations and ionic environment. The conformational ensemble of the DNA was explored by both conventional molecular dynamics and metadynamics simulations. HJH structure is dominated by two conformational sub-states defined as open and closed states. Metadynamics simulations allowed computing the free energy surface and populations of these states, providing a direct comparison between experiments and simulations. Molecular simulations showed good agreement with FRET measurements in the presence of both monovalent and divalent ions. Together, the integrated approach offers unprecedented detail into the energetics and atomic level mechanism of the conformational preferences of HJH constructs that help to understand the physical principles governing nucleic acids interactions and folding.

P-047

dsDNA Packed inside Phage Capsids: Structure and Defects Emergence

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DNA packaging and ejection are two critical moments in dsDNA bacteriophages lifecycle and their understanding is decisive for the effective application of phages as an alternative to antibiotics. The forces needed to pack the DNA molecule to near crystalline density (~0.5g/ml) combined with the geometrical constrains of the phage capsid determine the conformation of the confined DNA. Most theoretical studies that have been performed to better understand how is the conformation of the DNA inside bacteriophage capsids considered DNA as a perfect elastic rod and predict highly ordered structures. However, the emergence of more disordered conformations exhibiting defects such as knots, kinks, loops, … that hinder both the insertion and extraction of the DNA molecule in the phage capsid is also plausible. This raises the central question of the present work - how much order and disorder is reasonable (or required) when DNA is confined inside the phage capsid?

We have performed Molecular Dynamics simulations using oxDNA model for dsDNA and a purely repulsive harmonic wall representing the proteic capsid of the bacteriophage to mimic the packing process in phage ϕ29. We have thoroughly analyzed the DNA conformation by means of density profiles and correlation functions during packing finding different results depending on how fast the DNA is being inserted. DNA structure predicted by these simulations show patterns that agree with experiments, cryoEM and X-ray diffraction, but many features in a more realistic capsid model – presence of multivalent ions, torsional forces, and local attractive/repulsive sites in the capsid or an elongated shape - might contribute to the emergence of these or other characteristics.

P-048

Sequence-dependent regulation of the mechanical properties of double-stranded DNA and RNA at short length scales

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Sequence-dependent DNA conformation and flexibility play a fundamental role in specificity of DNA-protein interactions. Here we quantify the DNA crookedness: a sequence-dependent deformation of DNA that consists on periodic bends of the base pair centers chain. Using extensive 100 microsecond-long all-atom constant-force molecular dynamics (MD) simulations [1], we found that DNA crookedness and its associated flexibility are bijective: unveiling a one-to-one relation between DNA structure and dynamics [2]. This allowed us to build a predictive model to compute DNA stretch modulus from solely its structure. Sequences with very little crookedness show extremely high stiffness and have been previously shown to form unstable nucleosomes and promote gene expression. Interestingly, the crookedness can be tailored by epigenetic modifications, known to affect gene expression. Our results rationalize the idea that the DNA sequence is not only a chemical code, but also a physical one that allows to finely regulate its mechanical properties and, possibly, its 3D arrangement inside the cell.

Mechanical properties also play a key role in many biological functions of double-stranded (dsRNA) --like the interaction with proteins that regulate gene silencing--, but how sequence affects the global mechanical response has so-far remained unexplored. Using the same MD protocol, we find that the nucleotide sequence affects in a strikingly different manner the overall stretching and twisting flexibility of RNA and DNA duplexes [3]. For instance, poly-CG sequences soften the stretching response in dsRNA but in contrast they make the dsDNA duplex stiffer. At the dinucleotide level such disparities between dsRNA and dsDNA disappear and both molecules show a similar sequence-dependent flexibility. Our extensive simulations unveil how similar local base-pair motions can lead to divergent sequence effects in the global mechanical properties of DNA and RNA duplexes.

[1] A. Marín-González et al, PNAS) 114, 7049 (2017).
[2] A. Marín-González et al, Phys. Rev. Lett. 122, 048102 (2019).
[3] A. Marín-González et al, submitted (2019).

P-049

Non canonical interactions in artificial nucleic acids

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Advances in synthetic chemistry afford new artificial nucleic acids with intriguing properties. In this poster, we present our structural studies on chemically modified nucleic acids carried out by NMR spectroscopy. In particular, nucleic acids analogs containing 2'-fluoro-arabino (2'F-ANA) and 2'-fluoro-ribose (2'F-RNA) are interesting compounds for their potential applications in antisense and interference RNA therapy. In addition, locked nucleic acid (LNA) has become attractive modification in which the ribose moiety is modified with an extra bridge connecting the 2' oxygen and 4' carbon. The preferential conformations of these three analogs are diferent. Whereas 2'F-ANA is considered to be a DNA analog, 2'F-RNA and LNA exhibit a strong tendency to adopt a 3'-endo (North) conformation and, consequently, are considered RNA analogs. By changing the pattern of incorporation of these analogs in a particular oligonucleotide sequence, structure and stability, as well as binding affinity for RNA targets may be tuned. We discuss here the three-dimensional structure of several chimeric hybrid duplexes, whose sequences combine different patterns of LNA, 2'F-ANA and 2'F-RNA nucleotides, as determined by combining ^{1}H and ^{19}F NMR spectroscopy.

We will also present results on 8-amino-substituted guanine quadruplexes. Base-modifications in G-tetrads are usually not well-tolerated and have a destabilization effect in G-quadruplexes. One of the few exceptions is the substitution by 8-amino-guanines. 8-amino-Gs (8g) stabilize triplexes and parallel-hairpins. However, its effect on the stability on G-quadruplexes is not clear, and depends on the quadruplex topology and the sequence context. In the case of tetramolecular parallel quadruplexes, such as that formed by d(TGGGGT), we have found that substitution of the first guanine by a 8-amino-guanine provokes the formation of an unsual interlocked quadruplex dimer, stabilized G:T:G:T and 8g:T:8g:T tetrads. Dimerization of the two quadruplexes occurs in an antiparallel orientation through their 5'-side.

P-050

Sequence encoded DNA – DNA Interactions

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Sequence encoded DNA – DNA Interactions

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Ion mediated interactions between nucleic acids helices are essential for their efficient packaging within tight spaces such as viral capsids, sperm heads, nucleosomes and cell nuclei. However, the underlying physical principles governing these interactions at the molecular level are still debatable. In this study, we investigate the role of Mg^{+2} ions in DNA-DNA interaction within ordered dsDNA arrays, via an integrated approach of osmotic pressure measurements coupled with molecular dynamics simulations. Umbrella sampling simulations allowed computing the potential of mean force (PMF) between dsDNAs as a function of inter-DNA spacing and provided a direct comparison to osmotic pressure measurements. We studied poly(AT) and poly(A)-poly(T) sequences in varying cation concentrations. Our analysis revealed strong correlation between the structure of the counterion atmosphere and inter-DNA interactions. The interaction energy is found to be mediated by Mg^{+2} ions that show differences in localization in sequences studied. We further partition the interaction energy into thermodynamic potentials. Results suggest sequence specificity for solvent entropy which can have important implications in governing DNA assembly.

P-051

Conformational transitions in non canonical DNA structures.

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I-motif is a non-canonical DNA structure formed by intercalated C:C+ base pairs. It is believed that i-motifs are formed in regulatory regions of the human genome, playing a role in the regulation of gene expression. Since formation of C:C+ base pairs requires partial cytosine protonation, these kinds of structures are usually only stable at acidic pH. However, we have observed that i-motif can be formed at physiological pH provided the stack of C:C+ base pairs are flanked by two minor groove tetrads. This kind of tetrads results from the association of two Watson-Crick or G:T base pairs through their minor groove side, forming G:T:G:T, G:C:G:T or G:C:G:C tetrads.

We have explored the stability of different i-motifs with repetitive sequences with a very low content in cytosines, all of them stabilized by minor groove tetrads. We have obtained their structures by NMR methods. Among them, the one with tetrads G:C:G:C affords the most significant stabilization versus pH, resulting in effective pH_T values above 8.0. This peculiar structure requires the concurrent presence of neutral and protonated cytosines.

At pHs below 6.1 the cytosines involved in the G:C:G:C tetrads become protonated and are not able to form Watson-Crick pairs. However, the structure does not unfold but a drastic conformational change occurs, leading to an i-motif structure with four $C:C^{+}$ base pairs. Some the thymines that were in the loops at neutral conditions form G:T base pairs at acidic pH, giving rise to two G:T:G:T minor groove tetrads. The neutral and the acidic structures as well as the transition between them are discussed in the presentation.

P-052

Gemini/DOPE/PEGylated-based Lipoplexes for the efficient expression of *OPA1* mitochondrial protein in *in vitro* and *in vivo* experiments

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Recent advances on the mitochondrial morphology indicate that mitochondria are able to form a highly dynamic network that constantly fuse and divide. Fusion and fission balances are very important for normal cellular function. In mammalian cells, there are three main proteins involved in the mitochondrial fusion: Mfn1 and Mfn2 (OMM) and OPA1 (IMM). Deletion of any of them in mouse embryonic fibroblasts (MEFs) and CD-1 mouse models produces mitochondrial fragmentation, thus leading to mitochondrial diseases (MD), to which there is no cure at present. In this work, lipoplexes have been conceived as efficient therapeutic agents against MD. Lipoplexes, lipid/DNA highly packed complex, are composed by Imidazol Gemini/DOPE/PEGylated mixed cationic liposomes with a plasmid DNA coding on Opa1 mitochondrial protein. These synthetic and biodegradable lipoplexes are able to transport and efficiently delivery plasmid DNA into the cytoplasm. Then, the complementation of the mitochondrial phenotype in OPA1-KO MEFs produced by the deficiency on Opa1 protein was produced. These lipoplexes show a good viability and high transfection efficiency because the coexistence of two different hexagonal structures in the same formulation that produces a synergy in *in vitro* experiments. Besides, these lipoplexes show a great bioaccumulation and transfection efficiency in different organs of CD-1 mouse model without any toxicity over time after intraperitoneal (IP), Intracardiac (IC) and intramuscular (IM) administration, as was confirmed by different techniques.

Sunday 21st July

BIOPHYSICS OF CYTOSKELETON

P-053 (O-024)

Structure dissection of a contractile phage tail tube

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Bacteriophages are infectious nano-objects that inject their genetic material into the host via complex molecular machineries. To cross the biological barriers encompassing the extracellular space and the bacterial membrane, phages expose a tail, composed of several proteins, which executes numerous functions during infection. The particularity of the *Myoviridae* bacteriophages resides in the additional sheath, surrounding the inner tail tube. This contractile sheath pushes the inner tube across the host membrane acting therefore like a molecular syringe transferring the viral genome into the host cell. This complex machinery increases the infection efficiency in this type of phages, containing a higher amount of DNA content to transfer than other phage families.

We here elucidate the structural basis and the molecular mechanisms of a *Myoviridae* phage inner tail tube. Among them, the tail tube of bacteriophage Mu stands out because the molecular subunit protein of the tube assembly (gpM) fulfils its biological function with the minimal structural motif. We investigate the structural features of the Mu tail tube using a combination of solid-state NMR, liquid-state NMR and cryo-EM performed on in vitro assembled filaments. We show that the subunit protein gpM is partially folded in its monomeric state and adopts a globular beta-rich structure in a very rapid polymerisation process towards the native assembly. Our results suggest that the minimal construction motif in gpM conserves the structural features shared also by the *Caudovirales* phages. The apparent resemblance of these structures supports the idea that the 3D fold of the tail tube protein is conserved despite the poor sequence conservation.

P-054 (O-025)

Ultrafast tracking reveals the function of structural domains of single proteins

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Protein-protein interactions and their dynamics have been extensively studied mostly by fluorescence. Fluorescent labels are usually incorporated to discriminate biological processes under study from the complex background or visualize small molecules.

To overcome spatio-temporal limitations of fluorescence [1], we employ interferometric detection of scattering (iSCAT). iSCAT detects the light scattered on a protein molecule or a scattering label via its interference with a reference wave, e.g. light partially reflected at a glass coverslip. By these means, it is possible to image very small scattering labels [2] or even unlabeled proteins [3].

PRC1 (protein regulator of cytokinesis 1) belongs to the Ase1/MAP65/PRC1 family of microtubule-associated proteins (MAPs) and plays an important role in cytokinesis. These proteins serve as rigid connections between MTs and can interact with other proteins. Each structural domain of the PRC1 protein seems to play its role but details remain unclear [4].

We studied two different domains via a specific attachment of scattering labels. 3D maps of PRC1 on a single microtubule were measured and analyzed. We observe that each domain has a different behavior and we propose a new functional model of the interaction.

References

[1] S. Weisenburger and V. Sandoghdar, "Light microscopy: an ongoing contemporary revolution," *Contemp. Phys.*, vol. 56, no. 2, pp. 123–143, 2015.

[2] K. Holanová, M. Vala, and M. Piliarik, "Optical imaging and localization of prospective scattering labels smaller than a single protein," *Opt. Laser Technol.*, vol. 109, pp. 323–327, 2019.

[3] M. Piliarik and V. Sandoghdar, "Direct optical sensing of single unlabelled proteins and super-resolution imaging of their binding sites," *Nat. Commun.*, vol. 5, p. 4495, 2014.

[4] R. Subramanian *et al.*, "Insights into antiparallel microtubule crosslinking by PRC1, a conserved nonmotor microtubule binding protein," *Cell*, vol. 142, no. 3, pp. 433–443, 2010.

P-055 (O-026)

Nucleotide and Osmolyte Induced Folding of FtsZ from *Staphylococcus aureus*

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The essential bacterial cell division protein FtsZ uses GTP binding and hydrolysis to assemble into filaments that treadmill around the Z-ring, guiding septal wall synthesis and cell division. FtsZ is a structural homolog of tubulin and a target for discovering new antibiotics. We have found that purified FtsZ monomers from the pathogen *S. aureus* (SaFtsZ) require bound nucleotide to keep a folded structure in solution. Nucleotide-less apo-SaFtsZ is essentially unfolded, as assessed by NMR and CD, and crystallizes in a non-native structure. Addition of GTP (≥ 1mM) dramatically shifts the equilibrium towards the folded protein, rendering SaFtsZ active for assembly. Other mesophile FtsZs are also stabilized by nucleotide binding. Apo-SaFtsZ also folds in CD with glycerol, and other stabilizing osmolytes such as TMAO, ethylene glycol, betaine and proline, which enable high-affinity GTP binding (K_d 20 nM in 3.4 M glycerol, determined by ITC) similar to thermophile stable FtsZ. We have devised a competition assay to detect any molecules that bind overlapping the nucleotide site of SaFtsZ or EcFtsZ, employing glycerol-stabilized apo-FtsZs and the specific fluorescence anisotropy change of *mant*-GTP upon dissociation from the protein. This robust assay provides a basis for robotic screening for high affinity GTP-replacing ligands, which combined with phenotypic profiling, may yield the next generation of FtsZ-targeting antibacterial inhibitors. Finally, by solving several SaFtsZ crystal structures, we identified a cavity behind the nucleotide-binding pocket that harbors distinct compounds, opening the way for designing extended inhibitors.

P-056

Unfolding of I91 protein under constant force follows continuous time random walk sub-diffusive dynamics

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Using large statistics, unfolding of ubiquitin under constant force displayed nonexponential dwell-time distribution, which was explained in terms of a form of glassy transition between the folded and unfolded states. This means that the unfolding process occurs over a distribution of activation barriers, and thus can be best described as a disordered process, and characterized with a stretched exponential (Weibull) distribution. Considering unfolding as a diffusion driven process over an activation barrier, we studied protein unfolding under constant force by attaining the time-averaged mean square displacements (TA-MSD) of the unfolding dwell time distributions within the framework of continuous time random walks (CTRW). To this end, we measured the unfolding dwell-time distributions of I91 protein using atomic force microscopy under a constant force of 180 pN, which similarly to ubiquitin exhibited nonexponential unfolding behavior. According to the CTRW approach, unfolding can be represented by a joint probability density function $P(x,t)$ that can describe the transition over several length or time scales. Using asymptotic algebraic (power-law) decay with a long time behavior of the form $\sim t^{-\alpha-1}$ proves to be more suitable to describe the observed dwell-time distribution. The TA-MSDs alluded to weak ergodicity breaking, and a long-tail power-law dependency of the unfolding dwell-time distributions with an exponent of $\alpha \sim 0.85$, with a cutoff set by experimental limitation.

P-057

Active organization and mechanical properties of membrane bound minimal actin cortices

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The cell cortex is a highly regulated network, which is attached to the plasma membrane. This network consists of filamentous actin (F-actin), which forms a thin actin sheet on the inner face of the cell membrane composed of single and bundled filaments. In this cortex, the F-actin is regulated by a vast array of different actin binding proteins (ABPs) like cross-linkers. The actin cortex is bound via linker-proteins like ezrin, radixin and moesin (ERM-family) to the membrane receptor-lipid phosphatidylinositol-4,5-bisphosphate (PtdIns(4,5)-P_2). The direct connection between the cortex and the membrane is necessary to convert forces among both structures. The motor protein myosin II is able to reorganize the actin cortex and hereby to apply mechanical forces via the actin-membrane connection to the cell membrane. With this direct connection, F-actin is able to vary membrane tension, modulate the cell shape and to respond on external forces [1].

This work addresses the organization and reorganization of a membrane bound minimal actin cortex (MAC), using physiological binding conditions. Several model systems for MACs have been used to study the active behavior of membrane bound actin-networks in two- (2D) and three-dimensional (3D) systems [2]. In this work, a 2D *in vitro* bottom up approach is used, due to the higher efficacy for small scale effects like on single filaments. The MAC is physiologically bound to supported lipid membranes via the receptor-lipid PtdIns(4,5)-P_2 and the linker-protein ezrin [3]. The myosin induced dynamics are analyzed by means of high-resolution confocal laser microscopy (CLSM). Besides the overall network reorganization by myosin, the underlying binding behavior between actin and myosin is investigated for a more detailed insight of the reorganization mechanism. Furthermore, myosin induced shifts of the receptor-lipid distribution will be studied. The investigation of the mechanical properties, provided by F-actin and actomyosin-networks, will be carried out on pore-spanning lipid bilayers (PSLBs) by means of atomic force microscopy (AFM).

[1] Chugh et al., „Actin Cortex Architecture Regulates Cell Surface Tension".
[2] Schön et al., „Influence of cross-linkers on ezrin-bound minimal actin cortices".
[3] Nöding et. al., „Rheology of Membrane-Attached Minimal Actin Cortices".

P-058

Microtubule depolymerisation leads to changes in mechanical and adhesive properties of endothelial cells

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Mechanical forces such as shear force, pressure, friction and more are ubiquitous in biological systems. Eukaryotic cells are complex systems, composed of many different materials at different length scales and with differing mechanical properties. These cells are able to feel, transduce and exert forces, making the study of their mechanical properties interesting for better understanding underlying mechanisms (1). Up to date, there is no full understanding of the complex interplay of cellular components in defining mechanical properties. What is known is that mostly the cytoskeleton, the nucleus and the membrane (with the glycocalyx) are responsible for the apparent mechanical response (2). In this study, the influence of microtubules on mechanics and adhesion of endothelial cells was tested. This was done using atomic force microscopy (AFM) together with fluorescence microscopy techniques. AFM was used for nanoindentation to quantify cell mechanical properties like Young's Modulus, relaxation time, adhesive work and rupture events during retracting the AFM-tip from the cell (3, 4). Confocal Laser Scanning Microscopy (CLSM) was used to follow the dynamics of the cytoskeleton. Results showed that depolymerization of microtubules with colchicine does indeed lead to significant changes in nearly all the studied properties over the time.

References

(1) Jonas O, Duschl C. Force propagation and force generation in cells. Cytoskeleton. 2010; 67(9):555-563. Available from: doi:10.1002/cm.20466
(2) Fletcher D. A. Cell mechanics and the cytoskeleton. Nature. 2010; 463(7280): 485-492. Available from: doi:10.1038/nature08908
(3) Haase, K., Pelling, A.E. Investigating cell mechanics with atomic force microscopy. Journal of the Royal Society Interface. 2015. 12(104): 970-986. Available from: doi.10.1098/rsif.2014.0970
(4) Sun, M., et al. Multiple membrane tethers probed by atomic force microscopy. Biophys J. 2005, 89(6): 4320-4329

P-059

A HaloTag-TEV genetic cassette for mechanical phenotyping of native proteins

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Single-molecule methods using recombinant proteins have generated transformative hypotheses on how mechanical forces are generated and sensed in biological tissues. However, testing these mechanical hypotheses on native molecules in their natural environment remains inaccessible to conventional genetics, biophysics and molecular biology tools. To overcome these limitations, here we demonstrate a genetically engineered knock-in mouse model carrying a HaloTag-TEV insertion in the protein titin, the main determinant of myocyte stiffness. Using our system, we have specifically severed the titin filament by digestion with TEV protease, and found that the response of muscle fibers to length changes requires mechanical transduction through titin's intact polypeptide chain. HaloTag-based covalent tethering has enabled directed examination of the dynamics of native titin under physiological forces using recently developed magnetic tweezers. At physiological pulling forces lower than 10 pN, titin domains are readily recruited to the unfolded state, and produce 41.5 zJ mechanical work during refolding. Our results support an active role of titin in muscle contraction in coordination with actomyosin motors. Insertion of the HaloTag-TEV cassette in proteins with mechanical roles opens new grounds to explore the molecular basis of cellular force generation, mechanosensing and mechanotransduction.

P-060

How Does Cyclase-Associated Protein Modulate the Structure and Dynamics of Actin?

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Actin polymerization is the engine behind cell motility, morphogenesis, and endocytosis. It is associated with many disorders ranging from cancer to immunological and neurological diseases. Both polymerization and depolymerization of actin filaments are active processes requiring ATP. While ATP-loaded actin associates with filaments to extend the filament on one end, older actin within the filament hydrolyzes its ATP and dissociates from the other end of the filament. Given this, the continuous polymerization depends on re-charging of ADP-bound actin with ATP that is known as nucleotide exchange. In this context, only two proteins (cyclase-associated protein (CAP) and profilin) have been implicated in the nucleotide exchange. Due to its stronger affinity for actin and greater robustness under various *in vitro* conditions, CAP emerges here as the best candidate for facilitating the re-charging process *in vivo*. The recently determined X-ray structure of globular actin in complex with the C-terminal CARP domain of CAP has shed light on to various aspects of the molecular mechanism behind the actin recharging activity of CAP. The complex features a dimer of CARP domains sandwiched between two actin monomers and reveals key interactions between the CARP domain and the functionally important D-loop and the nucleotide-sensing regions of actin.
To explore how CARP binding influences the conformational state of actin and how it promotes nucleotide exchange, we performed atomistic molecular dynamics simulations of the crystallized complex. We also considered large segments of unstructured regions to assess their role in actin-CARP interactions. The simulations revealed how CARP binding alters the dynamical coupling between the functional domains of the actin monomer and the mechanistic role played by different regions of the CARP domain. The simulations further highlighted that CARP domain stabilizes the open conformation of actin and destabilizes the bound nucleotide. This effect of the CARP domain on the actin conformation can explain how it facilitates and promotes the re-charging of actin.

P-061

Single molecule mechanics of talin-integrin bond

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Integrins are large heterodimeric proteins that play an important role in force transduction across cell membrane. Each α and β subunit has a large ecto-domain, continued by a single transmembrane helix and terminated by a short cytoplasmic tail. The integrin ecto-domains can switch between an inactive folded conformation and an activated open form that is able to bind the extracellular matrix. Inside-out mediated activation of integrins occurs through the binding of talin, a big cytoplasmic protein (250 kDa) that connects the integrin receptors to the actin cytoskeleton. Talin contains an N-terminal head region, which is comprised of four FERM subdomains arranged in a linear configuration. The binding of talin N-terminal F3 domain to the integrin β-tail is the minimal interaction necessary for inside-out activation of integrins.
Despite its critical role in force transduction, the interaction between integrin β-tail and talin F3 domain is suprinsingly weak for all isoform pairs (K_d>10 μM). This raises the question how such a weak bond is able to provide a stable connection across the cell membrane. To answer this we designed fusion constructs between talin F3 domain (but also talin full head) and β-integrin cytoplasmic tail. Using a dual-beam optical tweezer setup we were able to probe the mechanics of the talin-integrin bond at the single molecule level and obtain important kinetic and thermodynamic data. Our results confirm the dynamic character of the talin-integrin bond, which shows an unbinding-rate of ≈ 50/s. The binding energy of the talin-integrin bond is also weak, of only 4-6 k_BT, the bond being mainly in an open state at forces higher than 5 pN.
Nevertheless, we discovered that the talin-integrin bond is stabilized by the presence of kindlin. Kindlin, another FERM-domain cytosolic protein, is known to play an important role in integrin activation. When measuring the talin-integrin interaction with kindlin in solution, longer talin-integrin bound states are observed. Suprisingly, the length of these longer states proves to be independent of the applied force. This kindlin induced stabilization effect might be of crucial importance for integrin clustering and focal adhesion formation.

P-062

Structure and mechanics of strained membrane-bound vimentin filaments

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In eukaryotic cells, the shape and their mechanical properties are determined by the cytoskeleton, a highly intertwined network which includes three types of biopolymers: actin filaments, microtubules and intermediate filaments. Importantly, intermediate filaments are considered to be the main determinants of cell stiffness and strength. The reason for this is that they can withstand much larger deformations - in contrast to both other cytoskeletal components. Thus, they are believed to dominate the mechanical response of cells at higher strains, even though they tend to be softer at low strain. Therefore, the organization of intermediate filaments at the plasma membrane and their influence on the mechanical properties of the cells are important to investigate, especially under higher strains.
Hence, in our *in vitro* model system, we aim at the development of a model system to mimic the composition of the plasma membrane under strain by pursuing a bottom-up approach. Biotin-labeled vimentin filaments are attached to a biotin-decorated lipid bilayer *via* neutravidin that prevents nonspecific binding due to its high affinity to biotin. Since the composite system needs to be laterally stretchable by a motor-driven stretching device, the lipid bilayer is prepared on oxidized, elastic polydimethylmethoxysilane (PDMS) by vesicle fusion and spreading. Fluorescent beads are embedded in the PDMS to accurately calculate the expansion of the system under strain. We found that around 66 % of the applied strain is actually being transferred to the system. However, crack formation in the oxide layer of the PDMS is an unwanted side effect, which may be prevented by chemical modification (*e.g.* MPTMS) before oxidizing. Concerning the properties of the membrane while stretching, two different behaviors of the membrane are revealed: no-slip and sliding, which depends on the oxygen plasma exposure time and therefor on PDMS adhesiveness. In our case, we need a no-slip membrane and a vesicle pool to refill membrane ruptures in order to enable stretching of the membrane more than the common 5 % and to observe changes in the vimentin organization.

P-063

Intracellular ion concentration dependent remodelling of bacterial MreB assemblies

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In many bacteria cell wall synthesis is orchestrated by the actin homolog protein, MreB. Limited information are available regarding the variations of salt concentrations in bacteria and the salt dependence of the polymerization MreB. Here we measured the intracellular salt concentrations of Gram negative and positive bacteria and described their effects on the polymer formation of MreB. We used ionselective electrodes to measure the intracellular salt concentrations in *Leptospira interrogans*, *Bacillus subtilis* and *Escherichia coli* cells. MreBs of *Leptospira interrogans*, *Rickettsia rickettsii* and *Escherichia coli* were purified from *E.coli* cells. Polymers were prepared and filtered with a novel method. The examination of the salt dependence of the structure and stability of Alexa-488 labelled MreB polymers was carried out with confocal microscopic methods. MreB polymerization rate was measured using light scattering and fluorescent emission signals. The intracellular ionic strength varied in both Gram negative and Gram positive bacteria. The alterations fell into the hundreds of millimolar range. The potassium ions were in tens of millimolar concentrations in these cells and were mostly responsible for the intracellular changes reacting to extracellular effects. Magnesium and calcium ions, responsible for the regulation of the cytoskeleton, were in millimolar and submillimolar concentrations, respectively. The MreB polymerisation was the fastest in the monovalent cation concentration range of 200-300 mM. The MreB filaments were stabilized in this concentration range and formed large assemblies of tape like polymers that transformed to huge sheets under higher ion concentrations. Change of calcium concentration from 0.2 mM to 0 mM, and then from 0 mM to 2 mM initialized a rapid remodelling of MreB polymers. All these observations indicate that bacteria are sensitive to their environment and one of the key component of their communication with the extracellular space is manifested through the changes of the concentration of various ions.

P-064

Splicing alterations and thermodynamic instability: protein haploinsufficiency drivers to define pathogenicity of missense mutations in cardiac myosin binding protein C

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Mutations in cardiac myosin-binding protein C (cMyBP-C) are a leading cause of Hypertrophic cardiomyopathy (HCM), the most common inherited cardiac disease. The pathogenicity of many genetic variants identified in HCM patients remains challenging to define and the molecular mechanisms by which these variants lead to HCM remain elusive. Essentially, the variants with uncertain pathogenicity limit the reach of genetic testing in clinical practice.

We analysed pathogenicity drivers in a group of clinically annotated exonic variants and variants of uncertain significance (VUS) of MYBPC3, the gene encoding cMyBP-C. We integrated bioinformatics evaluation and functional studies of RNA splicing and protein thermodynamic stability. RNA splicing of the MYBPC3 gene was analysed using peripheral blood from variant carriers, whereas circular dichroism measurements were carried out on purified recombinant proteins.

We find that around 50% of pathogenic exonic mutations alter RNA splicing or protein thermodynamic stability, both of which can lead to cMyBP-C haploinsufficiency. These molecular phenotypes are not found in control, non-pathogenic variants. Remarkably, the remaining pathogenic missense mutations appear to result in stable proteins.

We propose that examination of protein haploinsufficiency drivers defines pathogenicity of genetic variants associated with HCM, decisive for the clinical management of patients and their families.

P-065

Insights into mechanisms for microtubule dynamic instability from computational analyses of tubulin protofilament mechanics

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Tubulins are essential and some of the most abundant proteins in eukaryotic cells. In live cells and in vitro, tubulin heterodimers bound to guanosine triphosphate (GTP) self-assemble into microtubules, which represent hollow cylindrical structures, usually made of 13 parallel strands, or protofilaments. After incorporation into the microtubule lattice at the ends of protofilaments, GTP molecules experience hydrolysis, so the majority of tubulins in microtubule lattice, except for a few terminal layers, transform to guanosine diphosphate (GDP) form. It is not currently understood why, but GDP-tubulin lattice turns out to be less stable than GTP-tubulin lattice. Therefore, when a critical number or density of GTP-tubulins is lost from the microtubule tip, remaining GDP-tubulin lattice depolymerizes by losing tubulin oligomers from the disassembling end. Recent cryo electron microscopy reconstructions of microtubules in the GDP-state and GTP-like state (in presence of a slowly hydrolyzing GTP analog, GMPCPP), have provided the first inhibitor-free structures of tubulins and confirmed an older underappreciated finding that tubulin dimers experience a small but highly reproducible longitudinal compaction upon GTP hydrolysis. The mechanistic role of this inter-dimer compaction and its relevance to microtubule dynamics has remained elusive. Here we have carried out several microseconds of all-atom explicit solvent molecular dynamics simulations of tubulin oligomers to investigate effects of nucleotide phosphorylation state on tubulin mechanics. We found that initially straight tubulin tetramers relax to very similar non-radially curved conformations independent of the nucleotide. Strikingly, GTP hydrolysis dramatically affected the flexibility of the inter-dimer interface, without a strong impact on the shape or flexibility of tubulin dimers. Inter-dimer interfaces in presence of GTP were significantly more flexible compared to intra-dimer interfaces. We argue that such a difference in flexibility could be key for distinct stability of opposite microtubule ends. Overall, our data support a model, in which extended interface between GTP-tubulin dimers is softer, compared to that between compacted GDP-tubulin dimers. Hence, GTP-tubulin protofilaments are less energetically costly to straighten and incorporate into microtubule lattice, at least partially accounting for the mechanism of microtubule dynamic instability.

P-066

Analysis of Time-Dependent Microtubule Tip Reshaping Both In Silico and In Vitro

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An essential but not yet fully understood feature of cytoskeletal microtubules is their unique ability to switch from elongation to shrinkage and back. These transitions, termed catastrophes and rescues, respectively, are crucial for multiple microtubule functions, including search, capture and segregation of chromosomes during mitosis. One of the obscure aspects of microtubule elongation process is a gradual increase of the probability of microtubule catastrophe over time. This phenomenon, referred to as microtubule aging, is thought to be responsible for non-exponential distribution of microtubule lifetimes and lengths, helping cells to maintain a large proportion of microtubules at a certain length. One of the plausible hypotheses, which has been proposed to explain microtubule aging, suggests that it may be caused by progressive tapering of the growing microtubule tip, eventually making the microtubule configuration less stable over time. Here we use a well-characterized computational model to systematically analyze parameter dependence of microtubule tapering and identify regimes where microtubule taper is maximized, while its kinetics is slow enough to be compatible with the experimentally observed rate of microtubule aging process. We found that microtubule tapering in the model is favored when longitudinal bonds are stronger than a certain threshold, and when tubulin association rate constant is low. Modeling further predicts that the magnitude of taper is usually limited and it rapidly comes to a plateau at most sets of parameters. This is in contrast to existing experimental reports, based on TIRF-microscopy, suggesting that the tapering may progress during microtubule assembly for minutes and achieve several hundred of nanometers. Shedding light on this contradiction, control experiments with stable microtubules in the absence of soluble tubulin, suggest that the observed microtubule length-dependence of apparent tip taper could be attributable to TIRF-microscopy limitations. Staggered TIRF- and EPI-fluorescence microscopy measurements revealed no linear increase in the extent of the tip tapering over time judging by fluorescence intensity decay at the microtubule extremity, which favored no persistent changes in the tip structure to rationalize aging. Together, our results provide alternative explanation of the given theoretical and experimental data while appealing to new investigations to elucidate microtubule aging mechanism.

P-067

Viscoelastic properties of crosslinked actin filament networks

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Actin filaments (F-actin) that locate just beneath the cell plasma membrane could be crosslinked into a thin film network by actin binding proteins (ABPs). Among these different actin binding proteins, filamins prefer to crosslink actin filaments into a complex orthogonal network structure. As a crucial substructure of cell cytoskeleton, the crosslinked actin filament network (CAFN) plays a major role in different cell functions, such as cell migration, cell division and signal transduction. In physiological conditions, the extra cellular matrix (ECM), cells and cytoskeleton could deform largely in response to external force or internal contraction, and thus show obvious viscoelastic properties. However, the viscoelastic bahaviour of crosslinked actin filament network is not well understood because of the extreme complexity of its structure. In order to study the viscoelastic properties of crosslinked actin filament networks, a three dimensional representative volume element (RVE) model is developed by finite element method (FEM) according to the physiological conditions. Periodic boundary conditions and free boundary conditions are adopted in this model. The creep and relaxation behaviour of crosslinked actin filament network are studied by conducting numerical simulations and results show good agreement with experimental measurements. Moreover, the influences of load frequency and amplitude on the dynamic shear modulus of crosslinked actin filament network are also obtained and compared with results reported in literature.

P-068

The role of the amino terminal domain in modulation of connexin36 gap junction channels by intracellular pH and magnesium ions concentration

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Connexin36 (Cx36) form gap junction (GJ) channels in the pancreatic beta cells and is also the main connexin (Cx) forming electrical synapses between neurons in the mammalian CNS. As compared to other Cxs, Cx36 GJ channels exhibit a distinctive response to intracellular pH (pH_i) and Mg^{2+} ion concentration ($[Mg^{2+}]_i$) . In this study, we used dual whole-cell patch clamp technique and site-directed mutagenesis to evaluate the role of amino terminus (NT) domain in Cx36 GJ channel modulation by pH_i and $[Mg^{2+}]_i$. Substituting negatively charged glutamates at the 3rd and 8th positions of Cx36 to neutral amino acid glutamine resulted to increased sensitivity of junctional conductance (g_j) to $[Mg^{2+}]_i$ and pH_i. Electrostatic analysis of homology structural models suggested that these mutations reduce negative electrostatic potential at the entrance into the channel pore at cytoplasmic side. Presumably, this could restrict the influx of Mg^{2+} ions into the channel pore and limit its concentration near a putative binding site at 47th position in extracellular loop. Moreover, we demonstrated that substitution of neutral amino acids at the 13th and 18th positions to a positively charged lysine produced a decreased sensitivity to pH_i and $[Mg^{2+}]_i$ as compared to wild-type Cx36. Electrostatic analysis showed that these substitutions shift the surface potential of channel pore towards positive side. We hypothesize that these changes in surface potential near the pore entrance could act as an electrostatic barrier which would disturb passage of Mg^{2+} and H^+ through the channel pore. Furthermore, control experiments in mutants with substitutions of neutral-to-neutral residue at the 5th and 18th positions did not show significant changes in g_j sensitivity to pH_i and $[Mg^{2+}]_i$. Thus, our electrophysiological and modelling data suggest that amino acids in NT domain could play an important role for pH_i and $[Mg^{2+}]_i$ sensitivity of Cx36 channels through electrostatic mechanisms by the regulation of ion permeation near the pore entrance at cytoplasmic side.

P-069

Ionizing radiation effects on human cell cytoskeleton

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The aim of this investigation is to study the structural damages borne to the cell cytoskeleton by ionizing radiations (IR) such as X-rays and protons. The study wants to bring on the same page DNA and cytoskeleton as targets of IR damages. To figure out how IRs behave, radiobiologists have always looked at genomic DNA, as main IRs biological target and many assays have been depicted to connect the such damages with the IRs effects on the cells. In this work, we would like to correlate the different Relative Biological Effectiveness (RBE) between X-rays and protons with new proposed integrity parameters. RBE values connected to cell inactivation are trustable to predict tumor control probability, but they prove to be inadequate in case of normal tissue behavior. For this reason, our work is mainly focused on the Hs 578Bst, a non cancer, non immortalized, human breast epithelial cell line. The cell line is irradiated with protons at the Proton Therapy Centre of Trento (Italy) and with X-rays at Trento Institute for fundamental physics applications – TIFPA (Italy). Both the irradiations were performed 24h after cell seeding. Then cells are fixed and the cytoskeleton stained to be investigated by Atomic Force Microscopy (AFM) and Stochastic Optical Reconstruction Microscopy (STORM). Results about the effects of 4 and 8Gy absorbed doses delivered by protons and 4, 8 and 25Gy absorbed doses delivered by X-rays, are presented.

P-070

Ultrafast imaging and tracking of protofilaments during the microtubule disassembly

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Microtubules are highly dynamical cellular structures governing range of functions including motion and intracellular transfer. The dynamics of microtubules is associated with alternating periods of assembly and disassembly. Electron micrographs provide still images of the disassembling microtubule suggesting a structural transition from straight protofilament embedded in the microtubule lattice to curled protofilament at the microtubule tip sometimes referred to as "ram's horns" [1]. Despite the focused effort of dynamics tracking, force analysis and molecular dynamics simulations no method has directly visualized protofilaments during microtubule disassembly at sufficient spatiotemporal resolution to understand the formation of the elusive ram's horns. Our experimental approach is direct optical tracking of 3-dimensional position of a single protofilament rendered by a small scattering label attached to a single tubulin. In the tracking experiment we use interferometric detection of scattering (iSCAT) [2] allowing us to localize scattering labels of 30 nm diameter with precision better than 2 nm [3] in all three dimensions [4] and with temporal resolution of 22 µs.

We show the complex dynamics of protofilament peeling involving series of sub-millisecond excursions of protofilaments in a rapidly evolving interaction potential taking place hundreds of milliseconds prior to final tubulin release.

References

[1] T. Müller-Reichert, D. Chrétien, F. Severin, AA Hyman, Structural changes at microtubule ends accompanying GTP hydrolysis: information from a slowly hydrolyzable analogue of GTP, guanylyl (alpha,beta)methylenediphosphonate, Proc Natl Acad Sci U S A. vol. 95, p.3661, 1998.
[2] M. Piliarik and V. Sandoghdar, "Direct optical sensing of single unlabelled proteins and super-resolution imaging of their binding sites," *Nat. Commun.*, vol. 5, p. 4495, 2014.
[3] K. Holanová, M. Vala, and M. Piliarik, "Optical imaging and localization of prospective scattering labels smaller than a single protein," *Opt. Laser Technol.*vol **109,** p. 323, 2019.
[4] R. W. Taylor, R. G. Mahmoodabadi, V. Rauschenberger, A. Giessl, A. Schambony, V. Sandoghdar, Interferometric scattering microscopy reveals microsecond nanoscopic protein motion on a live cell membrane, bioRxiv 401133;

P-071 (O-030)

Activation of human aortic valve interstitial cells by local stiffness in Calcific Aortic Valve Disease

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Differentiation of valve interstitial cells (VICs) into pro-calcific cells is one of the central events in calcific aortic valve (AoV) disease (CAVD). A contribution of mechanical factors to such disease has been recently hypothesized due to the cyclic load that valve tissue withstands during cardiac cycle and the susceptibility of VICs to mechanical forces supporting their role as tissue **'**mechanosensors**'**. Here, we investigated the role of mechanical compliance on activation of VICs obtained from patients with valve stenosis and valve insufficiency. We employed a 2D culture system onto polyacrylamide gels with atomic force microscopy (AFM) controlled elastic modulus to establish correlations between nuclear translocation of mechanically-activated transcription factor YAP and substrate mechanical compliance. We found that high stiffness levels determined YAP nuclear translocation with a different dynamics in cells from the two pathologic settings, thus revealing a pathology-specific VICs response to mechanical cues. In stenotic VICs, YAP nuclear translocation was associated to stiffness-dependent formation of stress fibers, adhesion complexes, and loading of **α**SMA onto F-Actin cytoskeleton. AFM force mapping performed along radial sections of human calcific valve leaflets identified, finally, areas with high and low levels of rigidity within a similar range to those controlling YAP nuclear translocation in vitro. Since VICs juxtaposed to these areas exhibited nuclear localized YAP, we conclude that subtle variations in matrix stiffness are involved in mechanosensing-dependent VICs activation and pathological differentiation in CAVD.

Sunday 21st July

MECHANOBIOLOGY

P-072 (O-031)

Role of Rab8A and Caveolin-1 in the interplay between cell mechanotransduction and cholesterol trafficking

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Rab8A is a small GTPase involved in the regulation of intracellular traffic and cell shape. Preliminary data from our lab and others showed that Rab8A and Caveolin-1 participate of complementary traffic pathways. Given the key role of Caveolin-1 in mechano-transduction, we hypothesized that Rab8A activity and localization might be sensitive to changes in membrane tension. Using cell stretching and osmotic shock experiments, we demonstrate that Rab8A is recruited to the plasma membrane upon changes in membrane tension. Moreover, PM rupture induced by laser ablation promoted Rab8A recruitment to the damaged site. Since Rab8A is involved in cholesterol efflux, we are exploring its function in lipid homeostasis, and the potential relevance of such function for mechanoadaptation. Preliminary data obtained from CRISPr/Cas9-edited Rab8AKO cells suggest that feedback systems regulating cholesterol homeostasis require Rab8A. Rab8AKO cells exhibit a blunted response to cholesterol deprivation (i.e. reduced induction of SREBP2 activation and of the upregulation of the mevalonate synthesis pathway). Further supporting an important role for Rab8A in the regulation of cholesterol trafficking, Rab8 over-expression *per se* normalizes cholesterol levels at the endosomal compartment in Cav1KO cells. Our observations support a model whereby Rab8 plays a key role in both mechano-transduction pathways and cholesterol homeostasis. We propose that Rab8A is a candidate regulator coupling mechanoadaptation to cholesterol homeostasis through mechanisms intersecting with Cav1 function.

P-073 (O-032)

The Mechanical Work of Vinculin Binding to Talin Regulates Vinculin Activation

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Talin is a multidomain protein that bridges the extracellular matrix with the active cellular cytoskeleton in focal adhesions; hence, talin is a molecular adaptor that works as a force sensor to allow the cell to communicate mechanically with its environment. Talin is a promiscuous protein, and a multitude of molecular partners are known to bind talin at different stages of the mechanosensitive signaling pathways. Among them, vinculin stands particularly out, given that it binds to at least 11 of the talin helices to recruit the actin filaments, and that all of these sites are cryptic; talin must be mechanically unfolded in order for vinculin to bind. Therefore, the interaction between talin and vinculin requires of force spectroscopy techniques to be studied. Previous work has demonstrated that vinculin locks talin in an unfolded state, and that this state can be reversed if the force is increased, suggesting a feedback regulatory mechanism in this mechanosensing pathway. However, the origin of this effect is unknown, and the force range under which the talin-vinculin complex is stable has not been accurately measured. Here, we focus on the R3 domain of talin, and use our magnetic tweezers force spectrometer to directly observe the binding of vinculin to talin over a large range of forces and timescales. Our experiments demonstrate that vinculin binding is always preceded by a ~3 nm contraction of the unfolded talin polypeptide, which, interestingly, does not occur steeply, but rather with a slow ~500 millisecond-long time-scale. This observation implies that vinculin binding to talin does mechanical work, which might be the cause for the inhibitory effect at high forces. We measure the probability of vinculin binding in the range from 4 to 50 pN, and determine that binding occurs optimally between 8.5 and 15 pN. Our results are accurately represented by a simple analytical theory that combines the unfolding dynamics of talin, and the mechanical work necessary to contract a stretched polymer, which establishes the physical grounds for the formation of this complex. We propose this to be a universal mechanism for ligand binding to substrates extended under force.

P-074

AFM cantilever with integrated microfluidics for probing single-cell mechanosensitivity and membrane fusion

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Both micropipettes and AFM force spectroscopy have brought many new insights in membrane and single-cell biophysics. However, both techniques have inherent drawbacks, like the missing force feedback for micropipettes, and the limited probe attachment possibilities for AFM. Here, we introduce fluidic force microscopy, short FluidFM, that overcomes both of those limitations, and present its applications for the study of single-cell mechanosensitivity and membrane fusion.

FluidFM makes use of AFM cantilevers with integrated microchannels that end in a nano- to micron-sized aperture. Hereby, we are able to exert and control mechanical forces and pressures simultaneously. On one hand this tool is used as a force-controlled micropipette, e.g. for better control in cellular mechanosensitivity measurements. On the other hand, the microfluidics is used for versatile probe attachment to the AFM cantilever, enabling e.g. force spectroscopy with lipid vesicles.

In the study of single-cell mechanosensitivity, the FluidFM cantilever is brought into force-controlled contact with adherent cells, and pressure pulses are applied through the microchannel. By using a calcium sensitive fluorescent dye inside of the cells, we can detect the opening of mechanosensitive ion channels by the influx of calcium into the cell. With this method we investigated various cell lines such as Piezo 1 overexpressing and knock-out HEK cells, C2C12 cells and fibroblasts. So far, we showed the influence of the contact force on the cellular response to applied pressures and could detect differences in mechanosensitivity between cell types.

For studying membrane fusion, giant unilamellar vesicles (GUVs) are attached to the tip of the cantilever by applying constant negative pressure to the microchannel. These AFM probe-attached vesicles can then be brought into force-controlled contact with supported lipid bilayers, immobilized GUVs, and adherent cells. By incorporating fluorescent probes into the lipid bilayer, several microscopy techniques such as total internal reflection fluorescence (TIRF) microscopy and fluorescence recovery after photobleaching (FRAP) can be used to study bilayer fusion as a function of contact force and time.

All in all, the application of FluidFM for studying mechanosensitivity and membrane fusion promises new insight in both biophysical fields by adding force as a controllable parameter.

P-075

Piezo proteins as molecular correlates of mechanosensitive ion channels in human endometrial-derived mesenchymal stem cells

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Human adult mesenchymal stem cells derived from desquamated endometrium (eMSCs) are promising candidates for use in regenerative medicine due to their availability and non-invasive isolation protocols. Mechanical signals were shown to control a number of crucial cellular reactions in stem cells including proliferation, gene expression, growth, migration, differentiation, and signal transduction. One of the major players in the processes of membrane mechanotransduction are mechanosensitive non-selective Ca^{2+}-permeable channels whose activity is controlled by plasma membrane stretch (stretch-activated channels, SACs). Mechanosensitive channel proteins of Piezo family were shown to represent molecular correlates of native SACs in various cells and tissues, including several types of stem cells. In our previous study on eMSCs, we registered the activity of SACs having biophysical characteristics close to Piezo channels. Current work was aimed at confirmation of functional expression of Piezo channels. RT-PCR data allowed to detect PIEZO1 and PIEZO2 transcripts in eMSCs. Consistently, immunofluorescent staining confirmed the presence of Piezo1 and 2 proteins. Importantly, calcium imaging using ratiomeric dye Fura2AM demonstrated, that Yoda1, a selective chemical Piezo1 agonist, induced bi-phasic Ca^{2+} entry in eMSCs indicating functional expression of Piezo1 channels in the plasma membrane. Further experiments will be aimed at revealing the role of Piezo1/2 channels in physiological reactions of eMSCs. The work was supported by RSF grant №18-15-00106.

P-076

The mechanobiological basis of adipocyte expansion

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Adipocytes work as dynamic lipid stores, undergoing remarkable changes in cell volume in order to meet the energetic needs of the organism. Adipose tissue stiffness has been postulated as a modulator of cardiovascular risk factors and tumour progression, stressing the relevance of the adipocyte mechanical microenvironment in adipose tissue function and organismal homeostasis. Loss of caveolin-1, the main component of caveolae, an established membrane mechanosensor, results in adipose tissue dystrophy in murine models and human patients, suggesting a crucial role for mechanical factors in adipose tissue development and physiology. Histopathological analysis revealed a progressive increase in interstitial fibrosis in Cav1-null adipose tissue, concomitant with a decrease in adipocyte size and an increase in adipocyte circularity, evidencing reduced adipocyte expansion ability and shape plasticity. Using Atomic Force Microscopy, we measured the stiffness of visceral adipose tissue explants and *in vitro* differentiated adipocytes, finding altered biomechanical properties in Cav1-null adipose tissue, which where independent from the development of fibrosis. Our preliminary data suggest that these mechanical alterations can modulate adipocyte size, confirming that changes in adipocyte mechanical environment can result in a significant impact in adipose tissue functionality.

P-077

***In vivo* titin oxidation as a regulator of muscle elasticity**

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Titin is the sarcomeric protein responsible for the passive elasticity of striated muscle. Its mechanical properties are determined by unfolding and refolding equilibria of the immunoglobulin-like (Ig) domains of the I-band. Recent studies have shown that redox posttranslational modifications of cysteines located in these domains modulate the elasticity of titin *in vitro*. However, the identity, extent and specific residues targeted by these modifications *in vivo* remain unexplored. Here we show for the first time that titin is oxidized *in vivo* and that oxidative modifications target conserved cysteines of the mechanically active Ig domains. We have optimized a method for in-gel determination of oxidized thiols by fluorescent labelling, which has allowed us to prove that titin´s cysteines are remarkably oxidized when compared to other sarcomeric proteins, such as myosin. By mass spectrometry, we have also found that conserved cysteines previously described *in vitro* as mechanically relevant are in fact oxidized *in vivo*. The characterization of redox posttranslational modifications of titin opens a new avenue to a better understanding of the modulation of muscle elasticity in health and disease, and could explain the pathological effects of an altered redox status, such as during myocardial infarction and ischemia.

P-078

Impact of lipid composition and ion channel on the mechanical behavior of axonal membranes: a molecular simulation study

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Traumatic brain injury is one of the most frequently occurring types of brain injury in which a mechanical force injuries the brain. It is currently thought that the mechanism behind traumatic brain injury is a supra-threshold deformation of the axons. Axonal membrane integrity is critical for axons functionality and its damage might lead to neurological disease. Naturally occuring membranes contain hundred types of different lipids and are crowded with proteins. In particular, voltage-gated ion channels are embedded in the axonal membranes to promote ion conductance through the axon.

Here we aim to elucidate the role of lipid content and embedded proteins on the mechanical behavior of lipid bilayers. To achieve this, we use molecular dynamics simulations together with an atomistic and coarse-grained descriptions of the molecular system. We simulate the lipid bilayers in presence or absence of proteins at equilibrium and under deformations. We have used both simple three component lipid bilayers and complex bilayers accounting for asymmetry between two bilayer leaflets and containing 63 different types of lipids in plasma membrane.

Sphingomyelin/phospholipid/cholesterol and galactosylceramide/phospholipid/cholesterol bilayers were used to distinguish the role of sphingomyelin and galactosylceramide lipids, which represent the main difference in lipid composition between plasma membrane and myelin sheaths. The result shows that galactosylceramide lipids increase the stiffness of the membrane compared to sphingomyelin and/or phospholipids, thanks to the sugar headgroup interactions and H-bond network with phospholipids. Both galactosylceramide and sphingomyelin make membranes more resistant to water penetration than phospholipids.

A plasma-type membrane model was used to study mechanoporation of the axonal membrane in presence and absence of sodium channel protein type 1 subunit alpha (Nav1.1). The protein was modelled using the cryo-electron microscopy structure of NaPaS as template. We have found that both the system stiffness and mechanoporation are affected by the presence of the protein: membrane containing Nav1.1 can withstand 38% higher strain than without protein. Moreover, the poration occurs in bilayer region lacking galactosylceramide lipids. Finally, by combining molecular model of membrane and continuum model of axon, we provide a realistic model for studying the axonal injury mechanism.

P-079

Role of glomerular biophysics in regulating diabetic kidney injury

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Background: In the last decade there has been a rapid increase in the patients with diabetic nephropathy. A major site for diabetic damage in the kidney is the glomerulus, a specialized tuft of capillaries that serves as a blood filter. Scarring is an important and common manifestation of diabetic glomerular injury. Produced primarily by fibroblast-like mesangial cells, this scar tissue can over time obliterate and compress the surrounding capillaries. The end result of this scarring process is reduced blood filtration and kidney failure. Studies of mesangial cell activation have historically focused on biochemical stimuli (eg. high glucose, transforming growth factor-β, angiotensin II). However, increasing evidence suggests that biomechanical stimuli such as extracellular matrix stiffness can also play important roles in regulating fibroblast activation. In particular, much attention has recently focused on the mechanosensory transcription co-factors YAP and TAZ in linking matrix stiffness to fibrogenesis. Interestingly, recent studies have suggested that glomerular stiffness changes following injury, although to date glomerular stiffness has not been studied in the diabetic kidney.

Hypothesis: Glomerular stiffness increases following injury, and this stiffening leads to mesangial cell activation.

Results: Using atomic force microscopy (AFM), I have shown in experimental diabetic nephropathy that glomeruli stiffen as diabetes progresses. At both early and late stages of diabetic kidney injury, glomerular stiffness correlated with the degree of glomerular scarring. Glomerular cell YAP/TAZ activity was also increased in diabetic animals when compared to their wild type littermates, as evidenced by increased YAP/TAZ nuclear localization within glomerular cells.

Conclusion: Taken together, these data suggest that stiffness may be an important determinant of mesangial cell activation and glomerular scarring.

P-080

The Effect of PF74 on HIV-1 capsid stability and reverse-transcription induced disassembly

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THE EFFECT OF PF74 ON HIV-1 CAPSID STABILITY AND REVERSE TRANSCRIPTION-INDUCED DISASSEMBLY

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The RNA genome of human immunodeficiency virus type 1 (HIV-1) is enclosed inside a capsid shell that disassembles within a cell in a process known as uncoating. Using time-lapse atomic force microscopy of purified HIV-1 cores, we recently showed that the pressure inside the capsid increases during reverse transcription, which leads to a complete rupture of the capsid near the narrow end of the cone. High-resolution mechanical mapping revealed the formation of a stiff coiled filamentous that disappears when the stiffness of the capsid drops. If the capsid stability is too high (e.g. with the E45A CA mutant), the force generated during reverse transcription is not sufficient to breach the capsid structure. We now show that binding of the CA-targeting inhibitor PF74 to capsids assembled from CA protein *in vitro* and to HIV-1 isolated cores increases the stability of the capsid in a concentration-dependent manner. At a PF74 concentration of 10 μM, the mechanical stability of the core is elevated to a level similar to that of the hyperstable capsid mutant E45A. In contrast to HIV-1 cores without PF74, which undergo complete disassembly after 24 h of reverse transcription, cores with PF74 only partially disassemble: specifically, the main body of the capsid remains intact and stiff, but a cap-like structure dissociates from the narrow end of the core. Moreover, the internal coiled structure is formed and persists throughout the entire duration of the measurement (~24 h). Our results provide direct evidence that PF74 directly stabilizes the HIV-1 capsid lattice, thereby permitting reverse transcription while interfering with a late step in uncoating.

P-081

Effects of experimental antiviral compounds on the mechanical properties and equilibrium dynamics of the human immunodeficiency virus capsid lattice

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Recent findings indicate that the infectivity of viruses may be dependent on their degree of mechanical stiffness. Moreover, we have just found that certain compounds binding to hydrophobic pockets in the rhinovirus capsid exert their antiviral effect by stiffening the virus particles. We have undertaken a detailed biophysical exploration of the effects of experimental antiviral agents on the mechanical properties and equilibrium dynamics of a virus capsid. As a suitable model we have used the mature capsid of the human immunodeficiency virus (HIV) assembled as a bidimensional protein lattice on a flat solid surface. Results so far using atomic force microscopy reveal that mechanical stiffness, strength against mechanical disruption and equilibrium dynamics of the HIV capsid lattice may be significantly altered in complex ways in the presence of small antiviral compounds that were previously known to interfere with the assembly and/or stability of the HIV capsid. The biomedical and nanotechnological implications of these results will be discussed.

P-082

Shear flow promotes isotropic redistribution of fibrin fibers inside glycosylated fibrin networks

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Fibrin networks form at the border of injured blood vessel wall, preventing bleeding and promoting wound repair. *In vivo,* the formation of fibrin clots takes place in the presence of flowing blood that exerts a continuous shear force on the structure. While it is commonly known that the external force affects mechanical properties of fibrin clots, the exact role of shear flow in the bulk organization of fibrin networks and the response of individual fibrin fibers within fibrin clots still remains poorly understood. Here, by using combined mechanical (parallel-plate flow chamber) and optical (confocal microscopy) methods we follow *in situ* changes in the spatial organization of individual glycosylated fibrin filaments in the presence of shear flow. Glycation of fibrinogen, and subsequently fibrin, is a natural process taking place under normal physiological conditions. However, excess glycation, as observed in diabetes states, yield formation of modified fibrin clots with more compact and difficult to lyse structure. We show that shear flow deformations can directly affect orientation of individual glycosylated fibrin fibers by promoting their highly isotropic distribution within fibrin clots. Such shear flow-induced reorientation of fibrin fibers may impact the bulk permeability of the whole clot, and consequently influence the diffusion of blood cells and/or thrombolytic components through fibrin mesh. Our results highlight thus the direct role of shear flow in reorganization of fibrin networks at the level of individual fibrin filaments, and reveal its importance in modulating the response of fibrin clots in diabetic conditions.

P-083

Supracellular Mechanical Architecture of the Intact Bone Microenvironment

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We introduce an improved platform based on atomic force microscopy (AFM) to quantify the mechanical architecture of the intact bone microenvironment (BMev) within regions of interests (metaphysis, cortical bone, marrow and growth plate). The elastic modulus of all regions was found to be highly heterogeneous on supracellular scale, ranging over 3 to 4 magnitudes, and could be down to only few Pas. Such unique architecture provides 'soft channels' for cell migration and consequently may impact a substantial number of active biological processes involving cell migration, such as bone remodelling and cancer metastasis.

We collected the force generated by indentation of the fresh (un-fixed) murine bone tissue in physiological buffer and the subsequent relaxation process, on either individual points distributed over the bone surface or a micro scale array (*i.e*. AFM force map). The Young's moduli, extracted from weighted Sneddon model fit, of all BMev regions are overall much lower than the values from individual cells or bulk tissues. This is likely due to the tissue hydration and natural supracellular structures maintained relatively intact in our study. The viscoelastic model describes the mechanical response better than elastic model, and indicates that the instantaneous elastic response can be neglected in almost all BMev regions. Both elastic and viscoelastic properties were found to be significantly different between the various BMev regions as well as between bones from young and mature mice.

High resolution AFM force maps show highly heterogeneous mechanical properties and corresponding morphology at a supracellular level, in particular in the metaphysis and cortical bone. We also demonstrate the ability to further correlate such maps with tissue components by combining *in-situ* and *ex-situ* fluorescent BMev images. This improved AFM based system is powerful for further characterisations of bones in the presence of stimuli (*e.g*. hormones, cancer cells and drugs) or other complex tissues.

P-084

Super-resolution microscopy reveals nano-hubs of spatially segregated proteins within focal adhesions

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Focal Adhesions (FA) are mechanosensitive complexes that connect the extracellular matrix (ECM) with the actin cytoskeleton. This is achieved through clustered integrins, their ligands in the ECM, and an internal dynamic protein complex linking the cytosolic domain of integrins with the actin cytoskeleton. In contrast to the established view that FAs are homogenous micron-scale protein assemblies, recent super-resolution imaging and single molecule dynamic approaches are challenging this view. These studies suggest that FA molecular components are highly organized in the axial direction establishing segregated layers of functional activity. Recent data also indicates that similar type of nanoscale modularity might exist in the horizontal plane of FAs. Here, we present a set of experiments aimed at dissecting the lateral nanoscale organisation of different proteins as a function of FA maturation. By combining quantitative multicolour STED and STORM nano-scopy we revealed that main protein actors involved in FAs, i.e. paxillin, talin and vinculin, are organised in segregated nanoclusters within FAs. Whereas paxillin nanoclusters are mostly concentrated inside FAs, both vinculin and talin nanoclusters were found inside and outside mature FAs, albeit at different densities and molecular packing. Paxillin and vinculin nanoclusters inside FAs are spatially segregated at around 80nm while talin nanoclusters were found to be more sparsely distributed and exhibit a larger variation in nanocluster sizes and molecular density. This larger heterogeneity of talin organization might result from the highly dynamic spatiotemporal organization and segregation of inactive *vs.* active integrin nanoclusters within FAs. As a whole, our data indicate a highly complex spatiotemporal organization within FAs with different proteins forming nano-hubs of activity. The duration and strength of the protein interactions inside these hubs are highly regulated not only in the axial direction but, importantly, also in the horizontal plane of FAs.

P-085

Bacterial cell wall mechanical damage studied by simultaneous nanoindentation and fluorescence microscopy

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We have developed an experimental protocol to perform simultaneous AFM nanoindentation and fluorescence imaging on immobilized bacterial cells, with the goal of finding potential correlations between nanoindentation conditions and damage to the bacterial cell wall. The latter is assessed by quantifying the fluorescence enhancement kinetics arising from propidium iodide (PI), a marker for membrane integrity. Two types of bacteria with different cell wall composition, *E. coli* (Gram-negative) and *B. subtilis* (Gram-positive) are compared. Our main observation is that a correlation exists between the magnitude of the force applied to rupture the cell wall and the delay of the PI fluorescence response. We have also observed that there are other influential parameters in these experiments, such as the bacterial immobilization method or the cell cycle phase. Indeed, bacteria are easier to puncture during or just after replication. Other parameters, such as tip geometry or indentation pattern, are being investigated. While previous studies have shown that bacteria are rather resilient to AFM nanoindentation [1], our experimental strategy using simultaneous fluorescence detection of PI in a systematic and quantitative way may help to provide a deeper insight into the range of forces that are relevant to "mechano-bactericidal" mechanisms of action [2].

[1] Z. Suo, R. Avci, M. Deliorman, X. Yang, and D. W. Pascual, "Bacteria survive multiple puncturings of their cell walls," *Langmuir*, vol. 25, no. 8, pp. 4588–4594, 2009.
[2] C. Moraes, R. D. Rogers, P. Berton, N. Lin, and N. Tufenkji, "Nanodarts, nanoblades, and nanospikes: Mechano-bactericidal nanostructures and where to find them," *Adv. Colloid Interface Sci.*, vol. 252, pp. 55–68, 2017.

P-086

How do Cells Sense Physical Forces? Cellular Mechanosensing and Motility in Biomimetic 3D Environments

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The cellular microenvironment regulates processes such as cell division, cell migration and cancer progression. Cells *in vivo* constantly sense the physical properties of the environment such as adhesion and **mechanical confinement** and - accordingly to them - switch in between different migration modes. Embryonic progenitor stem cells derived from zebrafish embryos under mechanical confinement show **Myosin II** enrichment at the cell cortex. As a consequence cell contractility increases and eventually transforms cells from a non motile to a highly migratory phenotype, termed stable bleb (Ruprecht et al, 2015). This amoeboid transformation is highly conserved in between different cell types (Liu et al, 2015). However, how single cells are able to sense a physical force and how Myosin II is activated under confinement is still unknown. Here, by combining quantitative imaging with an interference approach, we identified a conserved minimal set of proteins necessary for **cell mechanosensation**. These two proteins allow the cells to sense the mechanical confinement and to differentiate between compression and inflation exemplified by hypotonic stress.

The tight interplay between **nuclear membrane tension** and **intracellular calcium levels** controls the mechanosensitive machinery which further activates myosin II and leads to the stable bleb motility transformation. We show that mechanical confinement induces nuclear membrane (INM) unfolding followed by INM tension increase and, in the presence of high calcium levels, cytosolic phospholipase A2 ($cPLA_2$) translocation to the inner nuclear membrane. $cPLA_2$ cleaves fatty acids, releasing arachidonic acid (AA) which further regulates TRPV4 channels and Ca^{2+} entry. Inhibition of TRPV4 related Ca^{2+} entry and $cPLA_2$ functioning completely blocks the mechanosensitive cell transformation. Altogether, the interrelated function of nuclear membrane tension and Ca^{2+} levels specify **a novel mechanosensation module** capable of reading mechanical force and osmotic inflation from the cell's microenvironment, with important impacts for amoeboid migration in diseases and cancer.

P-087

Active transport confers specific, tuneable, and reversible mechanosensitivity to nucleocytoplasmic shuttling.

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YAP is a transcriptional regulator with roles in development, cancer and regeneration, which has been described to be mechanosensitive. Forces applied to the nucleus increase the nuclear accumulation of YAP by enhancing its active import through nucleopores [1]. However, the mechanism by which forces to the nucleus affect the active import of YAP, or any other protein, is unknown. Here we studied how forces to the nucleus regulate the active transport of molecules through nucleopores. We combined static and dynamic measurements of nuclear import signals (NLS) and nuclear export signals (NES) of various binding constants to karyopherins. We plated mouse embryonic fibroblasts transfected with GFP-NLS on substrates of different rigidity, and observed that the nuclear to cytosolic ratio increased for high substrate rigidity (where the forces applied by cells to their nucleus are higher). However, the nuclear to cytosolic ratio decreased with rigidity for GFP-NES. When we exerted forces to the nucleus by a bead attached to an AFM cantilever, we measured that GFP-NLS and GFP-NES respectively increased or decreased their nuclear to cytosolic ratio, indicating that forces to the nucleus enhance active transport across nucleopores in both directions. We also measured the dynamics of active transport through the nucleopores on gels of different rigidities by optogenetically activating a basally caged NES within an NLS-containing construct. We observed that both import and export dynamics are increased with rigidity. Our study shows a general mechanism where rigidity increases active transport through nucleopores by exerting forces to the nucleus, which could explain how cells regulate the specificity of transcriptional program activation in response to mechanical cues.

P-088

Effect of therapeutic agents on tumor cell compartments studied by local measurement of cell stiffness via Scanning Ion-Conductance Microscopy

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Stiffness measurement of single cell via Scanning Ion-conductance Microscopy (SICM) is a novel method of studying cell mechanical properties. Due to the work principle of SICM [Korchev et al., 2009], which is allow to topography mapping with lateral and vertical nanoscale resolution. Also, it's possible to provide simultaneously stiffness mapping, due to applying low stress on cell surface [Clarke et al., 2016], whose nature is intrinsic colloidal pressure between nanopipette tip and cell membrane. Nanoscale diameter of nanopipette tip allows to obtain cell stiffness distribution on different parts of single cell. We report cell stiffness measurement of drug-induced alterations in cancer cell compartments studied by SICM, specifically, we measured fibrosarcoma cells (HT1080) transfectioned with Progerin, which is integrate in protein structure of nucleus membrane. Progerin was modified with GFP fluorescence dye (GFP-Progerin). Also, we analysed human prostate cancer cell line PC3 subjected with Paclitaxel for microtubulin stabilization and Cytochalasin-D for actin depolymerization. Experiments with GFP-Progerin were provided in heterogeneous population of HT1080 with control and GFP-Progerin transfectioned cells. Control cell stiffness measurement shows ~1.7kPa and ~0.7kPa, when GFP-Progerin treated cells increased value only on nucleus area (~2kPa). In control and treated PC3 cells we measured cell stiffness upon the nucleus area and cytoplasm area, which are show two different values in control cells (~1.3kPa and ~0.8kPa, respectively). Measured stiffness after Paclitaxel treatment shows significantly increased stiffness value on nucleus area and cytoplasm area (~4kPa and ~1.8kPa), whereas Cytochalasin-D treatment reduced cell stiffness only on cytoplasm area (~0.5kPa). As we can see, SICM-base measurement of stiffness shows different effects Paclitaxel, Cytochalasin-D, Progerin on cancer cell compartments, including actin, microtubulin and nucleus membrane, respectively. Drug-induced disruptions of these cell compartments lead to cell mechanical properties alteration, depending on inhibition mechanism.

P-089

Hierarchical biointerfaces as smart cellular mechanoselective surfaces

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The surface topography is crucial for biomedical implant effective implantation and tissue healing. This generally involves promoting cellular proliferation around the implant surface and simultaneously preventing possible infection by bacteria. Today this is commonly achieved by the co-administration of growth factors and antibiotics upon surgery. Nonetheless, a significant percentage of artificial implants still fail due primarily to aseptic loosening and infection [1]. The possibility to control the fate of both mammalian and bacterial cells simultaneously by topographical mechanoselective means have opened up new possibilities to solve these issues. Both mammalian cells [2] and bacteria [3] have seen previously to be responsive towards the surface topography, although at different micro and nano size ranges. This is due to the differences in physiology, morphology and size between bacteria and mammalian cells. As such, the design of effective topographical features, that not only prevent bacterial colonization, but also promote mammalian cell proliferation is extremely challenging. This work deals with this challenge by creating a convergent design of nano and micro hierarchical topographies. A novel fabrication process combines sequential nanoimprinting with optical lithography steps. This process allows for a simple and well-controlled hierarchical structures fabrication, where the nanofeatures cover the entire micropattern. These topographies have been fabricated onto biocompatible polymers. Hierarchical surfaces provide unique physical environments allowing for testing different biological scales at once. As a biological source, we employ mesenchymal stem cells because of their in vitro capacity to form differentiated cellular identities, which play a key role in tissue regenerative processes. Results have shown that bacteria are sensitive to the nano-scale where bacterial lysis is found. The impact of the hierarchical topography on mesenchymal stem cells surface topography in terms of morphology, cell growth and differentiation, is under study. Previous tests in mammalian cells are shown

References

[1] Raphel, J., Holodniy, M., Goodman, S. B., & Heilshorn, S. C. (2016). Biomaterials, **84**, 301-314.
[2] Viela, F., Granados, D., Ayuso-Sacido, A., & Rodríguez, I. Advanced Functional Materials, **26**(31), 5599-5609 (2016).
[3] Viela, F., Navarro-Baena, I., Hernández, J. J., Osorio, M. R., & Rodríguez, I. Bioinspiration & Biomimetics **13,** 026011 (2018).

P-090

Molecular reshaping of the plasma membrane in response to mechanical stress

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Cell dynamics largely relies on endogenous mechanical forces provoking large scale remodelling events of the plasma membrane, accompanied by changes in its exquisitely regulated nanoscale curvature. Exogenous forces (such as tensile or compressive stresses) perturb and drive this dynamics, also interfering with plasma membrane organization. In this study, we evaluated how a mechanical input drives membrane remodelling, and triggers a resulting biomolecular response. We used in-vitro and cell models consisting respectively of a synthetic lipid bilayer or the membrane of a single fibroblast, coupled to an area compression device. We then studied the response of a crucial player in membrane nanoshaping, a curvature-sensing protein (CSP), by taking the Amphiphysin BAR protein as an example. Bilayer reshaping and protein localization were followed over time by fluorescence microscopy. In the cell system, Amphiphysin overexpression increased the amount of compression-induced tubulation of initially small lipid buds. In-vitro, a synthetic lipid bilayer, formed from the deposition of negatively-charged liposomes, was also compressed and subsequently exposed to purified Amphiphysin. We used this simplified synthetic bilayer system for accurate biomolecular characterization of the reshaping events: compressed bilayers accommodated the excess area in the form of lipid tubes and buds, which were respectively pearled or elongated by the BAR protein. Computational simulations explicitly considering membrane mechanics and membrane/CSP mechanochemistry successfully recapitulated the protein binding mechanisms. By combining in-vitro and single cell experiments with computational studies, we show how force-induced remodelling of the plasma membrane provoked its reshaping by a BAR protein. Bearing in mind that cells are constantly submitted to stresses, this study puts forward an elegant example of mechanochemical coupling, which can potentially be translated into cellular signalling.

P-091

Probing Epithelial Cell Layers With a Thickness-shear Mode Quartz Resonator

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The epithelium is ubiquitous and essential in mammalian biology. Epithelial cells cover most internal and external body surfaces, including the lining of vessels and small cavities. They perform critical roles in the body. The mechanical properties of these layers are of great interest. To probe these properties, we use a thickness-shear mode quartz resonator. Typical shear-thickness quartz resonators have a resonance frequency in the range of 5 MHz which only sense and probe a few hundred nanometers into the cell layer. By decreasing the resonance frequency, the sensing range of the quartz is increased. This is why we use a crystal in the frequency range of 600 kHz that allows to sense and probe the viscoelastic properties of entire epithelial cell layers.

P-092

Gravitropic response in woody species: role of stem structural anisotropy

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In tree stems, mechanical stimuli induce reaction wood formation, characterized by changes in cell morphology and cell wall structural modifications. One of the crucial modifications is cell wall structural anisotropy, based on cellulose fibrils alignment and order. Structural order in cell walls of various woody species – spruce (*Picea omorika*), willow (*Salix viminalis*) and liana plant (*Dioscorea balcanica*) was studied using confocal microscopy and fluorescence detected linear dichroism (FDLD) microscopy, as well as fluorescence and FTIR spectroscopy. Responses to mechanical stimulation differed among the woody species with respect to changes in cell wall structural order. FDLD microscopy revealed that in juvenile spruce stems under bending stress, cellulose fibril order and thus cell wall order decreased in mechanically stimulated stems compared to control stems. In *D. balcanica* stems, there was no change in cellulose/cell wall order, based on FDLD results, between twined and straight stem segments. In willow, the cell wall structural parameters differed in tetraploid genotypes compared to diploids. FTIR and fluorescence spectroscopy showed that changes in cell wall organization, upon mechanical stimulation, are related to the changes in content, ratio and structure of the main building polymers.

P-093

Calcium-dependent elasticity of native titin filaments

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Titin, the sarcomeric giant protein, is one of the main determinants of muscle's elastic properties. While it mainly contributes to the development of passive tension upon muscle stretch, activation of the contractile apparatus may also have an impact on titin's mechanics. It has been suggested that sarcomeric calcium induces structural changes in titin by binding to glutamate rich motifs in its PEVK domain. To test how such calcium-responsive elements might alter the elastic properties of titin, we have manipulated individual full-length titin molecules in optical tweezers experiments using laminar-flow microfluidic system. The experimental setup allowed the efficient and rapid control of calcium concentrations during repetitive stretch-release cycles. When molecules were manipulated at high calcium concentrations (pCa 3), titin's apparent persistence length became reduced. As a consequence, titin molecules contracted into a more compact conformation, that resulted in the shortening of the polymer chain stretched by a given force. Our findings support that titin may act as a calcium sensitive, elastic parallel element of the sarcomere, that may contribute to sarcomeric force generation.

P-094

Fluid mechanics near lipid bilayers

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The mechanical and chemical interactions between cells and their surroundings dictate their behavior, such as motion, growth, and proliferation. Such interactions are mediated by the cell's membrane, a thin layer composed of a lipid bilayer and embedded proteins. The membrane's functions include the transport of molecules and ions, and sensing (or signaling); therefore, it is crucial for homeostasis, or the maintenance of a stable internal state. Controlled studies into membrane mechanics have been limited by complexities in geometry and local detection of forces at the scale of pico-Newtons.

To address these challenges, we use novel techniques with optical tweezers to probe the hydrodynamic flow around free-standing lipid bilayers within microfluidic channels. The planar geometry of the lipid bilayer facilitates interpretation of measurements using physical models. This technique is the first to combine multiple optical tweezers probes with planar free-standing lipid bilayers accessible on both sides of the bilayer. The aims of these measurements are to quantify fluid slip close to and transmission of hydrodynamic forces across the bilayer surface, building towards a fundamental understanding of the physical principles governing the hydrodynamics around and through membranes. Such findings may contribute to understanding how cells generate and detect forces, as well as providing a tool for designing and optimizing vesicles for drug delivery.

P-095

Investigating Dynamic Biological Processes with High-Speed, High-Resolution Correlative AFM-Light Microscopy

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The ability of atomic force microscopy (AFM) to obtain three-dimensional topography images of biological molecules and complexes with nanometer resolution and under near-physiological conditions remains unmatched by other imaging techniques. However, the typically longer image acquisition times required to obtain a single high-resolution image (~minutes) has limited the advancement of AFM for investigating dynamic biological processes. While recent years have shown significant progress in the development of high-speed AFM (HS-AFM), the ability to scan faster has typically been achieved at the cost of decreased scanner range and restricted sample size. As such, these HS-AFM systems have mainly been focused on studying single molecule dynamics and have been very limited in their ability to conduct live cell imaging. JPK BioAFM has developed a new NanoWizard® ULTRA Speed 2 AFM which not only enables high-speed studies of time-resolved dynamics associated with cellular processes, it's latest scanner technologies and compact design also allow full integration of AFM into advanced commercially available light microscopy techniques. Thus, fast AFM imaging of 10 frames per second can be seamlessly combined with methods such as epi-fluorescence, confocal, TIRF, STED microscopy, and many more. Furthermore, with the new NestedScanner technology, cells, bacteria or structured surfaces with sample heights of up to 8 µm can now be examined at the highest scan speeds. We will present how the latest advances in the ULTRA Speed 2 AFM can be applied to study a wide-range of biological samples, from individual biomolecules to mammalian cells and tissues in real-time, in-situ experiments. We will also describe how this unique system enables new research opportunities with high-speed, high-resolution correlative AFM-light microscopy.

P-096

Mechanical nanomanipulation of free-standing lipid bilayers accessible on both leaflets with optical tweezers

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Lipid tubes, tubules or nanotubes are highly curved lamellar structures in the nanometer-micrometer scale with a great importance in many biological processes. They play a vital structural role in different cellular organelles such as the endoplasmic reticulum, mitochondria and Golgi apparatus, but also in communication processes such as inter and intracellular exchanges and cellular migration.

Their biophysical study is often carried using vesicles, supported lipid bilayers or living cells. In these systems, it is challenging to achieve dynamic buffer control and zero curvature. Using of a freestanding lipid bilayer in a microfluidic device, these challenges can be solved and present additional advantages such as easy access to both sides of the membrane, possibility to create several membranes in a same device, possibility to circulate different solutions, and full compatibility with optical techniques.

In this work, we show the combination of these novel freestanding lipid membranes formed inside a microfluidic chip with optical tweezers for the study of lipid nanotubes. Nanotubes were formed by pushing beads through the membranes, with a 100% success rate and reaching lengths above half a millimeter. Through high-resolution measurements of the forces and displacements associated with the tube formation process, we quantify the tension of the membrane and the bending rigidity without the need of additional sensors apart from the optical tweezers. Our method provides a robust platform, not only for nanotubes studies, but also for further study of protein-membrane interactions under controlled conditions on each side of the membrane, and modulated membrane complexity.

P-097

HS1 protein role in regulating mechanical properties of Chronic Lymphocytic Leukaemia cells

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Chronic lymphocytic leukaemia (CLL) is one of the most common and incurable B cell leukaemia. CLL cells traffic between peripheral blood, bone marrow and secondary lymphatic tissues where interact with the microenvironment. These processes are affected by the mechanical-forces present in the environment and by the capability of the cells to sense the forces.

In this context we demonstrated that Hematopoietic-cell-specific Lyn-substrate-1 (HS1) protein is a cytoskeletal regulator and a prognostic factor in CLL. We proved that interfering with HS1 expression impacts on the progression and homing of CLL cells.

To further study HS1 role in CLL we knocked-down HS1 expression by CRISPR/CAS9 technology in a CLL cells line (MEC1). By RNAseq and network analysis on $MEC1^{HS1ko}$ vs $MEC1^{UT}$ we found that HS1-KO significantly affects the expression of molecules involved in: cell motility, adhesion, cell-cell communication, focal adhesion formation. In particular, we found LEF1, FAK and Cortactin genes are up-regulated in $MEC1^{HS1ko}$, suggesting their involvement in the mechano-signalling pathway. To study the nano-mechanical properties of those cells we are performing AFM measurements of single cell. The results provide an evaluation of the cell stiffness that is related to its deformation in response to an externally applied force. By AFM we found that $MEC1^{HS1ko}$ cells are less stiff if compared with $MEC1^{UT}$ cells. This results demonstrates a putative role for HS1 in regulating the mechanical properties of CLL cells. Due to the prognostic value of HS1 we are currently performing AFM measurement on selected patients and healthy B cells.

We are planning to study in depth the role of HS1 in regulating the mechanical properties of the leukemic cells and how this contributes to leukemia development, progression and resistance to therapy.

P-098

Mapping nanomechanical properties of proteins and polymers with bimodal AFM

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Fast, high-resolution mapping of the viscoelastic properties of soft matters, represents a major goal of atomic force microscopy (AFM) [1]. Bimodal AM-FM AFM is a suitable method for this purpose, since it allows the simultaneous acquisition of nanomechanical properties without losing in resolution and acquisition speed. This multifrequency configuration combines the robustness and simplicity of an amplitude modulation (AM) feedback in the first mode, with the sensitivity and a high signal-to-noise ratio of a frequency modulation (FM) feedback in the second mode. Finally, through the use of the appropriate contact mechanics model, it is possible to determine elastic and viscous properties of the analyzed sample [2].

Here we show how bimodal AM-FM is applied to extract, by means of the Hertz contact model, the elastic modulus and the true topography of a single protein in liquid, the 20S proteasome which in living organism plays a proteolytic role [3], and the viscoelastic properties, through the Kelvin-Voigt contact model, of two types of polymeric assemblies, a polymer blend, composed by polystyrene (PS) and low density polyethylene (LDPE), and a poly(styrene-block-methylmethacrylate) (PS-b-PMMA) copolymer [4].

[1] C. A. Amo, A. P. Perrino, A. F. Payam, and R. Garcia, ACS Nano **11**, 8650 (2017).

[2] E. T. Herruzo, A. P. Perrino, and R. Garcia, Nat. Commun. **5**, 3126 (2014).

[3] S. Benaglia, V. G. Gisbert, A.P. Perrino, C. A. Amo R. Garcia, Nat. Protoc. **13**, 2890 (2018).

[4] S. Benaglia, C. A. Amo, R. Garcia, in preparation.

Sunday 21st July

MEMBRANE STRUCTURE AND DYNAMICS

P-099 (O-036)

Adsorption kinetics of pulmonary surfactant complexes purified from bronchoalveolar lavages of porcine lungs and human amniotic fluid

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Surface tension forces are overwhelmed by lung surfactant complexes at the alveolar air-liquid interface. Specifically, type-II pneumocytes are the cells involved in synthesizing and assembling surfactant into dehydrated and highly packed multilamellar structures called lamellar bodies. Upon stimulation, alveolar type II cells secrete lamellar bodies to the alveolar air-liquid interface where they replace pulmonary surfactant spent during respiratory cycling. Owing to compression-expansion respiratory cycles, a wealth of membrane structures, which are thought to be related to surfactant metabolism, may be observed in alveolar spaces. In this context, lamellar bodies have been demonstrated to exhibit outstanding adsorption properties since they keep the molecular determinants defining surfactant activity. Nevertheless, the molecular mechanism of lamellar bodies adsorption to the interface is not well-understood yet. Additionally, lung surfactant preparations used for both research and clinical applications are routinely purified from bronchoalveolar lavages of animal lungs, thus they are mainly composed by already used pulmonary surfactant complexes in which lamellar bodies are a minority component. Alternatively, a pulmonary surfactant purified from human amniotic fluid has been recently demonstrated to keep structural features of a freshly secreted surfactant.

In the present work, we compare interfacial adsorption kinetics of a surfactant purified from porcine lungs with those of a surface-active preparation obtained from human amniotic fluid using a Wilhelmy surface balance. In addition, Langmuir interfacial films were transferred onto glass coverslips and observed under epifluorescence microscopy. We observed meaningful differences between the behaviour of both surfactants that may be related to their contrasting structural features.

P-100 (O-037)

Microfluidic platforms for the handling, manipulation, and analysis of model cells

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Biological cells in their natural environment experience a variety of external forces such as fluidic shear stress, osmotic pressures, and mechanical loads. How the cell membrane itself responses to such forces is of great interest. Synthetic membranes such as giant unilamellar vesicles (GUVs) offer a reduced cell model, whereby individual components can be isolated and studied without interference from cellular complexity (Robinson 2019). However, being able to handle and apply forces to these delicate objects in a controllable manner is non-trivial. Therefore we present several microfluidic methods to capture, analyse and apply a variety of forces to GUVs. First we present novel methods for the capture and isolation of vesicles for membrane pore analysis (Robinson 2013; Yandralli & Robinson 2019). Next we discuss a device that contains micro-patterned electrodes which allow the application of electric fields and observations of subsequent membrane fusion (Robinson 2014). While membrane proteins are a crucial part of the cellular response to external stimuli, lipid rafts are thought to play an important role in the spatial organization of membrane proteins. To this end, we produce vesicles with membrane domains to model them and explore their behaviour in response to external forces. We use a valve-based system to apply precise fluidic shear stresses to vesicles (Sturzenegger, Robinson 2016). The final device comprises an integrated micro-stamp which can mechanically compress GUVs to study the effects that deformation has on the membrane and domains (Robinson 2019 *submitted*).

Yandrapalli, N., and Robinson, T. (2019). Ultra-high capacity microfluidic trapping of giant vesicles for high-throughput membrane studies. *Lab Chip* 19, 626–633.

Sturzenegger, F., Robinson, T., et al. (2016). Membranes under shear stress: visualization of non-equilibrium domain patterns and domain fusion in a microfluidic device. *Soft Matter* 12, 5072–5076.

Robinson, T., et al. (2014). Controllable electrofusion of lipid vesicles: initiation and analysis of reactions within biomimetic containers. *Lab Chip*, 2852–2859.

Robinson, T., et al. (2013). Microfluidic trapping of giant unilamellar vesicles to study transport through a membrane pore. *Biomicrofluidics* 7, 44105.

Robinson, T. (accepted 2019). Microfluidic handling and analysis of giant vesicles for use as artificial cells. *Advanced Biosystems.*

P-101 (O-038)

Quantified Efficiency of Membrane Leakage Events Relates to Antimicrobial Selectivity

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In many therapeutic strategies like killing pathogenic microbes by antimicrobial peptides, drug delivery, endosomal escape, and in disease-related processes such as apoptosis, the membrane is permeabilized.

Our concept for quantifying the efficiency of individual membrane leakage events replaces less stringent descriptions of dye leakage. The concept is applicable to many types of leakage events including thinning, defects, (toroidal) pores, or channels.

We measure release and fluorescence lifetime of a self-quenching dye. Apart from the dose-response, our analysis also quantifies the efficiency of individual leakage events. Additionally, cumulative leakage kinetics can indicate certain membrane permeabilization mechanisms. For example, applying our concept to three series of antimicrobial peptide analogues shows how the leakage mechanism and leakage efficiency of a given compound change with lipid composition. Thus, lipids play an important role for the selectivity of membrane permeabilization.

I will also point out aspects to consider when comparing leakage in vesicle of various sizes or cells, the possible occurrence of more than one type of leakage event, and artefacts from vesicle aggregation or fusion.

The concept for the quantitative description of leakage behaviour and understanding of leakage mechanisms aids the design and improvement of membrane-active antimicrobials.

P-102

Transversal Rotation of Unsymmetrical Bolaamphiphile Molecules in Lamellar Structures

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Bipolar lipids found in archaebacterial membranes, generally termed bolaamphiphiles, induce increased stability in membranes exposed to environments such as acidic conditions, high temperatures, high salt concentrations and/or absence of oxygen. Several approaches to synthetic bipolar lipids have been performed in order to mimic archaeal membranes. In our laboratory dissymmetrical bolaamphiphiles bearing a neutral glycosidic polar head and an electropositive ammonium group at the opposite end of a polymethylene bridging spacer were efficiently synthesized. These bolaamphiphiles self-assemble into monolayer lipid membranes (MLMs), reproducing the unusual architecture of natural archaeal macrocyclic bipolar lipids. Such bipolar lipids offer several advantages for the construction of advanced liposomes that are characterized by high mechanical and thermal stabilities due to the organization of the membrane. This work presents the e.p.r. study of the transversal rotation (flip-flop) of a spin-labelled bolaamphiphile in medium-sized vesicles. The transversal rotation is slower than in phospholipid vesicles presumably because two polar heads of the same molecule should transverse the lipid barrier simultaneously during flip-flop.

P-103

The mechanism of antimicrobial peptide synergy

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We report our recent finding that a mixture of immune peptides that is previously known to kill bacteria more efficiently (synergy) presented an unexpected suppression of cytotoxicity when administered to eukaryotic cells, indicating the "double-benefit" of the peptide cocktail. Mechanistic studies suggest that these peptides assemble into a hetero-oligomer in membranes that exhibits functions that are different from individual peptides. One of the major bottlenecks for the broader applications of antimicrobial peptides (AMP) as an antibiotic alternative is their unpredictable side effects, because these peptides typically have several targets and functions inside our bodies. Therefore, lowering the dosage and clarifying the role of these peptides against our human bodies are the top priorities for enabling their major impact on the infection treatments. In 2000, Nagaoka and coworkers have reported that *Escherichia coli* and *Staphylococcus aureus* were killed much more efficiently when two types of peptides are mixed (synergy). In addition, last year my group has discovered that the cytotoxicity of these individual peptides is reduced when they are combined (antagonism). These discoveries suggest that we can "double-benefit" from the synergies for broadening the therapeutic window by orders of magnitude by mixing the right couple of antimicrobial peptides at the right ratio, opening a new horizon in the antimicrobial peptide research. However, the underlying mechanism of these synergies is completely left unexplored due to the limited available characterization tools that provide the information on the peptide-peptide interactions. How does exactly the same couple of peptides kill bacteria and protect the host cells more efficiently? In this work, we studied the mechanism of the antimicrobial peptide synergistic effects at the molecular level by employing electrochemical methods recently developed in my group, combined with circular dichroism, quartz crystal microbalance, isothermal titration calorimetry, and fluorescence recovery after photobleaching. Understanding the mechanism of the synergy will be a breakthrough for developing the peptide-based infection treatment and potentially can contribute to the current crisis of resistance.

P-104

The interaction of viral fusion inhibitor lipopeptides with biomembranes is improved by PEG linker length

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Human parainfluenza viruses (HPIV) and respiratory syncytial virus (RSV) are paramyxoviruses that are among the most common respiratory pathogens causing pneumonia and death among infants and children worldwide. Nowadays, acute respiratory infections are the leading cause of mortality in children under the age of 5, accounting for nearly 20% of childhood deaths worldwide (nearly 3 million children each year). There are no effective treatments available. Consequently, there is an urgent demand for efficient antiviral therapies. Infection of healthy cells by these respiratory viruses requires fusion of the viral membrane with the target cell membrane, a process mediated by the trimeric viral fusion (F) protein. Fusion inhibitor peptides inhibit viral fusion by binding to F's transient intermediate, preventing it from advancing to the next step in membrane fusion. Here, we evaluated modifications of lipid-tagged F-derived peptides with different lipid moieties and different PEG linker length to search for properties that may associate with efficacy and broad-spectrum activity. Fluorescence spectroscopy was used to study the interaction of the peptides with biomembrane model systems, using partition assays. Using acrylamide, a quencher of tryptophan fluorescence, it was possible to understand the preferential localization of the peptides in lipid bilayers. The interaction of the peptides with human blood cells was evaluated using the dipole potential probe di-8-ANEPPS and atomic force microscopy. The interaction of the peptides with biomembranes seems to be influenced by PEG linker length. These data provide new insights about the dynamics of peptide-membrane interactions of these peptides, known for their potential as antiviral drugs.

P-105

nMERLIN: novel Mitochondria-ER Length Indicative Nanosensor based on BRET

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The contacts between the endoplasmic reticulum (ER) and mitochondria play a key role in cellular functions like the exchange of lipids and calcium between both organelles, as well as in apoptosis and autophagy signaling. The molecular architecture and spatiotemporal regulation of these distinct contact regions remain obscure and there is a need for new tools that enable tackling these questions. Here we present a new Bioluminescence Resonance Energy Transfer (BRET)-based biosensor for the quantitative analysis of distances between ER and mitochondria that we call MERLIN (Mitochondria ER Length Indicator Nanosensor). The main advantages of MERLIN compared to available alternatives are that it does not rely on the formation of artificial physical links between the two organelles, which could lead to artifacts, and that it allows to study contact site reversibility and dynamics. We show the applicability of MERLIN by characterizing the role of the mitochondrial dynamics machinery on the contacts of this organelle with the ER.

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The effect of membrane curvature on the formation of asymmetric phosphatidylserine-containing vesicles

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Biomembranes are asymmetric in the distribution of lipids in two leaflets which is related to the physiological functions of membranes such as the cell apoptosis. Our lab has proposed a novel method to produce controllable asymmetric lipid vesicles using Ca^{2+}. The enrichment of phosphatidylserine (PS) molecules in the inner leaflet is found not due to charge–charge attraction, but rather a modulation effect on the occupying size of the headgroups of PS molecules by Ca^{2+} which makes it flip to the negative curvature inner leaflet.[1] To further understand this formation process of asymmetric vesicles, we investigate the effect of membrane curvature on it in this study. The asymmetric lipid vesicles with different size were prepared by our method and we compared the forming process of them. From Nano DSC results, with the size of vesicles reducing from 400 nm to 100 nm, the main phase transition peak moved to higher temperature and became broader. However, when the size decreased to 50 nm, there is a shoulder peak occurring during phase transition suggesting the smaller vesicles experienced a more drastic redistribution (flip-flop) of lipids during the formation of asymmetric vesicles. Additionally, combined with the fluorescence quenching assay, we calculated the rate constants of the flip (k_{in}) and flop (k_{out}) using the kinetic model we established. The results showed that both k_{in} and k_{out} increased with the decrease of vesicles size but the increase of k_{in} is larger which lead to a higher asymmetry degree of vesicles. All the results above illustrated that membrane curvature could influence the formation process of asymmetric vesicles and the vesicle with larger membrane curvature would have higher asymmetry degree.

References

[1] Sun HY, Deng G, Jiang YW, Zhou Y, Xu J, Wu FG, and Yu ZW. Chemical Communications, 2017; 53: 12762.

P-107

Effect of butein (hydroxy-derivative of trans-chalcone) on a structure of poly-L-lysine and DPPC/DPPG membranes.

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Butein is a hydroxyl derivative of trans-chalcone and as polyphenols exhibits important biological functions. A molecular mechanism by which butein acts is not fully understand, and a character of interactions between this compound and proteins or lipid membranes still needs to be investigated. The effect of butein on a secondary structure of free and membrane-associated poly-L-lysine (PLL) polypeptides as well as on a structure of free and PLL-associated dipalmitoylphosphatidylcholine/dipalmitoylphosphatidylglycerol (DPPC/DPPG) membranes was investigated using Fourier-transform infrared (FTIR) spectroscopy. Butein-induced changes in conformational (*trans*/*gauche*) state of hydrophobic region of lipid membranes and in a hydration of DPPC/DPPG membrane interface as well as in mutual interactions between electrostatically associated PLL molecules and DPPC/DPPG membranes were investigated using FTIR, vibrational dichroism spectroscopy (VCD) and transmission electron microscopy (TEM). Additionally, an influence of butein molecules on fibrillogenesis of PLL related to the α-helix-to-β-sheet and PPII-to α-helix transitions in secondary structure of PLL was determined for different external conditions.

P-108

Lipid scrambling induced by membrane active substances

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Membrane leakage assays based on vesicles loaded with self quenching dyes have been widely used for quantifying the activity of membrane active substances that induce membrane pores or leaks. Time-resolved fluorescence decays of calcein-loaded liposomes allow a parallel quantification of the free and entrapped dye fractions and their effective local concentrations.[1] Thus, different types and mechanisms of membrane leakage to aqueous solutes can be distinguished by this method. However, much less is known about another way of membrane permeabilization: the enhancement of lipid flip flop that causes a scrambling of the lipids between the leaflets. Since virtually all biological membranes show an asymmetric distribution of lipids between the two leaflets, such a scrambling should have massive impact on the cell even if it proceeds without leakage to aqueous solutes. In order to assess such activity, we utilized a recent protocol[2] to produce asymmetric vesicles with anionic diacyl phosphatidyl glycerol (POPG) in the outer layer exclusively. Addition of lipid scrambling agents permits the POPG to flip to the inner leaflet, which can be monitored in terms of a less negative zeta potential of the liposomes. The study compares the activities of nonionic detergents including n-Dodecyl β-D-maltoside, n-Octylglucoside and $C_{12}EO_8$ to induce lipid scrambling and pore formation, respectively.

References:

1 H. Patel, C. Tscheka, H. Heerklotz; *Soft Matter*, 2009, 5, 2849-2851.
2 M. Markones, C. Drechsler, M. Kaiser, L. Kalie, H. Heerklotz, S. Fiedler; *Langmuir*. 2018, 34(5), 1999-2005.

P-109

Membrane-permeabilizing activity of NSAIDs: a role in cardiotoxicity?

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The cardiovascular (CV) toxicity of nonsteroidal anti-inflammatory drugs (NSAIDs) was first highlighted by the development of coxibs, particularly rofecoxib, which was associated with an increase of myocardial infarction events.[1] Afterwards, the CV risk-benefit profile of NSAIDs has been reassessed, and cardiotoxicity is today recognized as a class effect of NSAIDs.[2] However, their mechanisms of CV toxicity are not understood until today, since different compounds with similar mechanisms of action exhibit distinct cardiotoxicity. For instance, diclofenac is among the most cardiotoxic compounds, while naproxen is associated with low CV risk.[3]

Since CV diseases have been related to alterations in membrane structure and composition,[4,5] and mitochondrial disfunction is related to cell death in myocardium,[6] this study aims at describing the interactions of NSAIDs with membrane lipids of the cell membrane and the mitochondrial membranes. The cell membrane was mimicked by liposomes made of 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC) and POPC:cholesterol (4:1). The inner and outer mitochondrial membranes were mimicked by liposomes made of POPC:cardiolipin (8.5:1.5) and POPC:phosphatidylinositol (8.5:1.5), respectively. Diclofenac and naproxen were tested to eventually correlate the drugs' effects on membrane lipids with their cardiotoxicity. The drugs' affinity for the membrane models was assessed through the partition coefficient by derivative spectrophotometry. Moreover, the drugs' effects on membrane permeability were evaluated by a leakage assay.

The affinity of the drugs for the model membranes was dependent on the structure of the compounds and on the lipid composition of vesicles but both drugs exhibited high affinity for all membrane models. Moreover, both diclofenac and naproxen induced permeabilizing effects, particularly on the models of the mitochondrial membranes. These data suggest that the cardiotoxicity mechanism of NSAIDs may be related to the disruption of the mitochondrial membranes, eventually leading to cell death.

References

1. Bresalier RS, et al. New Engl J Med.2005;352(11):1092-102.
2. Varga Z, et al. Cureus.2017;9(4):e1144-e.
3. Martin Arias LH, et al. J Clin Pharmacol.2019;59(1):55-73.
4. Escriba PV. Trends Mol Med.2006;12(1):34-43.
5. Pereira-Leite C, et al. Prog Lipid Res.2013;52(4):571-84.
6. Orogo AM, et al. IUBMB Life.2013;65(8):651-6.

P-110

Planar plasmonic antenna arrays resolve transient nanoscopic heterogeneities in biological membranes

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Resolving the various interactions of lipids and proteins in the eukaryotic plasma membrane with high spatiotemporal resolution is of upmost interest [1]. Here, we present planar plasmonic antenna arrays with different nanogap sizes (10-45 nm) combined with fluorescence correlation spectroscopy (FCS) to resolve dynamic nanoscopic heterogeneities in mimetic and living plasma membranes. Our innovative approach confines the excitation light within the fully accessible planarized hotspot region of the nanoantennas yielding giant fluorescence enhancement factors of up to 10^4-10^5 times together with nanoscale detection volumes in the 20 zeptoliter range [2]. We exploit these planar nanoantenna arrays to investigate the dynamics of individual fluorescently labelled lipids in membrane regions as small as 10 nm in size with microsecond time resolution. The existence of nanoscale assemblies of sterol and sphingolipids on mimetic as well as on living cell membranes has been questioned due to their highly transient and nanoscopic character. Our results on model lipid membranes reveal the coexistence of transient nanoscopic domains in both microscopically phase-separated regions with characteristic sizes < 10nm and lifetimes between 30 µs to 150 µs [3]. Currently, we are increasing the complexity of our mimetic system by incorporating hyaluronic acid (HA) to our multi-component lipid membranes. HA is an abundant glycoprotein of the extracellular matrix and recent work points to the glycocalyx as an important local organizer of the biological membrane [4]. Our current experiments combining atomic force microscopy and plasmonic nanoantennas aim at deciphering how HA contributes to phase segregation and nanoscale dynamic partitioning of mimetic biological membranes.

[1] D. Lingwood, K. Simons, *Science* **327**, 46 (2010).
[2] Flauraud, V. et al., *Nano Lett.* **17**, 1703−1710 (2017)
[3] Winkler, P.M. et al., *ACS Nano* **11**, 7241–7250 (2017)
[4] Subramaniam, A.B. et al., *Nat. Mat.* **12**, 128–133 (2013)

P-111

Edge tension and elasticity of cell-mimetic membranes and the effect of sugars

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The wide application of carbohydrate molecules in biomedicine underlies the interest towards investigating the molecular mechanisms of sugar-membrane interactions. Evidences were provided that the sugar molecules interact with the lipid bilayer, thus affecting its mechanical and electrical properties (Vitkova et al. Mol. Cryst. Liq. Cryst. 449:95, 2006; Vitkova et al. *Colloid Surf. A-Physicochem. Eng.* 557:51, 2018). Here, we report results for the edge tension of model lipid membranes in sugar-containing aqueous environments. Closed lipid bilayer structures named giant unilamellar vesicles (GUVs) with nearly spherical shape and diameters of tens of microns are used as a common biomimetic system for studying the physical properties of biomembranes. Combining fast phase-contrast imaging, digital-image analysis and membrane electroporation, we were able to monitor the rate of pore formation and closure and deduce the edge tension of membranes. The edge tension of palmitoyl-oleoyl phosphatidylcholine bilayers in the presence of sugar was obtained from the pore-closure analysis after electroporation of GUVs with strong electrical pulses (5 ms; 60-80 V/mm). Pore resealing was studied for vesicles in medium containing sucrose and/or glucose at concentrations from 0 to 0.4 mol/L. The edge tension is observed to decrease with increasing the sugar content in the aqueous surroundings. Our finding corroborates the stabilizing effect of sugar molecules on the hydrophilic edge in accordance with the concentration-dependent sugar binding to the membrane reported in the literature.

P-112

Spatial organization of lipid membrane inclusions is driven by membrane undulations

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Spatial ordering in cellular membranes and model systems is a widespread phenomenon. It is observed in multilamellar systems, where it leads to the spontaneous formation of domain stacks. Within a lipid bilayer, it manifests in the alignment of ordered domains (rafts) from the opposite monolayers. Accordingly, alignment is believed to be essential for transmembrane signal transduction. Hydration differences between the different lipid phases and interactions between different phases in opposite monolayers were hypothesized to act as driving forces for interbilayer ordering and intrabilayer domain alignment, respectively. Yet, the underlying molecular mechanisms are unknown. Here we show that shape fluctuations of lipid membranes promote the spatial organization of various membrane inclusions in both environments: stiff regions in opposing monolayers or bilayers attract each other because their alignment allows maximizing entropy (1). That is, alignment minimizes restraints on membrane undulations. Accordingly, fluid membrane regions harbour lipids that offer little resistance to bending and may thus undulate with higher curvature. The coupling energy is proportional to the domain area. It may not be sufficient to ensure alignment of very small domains (tens of nm in size). Alignment of these domains is driven by line tension, i.e. by the gain in energy that is provided by minimizing the hydrophobic mismatch at domain boundary (2) and minimizing curvature strain at the edge of the domain (3). This energy gain linearly depends on domain radius, i.e. it is less important for larger domains (>50 nm radius). Thus, the concerted action of both line tension and undulations leads to the spatial organization of membrane inclusions. The driving forces have their origin in the spatial inhomogeneities of membrane elastic properties. The work was supported by the grant of the President of the Russian Federation MK-3119.2019.4.

1. Galimzyanov et al. *Biophys. J.* **2017,** *112* (2), 339.
2. Galimzyanov et al. *Phys. Rev. Lett.* **2015,** *115* (8), 088101.
3. Galimzyanov et al. *Phys. Rev. Lett.* **2016,** *116* (7), 079802.

P-113

Impact of an antimicrobial peptide on the membrane fluidity of host membranes: influence of cholesterol and a hopanoid.

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The effect of the antimicrobial tetradecapeptide Polybia-MP1 on the dynamics, viscosity, and bending of the host membrane has been investigated using different membrane compositions. Membranes composed of pure POPC, and binary mixtures with cholesterol (Chol) or the hopanoid diplopterol (Dip) were selected, and the interaction of Polybia-MP1 with lipid monolayers and bilayers (GUVs and LUVs) was studied. Hopanoids are proposed sterol-surrogates in sterol-lacking bacteria. The inclusion into POPC/Chol occurs in a lesser extent and inducing less disorder than in the other membranes. This is intriguing because Dip has been proved to induce similar order than chol in model membranes and in bacteria. Despite the hopanoid, MP1 induced similar dye release from LUVs of POPC and POPC/Dip. The effect of the peptide on the membrane flexibility was investigated by generating nanotubes from single GUVs using optical tweezers. Analysis of the nanotube refraction showed that the membrane rigidity decreased with time and in the following order POPC<POPC/Dip<POPC/Chol. The differential interaction and consequent effect promoted by the peptide in membranes with Dip and Chol is a promising starting point for targeting antimicrobial peptides to bacterial membranes. Although electrostatic interactions are likely to be important in the mechanism of an antimicrobial peptide-induced defect in membrane integrity, the elasticity and fluidity modulated by the presence of sterol-surrogate diplopterol can also be important for Polybia-MP1 selectivity to these cells.

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Prediction of Chlorosulfolipid Membrane Structures Using Hybrid Molecular Dynamics Simulations

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Prediction of Chlorosulfolipid Membrane Structures Using Hybrid Molecular Dynamics Simulations

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Chlorosulfolipids (CSL) are the major components of flagellar membrane in sea algae. Unlike typical biological lipids with a hydrophilic head group and hydrophobic hydrocarbon tail, CSLs are well known as lipids containing the sulfonate group and chlorines in the head group and hydrocarbon tail group. Among the CSLs, Danicalipin A, Malhamensilipin A, and Mytilipin A are representative lipids. However, it was impossible to isolate Danicalipin A from the alga because of the lack of technology when it was first identified. 40 years later, the research on CSLs have attracted renewed attention by several groups because of their toxicity. It was reported that Malhemensilipin A inhibits bacterial growth and the lysis of mammalian erythrocytes. Mytilipin A is also known as the poison such as yessotoxin that is responsible for human seafood poisoning. Until now, however, there is not enough structural information of CSLs that reveals how the CSLs play biological roles. Thus, we combined coarse-grained (CG) and atomistic molecular dynamics (MD) simulations to obtain some insights into the CSL membrane structures. First, The CG model based on Martini force fields was used to predict the mesoscopic membrane structure of CSLs. We observed that unlike the other CSLs, Danicalipin A forms a stable monolayer membrane structure. Based on these results, we performed atomistic MD simulations of the corresponding atomistic model and obtained a stable CSL membrane structure, in which the CSL lipids adopt a bent structure. In addition, we calculated the membrane thickness, area per lipids, and order parameter to show the membrane integrity. These results provide the significant information to understand the CSL membrane structure.

P-115

Curving in or out: membrane remodeling by ions and molecules as assessed on giant vesicles

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Giant vesicles, with sizes in the 10-100 μm range, are a fascinating model membrane system, which has been initially established and used as a workbench for studying basic properties of simple lipid bilayers (Dimova, *Annu. Rev. Biophys*., 2019). Nowadays, they are increasingly employed by biophysicists to unravel the mechanisms driving various biological processes occurring at the level of the cell membrane. Here, we will introduce approaches employing giant vesicles to assess mechanical properties of membranes such as bending rigidity and spontaneous curvature. The latter is readily generated by asymmetries across the membrane and might be a governing factor in defining shapes of membrane organelles and in remodeling them. Several examples for the generation of spontaneous curvature will be considered: asymmetric distribution of ions on both sides of the membrane (Karimi et al. *Nano Lett*. 18:7816, 2018), insertion and desorption of the ganglioside GM1 from the bilayer leaflets (Dasgupta et al., *Proc. Natl. Acad. Sci. USA*. 115:5756, 2018; Bhatia et al., *ACS Nano* 12:4478, 2018), and asymmetric adsorption of poly(ethylene glycol) (Li et al., *Proc. Natl. Acad. Sci. USA*. 108:4731, 2011; Liu et al., *ACS Nano* 10:463, 2016). All of these factors can drive the spontaneous formation of cylindrical or necklace-like lipid nanotubes which evidence the generation of membrane spontaneous curvature. The presented examples will demonstrate that simple physicochemical effects can easily lead to membrane remodeling to a similar extent as when driven by proteins and active processes which were recently reviewed in the Biomembrane curvature and remodeling roadmap (Bassereau et al., *J. Phys. D: Appl. Phys*. 51:343001, 2018).

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Designing supramolecular guanidinium-based non-viral vectors: binding to DNA

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Functional supramolecular systems based on host-guest interactions of adamantyl guanidines (AG) with phosphatidylcholine liposomes and amphiphilic β-cyclodextrin vesicles were prepared and characterized. Size (~150 nm) and surface charge of these nanovesicles were determined after host-guest complexation with a series of AGs, whose incorporation efficiency was also tested. In order to evaluate the potential of the system as a gene vehicle, we assessed the binding of fluorescently labeled DNA to AGs presented by the nanovesicles. Fluorescence correlation spectroscopy (FCS) is used for diffusion time measurement of rodlike, double stranded DNA oligos (120 bp, 40 nm), at concentrations of 10-20 nM (oligos). DNA was titrated by nanovesicles (concentrations 1-10 nM). While free DNA diffusion time is ~400 μs, upon nanoparticle addition the observed diffusion time would increase to 10 ms which corresponds to a calculated diffusion time for a 150 nm nanoparticle. That is, above threshold concentrations (dependent on AG derivative) of nanovesicles they would remove all free DNA from solution. FCS would then detect the diffusion time (~10 ms) of the DNA-nanoparticle complex. There was a stark contrast in binding stoichiometry for nanovesicles that feature different types of AG derivatives. This result strongly indicates a different steric or Coulomb interaction on behalf of the adamantane-substituted guanylhydrazones vs. adamantyl aminoguanidines. Eventually, the change in the surface charge of nanovesicles due to complexation would, in some cases, lead to aggregation that was observed as extremely high characteristic diffusion times (~1 s).

Special thanks to Prof. Dr. Kata Mlinarić-Majerski (Ruđer Bošković Institute, Zagreb) for providing adamantyl guanidines samples and to dr. Ajasja Ljubetič (National Institute of Chemistry, Ljubljana, Slovenia) for providing access to the FCS instrument and training to AŠ and TV.

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EIS AND SERS ANALYSIS OF ANCHOR MOLECULES FOR TETHERED BILAYER LIPID MEMBRANE FORMATION

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The tethered bilayer lipid membranes (tBLMs) are considered as perspective experimental platforms for membrane biosensors and may be suitable for a broad spectrum of biophysical experiments such as peptide/membrane interactions, protein/membrane interactions, lipid phase transitions and others. tBLMs attachment to the silver surface allows the monitoring of biologically relevant events with electrochemical impedance (EIS) and surface enhanced Raman (SERS) spectroscopical techniques.

It is known that structure of the self-assembled monolayers (SAMs) used to anchor phospholipid bilayers to surfaces affects the functional properties of the tethered bilayer lipid membrane.

To evaluate the differences in tBLM formation on flat silver surface two types of anchor molecules were chosen to form SAMs – long strand thiolipid Wilma's compound (WC14) and four different length backfillers 3-mercapto-1-propanol (3-M-1-P), 4-mercapto-1-butanol (4-M-1-P), 6-mercapto-1-hexanol (6-M-1-H) and 9-mercapto-1-nonanol (9-M-1-N). EIS results showed the ability to successfully form functional SAMs and tBLMs on the plain silver surface, which leads to further experiments with this system using the SERS technique on nanostructured silver surface.

For the SERS analysis the same backfillers (3-M-1-P, 4-M-1P, 6-M-1-H and 9-M-1-N) were used in a pair with WC14 compound to form SAMs with different structural properties. Analysis of SERS spectra form anchor molecules showed, that WC14 and all of the tested short strand backfiller molecules are suitable for immobilization on nanostructured silver surface. Furthermore, by using different length backfiller molecules it is possible to control long strand anchor molecules conformation on the surface.

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Controlled adhesion, membrane pinning and vesicle manipulation by Janus and homogeneous particles

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Janus particles, giant vesicles, magnetophoresis, engulfment, penetration depth

Studying the adhesion and engulfment of particles by biomembranes is important for a wide range of processes, such as drug delivery, biomedical imaging and cytotoxicity. Many previous studies have illustrated the effect that properties such as particle surface chemistry, responsible for the adhesion strength in the particle-membrane interactions, or the particle size, can have on interactions. Previously we have presented how nano-particles interact with biomimetic membranes, giving insights into their function as anti-microbial agents (Ewins, et al. Adv.Sci., 2019;6;4). Here, we demonstrate that the geometrical asymmetry of the particle surface is also responsible for how particle-membrane interactions proceed. We use metallo-dielectric 'Janus' particles having two regions of distinctly surface properties. Other studies have demonstrated their potential applications, such as the broken symmetry enabling imaging/quantification of rotational dynamics, or the ability to combine two incompatible properties on single-particles. We investigate the wrapping behaviour of biomembranes in contact with such particles.

We use micron-sized particles; the metallic-hemisphere coating is prepared via metal vapour deposition on a dielectric polystyrene particle monolayer (Bharti, et al. J.Am.Chem.Soc, 2016;138;45), while non-coated particles provide a reference. The biomembrane system consists of giant unilamellar vesicles (GUVs), a convenient model system as they mimic the size and curvature of plasma membranes, while simultaneously offering direct visualization of the membrane under the microscope. Incubating the GUVs with the particles results in a pinning of the contact line for membranes in contact with Janus particles, and complete wrapping for homogeneous particles. The degree of particle penetration is characterised as a function of adhesion energy. Fluorescent LUV adhesion to the particle surfaces, in the presence of different salt concentrations, reveals the electrostatic attraction between the membranes and particles is only partially responsible for adhesion. We demonstrate a potential use of these metal-coated Janus particles as microbots to manipulate and transport vesicles, due to the magnetophoretic behaviour of the iron patch.

Finally, we investigate the role that membrane spontaneous curvature can have on the engulfment behaviour of particles, as has been investigated and modelled previously using a theoretical approach (Agudo-Canalejo, Lipowsky, ACS Nano, 2015;9;4).

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How Nanoscale Protein Interactions Determine the Mesoscale Dynamic Organisation of Membrane Proteins

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The spatiotemporal organization of membrane proteins is often characterised by the formation of large protein clusters and complex mixtures of lipids. Modelling the nanoscale heterogeneities within physiological membranes in molecular detail, has been confounded by the inherent difficulties of simulating large numbers of proteins over meaningful timescales. We have developed and a mesoscale model that can incorporate 1000s of proteins, trained on the results of coarse-grained molecular dynamics simulations[1]. We achieve simulations over timescales that allow direct comparison to experimental data, in particular, the single molecular tracking of bacterial outer membrane proteins (OMPs). In *Escherichia coli*, OMP clustering leads to OMP islands, the formation of which underpins OMP turnover[2] and drives organization across the cell envelope[3]. Using molecular dynamics and mesoscale simulations we show that specific interaction surfaces between OMPs are key to the formation of OMP clusters, that OMP clusters present a mesh of moving barriers that confine newly inserted proteins within islands, and that mesoscale simulations recapitulate the restricted diffusion characteristics of OMPs in vitro. We extend the mesoscale model to explore the impact of lipid composition and protein activity on the organisation of receptors in mammalian cell membranes. The mesoscale model provides a powerful integrated approach to understand physiologically accurate biological membranes at mesoscale length and time scales.

References

1. Chavent *et al, Nat Comms*, 9, 2846, **2018**
2. Rassam *et al*, *Nature*, 253, 333-6, **2015**
3. Rassam *et al, Nat Comms*, 9, 1082, **2018**

P-120

Protomembranes at the origin of life

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Given the probable extremely contrasted environmental conditions at the origins of life (high temperature, pressure and pH), the origin and nature of the first cells membranes is still an open question. Due to complex organic carbon limitations, the first membranes were most likely composed of simpler, single chain fatty acids, which raises questions as how they could withstand the very variable and extreme surrounding environment. A novel membrane architecture has recently been proposed to explain the stability of contemporary poly-extremophilic archaea, in which apolar alkanes might be present in the midplane of the lipid bilayer. By populating the midplane, the alkanes should shift the functional domain of the membrane towards higher pressure and temperature.

Following the same line of argumentation, the current project proposes a similar architecture for protocell membranes. In this model, the bilayer is made of short single chain amphiphiles (e.g. decanoic acid - decanol), with an apolar lipid (e.g. eicosane) inserted in its midplane. If the functional predictions are demonstrated, this will represent a possible strategy to explain the survival of the membranes of the first forms of life to the extreme conditions of the early-Earth.

Several complementary techniques have been employed to characterize the model single-chain amphiphiles vesicles at different membrane compositions (apolar lipid presence / amount / type). Among them, Static / Dynamic Light Scattering allowed to observe vesicle appearance, characteristics and time stability. Differential Scanning Calorimetry was employed to detect the membrane phase transitions and stability with temperature. Fourier Transform Infrared Spectroscopy, able to detect changes in the vibrational mobility of the amphiphiles acyl chains, was done by following variations with both temperature (20 - 90 °C) and pressure (1 - 1000 bar). Furthermore, Fluorescence Spectroscopy using the Laurdan fluorophore was used to study the membrane fluidity with temperature.

Neutron scattering has been of particular importance to answer the questions arising from the investigation of the protomembrane model. In particular, Neutron Membrane Diffraction and Small Angle Neutron Scattering gave very promising results, with evidences on the actual localization and the stiffening effects of the apolar lipids intercalated in the membrane. The latest results, obtained with the above mentioned techniques, will be presented.

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Lipid mixing and titration in polymer-stabilized lipid nanodiscs

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"Lipid mixing and titration in polymer-stabilized lipid nanodiscs"

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Hydrolysed copolymers of styrene and maleic anhydride (SMA) are able to extract proteins from biological membranes, without the use of conventional detergents[1,2]. The discoidal structures formed by this process, termed Lipodisq nanoparticles (also known as Lipodiscs) are approximately 10 nm in diameter[1]. Recent publications have indicated that these nanoparticles are susceptible to collisional lipid transfer, and that the rate of lipid mixing is dependent on the conditions used[3]. The kinetics of lipid mixing between Lipodisqs of different composition, are quantified using NMR spectroscopy and FRET, demonstrating that the collisional lipid transfer in these systems rapidly destroys any spatial order in the extracted membranes (on a timescale of seconds to minutes at temperatures above the lipid phase transition). Here we exploit the property of lipid mixing to titrate specific lipids into Lipodisqs nanoparticles containing cytochrome c oxidase (CcO), the activity of which is enhanced by the presence of cardiolipin[4]. By following the oxidation of reduced cytochrome c, we can measure the effect of changes in lipid composition (delivered by collisional transfer) on CcO enzyme activity and demonstrate that lipid-polymer nanoparticles can be used for the study of specific protein-lipid interactions within a native-like membrane system.

(1) Orwick, M. C. *et al.* Detergent-Free Formation and Physicochemical Characterization of Nanosized Lipid-Polymer Complexes: Lipodisq. *Angew. Chemie - Int. Ed.* **2012**, *51* (19), 4653–4657.
(2) Long, A. R. *et al.* A Detergent-Free Strategy for the Reconstitution of Active Enzyme Complexes from Native Biological Membranes into Nanoscale Discs. *BMC Biotechnol.* **2013**, *13* (1), 41.
(3) Grethen, A. *et al*. Role of Coulombic Repulsion in Collisional Lipid Transfer Among SMA(2:1)-Bounded Nanodiscs. *J. Membr. Biol.* **2018**, *251* (3), 443–451.
(4) Sedla, E. *et al.* Phospholipase A 2 Digestion of Cardiolipin Bound to Bovine Cytochrome c Oxidase Alters Both Activity and Quaternary Structure †. **1999**, 14966–14972.

P-122

In vitro reconstitution of double membrane fission

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Mitochondria self-assemble into a network with a rich dynamical behaviour. Here, they split apart or merge together to adapt to the energetic demands of the cell. Particularly, mitochondrial division (MD) has attracted much attention due to the its implication in aging, neurogenerative diseases and acute brain injury among other pathologies. MD requires fine kinetic control over topological transformations of both, the inner and outer mitochondrial membranes, achieved largely through synergistic action of proteins from the dynamin superfamily. However, the molecular description of the process is still lacking mainly due to the absence of an *in vitro* system mimicking the correct topology of MD.

We have recently developed an *in vitro* system that allows the reconstitution of the MD process with its correct topology. The double membrane nanotubes (dNTs) are formed between polymer micro-pillars standing on a coverglass bottom of a microfluidic chamber. Such design allows controlling dNTs length and topology as well as to perform controlled perfusion with desired solutions. Due to close proximity to the coverglass, the dNTs can be observed in real time with high power optical objectives of a fluorescence microscope.

Using the dNT system we reconstituted the process of double membrane fission induced by a bulk force and by dynamin 2, a protein implicated in the MD. Our data show for the first time how membrane mechanics affects the process of double membrane fission.

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X-RAY STUDIES OF ANTIMICROBIAL PEPTIDE ACTIVITY IN ASYMMETRIC INNER AND OUTER MEMBRANE MIMICS OF GRAM-NEGATIVE BACTERIA

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Infectious diseases caused by multi-resistant pathogenic bacteria are rapidly gaining grounds world-wide. One highly promising strategy to combat infectious diseases is based on antimicrobial peptides (AMPs), effector molecules of innate immunity, mostly acting on the cell envelope. The majority of peptide/lipid interaction studies to gain information on the molecular mechanism(s) of AMPs have been performed using model membranes with a symmetric lipid distribution.

The outer membrane (OM) of Gram-negative bacteria however, is well-known for its asymmetric distribution of lipopolysaccharides (LPS) and phospholipids. Based on cyclodextrin-mediated lipid exchange [1] we were able to produce OM mimics in form of asymmetric large unilamellar vesicles (aLUVs) with an outer leaflet enriched in LPS and an inner leaflet composed of a mixture of palmitoyloleoyl-phosphatidylethanolamine (POPE) and -phosphatidylglycerol (POPG).

In addition, inner membrane (IM) mimics were investigated. Evidence of asymmetry within the cytoplasmic membrane of Gram-positive bacteria has existed for some time [2], hinting that this may also occur for the IM in Gram-negative bacteria. This is central to the activity of AMPs, since their activity and in turn mode of action is governed by the lipid composition [3]. Therefore, aLUVS consisting of a mixture of POPE, POPG and tetraoleoyl-cardiolipin (TOCL) with a reduced POPE content on the outer leaflet of the bilayer were used to mimic the IM.

Structural and thermodynamic properties of IM and OM mimics were studied using different techniques, including small angle scattering (SAS), differential scanning calorimetry (DSC) and dynamic light scattering (DLS). This provides us with a valuable platform for interrogating specific interactions of antimicrobial peptides with either membrane.

[1] M. Doktorova et al., Nature protocols (2018), 13(9), pp 2086–2101
[2] J. E. Rothman and E. P. Kennedy, J. Mol. Biol. (1977), 110, pp 603-618
[3] K. Lohner, Curr. Top. Med. Chem. (2017) 17, pp. 508-519

P-124

Coupling of Leaflet Structure in Asymmetric Lipid Vesicles

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Lipid asymmetry is a hallmark of biological membranes [1]. In particular, prototypical mammalian plasma membranes are known to be composed of an outer leaflet enriched in cholinephospholipids, while the majority of the aminophospholipids are confined to the inner leaflet [2]. One of the enduring questions concerning plasma membrane architecture and lipid asymmetry is the possibility of bilayer leaflets being coupled to each other, which may influence a number of physiological processes that require communication between interior and exterior of the cell [3]. A relatively new model system to study transbilayer coupling mechanisms are asymmetric large unilamellar lipid vesicles (aLUVs), which are produced via cyclodextrin-mediated lipid exchange [4]. These systems were shown to be stable over several days [5] and have already been investigated by elastic scattering techniques (small-angle neutron and X-ray scattering; SANS/SAXS), providing insight into structural coupling of the leaflets [6]. The techniques provide static structural features of each leaflet (thickness, area per lipid), including the absence/presence of partial hydrocarbon chain interdigitation at the interface between the two leaflets. To study in detail the effect of hydrocarbon chain composition, we use combinations of dipalmitoylphosphatidylcholine (DPPC) in the inner leaflet and mixed chain lipids in the outer leaflet, in particular C16:0/C18:1 PC (POPC), C18:0/C18:1 PC (SOPC), C18:0/C14:0 (SMPC), C14:0/C18:0 (MSPC) and C16:0/C14:0 PC (PMPC). We present leaflet specific structural data for these systems.

[1] a) A. J. Verkleij et al., Biochim Biophys Acta 323,178 (1973); b) M. S. Bretscher, Nat New Biol 236,11 (1972).
[2] P. F. Devaux and R. Morris, Traffic 5, 241 (2004).
[3] K. Simons and D. Toomre, Nat Rev Mol Cell Biol 1, 31 (2000).
[4] M. Doktorova et al., Nat prot 13.9, 2086 (2018).
[5] a) F. A. Heberle et al., Langmuir 32, 5195 (2016); b) D. Marquardt et al., Langmuir 33, 3731 (2017).
[6] a) B. Eicher et al., J Appl Crystallogr 50, 419 (2017); b) B. Eicher et al., Biophys J 114, 146 (2018).

P-125

The Development of Nanotechnologies to Study Surfactant-Membrane Interactions

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In this research the kinetics of small molecules crossing through model bio-membranes upon simulated disruption is explored. The short and long range order of lipid assemblies and their interactions with surfactants were investigated as a function of temperature, time, surfactant concentration and bio-membrane composition using a variety of techniques including Wide and Short Angle X-Ray Scattering (SAXS / WAXS) at the Diamond Light Source Synchrotron, UK, SS NMR and fluorescent microscopy. These studies have led to an increased understanding of the mechanisms and kinetics technique behind complex biologically relevant membranes and surfactant assemblies.

P-126

Effects of phase state and coexistence on membrane fusion

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Cell membranes are believed to be heterogeneous and exhibiting lipid domains (or rafts) of coexisting phases. The phase heterogeneity can govern cellular functions and processes. In this work, we address the question how the process of fusion is affected by the membrane phase state (fluid or gel) and by phase coexistence. We also investigate whether it is possible to confine fusion into specific regions of the membrane by using membranes displaying phase separation and whether gel domains can be dissolved upon fusion. Our system is based on small fusogenic liposomes (100 nm) and giant unilamellar vesicles (GUV, 10-50 μm), the latter being commonly used for investigating membrane phase coexistence with optical microscopy. The fusogenic liposomes are cationic large unilamellar vesicles (LUVs) and the GUVs are neutral or negatively-charged. By choosing the lipid composition of the GUVs, we modulate the phase state and the charge of the different phases (charged or neutral, fluid or gel). Fusion efficiency is characterized using microscopy-based fluorescence resonance energy transfer (FRET) and content mixing assays. For homogenous GUVs in the gel phase, no fusion occurs regardless of the membrane charge, whereas we observe high fusion efficiency for fluid negatively charged vesicles (see also Lira et al. Biophys. J. 116:49, 2019) and only docking and hemifusion for neutral fluid GUVs. For phase-separated GUVs exhibiting charged-gel and neutral-fluid domain coexistence, we observed docking at low LUVs concentration, whereas higher LUV concentration leads to an increase in membrane area of the vesicle (due to LUV lipid transfer) and dissolution of the gel domains. For GUVs with neutral-gel and charged-fluid phase coexistence fusion proceeds instantaneously and is also efficient at low concentration of LUVs. These findings have the potential for unravelling important role on the regulation of the interactions between cells and liposomes used in drug delivery systems.

Financial Support: FAPESP 18/08014-4

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Selective membrane permeabilization induced by synthetic antimicrobials

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Natural or synthetic antimicrobial agents (AMPs or smAMPs) acting on the cell membrane are promising alternatives to classical antibiotics because they are less prone to resistance. Common to all antimicrobial treatment is the need for selectivity.

A series of smAMPs with varying antibacterial gram-selectivity is discussed. Sophisticated analysis of vesicle leakage mechanisms and kinetics reveals how the combination of smAMP design and lipid mixture can determine the mode of action. Activity and selectivity relate to molecular mechanisms like asymmetry stress or rare and strong leakage events. These rare events could involve stabilization of local curvature (as in toroidal pores) or electrostatic lipid clustering. We also discuss how vesicle leakage experiments relate to biological activity. A general view on membrane leakage and antimicrobial activity and selectivity will aid future design of antimicrobials as well as improvement of model for *in vitro* studies.

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Mechanism and pathways of structural instability completing membrane fission.

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Membrane fission occurs throughout each eukaryotic cell, underlying intracellular trafficking and organelles morphogenesis. To undergo scission a short membrane neck connecting two dividing compartments needs first to be squeezed down to the lipid molecule scale. The accumulation of bending stress in a membrane of a highly-curved neck leads to bilayer structure instability, resolved through membrane fission at finite curvature. The elastic properties of a membrane at such curvatures as well as the molecular pathways of lipid bilayer structural reorganization induced by bending stress has not been directly assessed yet. Here we introduce a novel approach to probe the elastic response of a membrane of ultra-short (~100 nm) cylindrical lipid nanotubes, pulled from reservoir membranes. By applying nanoscale electro-actuation on a NT, we could directly assess the material parameters of its membrane, during the fission process produced by different force factors as well. We found that the NT membrane retains linear elasticity characterized by macroscopic (bulk) bending rigidity over the entire range of curvatures up to the loss of stability. It was the high bending stress accumulated in the NT membrane that provoked the stochastic destabilization of the lipid bilayer associated with the transient formation of lipids packing defects in it. The particular intermediates through which the structural reorganization of the membrane takes place then depended on the bending rigidity of the membrane. A membrane of NTs pulled from soft lipid bilayers (having physiologically relevant bending moduli) came to critical values of bending stress at curvatures compared with molecular dimension and its scission was seamlessly realized through a hemifission intermediate (local self-closure of internal monolayer). While stiffer membranes (but still physiologically relevant) attained bending stress sufficient to induce membrane instability at significantly lower curvatures resulted in membrane poration leading to either fission or rupture of both the NT and the parent membrane. Material compliance thus emerges as a critical regulator of the topology of cellular membrane remodelling.

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Functional nanostructure of NhaA protein in tethered lipid bilayer membranes

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The study of active membrane proteins requires an environment which is as close as possible to their natural environment to retain the protein function, while at the same time keeping the system as simple as possible to allow for an experimental characterization and to be able to identify factors which influence the system. Tethered lipid bilayers (tBLMs) represent an experimentally accessible and stable model for biological membranes that offers a high level of control over the structure. Due to their connection to a planar surface they can form a more natural environment for membrane protein incorporation than the widely used solid supported bilayers and are stable for months [1]. We report the use of such a tBLM system to investigate how the structural factors of the surrounding membrane influence the incorporation and subsequently the activity of the NhaA protein, which is the main sodium proton antiporter of *Escherichia coli*. NhaA serves as the means for *E. coli* to maintain sodium homeostasis and for pH control [2]. Here we present a study on the incorporation of NhaA into PEG-tBLM on gold surfaces. We show the nanostructural characterization of highly covering tBLMs of different lipid composition with large fractions of incorporated NhaA by neutron reflectometry (NR) and how electrochemical impedance spectroscopy (EIS) can be used to investigate its activity.

NR allowed us to determine the nanostructure of the membrane/protein system to monitor structural variations and to precisely determine the amount of incorporated NhaA protein. Using EIS we obtained functional characteristics. It provided electrophysiological properties related mainly to ion permeability and indicated NhaA activity, as this is associated to an ionic current across the bilayer.

The combination of these two methods enables us to correlate structural and functional information of the NhaA-membrane system in order to understand the mechanisms behind these dependencies.

[1] M. Maccarini, L. Gayet, J.-P. Alcaraz, L. Liguori, B. Stidder, E. Watkins, J.-L. Lenormand, D. Martin, *Langmuir*, **2017**, *33*, 9988-9996.
[2] Etana PADAN, *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, **2014**, 1837. Jg., Nr. 7, S. 1047-1062.

P-130

Electrical phenomena associated with release of protons on the surface of bilayer lipid membrane

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The changes of the capacitance and electrostatic potential of bilayer lipid membrane (BLM) initiated by fast release of protons on its surface have been studied. The protons were released after photolysis of "Caged-H^+" - 2-methoxi-5-nitrosulphate (MNPS) bound on the membrane. This binding was controlled by measuring of the boundary potential (BP) by Inner Field Compensation (IFC) method developed by us earlier (Sokolov and Kuz'min, 1980, Biofizika, 25:170-172) or as the change of ζ-potential of liposomes measured by dynamic light scattering method. The illumination of BLM with bound MNPS by UV light resulted to small increase of its capacitance as well as to appearance of positive charge of its surface detected either as a fast shift of open circuit potential or as a slow BP shift measured by IFC method. The shifts of the membrane capacitance and BP decreased with decrease of pH and with increase of the concentration of buffer in the solution. The effect of buffer on the BP shift was not full and saturated at concentrations above 1 mM. It allows concluding that the BP change is assigned partially to decrease of the amount of the MNPS anions bound on surface of BLM due to their photolysis, partially – to binding of protons with the membrane. The shifts of the membrane capacitance and BP were observed on BLM formed from phospholipids (phosphatidylcholine or phosphatidylserine) as well as on BLM formed from neutral lipid glycerylmonooleate. The restoration of the capacitance and BP after the light flash took about ten seconds. It indicates that the binding of protons with the membrane leads to forming a long lived state, in which essential role play water molecules oriented on the membrane surface.

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Lipid-ordered domain boundary acts as attractor for curvature-inducing membrane inclusions

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Cell membranes represent a complex assembly of various lipids interacting with membrane proteins. Lipid diversity results in the formation of separate domains, also called lipid rafts, easily identified by in vitro experiments. Raft and surrounding membrane have different thicknesses of the lipid bilayer. On their boundary membrane deformations occur in order to compensate the thickness mismatch. Membranes may contain plenty of inclusions, inducing elastic stress: transmembrane proteins, peripheral proteins, nonlamellar lipids, etc. Deformations, induced by inclusions, can interfere with deformations, that arise near the domain boundary, leading to the specific arrangement of inclusions with respect to the boundary. To analyze such membrane-mediated interaction we utilized the linear theory of elasticity of lipid membranes. In the framework of the theory, elastic deformations of splay, tilt, lateral compression-stretching and lateral tension were taken into account. We revealed, that the domain boundary can serve as attractor for almost all types of membrane inclusions. By varying the lateral position of membrane inclusions we demonstrated that the total elastic energy of the system reaches its minimum when the inclusions are located in the vicinity of the domain boundary. The magnitude of the potential well was 0.4–0.5 k_BT/nm for membrane proteins, amphipathic peptides and hydrophobic molecules. For nonlamellar lipids, possessing spontaneous curvature of ±0.25 nm^{-1}, the depth of the energy well was about 0.15–0.2 k_BT/nm; the energy values are related to 1 nm along the domain boundary. We hypothesize that the attraction of membrane inclusions to the domain boundary may have an impact on cell signal transduction pathways, viral-induced membrane fusion, aggregation of amphipathic peptides.

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Nanoscale structure of biomembranes.

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Despite the biological significance of membrane nanodomains, details on their structure remain elusive.

Molecular dynamics (MD) simulations have increasingly become a powerful tool to provide spatial and temporal information of membrane substructures. However, all-atom (AA) approaches suffer from restrictions on accessible time scales and system sizes. In contrast, more efficient coarse-grained (CG) force fields remain insufficiently accurate to achieve correspondence with experiments.

Here, we present a combined CG/AA simulation approach in the study of ternary and quaternary lipid mixtures comprising cholesterol and/or sphingomyelin. Our simplified, yet biologically relevant, lipid compositions form coexisting liquid-ordered/liquid-disordered (L_o/L_d) phases on a multi-μs time-scale at atomistic resolution. In all studied case, the L_o domains are characterized by substructures of hexagonally packed saturated hydrocarbon chains nanoclusters, separated by interstitial regions enriched in cholesterol. Moreover, our simulations provide atomic-details insights on spontaneous cholesterol trans-bilayer motion (flip-flop) and its dependence on the lateral heterogeneity of the membranes.

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The interaction of personal care formulations with skin mimetics

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The outermost layer of human skin, the stratum corneum (SC), is an assembly of corneocyte cells embedded in a lipid matrix. The SC forms a highly effective, yet selective barrier, preventing pathogens and many other harmful agents from entering the body. Importantly, the SC is also the body's fundamental interface with topical formulations, with the lipid matrix thought to control the uptake and delivery of passive skin creams, excipients and active pharmaceutical ingredients.

In this work we aim to understand the molecular and assembly scale structural interactions between topical formulations and skin lipid mimetics. Understanding the physical interactions between stratum corneum lipids and formulations components is critical to the design of effective topical formulations. Choline geranate, a class of ionic liquid, is of high interest due to its effective transdermal drug delivery and antimicrobial properties. However, the mechanism of its penetration through bacterial membranes and human skin is not fully understood.

We have utilised a simplified, synthetic stratum corneum lipid mimic, composed of egg ceramide, cholesterol and free fatty acids, that is an effective and controllable model for investigating the specific molecular interactions that underpin physical and structural changes induced by the application of topical formulations, such as choline geranate.

We show that the hydration of choline geranate causes changes in its structural organisation from a simple lamellar structure to micellar assembly and finally an emulsion phase. Furthermore, we have also been able to show that choline geranate causes significant disruption to the meso-structure of model skin lipids. In addition, we also show insight to choline geranate's antimicrobial properties by disrupting stable DPhPC (1,2-diphytanoyl-*sn*-phospocholine) lamellar bilayers. These phase transitions and structural organisation have been investigated using several biophysical techniques including differential scanning calorimetry, small and wide-angle x-ray diffraction, solid-state NMR and light microscopy.

The outcome of this project will have a significant impact on the topical formulations industry as well as our understanding of the complex structure of the body's skin barrier.

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1D potential well describes the interaction of amphipathic peptides mediated by membrane elastic deformations

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Amphipathic alpha-helical peptides are considered as effective antimicrobial agents. In such peptides one side-surface of the helix is enriched by hydrophobic aminoacids, while polar or charged amonoacids are exposed on the opposite side-surface. Amphipathic peptides can electrostatically interact with lipid membranes. Upon adsorption, their hydrophobic aminoacids can be buried into the hydrophobic core of lipid monolayer. Such partial incorporation causes elastic deformations of the membrane in the peptide vicinity. If two peptides are far separated, their induced deformations are independent, and the corresponding elastic energy is additive. When the peptides come closer, the deformations overlap, leading to effective lateral interaction. Utilizing the theory of elasticity of liquid crystals adapted to lipid membranes, we calculated the energy of elastic deformations for discrete set of arbitrary configurations of two amphipathic peptides adsorbed to the membrane. The global minimum of the energy is achieved in the configuration of aligned parallel peptides separated by about 6 nm. We analytically obtained the expression for the interaction potential in unidimensional approach. The potential well approximates the interaction energy profiles, obtained numerically for peptides of different lengths arbitrary oriented with respect to each other. The effective interactional length of aligned parallel peptides for the unidimensional approach slightly exceeds their actual length. When the peptides are mutually shifted along their axis, the effective interaction length appears to be close to the length of projection of one peptide onto the other. If longitudinal axes of the peptides cross at substantial angle, the interaction is mainly governed by the peptide edges; the effective interaction length in this case is determined by characteristic length of deformation decay, i.e. by the membrane elastic properties rather than peptide geometry. We thus conclude that deformation-mediated interaction of membrane inclusions can be adequately described by the potential calculated in the unidimensional approach.

The work was supported by the Russian Foundation for Basic Research grant #17-04-02070.

P-135

Self-assembly of 10-N-nonyl acridine orange (NAO) creates molecular zippers promoting cell death by adhesion of membranes

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The fluorescent dye 10-N-nonyl acridine orange (NAO) is widely used as a mitochondrial marker. Very early, NAO was reported to have cytotoxic effects in cultured eukaryotic cells when incubated at high concentrations. Although the biochemical response of NAO-induced toxicity was well identified, the underlying molecular mechanism has not been yet explored in detail. Here, by using giant unillamelar vesicles and fluorescence confocal microscopy, we have obtained direct evidence that NAO promotes strong membrane adhesion of negatively charged vesicles. The attractive interactions between adhering membranes derive from van der Waals interactions between antiparallel H-dimers of NAO molecules from opposing bilayers, as revealed by fluorescence lifetime imaging microscopy. Semiempirical calculations have confirmed the supramolecular scenario by which antiparallel NAO molecules form a zipper of bonds at the contact region. The membrane remodelling effect of NAO, as well as the formation of H-dimers, was also confirmed in cultured fibroblasts, as shown by theultrastructure alteration of the mitochondrial cristae. We conclude that membrane adhesion induced by NAO stacking accounts for the supramolecular basis of its cytotoxicity.

P-136

Structural changes in biomimetic myelin membranes induced by Myelin Basic Protein

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The myelin sheath plays an important role in nerve signal conduction. It acts as an insulating layer around axons which enables fast signal transport by reducing conduction losses. In demyelinating diseases like multiple sclerosis, this membrane is damaged which leads to severe problems in nerve conduction. In literature, different values for the lipid composition of healthy and modified membranes have been found. Based on these results we investigate the membrane structure of the respective lipid compositions. As next step we add Myelin Basic Protein (MBP) to the membranes and investigate the induced structural changes. Small angle neutron scattering (SANS) and cryo-transmission electron microscopy (cryo-TEM) data show the structure of vesicles with healthy and modified membrane composition and the strong structural change induced by MBP. In detail, we see as well multilamellar structures as vesicle fusion and aggregation. Neutron Reflectometry (NR) data shows how the interaction of healthy and modified myelin membranes with MBP differs, respectively.

P-137

NMR Studies of Phospholipid Motion using Lanthanide Induced Shifts

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Biological membranes are made up of phospholipids, which form a bilayer structure along with proteins and carbohydrates. In order to study the behaviour of these membranes, phospholipid vesicles have long been used as models. Whilst they may be a far cry from the complexity of a cellular membrane, their simplicity allows researchers to study individual biophysical effects in isolation. However, NMR of phospholipid vesicles presents many challenges, one of the most notable is the difficulty of distinguishing between the inside and outside leaflets of the bilayer. To address this, we utilised ytterbium ions to induce a pseudocontact shift on the lipids, which results in two separate signals for the head group of the lipid. This was then used to differentiate between the outer and inner leaflets of a unilamellar vesicle, and observe molecular motion using ^{1}H NMR. Specifically of interest is trans-bilayer movement, which is fundamental to biological activity, but remains difficult to observe and characterise. It was found that the ratio of the lanthanide to lipid was shown to influence the splitting, and the magnitude of the splitting was also dependent on the lipid. By introducing lyso lipids, the membrane was perturbed and increasing the temperature past the transition temperature showed merging of signal from the head group, which we believe to indicate trans-bilayer movement. This work demonstrates the utility of the lanthanide shift reagents. It has been shown that they are sensitive to differences in lipid formulations and capable of elucidating molecular motion previously unobserved by NMR.

P-138

Lipid dynamics in nanodiscs probed by solid-state NMR

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Lipid dynamics in nanodiscs probed by solid-state NMR

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Nanodiscs provide a novel tool to embed membrane proteins into native-like lipid bilayers environment allowing high-resolution biophysical studies while maintaining their structure and function. Nanodiscs are composed of a small patch of a lipid bilayer surrounded by a membrane scaffold protein (MSP) creating a belt like shape. Different scaffold proteins can be used resulting in nanodiscs of various diameters and properties. It still remains unclear how lipids dynamically organize inside a nanodisc scaffold compared to their behavior in cellular membranes.

Recently, we compared lipid dynamics in nanodiscs and in liposome [1]. In the present work, we use deuterium solid-state NMR spectroscopy to investigate the organization and dynamic of lipids in various nanodisc constructs with an average size from 8 to 13 nm, assembled in different lipids composition (DMPC, DPPC). The thermotropic behavior, ordering and thickness of lipid membrane in nanodiscs were assessed. The results point out highly variable gel-to-fluid phase transitions and lipid ordering as a function of the nanodisc diameter, suggesting that the scaffold design have a profound impact of the dynamic organization of the lipid bilayer.

1. Martinez, D. *et al., ChemPhysChem* **18**, 2651-2657 (2017).

P-139

Comparative biophysical study of SARS-CoV Spike membrane-active domains: Implications for membrane fusion

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The S2 subunit of the Severe Acute Respiratory Syndrome (SARS) Spike protein features membrane-active domains that play critical roles in viral entry and infection. Interaction of these functional domains with membranes drives large conformational changes in the viral glycoprotein that lead to the merge of the virus envelope with the cell membrane. Here we present a series of spectroscopic, calorimetric, and optical microscopy experiments to probe peptide-membrane interactions of four SARS segments corresponding to the putative fusion peptides (FP1 and FP2), and to internal segments near the FPs (IFP and fIFP). Our results show that all peptides acquired α-helical secondary structure in micelles and induced membrane permeabilization, aggregation, and lipid mixing of phospholipid model membranes in a concentration- and pH-dependent manner and at different extents. The peptide fIFP was the most effective in promoting lipid perturbation and mixing, while FP2 presented the lowest fusion activity and the lowest effect on the electron spin resonance (ESR) line shape of nitroxide-labeled lipids embedded in lipid model membranes. ESR showed that FP1, IFP, and fIFP substantially increased lipid packing and head group ordering only in the presence of negatively charged lipids, which might be related to bending moment induction in the bilayer and membrane dehydration. On the other hand, all peptides exhibited low hemolytic activity, but both FP2 and fIFP promoted higher hemagglutinating activities than FP1 and IFP. In addition, the peptides only slightly changed the melting behavior of ghost erythrocyte proteins as seen by differential scanning calorimetry. Taken together, our results highlight the importance of the lipid composition of the membranes and the environmental pH in modulating the biological activities of the peptides and hypothesize the role played by different membranotropic domains of the Spike S2 subunit. Depending on the environment, different membrane-active domains may act independently and help to overcome the kinetically unfavorable task of bringing together two apposed membranes to induce fusion.

P-140

Lipid lamellar phases at cryogenic temperature: electron spin echo studies.

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Lipid lamellar phases at cryogenic temperature: electron spin echo studies.

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Electron spin echo methods of pulsed electron paramagnetic resonance spectroscopy are used to study fully hydrated lamellar phases of phosphatidylcholine lipids in the frozen state. Two-pulse echo-detected EPR spectra of chain labelled lipids are indicative of librational dynamics in the low-temperature phases of the phospholipid membranes. The characteristics of the librational lipid-chain motion, i.e., mean-square angular amplitude and rotational correlation time, depend on the linkage between the nonpolar chains and the glycerol backbone (ester- *vs* ether-linkage) and on the degree of chain unsaturation (saturated *vs* mono- and di-unsaturated chain) of the lipid molecules. Fast (in the subnanosecond-nanosecond time range) segmental librational oscillations of small amplitude and the dynamical transition around 200 K are detected in any membrane sample. Experiments of three-pulse electron spin echo envelope modulation by D_2O of site-specifically spin-labeled phospholipids throughout the chain reveal a uniform profile of solvent (D_2O) penetration across the hydrocarbon region of ether-linked dialkyl lipids with interdigitated chains. This profile is markedly different from the sigmoidal transmembrane profiles of solvent accessibility into bilayers of ester-linked diacyl phosphatidylcholines. These results obtained in lipid membranes at cryogenic temperature are also relevant at higher temperatures. The differences in dynamics and polarity evidenced in the considered linear-chain phosphatidylcholine membranes should be expected to affect the functional behavior of the corresponding biological membranes.

Sunday 21st July

CELLULAR PROLIFERATION

P-141 (O-042)

Cell-size regulation induces long-term oscillations in population growth rate

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There are negative correlations between the generation time of a bacterial cell and those of its descendants. If a cell grows for a longer time than expected, its daughter cells (and subsequent descendants) will be larger at birth and have to compensate for their sizes by dividing slightly earlier than expected. This process is known as cell-size regulation. In this talk, I discuss the effect of these correlations on the dynamics of population growth of microorganisms. I show that any non-zero correlation that is due to cell-size regulation can induce long-term oscillations in the population growth rate. The population only reaches its steady state due to the often-neglected variability in the growth rates of individual cells. The relaxation time scale of the population to its steady state is determined from the distribution of single-cell growth rates and is surprisingly independent of the details of both the division process and the cell-size regulation. I propose an experimental method to measure single-cell growth variability by observing how long it takes for the population to reach its steady state, a measurement that is significantly easier and less biased than single-cell measurements.

P-142 (O-043)

Molecular architecture of bacterial amyloids in Bacillus biofilms

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The formation of biofilms provides structural and adaptive bacterial response to the environment. In *Bacillus* species, the biofilm extracellular matrix is composed of exopolysaccharides, hydrophobins and several functional amyloid proteins.

We report the molecular architecture of *Bacillus subtilis* and pathogenic *Bacillus cereus* functional amyloids, using multi-scale approaches such as solid-state NMR, electron microscopy, X-ray diffraction, DLS, ATR-FTIR and immune-gold labeling. Solid-state NMR data reveal that the major amyloid component TasA in its fibrillar amyloid form contain β-sheet and α-helical secondary structure, suggesting a highly non-typical amyloid architecture, and species variability between *B. subtilis* and *B. cereus*. Proteinase K digestion experiments indicate the amyloid moiety is approximately ~100 amino-acids long, and subsequent solid-state NMR and FTIR signatures for *Bacillus subtilis* and *Bacillus cereus* TasA filaments highlight a conserved rigid amyloid core albeit with substantial differences in structural polymorphism and secondary structure composition. Structural analysis and cross-seeding data on the accessory protein TapA in *B. subtilis* and its counterpart CalY in *B. cereus* reveal a catalyzing effect between the functional amyloid proteins and a common structural architecture, suggesting a co-assembly in the context of biofilm formation.

Our findings highlight non-typical amyloid behavior of these bacterial functional amyloids, underlining structural variations between biofilms even in closely related bacterial species.

P-143 (O-044)

Microviscosity of bacterial biofilm matrix characterized by fluorescence correlation spectroscopy and single particle tracking

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Bacterial biofilms are surface-adherent communities of bacteria surrounded by an extracellular polymeric substance (EPS) consisting of secreted polysaccharides and other biomolecules. In healthcare settings bacterial biofilms represent a severe threat, causing chronic infections and contamination of medical devices. To remove biofilms, multiple strategies have been developed, e.g. treatment with antibiotics or bacteriophages, which are advantageous to specifically target bacterial species. In this context, it remains unclear to which extent the EPS matrix imposes a physical barrier to the transport of bacteriophages through the biofilm. To address this question, we have reconstituted the EPS matrix of the bacterium *Pantoea stewartii*, responsible for a severe disease of corn plants, and investigated the diffusion properties of fluorescent particles using fluorescence correlation spectroscopy and single particle tracking. This approach allows to study the EPS spatial organization under various physico-chemical conditions. We show that small probes diffuse freely in the EPS with diffusion coefficients similar to those measured in water. In contrast, large probes are drastically slowed down, showing anomalous subdiffusion. The degree of confinement increases with EPS concentration. Tracking single fluorescently labeled bacteriophages at physiological concentrations, we observe a population of strongly confined particles, showing distinct subdiffusive dynamics with anti-correlation of successive steps. To overcome the physical barrier imposed by the EPS, bacteriophages are equipped with EPS degrading enzymes. By treating the EPS with purified bacteriophage enzymes, we show that upon EPS degradation strongly confined diffusion rapidly turns to free diffusion. Thus, our approach allows the investigation of dynamic changes of the biofilm microviscosity and demonstrates that the EPS matrix imposes a probe size dependent diffusion barrier under physiological conditions. Our data suggests that the ability to degrade the EPS provides a key for bacteriophages to evade trapping in the biofilm.

P-144

EFFECT OF ULTRAVIOLET RADIATION ON HUMAN HEALTH: STUDY OF A SAMPLE OF SKIN CANCER PATIENTS IN ALGERIA.

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Skin cancer generally is a cumulative effect of solar ultraviolet radiation exposure and is a public health problem; solar ultraviolet radiation is a major risk factor for this disease. This epidemiological study was carried out by different methods and techniques and especially treatment of medical records at the level of the Chlef region from Algeria. The study was more than 70 cases of female and male skin cancer. This study can provide much information on the biophysical and epidemiological aspects, where the incidence and the average age were among parameters of these patients and population, the distribution of patients according to incidence and some risk factors were estimated according to this study during five years from January 2014 to December 2018. The use of sunscreens is often advocated to prevent the cumulative effects of sun exposure. The objective of this work is the development of the cancer registry of Chlef region in Algeria.

P-145

Anticancer activity of a novel cell-penetrating peptide, vCPP2319, and its interaction with breast cancer cells' derived exosomes

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Despite improvements in breast cancer treatment there is increased incidence of a metastatic disease. While systemic therapies control the primary tumor allowing breast cancer patients to achieve long-term survival, lesions occur in metastatic sites. Unspecific cell targeting and resistance are associated to conventional treatments and render metastatic breast cancer (MBC) with a poor prognosis. New and innovative therapeutic strategies to control breast cancer disease while simultaneously targeting and/or preventing metastases are urgently needed.

Exosomes are small extracellular vesicles naturally found in body-derived fluids. These vesicles play an important role in intercellular communication. Due to their low toxicity and immunogenicity and their potential to transpose biological barriers, such as the blood-brain barrier, exosomes have been referred to as ideal stealth drug nanocarriers. Nonetheless, the use of exosomes as drug delivery system (DDS) demands a thorough characterization of the vesicles and would benefit from post-isolation modifications. In parallel, peptide-based therapies have been evolving as important therapeutic strategies to surpass lack of specificity and circumvent resistance issues. Cell-penetrating peptides (CPPs) are small molecules with high ability to internalize cells and their use as delivery vectors has been broadly studied.

In this work, the anticancer activity of a CPP from viral origin, vCPP2319, was studied, along with its selectivity for MBC cells. The peptide's mode of action was investigated using different biophysical techniques, namely zeta potential determination, fluorescence spectroscopy, and atomic force microscopy (AFM). The obtained results reveal the potential of vCPP2319 as an anticancer peptide drug lead and suggest it has an intracellular target. The interaction between vCPP2319 and MBC-derived exosomes was also evaluated using zeta potential determinations and surface plasmon resonance (SPR). Exosomes were isolated from MBC and human healthy breast cells and characterized by flow cytometry, transmission electron microscopy (TEM) and AFM. The results showed a strong peptide-exosome interaction paving the way to the development of a promising vCPP2319-loaded exosomal-based DDS to be used in MBC treatment.

P-146

BK channels in cell cycle progression and proliferation of mesenchymal endometrial stem cells

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Ion channels in stem cells participate in numerous cellular reactions including differentiation, proliferation, cell migration etc. Previously we have identified Ca^{2+}-dependent potassium channels of big conductance (BK) in human mesenchymal endometrial stem cells (eMSCs). The Ca^{2+}-mediated coupling between the activity of BK-and mechanosensitive channels was determined. Due to high single channel conductance and level of functional expression BK channels could significantly contribute to different signaling processes in eMSCs via setting and controlling the membrane potential during cell cycle progression. Here, we aimed at revealing the putative impact of BK channels to cell cycle transitions of eMSCs. The functional expression of native BK channels was confirmed using patch-clamp and immunocytochemistry methods. In cell-attached patch-clamp configuration a characteristical fingerprints of BK-mediated channels that are voltage sensitivity and current saturation were observed.

Importantly, immunofluorescent analysis revealed that the fraction of unsynchronized eMSCs were negatively stained with specific antibodies against extracellular epitope of pore-forming alpha BK subunit. The observed effect could not be explained by heterogeneity of cell culture, apoptosis or their differentiation status. Using cell synchronization, we found that the presence of BK channels in plasma membrane was cell cycle-dependent and significantly decreased in G2M phase. However, inhibition of BK channels with specific blockers iberiotoxin (ibTx, 100-200 nM) or charybdotoxin (chTx, 100-200 nM) did not affect cell cycle progression thus indicating a lack of functional importance of BK channels as selective potassium-transporting pathway for the regulation of cell proliferation. At the same time, we propose that the dynamics of the presence of BK channels on plasma membrane of eMSCs can be a novel indicator of cellular proliferation.

P-147

Development of a new microfluidic device for observing dense bacterial populations in a controlled environment

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Recently, roles of mechanical interaction between bacterial cells have been widely discussed for understanding macroscopic features of microbial communities. For example, cell-elongation-induced stresses are thought to be an important factor for nematic alignment [1], spatial competition inside a colony [2] and verticalization during biofilm formation [3]. Although those studies considered stable environments, the real environment surrounding actual bacterial populations is basically fluctuating. Therefore, it is desired to investigate how spatial structure of dense bacterial colonies react against changes in the culture condition.

In order to analyze reaction of spatial structure against environment change, we developed a new microfluidic device, which we call "extensive microperfusion system" [4]. This device allows us to observe dense bacterial suspensions in two-dimensional space under uniform environment, which has been difficult by existing methods. In our system, cells are trapped in a designed geometry and continuously fed with fresh medium through a porous membrane placed above the observation region. The culture condition can also be changed uniformly in space.

In the presentation, we will show several experimental results of performance evaluation of our experimental system. We demonstrate that, by switching the culture medium, we can indeed control growth of *E. coli* populations confined in a two-dimensional well. We also checked how the medium is replaced by observing dynamic profile of fluorescent dye by a confocal microscope. We confirm that one can switch the culture condition inside the well within a few minutes, which is shorter than typical division time of bacteria in ideal conditions. We will also show some preliminary observations obtained by this new system. We would like to discuss what kind of problems is possibly interesting to address with our extensive microperfusion system.

[1] Z. You et al., Phys. Rev. X **8**, 031065 (2018).
[2] W. P. J. Smith et al., PNAS **114**, E280 (2017).
[3] F. Beroz et al., Nat. Phys. **14**, 954 (2018).
[4] T. Shimaya et al., in preparation.

P-148

Quantitative imaging of efflux pumps in planktonic and biofilm-associated bacteria through single-molecule localization microscopy

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Many bacteria have the ability to switch from a planktonic to a biofilm-associated lifestyle in response to a variety of environmental conditions. This process involves an extensive remodeling of gene expression: in particular, biofilm growth has been associated with the upregulation of stress-response genes, including efflux pumps. Efflux pumps are active transporter proteins capable of extruding a wide range of molecules from bacteria, such as harmful metabolic products or xenobiotics. Multidrug efflux pumps such as the AcrAB-TolC complex in gram-negative bacteria are notable for having an especially broad spectrum of substrates, which includes several antibiotics. Therefore, the increased expression of such genes is believed to be one of the leading causes behind the increased resistance of biofilms to antimicrobial compounds. Although the expression of efflux pumps has been previously investigated through standard gene expression assays on bulk biofilms, quantitative single-cell data is still lacking. Biofilm-associated bacteria are known to be functionally heterogeneous, with different subpopulations of isogenic cells assuming different roles characterized by different gene expression patterns. As such, the distribution and expression level of efflux pumps in single bacterial cells within biofilms is a crucial piece of information that could shed light on the internal organization of these complex microbial communities. Here, we implement a method involving precise genome editing of E. coli through CRISPR/Cas9-assisted recombineering coupled with single-molecule localization fluorescence microscopy to obtain quantitative data on the expression and distribution of the AcrB efflux pump component in single bacterial cells, both planktonic and embedded in a biofilm.

P-149

RECIPROCAL EFFECT OF CHELIDONE ON TYROSINE AND SERINE PHOSPHORYLATION OF STAT3 IN UVEAL MELANOMA CELLS

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STAT3 is a transcription factor with a critical role in the regulation of cell cycle, cell proliferation/survival and cell migration. As a consequence, it is a promising target in antitumor therapies. Canonical function of STAT3 demands phosphorylation of a Tyr705 residue followed by dimerization and nuclear translocation. STAT3 can also be phosphorylated on Ser727, with a putative role in fine tuning STAT3 activation.

One of the major activators of STAT3 is interleukin-6, a cytokine present in an elevated concentration in various tumors, including uveal melanomas. Herein we aimed to study whether chelidonine, a potential antitumor agent, interferes with the IL-6/STAT3 pathway in human uveal melanoma cells. According to previous data, this alkaloid provokes cell death in numerous cancer cells, inhibits microtubule assembly and affects cell cycle progression.

Using flow cytometry and confocal microscopy experiments we have demonstrated that chelidonine increases the basal level of pS-STAT3 in a significant fraction of cells. This effect was accompanied with abrogation of IL-6-induced STAT3 activation, which developed in a time-dependent fashion. Reduced efficiency of IL-6 induced STAT3 activation was also observed in cells with unaffected level of pS-STAT3. Chelidonine did not affect the level of IL-6Rα or total STAT3, but reduced significantly expression of gp130, the signaling subunit of functional IL-6R. According to our data chelidonine may interfere with STAT3 activation via two distinct mechanisms: one involves processes associated with serine phosphorylation, whereas the other one may be related to decreased expression of gp130.

P-150

Single-chain atomic crystals as extracellular matrix-mimicking material with exceptional biocompatibility and bioactivity

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Single-chain atomic crystals as extracellular matrix-mimicking material with exceptional biocompatibility and bioactivity

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In this study, $Mo_3Se_3^-$ single-chain atomic crystals (SCACs) with atomically small chain diameters of ~0.6 nm, large surface areas, and mechanical flexibility were synthesized and investigated as an extracellular matrix (ECM)-mimicking scaffold material for tissue engineering applications. The proliferation of L-929 and MC3T3-E1 cell lines increased up to 268.4 ± 24.4% and 396.2 ± 8.1%, respectively, after 48 h of culturing with $Mo_3Se_3^-$ SCACs. More importantly, this extremely high proliferation was observed when the cells were treated with 200 μg mL^{-1} of $Mo_3Se_3^-$ SCACs, which is above the cytotoxic concentration of most nanomaterials reported earlier. An ECM-mimicking scaffold film prepared by coating $Mo_3Se_3^-$ SCACs on a glass substrate enabled the cells to adhere to the surface in a highly stretched manner at the initial stage of cell adhesion. Most cells cultured on the ECM-mimicking scaffold film remained alive; in contrast, a substantial number of cells cultured on glass substrates without the $Mo_3Se_3^-$ SCAC coating did not survive. This work not only proves the exceptional biocompatible and bioactive characteristics of the $Mo_3Se_3^-$ SCACs but also suggests that, as an ECM-mimicking scaffold material, $Mo_3Se_3^-$ SCACs can overcome several critical limitations of most other nanomaterials.

Reference:
[1] Jin Woong Lee *et al*., *Nano Lett*. 2018, 18, 12, 7619-7627

P-151

Insights into the mechanism of action of pepR, a viral-derived peptide, against *Staphylococcus aureus* biofilms

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Bacterial infections are a major human health threat given both the increasing incidence of drug-resistant bacteria and the ability of bacteria to form biofilms. Biofilm-related infections are particularly difficult to treat due to their reduced susceptibility to conventional antibiotics. Given the increasing interest in the use of antimicrobial peptides (AMPs) as alternatives against bacterial biofilms, our work is focused on the key factors that govern the antibiofilm action of a model AMP at the molecular level. pepR, a peptide derived from the Dengue virus capsid protein, was selected as an AMP model because it abrogates biofilm formation and kills bacteria in preformed *S. aureus* biofilms. Using a combination of flow cytometry and confocal fluorescence microscopy assays, with quantitative imaging data treatment, we showed that the ability of pepR to prevent biofilm formation and act on preformed biofilms is directly related to bacterial membrane permeabilization. The effect of the peptide on biofilm-associated bacteria is dose and depth-dependent, and is controlled by its diffusion along the biofilm layers. Overall, our study contributes to shed light on the antibiofilm mechanism of action of AMPs, particularly regarding the importance of their diffusion through the biofilm matrix on their activity.

P-152

Hypericin - apomyoglobin an enhanced photosensitizer complex for the treatment of tumor cells

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The bioavailability of photosensitizers for photodynamic cancer therapy is often hampered by their low water solubility. Here we overcome this problem by using the water soluble protein apomyoglobin (apoMb) as a vector for photosensitizing molecule hypericin (Hyp). The Hyp-apoMb complex is rapidly taken up from HeLa and PC3 cells, at submicromolar concentrations. The fluorescence emission of Hyp-apoMb is exploited to localize the cellular distribution of the photosensitizer. The plasma membrane is loaded quickly and efficiently and the fluorescence is observed in the cytoplasm only later and to a lesser extent. The comparison with cells loaded with Hyp alone demonstrates that the absorption of the photosensitizer without the protein carrier is a slower, less efficient process, which involves the entire cell structure without preferential accumulation at the plasma membrane. Cell viability tests show that Hyp-apoMb has superior performance compared to Hyp. Similar results were obtained using tumor spheroids as three-dimensional cell culture models.

Sunday 21st July

NON-EQUILIBRIUM PHYSICS IN BIOLOGY

P-153 (O-048)

Non-Equilibrium Processes in Proteins Triggered by Light: Excited States Molecular Dynamics Perspective

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Classical molecular dynamics (MD) simulations of proteins are mainstream tools in biophysics. However, simulations of protein systems in which light absorption is taken into account are far from trivial. Many methods have been proposed so far [1]rydzewski nowak chapter], but there is no consensus on how to monitor rare events triggered by light absorption. Here, we present our results of protein structural rearrangements that are induced by absorbing photons in the chromophore parts of proteins. We adopt a simplified approach of switching the dynamics between electronic states, named the Landau-Zener model. We model fluorescent probes dynamics (Prodan, Aldan) embedded in apomyoglobin, the photodissociation of nitric oxide from a neuroglobin mutant [2], and structural changes induced by the cis-trans transition in proteins involved in the control of glucose level: EPAC2 and KATP Kir6.2/SUR1 channel [3,4]. We extract and indicate possible nano-mechanical events stimulated by light on a nanosecond time scale. Data show that systematic structural light-triggered effects in these proteins happen and that such motions may play a functional role. Our results indicate that developing light-controlled drugs related to diabetes treatment should be possible.

[1] J. Rydzewski, W. Nowak ¸"Molecular Dynamics Simulations of Large Systems in Electronic Excited States", in Handbook of Computational Chemistry, Springer, 1917-1941, 2016.
[2] J. Rydzewski, W. Nowak, Photoinduced Transport in an H64Q Neuroglobin Antidote for Carbon Monoxide Poisoning, J. Chem. Phys., 148(11), 2018, 115101,
[3] Broichhagen J, Schonberger M, Cork SC et al. Optical control of insulin release using a photoswitchable sulfonylurea. Nat Commun 2014; 5:5116. doi: 10.1038/ncomms6116.
[4] K. Walczewska-Szewc, W. Nowak, Spacial models of malfunctioned protein complexes help to elucidate signal transduction critical for insulin release. Biosystems. 2019 Mar;177:48-55. doi: 10.1016/j.biosystems.2018.11.001.

P-154 (O-049)

1/f noise in ion channels formed by the Classical Swine Fever Virus (CSFV) p7 protein

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1/F NOISE IN ION CHANNELS FORMED BY THE CLASSICAL SWINE FEVER VIRUS (CSFV) P7 PROTEIN

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Permeabilization of the endomembrane system by viroporins is instrumental in the progression of host-cell infection by many viral pathogens. Previous studies showed that CSFV viroporin p7 assembles into channels of nanometric dimensions. Here we analyze the power spectrum of current traces. We focus on the low-frequency range where the power spectrum shows typically $1/f^{\alpha}$ behavior with $0.5< \alpha < 2$ (the so called "pink noise"). We investigate several lipid mixtures to show that lipid composition determines not only the absolute value of current fluctuations but also the α parameter in the power spectrum of the current traces. Our findings give support to previous studies hypothesizing that p7 viroporin forms channels with at least two different types of pore architecture. Overall, our observations suggest that pink noise is caused by conductance fluctuations governed by equilibrium processes, in particular the conformational dynamics involving the proteins and lipids that assemble together to form proteolipidic pores.

P-155 (O-050)

Biophysical models of mRNA translation applied to ribosome profiling data

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Protein synthesis is regulated at many different levels including transcription of DNA into mRNA and translation of mRNA into protein. There is an accumulated evidence that synonymous codon choice affects mRNA translation, particularly the rate of protein production and the quality of the protein produced [1]. Dynamical details of this intricate process have recently become available thanks to rapid advances in DNA sequencing and single-cell imaging. However, existing biophysical models of mRNA translation have had limited success so far interpreting the new data because of our lack of mathematical tools to describe nonequilibrium systems [2]. I will present a newly developed method for solving the most commonly used models of mRNA translation, the ones that are based on the totally asymmetric simple exclusion process. The method assumes that translation initiation is rate-limiting for protein synthesis and predicts the rate of translation and ribosome density from translation elongation rates of individual codons. I will demonstrate how to use the method for analyzing ribosome profiling data and discuss what it teaches us about codon optimization.

[1] Brule CE, Grayhack EJ. 2017. Synonymous codons: choose wisely for expression. Trends Genet 33:283-297.
[2] Zur H, Tuller T. 2016. Predictive biophysical modeling and understanding of the dynamics of mRNA translation and its evolution. Nucleic Acids Res 44:9031-9049.
[3] Szavits-Nossan J, Cinadrini L, Romano MC. 2018. Deciphering mRNA Sequence Determinants of Protein Production Rate. Phys Rev Lett 120:128101.

P-156

Four state model for efficient simulation of gap junction channel voltage gating kinetics

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Gap junction (GJ) channels, formed of connexin (Cx) proteins, provide a direct pathway for metabolic and electrical cell-to-cell communication. These specialized channels are not just passive conduits for the passage of ions and metabolites, but have been shown to gate robustly in response to transjunctional voltage, V_j, the voltage difference between coupled cells and are regulated by various chemical factors. Voltage gating of GJs may play a physiological role, particularly in excitable cells which can exhibit large transients in membrane potential during the generation of an action potential. In this paper, we present a mathematical/computational model of GJ channel voltage gating based on assumption that two apposing hemichannels are connected in series, and activation energies for their gating transitions between an open and residual state linearly depend on voltage across each hemichannel. To demonstrate the viability of the model, we fit experimental data obtained from electrophysiological recordings in cell cultures transfected with Cx43 and Cx45, isoforms expressed in cardiac tissue. The results show that the model is capable of describing both steady-state and kinetic properties of homotypic and heterotypic GJ channels. Moreover, mathematical analyses showed that the model can be simplified to a reversible two-state system and solved analytically, using a rapid equilibrium assumption. Given that excitable cells are arranged in interconnected networks, the equilibrium assumption allows for a substantial reduction in computation time, which is useful in simulations of large cell clusters. Overall, this model can serve not just as a modeling tool, but also to provide a means of testing GJ channel gating behavior.

P-157

Study of β-Sheet Dynamics Using Isotope-Induced Frequency-Shifts of Vibrationally Coupled Modes

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Understanding the factors affecting β-sheet formation is of great scientific interest as many degenerative diseases have their pathology rooted in protein or peptide misfolding and aggregation, which often involves β-sheet formation. Infrared (IR) spectroscopy provides a useful means for attaining information about secondary structure elements, particularly β-sheet structures, and can also monitor localized conformational changes using site-selective isotopic labeling. Substitution of an amide C=O with ^{13}C leads to a downshift of its vibrational frequency of about 40 cm^{-1}. Labeling one peptide sequence with several ^{13}C=O groups on the one hand can enhance the intensity of the label band [1,2], but on the other hand, the arising coupling patterns might be difficult to interpret. Here, properties in equilibrium as well as dynamics of a three-stranded β-sheet peptide labeled at multiple locations were analyzed. As small β-sheet structures have limited stability in isolation (outside of a protein environment), formation of the three-stranded structure was promoted by stabilizing both turns with α-aminoisobutyric acid-glycine (BG) sequences [2]. In contrast to previous studies, which are mostly on β-hairpins [2], separate bands were observed for the individual labels of the peptide. The unfolding process of the peptide was monitored site-specifically by probing the individual bands with laser-excited temperature-jump spectroscopy [3]. Relaxation times of the single-labeled variants were found to be altered by the presence and location of additional labels. Differences in relaxation times were analyzed in regard to the coupling of those modes with the help of the density function theory calculations as well as molecular dynamics simulations.

[1] Brauner, J.W.; Dugan, C.; Mendelsohn, R., *J. Am. Chem. Soc.* **2000**, *122*, p. 677-683.
[2] Huang, R.; Setnicka, V.; Etienne, M.A.; Kim, J.; Kubelka, J.; Hammer, R.P.; J. Am. Chem. Soc. **2007**, p. 13592-13603.
[3] Scheerer, D.; Chi, H.; McElheny, D.; Keiderling, T. A.; Hauser, K., J. Phys. Chem. B **2018**, *122*, p. 10445-10454.

P-158

Design of polyQ peptides to study the dynamics of glutamine interactions spectroscopically

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Polyglutamine (polyQ) sequences play a key role in numerous proteins associated with neurodegenerative diseases. Those proteins form aggregated β-structures [1], a symptom found e.g. in Huntington's disease (HD). Previously we used polyQ peptides ($K_2Q_nK_2$ with n = 10, 20, 30) with different numbers of consecutive glutamines as a model system to study the aggregation process of polyQ fibrils. Oligomeric β-sheet structures were observed for a polyQ sequence with 20 Q repeats [2], however little is known about the formation of the β-structural motif within the fibrils. To analyse the unique role of glutamines, we aim to design soluble monomeric peptide templates and study their folding kinetics with time-resolved temperature-jump infrared spectroscopy. Monomeric peptides with defined hairpin structures were designed based on the tryptophan zipper Trpzip-2 (SWTWENGKWTWK-NH_2), which is stabilized by aromatic cross-strand interactions [3]. Threonines were substituted and glutamines were incorporated in each strand of the sequence (Trpzip-Q_n with n = 2, 6, 10). The structural changes of the templates were monitored by 2D NMR spectroscopy. NMR measurements indicate a monomeric structure for Trpzip-Q_2, which is similar to the template Trpzip-2. Trpzip-Q_6 is also monomeric with an ordered glutamine sequence but with less Trp-Trp cross-strand interactions. Trpzip-Q_{10} shows a strong structural change with no Trp –Trp cross-strand interactions anymore. The folding kinetics of the peptides occur on a microsecond time scale as revealed by T-jump IR. The templates will be used for selective isotope labelling in order to monitor conformational dynamics of the glutamine backbone and the side chains separately. In addition, we have designed a hairpin template, where glutamines are inserted alternately in the sequence, separated by other amino acids to improve the solubility and increase the number of cross-strand interacting side chains. This helps to understand the molecular mechanism underlying polyQ fibril formation and the contribution originating from glutamine side-chain interactions.

[1] T. Yushchenko, E. Deuerling, K. Hauser, *Biophys. J.* **2018**, *114*, 1847-1857.
[2] B. S. Heck, F. Doll, K. Hauser, *Biophys. Chem.* **2014**, *185*, 47-57.
[3] A. G. Cochran, N. J. Skelton, M. A. Starovasnik, *Pro. Natl. Acad. Sci. (USA)* **2001**, *98*, 5578-5583.

P-159

Mechanism of the electroneutral sodium/proton antiporter PaNhaP from transition-path shooting

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Na^+/H^+ antiporters exchange sodium ions and protons on opposite sides of lipid membranes. The electroneutral Na^+/H^+ antiporter NhaP from archaea *Pyrococcus abyssi* (PaNhaP) is a functional homolog of the human Na^+/H^+ exchanger NHE1, which is an important drug target. Here we resolve the Na^+ and H^+ transport cycle of PaNhaP by transition-path sampling. The resulting molecular dynamics trajectories of repeated ion transport events proceed without bias force, and overcome the enormous time-scale gap between seconds-scale ion exchange and microseconds simulations. The simulations reveal a hydrophobic gate to the extracellular side that opens and closes in response to the transporter domain motion. Weakening the gate by mutagenesis makes the transporter faster, suggesting that the gate balances competing demands of fidelity and efficiency. Transition-path sampling and a committor-based reaction coordinate optimization identify the essential motions and interactions that realize conformational alternation between the two access states in transporter function.

P-160

Droplet size regulation by non-equilibrium chemical reactions

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Biological cells use (liquid-liquid) phase separation to organize their interior in space and time. This leads to the formation of small droplets highly enriched with specific intracellular materials (e.g. proteins), which can act as nucleation centers. Most chemical reactions in cells are non-equilibrium processes driven by energy sources like ATP. We want to understand the interplay between non-equilibrium chemical reactions and phase separation, specifically how such reactions can be used to control the droplets.

We discuss a model where a protein can exist in a soluble and phase separating state, which can be converted into each other by (ATP-driven) chemical reactions. In this system, the droplet size can be controlled if the chemical reactions drive diffusive fluxes. We show using non-equilibrium thermodynamic that this requires an asymmetry in the transport properties between phases. Combining numerical and analytical calculations, we investigate how different asymmetries influence the droplet dynamics.

P-161

Measurement of noise on added size for *e coli* adder and sizer-like division strategies suggests a multi-step control.

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Recent experimental advances have suggested the *Adder* mechanism for *E. coli* division control. This means that bacteria grow, on average, a fixed size before division. Here we use new experiments to verify this mechanism with glucose as carbon source and explore deviations of the *adder* strategy, specifically, the division control of *E. coli* growing with glycerol as carbon source. In this medium, the division strategy is sizer-like, which means that the added size decreases with the size at birth. We propose a mechanistic model to explain our measurements and found that our model can explain not only the slope in the graph of added size vs size at birth but the noise of this relationship. We propose further experiments in order to distinguish between other possible explanations.

P-162

A proposed mechanism for the effect of extremely-low frequency magnetic fields on cancer cells apoptosis

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Regarding the observed effects of extremely low frequency weak electromagnetic fields (ELF-EMF) on biological systems, there have been ongoing efforts to explain how interaction with energy dozens of magnitudes below k_BT is not being masked by thermal noise. In a constant low-intensity magnetic field, the energy of the interaction with electron magnetic moments is many orders of magnitude less than the thermal fluctuation which is also the case for a quantum of energy in an AC field in this range of frequencies.

The cellular level of ROS, as a major component in cellular signaling pathways and particularly apoptosis, has been frequently reported to be affected by ELF. Here, grounded on the gated mechanism of Sarewicz et al., we introduce a scheme based upon the previously discovered phenomenon of "Radical Pair Mechanism" that suggests how applying low-intensity magnetic field of the order of a few tens of milliTeslas can increase the level of singlet oxygen reactive species of semiquinone at Q_o site of mitochondrial cytochrome bc1 and subsequently superoxide production rate.

Moreover, using a reaction-diffusion model developed for the simulation of the observed oscillation of ROS level in myocytes, we show how the mentioned superoxide production change can raise the whole cellular ROS level via a resonance phenomenon. Mitochondrial ROS production level is generally escalated in cancerous cells through mutations which in turn can contribute to the transformation of healthy cells into tumors. However, it puts them in dangerous territory, in terms of their vulnerability to therapeutic interventions that further stress their redox homeostasis. In our model, we set different mitochondrial network density and superoxide production rate for normal and tumor cells. By applying an ELF magnetic field with the frequency near the natural frequency of the system, we observed a resonance effect in cancerous but not in the normal tissue which resulted in a dramatic rise in cytosolic ROS concentration. In conclusion, this may explain how ROS level in tumor cells which survive at the edge of instability can be pushed beyond a critical level under the exposure of the ELF fields and eventually force these tissues to apoptosis pathways.

P-163

Distribution of membrane stress during cell division

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Distribution of membrane stress during cell division.

A problem of great interest is that of cell division. In this work, we present recent developments based on the distribution of stress during this phenomena. The elastic energy, is modeled by using the Helfrich energy whereas internal ordering on the membrane is modeled by the Frank nematic energy to take into account the splay, twist and bend the molecular nematic director. The cell division is modeled by perturbation of the shape membrane. Using the general expressions for the induced stres, we find analytical expressions for the stress generated by azimuthal perturbations, and show how the elastic and nematic stresses are distributed through the deformed membrane.

Sunday 21st July

TRAFFICKING AND SIGNALLING

P-164 (O-054)

TANGO1 Regulates Membrane Tension to Mediate Collagen Export from the Endoplasmic Reticulum

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Collagens are the main component of the extracellular matrix, a proteinaceous network that provides the structural integrity necessary for multicellularity. Collagens are bulky secretory proteins, which are *de novo* synthesized into the endoplasmic reticulum (ER), from where they are exported to the Golgi complex before being secreted. Despite their fundamental importance, the molecular and biophysical mechanisms of how collagens are exported from the ER still remain poorly understood. An ER-resident transmembrane protein, TANGO1, is required for the export of collagens by modulating and physically connecting the cytosolic COPII membrane-remodeling machinery to the collagens in the ER lumen. We recently monitored by super-resolution nanoscopy the organization of TANGO1 at collagen export sites, showing that TANGO1 assembles into rings around COPII proteins. How are these TANGO1 rings formed? How do they mediate collagen export from the ER? Here, we present a biophysical model in which TANGO1 forms a linear filament that wraps around COPII lattices to stabilize the neck of a growing carrier. Our model predicts how the different physical interactions of TANGO1 proteins regulate the size and shape of the rings. Moreover, our results indicate that the growth of a collagen-containing export intermediate may be driven by a local reduction of the ER membrane tension, which can be ascribed to the TANGO1-dependent tethering and fusion of ER-Golgi intermediate compartment (ERGIC) membranes to the sites of collagen export. Altogether, our results show that TANGO1 can induce the formation of transport intermediates by regulating ER membrane tension and thus controlling collagen export from the ER.

P-165 (O-055)

Facile membrane flow and tension equilibration at a presynaptic nerve terminal

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A crucial factor regulating synaptic transmission by neurons and hormone release by neuroendocrine cells is membrane tension, σ. Increased σ promotes fusion pore dilation leading to increased cargo release and strongly inhibits endocytosis, while decreased σ leads to transient pore opening resulting in incomplete cargo release and facilitates endocytosis. It has been proposed that a reduction in σ due to exocytic membrane addition to the presynaptic terminal surface could provide a mechanism that couples exo- and endocytosis. Such a coupling implies membrane flow from an exocytic site to an endocytic one, but such flows have never been demonstrated, and so the relationship between synaptic vesicle (SV) and σ dynamics is unknown. This is mainly due to challenges in the measurement of activity-dependent tension changes at nerve terminals, which are typically too small to allow direct access for tension measurements. We have overcome these challenges by using the giant synaptic terminal (~10 μm) of the goldfish retinal bipolar neuron. Such terminals are ideal for studying properties of presynaptic membranes, as each is filled with ~500,000 SVs, and respond to stimulation in a graded manner, increasing terminal area by up to ~15 % upon depolarization due to rapid exocytosis, followed by slower recovery due to endocytosis.

To measure σ, we use optical tweezers to manipulate a 1-3 μm bead, which is briefly brought into contact with a synaptic terminal, then pulled away to create a thin membrane tether between the bead and the terminal. The tether force reports σ. In addition, we manipulate membrane tethers to probe membrane flows and membrane-cortex drag.

We observe activity-dependent changes in σ consistent with exo- and endocytosis, as well as changes in tether force resulting directly from synapse depolarization– a mechano-electric effect. Unexpectedly, we also found that membrane tethers can be dragged across the terminal with little resistance, in sharp contrast to other cell membranes where cytoskeleton-PM interactions regulate σ and severely limit membrane flows. We conclude that exceptionally facile membrane flow and tension equilibration at the presynaptic plasma membrane is tuned for rapid turnover of synaptic vesicles, thus playing a key role in neurotransmission.

P-166 (O-056)

Allosteric regulation of small GTPases at the surface of membranes

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Lipidated small GTPases regulate most aspects of "cell logistics" including signaling, membrane traffic, cell shape and motility, and they are associated with myriad severe diseases. To function, they assemble multiprotein complexes at the surface of membranes to propagate actions in the cell, but an integrated understanding of their interactions with the lipid bilayer is still lacking. I will describe recent research in which we combined X-ray crystallography, SAXS, molecular dynamics, HDX-MS and fluorescence kinetics to understand the inner workings of small GTPases and their regulators on membranes, and how it led to new concepts in drug discovery.

References:
Aizel et al., Integrated conformational and lipid-sensing regulation of endosomal ArfGEF BRAG2. *PLoS Biol*. (2013) 11:e1001652.
Karandur et al. Multiple interactions between an Arf/GEF complex and charged lipids determine activation kinetics on the membrane. *PNAS* (2017), 114:11416-11421.
Nawrotek et al. PH-domain-binding inhibitors of nucleotide exchange factor BRAG2 disrupt Arf GTPase signaling. *Nat. Chem. Biol*. (2019) 15:358-366.

P-167

Modeling of morphological change during autophagosome formation

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Autophagy is an intracellular degradation process mediated by the autophagosome. Autophagosome formation involves development of a small membrane cisterna into a cup-shaped structure and a double-membrane spherical structure by closing the edge. In this presentation, we discuss the mechanism of autophagosome formation from a physical point of view. A flat cisterna has a highly-curved rim, which is energetically unstable. A physical model in this condition indicates an abrupt transition from a flat to closed shape along with the growth of the cisterna. However, live-imaging experiments have shown gradual formation of autophagosomes. In order to explain the gradual shape transition, we hypothesize the presence of "*curvature-generators*" that stabilize the highly-curved membrane, and consider a new physical model incorporating the effect. We show that the curvature-generators localize at the highly-curved rim and stabilize it. During expansion of the cisterna, intermediate cup-shape states appear. The closing dynamics becomes moderate and similar to that of autophagosome formation. Our model can quantitatively reproduce these dynamics during the autophagosome formation observed in vivo. Furthermore the model predicts that the amount of the curvature-generators regulates the size of autophagosome.

P-168

Probing synaptotagmin oligomerization by Atomic Force Microscopy

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Atomic Force Microscopy (AFM) is well-established methodology that can be used to observe the structure of biomolecules and measure their mechanical properties. However, to understand the molecular mechanism of dynamic biomolecules, the spatiotemporal resolution of conventional AFM had to be improved. In the last decade, High-Speed AFM (HS-AFM) has been developed and successfully applied to several cellular machineries, both in solution and bound to membranes. The molecular movies obtained by this method provide insights otherwise not accessible by other means to date.

Recently, we have applied HS-AFM to visualize the molecular mechanism of the Endosomal Sorting Complex Required for Transport-III (ESCRT-III) assembly formation. We observed the formation of spiraling filaments on lipid membranes and estimated that the accumulated elastic energy is sufficient to drive membrane deformation. In addition, we studied the molecular role of Vps4, an ATPase that it is known to drive the disassembly of persisting filaments of ESCRT-III, revealing a dynamic ESCRT subunit exchange.

Using similar methodological approach, we aim to visualize the organization of the synaptic exocytic machinery that enables rapid and synchronous neurotransmitter release. During this process, the exocytic machinery is 'clamped' in an intermediate, activated state until released by Ca^{2+} influx. The protein machinery involved is known, but the supra-molecular architecture and underlying mechanisms are unclear. Recently, we have demonstrated that Synaptotagmin-1, a key protein involved in this process, self-assembles into Ca^{2+}-sensitive ring-like oligomers on lipid monolayers. Functional and physiological analyses have revealed that these oligomeric structures are critical for Ca^{2+}-regulated exocytosis. Hence, we hypothesize that Synaptotagmin-1 oligomeric structures formed at the vesicle-plasma membrane interface organizes the exocytic machinery to regulate neurotransmitter release. To test this hypothesis, we aim to employ correlative HS-AFM and super-resolution optical microscopy to delineate the molecular structures formed at synaptic vesicle-plasma membrane junction in reconstituted system, using purified proteins. HS-AFM will provide sub-second temporal resolution and nanometer spatial resolution of label-free complexes assembly in the lipid membrane and allow us to map the nanomechanical properties of any supra-molecular structures formed on the membrane. This study will provide both structural and mechanical insights into a fundamental physiological process.

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TRPV5 – Calmodulin interaction: surfaces involved and calcium dependency

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Introduction

The epithelial calcium (Ca^{2+}) channel TRPV5 is the gatekeeper of Ca^{2+} reabsorption in the kidney, which is essential to maintain plasma Ca^{2+} levels within a narrow range. Alterations in the Ca^{2+} balance are related to bone disorders, tetany, cardiac aberrations and kidney stone formation. In contrast to its other TRPV family members, TRPV5 and its homologue TRPV6 are characterized by a high selectivity for Ca^{2+}. Channel activity is regulated by a negative feedback via a dynamic interaction with Ca^{2+}/calmodulin (CaM). CaM is a Ca^{2+}-binding protein that consists of two lobes (N- and C-lobe) that each contain two Ca^{2+} binding sites. Despite recent structural insight into the TRPV5-CaM complex, the functional link towards channel regulation is not well understood. By using the fluorescence life time imaging microscopy (FLIM)-based FRET technique, the interaction of mCherry-tagged TRPV5 with GFP-tagged CaM was studied. The Ca^{2+}-dependency of this interaction was investigated by studying the interaction of TRPV5 with Ca^{2+}-binding mutants of CaM. Moreover, various TRPV5 residues that were shown to play a role in the CaM interaction were mutated and studied according to the recent TRPV5 structures obtained by cryo-electron microscopy. The link with TRPV5 channel activity was examined by Fura-2-AM fluorescent Ca^{2+} imaging and electrophysiological analyses. Our study further substantiates the interaction of TRPV5 with wildtype CaM, and demonstrates that the channel is also able to interact with a Ca^{2+}-deficient CaM mutant (apoCaM). Further FLIM-FRET analyses disclosed intermediate states of TRPV5-CaM interaction for lobe-specific Ca^{2+}-binding mutants. Additionally, significant differences in FRET efficiency of the TRPV5 mutants with wildtype CaM were identified, and thereby the interaction interface of TRPV5-CaM as well as the Ca^{2+}-dependency of interaction was unraveled. Preliminary data of Fura-2-AM imaging suggests a link between different CaM binding states and TRPV5 channel function. By using a combination of fluorescent microscopy and functional assays, this study revealed the dynamics of TRPV5-CaM binding and provides a model for CaM-dependent channel inactivation. Future work should identify whether our model is unique to the Ca^{2+}-selective TRPV5 and TRPV6 or can be extrapolated to other TRPV channels.

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Nuclear transport receptors play a role in maintaining the RanGTP/RanGDP gradient

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Nucleocytoplasmic transport (NCT) is a fundamental biological process in eukaryotic cells. This is facilitated by nuclear transport receptors known as importins and exportins that traffic specific cargoes through nuclear pore complexes (NPCs). Recently, we found that a key importin, Kapβ1, is highly enriched in NPCs and in doing so functionally reinforces the NPC selective barrier [1-3]. The bound-fraction of NTRs at the NPC is governed by their affinity towards FG Nups and cellular concentration. Here, we show now that cellular apoptosis susceptibility protein (CAS, known also as Xpo2 or Exportin2), interacts differently with the phenylalanine-glycine nucleoporins (FG Nups) and consequently is less enriched at NPCs. Immunostaining reveals that CAS accumulates mostly inside the nucleus in contrast to Kapβ1. In addition to its interaction with the FG Nups, this suggests that an additional mechanism is required to regulate the nuclear localization of CAS. Indeed, CAS functions to export Kapα, an adaptor protein required for the specific cargo import. Furthermore, import and export processes are dependent on the RanGTP/RanGDP gradient that is established at the NPC being generated by RanGAP/RanBP1 and RanGEF [4]. We show here that an NPC transport barrier can be also selective for such small cargos. As in the present case, RanGTP or RanGDP interact with transport receptors within the NPC and this in turn can play a role in maintaining the RanGTP/RanGDP gradient.

RanGTP/RanGDP gradient

[1] Lim R.Y.H., Binlu Huang, Kapinos L.E., (2015) *Nucleus,* **6***, 366*

[2] Schoch R. L., Kapinos L.E., Lim *(2012) Proc Natl Acad Sci USA,* **109***, 16911*

[3] Kapinos L.E.et al., (2014) *Biophys. J,***106***, 1751*

[4] Kapinos L.E.et al., (2017) *JCB,* **216***, 3809*

P-171

A potent partial agonist of PKC orients in membranes like the biological activator diacylglycerol

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The enzyme protein kinase C (PKC) has a plethora of roles in human physiology, and thus plays also a significant role in pathologies of many diseases, such as cancer and Alzheimer's disease [1, 2]. This enzyme is activated when its C1 domain interacts with an intracellular membrane that incorporates the lipid second messenger diacylglycerol (DAG). In cancer research PKC was previously thought to be an oncoprotein, however, upon discovering that PKC associated cancer activity is in fact associated with loss-of-function mutations in PKC, moderate activation could actually be tumour suppressing [2]. The PKC enzyme's activation is also involved in learning and memory formation, and therefore drugs that modulate PKC could additionally be used to treat Alzheimer's disease.

In studies performed by R. Provenzani et al. isophthalate derivatives and pyrimidine analogs, respectively, were tested to target PKC [3]. Both sets of compounds were predicted to bind well to the C1 domain based on their structure, however, only isophthalate derivates showed in vitro binding in a standard assay with phosphatidylserine membrane present.

These behaviors are not completely explained by just the fit of the molecule to the binding site of the PKC C1 domain, and thus we hypothesized that the interaction of the drug molecules and the respective inner plasma membrane is of importance for activation. To answer this, we simulated one isophthalate and a respective pyrimidine compound in the PS binding assay environment.

Analysis of the molecular dynamics simulations indicate that isophthalate compound quickly equilibrates to DAG-like orientation, but the pyrimidine compound fails to do so, while also sinking deeper into the membrane. This results to the pyrimidine compound being inaccessible for the enzyme at the interface. These factors begin to explain the difference in experiments, and highlight the importance of environment effects in drug design.

References

[1] V. Talman et al., Basic & Clinical Pharmacology & Toxicology, vol. 119, no. 2, pp. 149–160 (2016).

[2] A. C. Newton et al., Trends in Pharmacological Sciences, vol. 38, no. 5, pp. 438 – 447 (2017).

[3] R. Provenzani, et al., Plos One, 13(4) (2018)

P-172

Dynamic recruitment of BAR domain proteins by Fluid-FM combined with fast-scanning confocal microscopy

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Cell responses to external stimuli and many vital processes, such as nutrient uptake and receptor turnover, are mediated by cell membrane and endocytosis. Endocytosis occurs through a variety of pathways, broadly classified into clathrin-mediated and non clathrin-mediated[1-2]. While clathrin-mediated endocytosis is well characterized, most of non-clathrin mediated endocytic pathways are poorly understood from a mechanistic point of view. In this context, BAR (Bin/amphiphysin/Rvs)-domain proteins play a key role in membrane curvature induction/recognition, and some of them, particularly the endophilin subclass, have been found to be involved in non-clathrin dependent endocytosis of several cargoes, such as β-adrenergic receptors, IL-2 receptor or bacterial toxins[3-5]. Nevertheless, the dynamics of endophilins participation in receptor-endocytosis is still not clear, as well as many aspects of the molecular mechanisms.

We present an original single-cell approach, using the combination of Fluidic Force microscopy (Fluid-FM) and fast scanning confocal microscopy. This set-up allows the dynamic imaging of endophilin recruitment to be followed directly at the cell membrane in response to local extracellular stimulation. Fluid-FM combines microfluidics to accurate force control by using microchanneled cantilevers connected in parallel to a vacuum pump and to an atomic force microscope (AFM). In our approach, we tested the induction of endophilin-mediated endocytosis from several ligands that have been immobilized on the surface of sub-micron beads and trapped at the aperture of Fluid-FM cantilevers. By approaching the cantilevers to cells expressing fluorescently-labeled endophilins we achieved spatially and temporally resolved extracellular stimulation at controlled force, while monitoring simultaneously the dynamic movement of the endophilins in response to the local extracellular stimulus via fast-scanning confocal microscopy. Although at its infancy, this new approach holds the potential of monitoring, *in situ* and with high spatial and temporal resolution, variations of cell mechanical properties and intracellular trafficking in response to extracellular signalling, while measuring simultaneously interaction forces, thus enabling to tackle a broad range of unsolved biological questions.

References

1. Kaksonen, M. et al. Nat Rev Mol Cell Biol. (2018)
2. Mayor, S. et al. Nat Rev Mol Cell Biol. (2007)
3. Boucrot, E. et al. Nature (2015)
4. Chan Wah Hak, L. et al. Nat Cell Biol (2018)
5. Renard, H.F. et al. Nature (2015)

P-173

Gag polyprotein of HIV-1 shows preference to membrane curvature

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Enveloped viruses presents a large family of pathogens including such dangerous ones like HIV, Ebola, Zika, etc. These viruses have their genome wrapped in a protein scaffold, which, in turn, is surrounded by the bilayer lipid membrane. Viral membrane is formed by lipid molecules captured by the virus during its budding from the plasma membrane of the infected cell. It is of great interest to investigate, which of the viral proteins is responsible for the formation of the curvature required for bud initiation and whether viral proteins have sensitivity to membrane curvature. Using a system of lipid nanotubes, which allows the creation of highly curved lipid surfaces, we investigated the self-organization of the human immunodeficiency virus (HIV-1) polyprotein Gag and showed that this protein tends to self-organize in curved sections of the membrane.

P-174

Basal actin cytoskeleton-dependent siglec-1 nanoclustering facilitates the formation of HIV-sac compartments in mature dendritic cells

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Dendritic cells (DC) are antigen presenting cells that play essential roles in the development of innate and adaptive immunity against pathogens. However, in the context of HIV infection, viruses can exploit DCs as vectors to spread the infection to CD4+ T lymphocytes, a process which is specially enhanced in DCs exposed to maturation stimuli. Mature DCs (mDCs) have increased expression levels of the cellular lectin Siglec-1, which mediates HIV capture by interacting with sialyllactose containing gangliosides in the viral membrane. Interestingly, HIV capture by siglec-1 is not followed by an endocytic process. Instead, viral particles are accumulated in an open, viral containing compartment (VCC), preventing mDCs to become infected and averting HIV from endocytic degradation. We combined high-end quantitative fluorescence approaches, including super-resolution microscopy, to show that in mDCs siglec-1 forms basal nanoclusters at a higher frequency than in immature DCs. These nanoclusters appear specially enriched in regions with high density of cortical actin dependent on formins activity. Moreover, siglec-1 exhibits a higher immobile fraction and hindered diffusion, suggesting that siglec-1 nanoclustering in mDCs occurs by spatiotemporal confinement of the receptor due to the underlying actin cytoskeleton. Importantly, this actin-dependent Siglec-1 enrichment facilitates active clustering of the receptor upon HIV engagement, which precedes HIV-induced filopodia growth and actomyosin II contraction leading to the formation of the VCC. Altogether, our results provide new insights on the pathogenesis of HIV on mDC and highlight the essential role of Siglec-1 nanoclustering for evading HIV degradation via the formation of viral compartments.

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Impact of Ca^{2+} induced $PI(4,5)P_2$ clustering on the dynamics of $PI(4,5)P_2$ binding proteins

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Despite its low abundance, phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) is a key modulator of membrane associated signalling events in eukaryotic cells. Temporal and spatial regulation $PI(4,5)P_2$ concentration can achieve localized increases in its concentration which are crucial for activation or recruitment of proteins to the plasma membrane. The recent observation of the dramatic impact of physiological divalent cation concentrations on $PI(4,5)P_2$ clustering, suggests that protein anchoring to the plasma membrane through $PI(4,5)P_2$, is likely not defined solely by a simple (monomeric $PI(4,5)P_2$)/(protein bound $PI(4,5)P_2$) equilibrium, but instead depends on complex protein interactions with $PI(4,5)P_2$ clusters. Nevertheless, the impact of the organization of $PI(4,5)P_2$ at the plasma membrane on its biomolecular interactions with $PI(4,5)P_2$ binding proteins is largely unknown.

Using different advanced spectroscopic (Förster Resonance Energy Transfer, Fluorescence Correlation Spectroscopy and Photon Counting Histogram) and molecular dynamics (Martini forcefield coarse-grained simulations) methodologies, we characterized the impact of calcium on $PI(4,5)P_2$ – protein interactions and membrane organization. We show that in Giant Unilamellar Vesicles (GUVs) presenting $PI(4,5)P_2$, the membrane diffusion properties of pleckstrin homology (PH) domains tagged with a fluorescent protein (FP) are affected by the presence of Ca^{2+}, suggesting interaction of the protein with $PI(4,5)P_2$ clusters. Importantly, PH-FP is found to dimerize in the $PI(4,5)P_2$-containing membranes in the absence of Ca^{2+} and this oligomerization is inhibited in the presence of physiological concentrations of the divalent cation. Furthermore, it was also found that Ca^{2+} induced clustering of $PI(4,5)P_2$ could modulate $PI(4,5)P_2$ binding protein affinity and enhance protein sequestration of the phosphoinositide, significantly depleting the levels of free $PI(4,5)P_2$. These results confirm that Ca^{2+}-dependent $PI(4,5)P_2$ clustering has the potential to dramatically influence affinity, oligomerization and organization of PI(4,5)P2 binding proteins in the plasma membrane.

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THE INTERACTION WITH PHOSPHOLIPIDS OF THE C2 DOMAIN OF PKCepsilon

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The ε isoenzyme of the protein kinase C (PKCε) belongs to the group of novel PKCs and is involved in the regulation of several biological processes, including neuronal differentiation, antiviral resistance, hormone secretion, regulation of transporters and integrin-dependent signaling. The regulatory part of PKCε consists of a C1 domain that binds to diacylglycerols and phorbol esters, and a C2 domain located at the N-terminal region that binds to membranes in a Ca^{2+}-independent manner. The three-dimensional structure of the C2 domain of PKCε (C2ε domain) was determined by crystallography and consists of a single Greek-key motif of 8 antiparallel β-strands assembled in a β-sandwich connected by surface loops and helices. It was demonstrated that C2ε domain mainly binds to negatively charged phospholipids; however also cardiolipin could play an important role in the binding to inner mitochondrial membrane. Indeed, despite it is still not known how PKCs may recognize the mitochondria, in the case of PKCε, the function of mitochondrial cardiolipin seems to be important to this end.

In this work, we wanted to obtain a deep insight about the effect of different lipid composition membranes on C2ε domain thermal denaturation, focusing in particular on the sequence of events that occur during the unfolding process. In addition, we wanted to investigate the role of cardiolipin on the domain-membrane binding. For this purpose, we used infrared spectroscopy (FTIR), generalized two-dimensional correlation analysis (2D-COS), solid state NMR spectroscopy (^{31}P MAS NMR) and model membranes with the following lipid composition: POPA, POPC/POPA (50:50) and POPC/POPE/CL (43:36:21) imitating the inner mitochondrial membrane. Our results confirmed the preference of the domain to form electrostatic interactions with negatively charged phospholipids, and indicated that there is no particular affinity for cardiolipin. Moreover, while the binding to vesicles containing PA induced a stabilization of C2ε domain, the presence of POPC/POPE/CL model membrane decreases its melting temperature. Finally, by using the 2D-COS analysis, we determined the sequence of the events that occur during the domain secondary structure thermal denaturation in the absence and presence of model membranes, highlighting some differences due to the different lipid compositions.

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Walking Step by Step Through the Unconventional Secretory Pathway of FGF2

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Fibroblast Growth Factor 2 (FGF2) is one of the most prominent examples of proteins secreted via the so-called unconventional secretory pathway. It is synthesized in the endoplasmic reticulum, but it can reach extracellular medium independently without a signal recognition peptide. FGF2 is critically important in physiological processes such as cell development, tissue regeneration, and wound healing. It controls normal cell growth and differentiation, and also plays a vital role under pathophysiological conditions by acting as a survival factor, inhibiting tumor cell apoptosis. Biochemical reconstitution experiments have shown that the unconventional secretory mechanism of FGF2 involves translocation of FGF2 across the plasma membrane. This process depends on the recruitment of FGF2 at the inner leaflet of the plasma membrane, which fosters FGF2 oligomer formation. Here, we used molecular docking studies and atomistic molecular dynamics simulations to shed light on the interaction of FGF2 with the plasma membrane and its oligomerization mechanism on the membrane surface. We first showed (1) that the key to the FGF2-membrane interaction is phosphoinositide $PI(4,5)P_2$. The simulations confirmed previous experimental findings that the binding pocket (K128, R129, and K133) interacts with the head group of $PI(4,5)P_2$. Further, the simulations revealed that FGF2 can simultaneously interact with several $PI(4,5)P_2$ molecules through additional residues (K34, K137, K143) that contribute to the high-affinity orientation of FGF2. Moving on, the critical step of FGF2 oligomerization is $PI(4,5)P_2$-dependent dimerization. Extensive simulations confirmed that the key to dimerization is the formation of the intermolecular C95-C95 disulfide bridge, which defines the primary dimerization interface. Molecular docking studies together with MD simulations further revealed (2) two candidates for the second FGF2 oligomerization interface.

1. J.P. Steringer et al., eLife 6, e28985 (2017)

2. F. Lolicato et al., manuscript under preparation (2019).

P-178

Membrane curvature generation by COPII proteins resolved by correlative light and electron microscopy

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Eukaryotic cells use vesicle intermediates to transport proteins between subcellular compartments. COPII machinery is responsible for the secretion of proteins from the endoplasmic reticulum (ER) to the Golgi complex, an essential route for 1/3 of eukaryotic proteome. Those proteins ready for exportation concentrate at ER exit sites (ERES) where scaffolding of COPII captures cargo and enforces membrane deformation to generate free vesicles in the cytoplasm. We know the protein components of this machinery, however our knowledge is limited with regard to how these proteins come together to overcome major energy barriers such as membrane stiffness and cargo crowding. This process is highly dynamic and vesicles are too small to be visualized with standard microscopy techniques. We investigate the ultrastructure of membrane during vesicle formation with a correlative light and electron microscopy approach. We have obtained detailed information about the stages of membrane deformation during COPII vesicle formation and identified free vesicles of 55 nm of diameter on average. Using yeast mutants with compromised membrane bending capacity we have found that different COPII coat subunits have specific roles in curvature generation which ultimately determine size and cargo packing of vesicles. Mutations reducing the stiffness of COPII coat increase the size of buds and vesicles, which could explain the leakage of ER resident proteins described for this strain. We have also explored a mutant of the small GTPase Sar1 with limited membrane deformation capacity in vitro and a delay in cargo secretion. Interestingly, this strain produces on average smaller but more abundant vesicles than wild type, which suggests membrane bending is not the main role of Sar1 in vivo. Our data supports a model for COPII vesicle formation where coat stiffness and control of membrane bending rigidity are required to generate highly curved and cargo crowded vesicles.

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Exploring the role of integrin glycosylation in wound healing at the single molecule level

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Cell migration is essential in a variety of physiological processes, including organ homeostasis, immunological response, cancer metastasis and wound healing. The engagement of the cell with the extracellular matrix (ECM) occurs through interactions between membrane receptors with ECM ligands, inducing the formation of complex multiprotein structures connecting to the actin skeleton.

Integrins are a class of proteins largely involved in cell adhesion and migration. They are glycosylated heterodimeric transmembrane receptors that interact with different components of the extracellular matrix. It is well accepted that integrin's ability to form functional dimers depends upon the presence of N-glycans, that can also modulate integrin's conformation. Consequently, cell migratory behavior may depend on molecular properties of these adhesion proteins, through their N-glycosylation that affects the binding to their ligand.

Although there are evidences that the integrin function can be regulated by changes in glycosylation, the mechanism underlying this effect on cell migration has not been thoroughly investigated. Since integrin diffusion has been shown to be a valuable proxy for the identification of integrin conformation and its linkage to actin cytoskeleton, we are using single particle tracking experiments to further investigate this effect and get insights at the single molecule level.

To this aim, we are studying the diffusion of $\alpha_5\beta_1$ integrins in human dermofibroblasts under resting and wound healing conditions and upon different treatments that impact the level of glycosylation. For this study, we have realized a half-antibody fragment that allow us to track single molecules without inducing artificial clustering and dimerization. Moreover, the use of quantum dots allows us to collect long single-particle trajectories and thus reveal characteristics of the motion with high spatio-temporal resolution. Preliminary control experiments show an integrin behavior in line with what previously reported for labeling with organic fluorophores [1], thus ensuring on the validity of the procedures. We are currently performing experiment in the presence of different treatments to fully characterize the effect of glycosylation. A better understanding of the molecular mechanisms underlying cell migration in wound healing could produce novel diagnostic and therapeutic approaches.

References:

[1] O. Rossier, et al. *Integrins β1 and β3 exhibit distinct dynamic nanoscale organizations inside focal adhesions.* Nature Cell Biology, **14**:1057 (2012).

P-180

Kinetics of synaptotagmins-1 vesicle docking.

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Upon Ca^{2+} influx, synaptotagmin-1 (the main Ca^{2+}-sensor during the neurotransmitter release) binds to the presynaptic membrane and promotes SNARE mediated fusion between the synaptic vesicle and the plasma membrane. Using a stopped-flow approach, we studied the kinetics of the synaptotagmin-1 membrane binding and synaptotagmins-1 vesicle docking. We show that, in the absence of Ca^{2+}, synaptotagmin-1 binds to $PI(4,5)P_2$ on the plasma membrane via its C2B polybasic patch and promotes docking of synaptic vesicles. Upon Ca^{2+} influx, the Ca^{2+} increases the affinity of synaptotagmin-1 to the plasma membrane decreasing the dissociation rate. Additionally, we studied the vesicle docking in the presence of Ca^{2+} and SNARE proteins. Both SNARE proteins and Ca^{2+}, tune the docking kinetics of synaptotagmin-1. Our results shed light on the molecular mechanism of synaptic vesicle and plasma membrane docking during exocytosis.

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Single-spanning transmembrane protein localization to Golgi membranes is independent of transmembrane domain length and composition

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The Golgi complex is the central organelle of the secretory pathway, where proteins are post-translationally modified and subsequently sorted for their export to other parts of the cell. It is a polarized organelle composed of a stack of flattened cisternae, each of which is endowed with a unique biochemical composition. Maintaining this polarity along the Golgi stack is crucial for the sequential modification of secretory proteins. However, how this compartmentation is achieved is not fully understood. In most cases, enzyme retention at specific Golgi cisternae seems to depend mainly on cytosolic and transmembrane domains (TMDs). Different retention mechanisms based on protein oligomerization, recognition of cytoplasmic retention motifs, or on the biophysical properties of the membranes have been proposed. In particular, the hydrophobic matching hypothesis proposes that single-spanning transmembrane proteins preferentially partition into membranes with a thickness that matches the hydrophobic length of the protein TMD. In fact, membrane thickness increases along the secretory pathway and correlates with the average length of the TMDs of the resident proteins. Thus, the segregation between Golgi-resident proteins and cargoes might occur due to different biophysical properties of the TMDs.

To test the role that the hydrophobic matching mechanism plays in controlling how Golgi-resident enzymes are retained and secretory cargoes allowed to exit, we focused specifically on studying the localization of the *trans*-Golgi enzyme sialyltransferase (ST), and TGN46, a protein that cycles between the *trans*-Golgi Network (TGN) and the plasma membrane. These two proteins localize in different domains of the *trans*-Golgi/TGN membranes and have TMD of different length and amino acid composition. Shortening the TMD of TGN46 does not affect its intra-Golgi localization or its glycosylation. Similarly, increasing the length of the TMD of ST does not change its localization. In fact, the intra-Golgi localization of TGN46 was not affected when its TMD was replaced by that of ST. Based on our findings, we suggest that the hydrophobic matching mechanism does not drive Golgi-specific transmembrane protein localization since stronger mechanisms might play a role in recognizing other intra-Golgi localization signals.

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Schistosoma mansoni STIM (SmSTIM) and ORAI (SmORAI) as potential targets in schistosomiasis

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Schistosomiasis is a chronic and debilitating neglected disease that affects over 240 million people in developing and underdeveloped countries. *Schistosoma mansoni* is the main causative agent of schistosomiasis in America. Praziquantel, the only commercially available drug against schistosomiasis, works by disrupting Ca^{2+} homeostasis in adult worms, highlighting the potential of calcium channel proteins as targets for new interventions. Here, we focused on the CRAC (calcium release-activated calcium channel) channel from *S. mansoni,* comprised by *S. mansoni* STIM (SmSTIM) and Orai (SmOrai) proteins. SmSTIM and SmOrai were cloned into mammalian vectors and expressed in HEK293 cells to assess their functionality as a calcium channel. SmCRAC was able to transport calcium and mutations in conserved regions of ORAI abolish its activity, thus displaying a behavior typical of CRACs. The role of the polybasic tail at the C terminus of SmSTIM was also consistent to what have been previously described, being essential for the targeting of ER-PM junctions by interactions with both plasma membrane lipids and SmORAI. However, heterologous expression of SmCRAC in human HEK293 cells resulted in a partially active system, with SmSTIM constantly present at the ER-PM junctions and constitutively activating SmOrai, independently of ER Ca^{2+} store depletion. This suggests that distinct regulatory elements that are absent in mammalian cells must exist in schistosomes. Importantly, we found that some human CRAC channel inhibitors, such as BTP2, showed limited efficiency in blocking schistosome CRAC channel, pointing to pharmacological differences that have potential to be explored in the search for new drugs that selectively target *S. mansoni* CRAC channel.

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Endocytosis across scales: from molecular structures to a functional process

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Endocytosis across scales: from molecular structures to a functional process

Clathrin-mediated endocytosis is a crucial cell biology process allowing internalization of many cell-surface proteins, and other cargo, in eukaryotes. Clathrin-coated vesicles (CCVs) are assembled with their cargo at the plasma membrane, then transport to the early endosome inside the cell. A CCV consists of a clathrin scaffold coating a lipid vesicle, in which the cargo is embedded, linked by adaptor proteins that are associated with effectors of CCV assembly, stability and disassembly. Owen's team recently determined that a single adaptor protein AP2 is sufficient to initiate and drive clathrin-coated bud formation on appropriate membranes, enriched in PtdIns(4,5)P2. [1,2]

In vivo, AP2 interacts solely with one leaflet of the cellular membrane. Therefore, an alternative valid model system is to explore clathrin assembly on a flat lipid surface (in our case a Langmuir monolayer). This allows us to probe the system with a set of state-of-the-art biophysical characterization methods, including neutron reflectometry combined with molecular dynamics simulations, interfacial rheology and imaging methods (AFM and fluorescence microscopy). We thus have been able to analyse the first stages of CCV assembly by using cargo embedded in a lipid monolayer [3]. We show here in particular the influence of AP2, and subsequently the clathrin scaffold, on the composition, structure and mechanics of the complex layer that self-assembles in stages.

References

[1] Miller SE, Owen DJ et al, (2011) *Cell.* **147**, 1118-31.
[2] Kelly BT and Owen DJ, *Science. (2014)* **345**, 459-63.
[3] Maestro A, Zaccai N, Campbell R, Gutfreund P, Owen D and Cicuta P, Submitted 2019.

P-184

Mechanims of activation of the mu-opoid and the vasopressin V2 receptors seen by liquid NMR.

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Opioid receptors and vasopressin receptors are members of the G protein-coupled receptor (GPCR) superfamily. Opioid receptors are involved in pain management and social behaviour whereas vasopressin receptors control water homeostasis. The structures of an inactive and active conformation of the μOR receptor bound to a G protein mimetic nanobody and to the Gi protein have been recently solved [1],[2] [3]. The structure of the vasopressin type 2 receptor (V2R) is still unknown. In both cases, much remains to be learned about the mechanisms by which different agonists can induce distinct levels of G protein activation and/or arrestin recruitment upon activation. Pharmacological and biophysical studies suggest that this versatility can be achieved through the structural plasticity of GPCRs[6].

In this work, we analyze the conformational landscape of the μOR and V2R in distinct pharmacological conditions using liquid-state NMR spectroscopy by monitoring signals from methyl-labelled lysines and methionines. We also investigate the structure and dynamics changes upon binding to different ligands ranging from agonist to antagonists. Our results outline common features as well as distinct particularities. We show that there is very weak allosteric coupling between the agonist binding pocket and G protein coupling interface (transmembrane TM 5 and 6). Furthermore, the analysis provides clues on the successive structural events leading to the full active conformation of μoR and V2R [5]. We know extend this approach to biased ligands, that are able to elicit G-protein activation without arrestin activation, and to partial ligands, that promote only partial activation of the G-protein[6]. In the case of V2R, whose structure is still unknown, NMR restraints help in deciphering the pose of the endogenous vasopressin ligand.

References:

[1] *Manglik A, et al. (2012) Crystal structure of the micro-opioid receptor bound to a morphinan antagonist.* ***Nature*** *485(7398):321-326.*
[2] Huang, W, et al. ***Nature****. 2015,524(7565):315-2*
[3] Koehl A, et al. **Nature**. 2018 558(7711):547-552.
[4] *Ghanouni P, et al. (2001) Functionally different agonists induce distinct conformations in the G protein coupling domain of the beta 2 adrenergic receptor.* ***J. Biol. Chem.*** *276(27):24433-24436.*
[5] Sounier, R., et al. (2015) ***Nature*** *524, 375-378.*
[6] in preparation.

Monday 22nd July

BIOMOLECULAR SIMULATION I

P-453 (O-060)

Automated cryo-EM structure refinement using correlation-driven molecular dynamics

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We present a correlation-driven molecular dynamics (CDMD) method for automated refinement of atomistic models into cryo-electron microscopy (cryo-EM) maps at resolutions ranging from near-atomic to subnanometer. It utilizes a chemically accurate force field and thermodynamic sampling to improve the real-space correlation between the modeled structure and the cryo-EM map. Our framework employs a gradual increase in resolution and map-model agreement as well as simulated annealing, and allows fully automated refinement without manual intervention or any additional rotamer- and backbone-specific restraints. Using multiple challenging systems covering a wide range of map resolutions, system sizes, starting model geometries and distances from the target state, we assess the quality of generated models in terms of both model accuracy and potential of overfitting. To provide an objective comparison, we apply several well-established methods across all examples and demonstrate that CDMD performs best in most cases.

P-454 (O-061)

Aβ peptides and β-sheet breakers. A coarse grained molecular dynamics approach using GO-Martini

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The problem of protein misfolding is at the origin of a class of pathologies called protein conformational disorders (PCD) to which all neuro-degenerative diseases belong. PCD's are characterized by the misfolding of proteins that grow in aggregates of fibrillar shape. Among them, Alzheimer Disease (AD) is one of the most studied for its high impact on the modern society. The plaques present in the brain of AD patients show deposition of fibril made of amyloid β (Aβ) peptides [1]. The process that leads to misfolding, aggregation and amyloid plaques formation is not yet fully elucidated. It seems, however, that the "trigger" of the process is an abnormal switch of the peptide secondary structure leading to β-sheet formation.

Several factors are known to affect Aβ aggregation processes. An important role seems to be played by metal ions that have been observed to be quite abundant in fibrils [2-4]. Recently, the observation that short synthetic peptides, called β-sheet breaker (BSB's), are able to directly interact with Aβ, precluding (or disfavouring) amyloid polymerisation. This finding has stimulated a lot of work in the direction of trying to understand the molecular mechanism by which BSB's are able to slow down or even prevent Aβ aggregation and fibrillation [5].

In this presentation we show how one can get a good understanding of the role that BSBs play in the aggregation process of Aβ peptides by means of *coarse-grained molecular dynamics* simulations based on the Martini force-field. Since the secondary structure switching is a crucial event for the successive aggregation process, we have extended the standard Martini approach to incorporate GO-Martini algorithm [6] that allows to properly model structural switches and study the secondary structure dynamical evolution of Aβ peptides in the presence of BSBs.

1. LC Serpell, *BBA,* 1502(1):16-30, 2000
2. E De Santis et al., ***JPCB*, 119, 15813-15820,** 2015
3. S Morante, GC Rossi, *Advance Alz Res,2:100-147,* 2014
4. F Stellato et al., *Biophys Chem, 229, 110-114,* 2017
5. V Minicozzi et al., *JBC*, 289, 11242-11252, 2014
6. AB Poma et al., *J Chem Theory Comput*, 13(3):1366-1374, 2017

P-455 (O-062)

Effect of pH on the influenza fusion peptide structure and activity: A constant-pH molecular dynamics approach

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Influenza pandemics are serious health threats of our time, in view of the limited treatments available. Research on the molecular mechanisms of infection by the influenza virus (IV) is needed to find new therapeutic targets. Inactivating the fusion of the viral and host membranes is considered a promising strategy, but this process is poorly understood at the molecular level.

The IV fusion process is promoted by the protein hemagglutinin (HA). The IV is uptaken by endocytosis and the low pH of the late endosome triggers a large conformational change of HA that initiates fusion. HA contains a region that is essential for this process: the fusion peptide (FP), which binds to the host membrane and promotes fusion. Interestingly, this peptide is able to induce fusion of lipid vesicles, even in the absence of the rest of the protein, making it a privileged model to study fusion.

In the last years, our group has studied the molecular determinants of the FP activity, showing that this peptide can adopt two different conformations in the membrane, which have different impacts on the membrane properties. Our work also shed light into the mechanisms by which the peptide perturbs the membrane, which include promoting lipid disorder and lipid-tail protrusion.

Given that fusion takes place when the virus is exposed to the low pH of the endosome, we are currently investigating the effect of pH on the influenza FP structure and membrane-interacting properties, by using an in-house developed constant-pH molecular dynamics method to shed light into this question. Our results show that lower pH stabilizes a deeper insertion of the peptide in the membrane and strengthens the interaction of the peptide with the lipids, which results in a higher fusogenic activity. By combining the simulation results with experimental studies performed by our collaborators, this study provides a detailed molecular characterization of the effect of pH on the influenza FP, which can be useful for the design of novel therapies against this devastating pathogen.

Monday 22nd July

MACROMOLECULAR COMPLEXES

P-185 (O-066)

Histone tails in nucleosome: fuzzy interaction with DNA

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New evidence from NMR spectroscopy suggests that histone tails remain highly dynamic even in the condensed state of chromatin. To probe the dynamic behavior of H4 histone N-terminal tail in greater detail, we prepared a sample of mononucleosome containing ^{15}N,^{13}C-labeled H4 histone. The HSQC spectrum of this sample features observable signals from the first fifteen residues in H4; half of these signals have been successfully assigned and used for site-specific ^{15}N relaxation measurements. The experimentally obtained chemical shifts and relaxation rates paint the picture of moderately mobile H4 tail with random-coil-like conformational properties. We have also recorded a μs-long MD trajectory of mononucleosome in the explicit TIP4P-D solvent, which has been designed specifically for (partially) disordered protein systems. This trajectory successfully reproduced the experimentally measured chemical shifts and relaxation rate constants. According to the MD data, the positively charged H4 tail hovers over the negatively charged ds-DNA, making transient contacts with both DNA backbone and major/minor grooves. This type of behavior, underpinned by electrostatic attraction and characterized by substantial mobility of H4 tail relative to the DNA chain, can be classified as "fuzzy interaction". The research was supported by RSF grant 15-14-20038 (modeling component) and NIH grant GM118664 (experimental component).

P-186 (O-067)

New protein-protein interaction modulators for the therapeutic regulation of synapse dysfunction in neurodevelopmental disorders and neurodegeneration

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New protein-protein interaction modulators for the therapeutic regulation of synapse dysfunction in neurodevelopmental disorders and neurodegeneration

The protein complex formed by the Ca^{2+} sensor neuronal calcium sensor 1 (NCS-1) and the guanine exchange factor protein Ric8a co-regulates synapse number and probability of neurotransmitter release, emerging as a potential therapeutic target for diseases affecting synapses [1]. In neurodevelopmental disorders, such as Fragile X syndrome (FXS) or Autism, neurons show an abnormally high synapse number. On the contrary, in neurodegeneration, such as Alzheimer´s, Huntington´s or Parkinson´s diseases, patients show a low synapse number. In the recent years, we have been investigating the structural basis of the NCS-1/Ric8a interaction and found out that the formation of this complex is essential to increase synapse number [1,2]. Therefore, an inhibition of the NCS-1/Ric8a complex would constitute a potential strategy to regulate synapse function in FXS and related disorders. Conversely, the stabilization of this protein-protein interaction could be key to regulate synapses in neurodegeneration. With this aim, virtual screenings and dynamic combinatorial chemistry approaches have been used to find out regulatory molecules of this protein-protein interaction. Further, a multidisciplinary approach including, biochemical, biophysical, crystallographic, cellular and *in vivo* studies have been performed to demonstrate the activity of the compounds, their therapeutic potential and molecular mechanism of action [3,4].

[1] Romero-Pozuelo, J. *et al.* (2014) *Journal of Cell Science* **127**, 4246-4259.
[2] Baños-Mateos, S. *et al.* (2014) *Acta Crystallographica* A**70**, 530-534.
[3] Mansilla, A. et al. (2017) *PNAS* **114(6)**, E999-E1008.
[4] Canal-Martín, A. et al. (2019) *Under review*.

P-187 (O-068)

Structural characterisation of tissue-derived, disease-associated polymers of alpha-1-antitrypsin using conformation-selective antibodies and single-particle reconstructions from electron microscopy images

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α_1-Antitrypsin is an abundant plasma inhibitor of neutrophil elastase, expressed at high levels by hepatocytes, and one of the causative agents of a class of conformational diseases termed serpinopathies. In its active state, α1-antitrypsin is in a kinetically stable, but thermodynamically unstable, configuration, rendering it susceptible to inappropriate conformational change. In individuals homozygous for the Z (E342K) mutation, α_1-antitrypsin accumulates in the liver as dense intracellular deposits, leading to a reduced level in circulation. These deposits are the consequence of an 'ordered aggregation' that yields linear, unbranched protein chains, termed polymers, that are both extremely stable and functionally inactive. The circulating deficiency results in a protease-antiprotease imbalance in the lung, predisposing affected individuals to emphysema and COPD, whilst the hepatic accumulation can lead to liver disease, including cirrhosis and hepatocellular carcinoma.

Our aim is to define the molecular details of the polymerisation pathway, in which α_1-antitrypsin passes through different conformational states as it transitions from the active monomer via one or more structural intermediates to a hyperstable polymeric form. Different models have been proposed for the terminal structure adopted by the pathological polymer; these are are largely based on characterisation of polymers produced under conditions mechanistically or biologically distinct from those existing in vivo, and as such their relevance to the pathological context has not been established. To probe the structural and energetic aspects of the polymerisation pathway, we have generated a molecular toolkit of conformation-specific monoclonal antibodies (mAbs), and mapped their epitopes. We have utilised these mAbs and applied single-particle reconstruction techniques to negative stain and cryo-EM images of polymers extracted from patient explant liver tissue. The resulting maps, in conjunction with molecular modelling, have allowed us to critically evaluate the proposed mechanisms of polymer formation.

P-188

The structure of full-length human phenylalanine hydroxylase in complex with tetrahydrobiopterin

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Phenylalanine hydroxylase (PAH) is a key enzyme in the catabolism of phenylalanine, and mutations in this enzyme cause phenylketonuria (PKU), a genetic disorder that leads to brain damage and mental retardation if untreated. Some patients benefit from supplementation with a synthetic formulation of the cofactor tetrahydrobiopterin (BH_4) that partly acts as a pharmacological chaperone. Here we present the first structures of full-length human PAH (hPAH) both unbound and complexed with BH_4 in the pre-catalytic state. Crystal structures, solved at 3.18Å resolution, show the interactions between the cofactor and PAH, explaining the negative regulation exerted by BH_4. BH_4 forms several H-bonds with the N-terminal autoregulatory tail but is far from the catalytic Fe^{II}. Upon BH_4 binding a polar and salt-bridge interaction network links the three PAH domains, explaining the stability conferred by BH_4. Importantly, BH_4 binding modulates the interaction between subunits, providing information about PAH allostery. Moreover, we also show that the cryo-EM structure of hPAH in absence of BH_4 reveals a highly dynamic conformation for the tetramers. Structural analyses of the hPAH:BH_4 subunits revealed that the substrate-induced movement of Tyr138 into the active site could be coupled to the displacement of BH_4 from the pre-catalytic towards the active conformation, a molecular mechanism that was supported by site directed mutagenesis and targeted MD simulations. Finally, comparison of the rat and human PAH structures show that hPAH is more dynamic, which is related to amino acid substitutions that enhance the flexibility of hPAH and may increase the susceptibility to PKU-associated mutations.

P-189

Fast and effective method for the purification of MBP fusion proteins

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Maltose binding protein (MBP) has a long history as an expression tag for the production of recombinant fusion proteins. The ability of MBP to increase the solubility of fusion proteins makes it a preferred option for the expression of recombinant proteins that are unable to fold properly or that tend to form aggregates. Moreover, the possibility of MBP to enhance the formation of crystal contacts and the fact that the MBP structure is already known provides us with an effective way to determine complex protein structures. Despite the relatively high expression yield of MBP fusion proteins with E.coli, a critical step for obtaining a sufficient amount of the desired protein is the purification step. Commercially available affinity chromatography for the purification of MBP fusion proteins uses polysaccharides, mainly amylose, as ligands responsible for the interaction with MBP. There are two main issues with this type of chromatography: (i) low affinity and (ii) limited number of uses due to the cleavage of polysaccharide matrix by the enzymes present in the crude cell extract. To avoid this problem, we developed an affinity chromatography model based on the protein-protein interaction. We use a specifically evolved DARPin (designed ankyrin repeat proteins), off7, which interacts with MBP with almost 1000 times higher affinity. To optimize the performance of "our" column, we tested several different conditions such as pH, detergents and polyols. The optimized affinity chromatography based on the MBP-off7 interaction enables the purification of the fusion proteins in one step procedure, and the column shows high stability and reproducibility. Our column provides an alternative approach to the purification of proteins containing MBP tag, with comparable or higher expression yield of purified protein than the commercially available affinity chromatographies.

P-190

Super-helical filaments at surfaces: dynamics and elastic responses

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Bio-filaments often behave in a way unexpected from the standard semi-flexible polymer chain model (WLC), when squeezed to a surface, confined in microfluidic channels or clamped by their end. This calls for the super-helical filament model, going beyond WLC, where the filament forms a helix much wider than its diameter. We study this model using Brownian dynamics simulations, focusing on filaments confined to a surface by a strong potential. We analyze shapes and shape fluctuations under tension where excited states comprising a number of inflection points (twist-kink) can be stabilized. Pulling/releasing experiments during a cycle of increasing/decreasing tension show hysteresis. We find that the excited state, once established, is long-lived and the life time grows with the filament length cubed. Twist-kink diffusion involves position (filament shape) dependent friction for which we provide analytical expression. Dynamic responses to tension are investigated via numerical simulations and several mechanisms of shape relaxation are found and rationalized.

P-191

Proton transfer in cytochrome *c* oxidase studied by multiscale simulations

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Cytochrome *c* Oxidase (C*c*O), a membrane-embedded enzyme complex, catalyzes the reduction of molecular oxygen to water. In each cycle it consumes four electrons and four protons from opposite sides of the membrane in which it is embedded. The energy released upon oxygen reduction is used to pump four additional protons across the membrane, thereby further increasing the proton electrochemical gradient, which drives the synthesis of the biological energy carrier ATP [1]. Here, we investigate the role of the controversial H channel in mammalian mitochondrial C*c*O and its yeast homologue. We apply a combination of path optimization methods for the explicit calculation of proton transfer events along the H channel and classical molecular dynamics simulations for the characterization of the conformation and hydration of the H channel in different redox states of wildtype and mutated enzymes [2,3,4]. Based on these results, we will present novel insights into H channel function and will discuss the molecular mechanism of redox-coupled proton pumping in C*c*O.

References

[1] M Wikström, K Krab, V Sharma, Chemical Reviews, **118**, 2469-2490 (2018).
[2] V Sharma, P Jambrina, M Kaukonen, E Rosta, P Rich, PNAS **114**, E10339-E10348 (2017).
[3] M Reidelbach, F Betz, RM Mäusle, and P Imhof, Chemical Physics Letters, **659**, 169-175 (2016).
[4] M Reidelbach, M Weber, and P Imhof, PLoS One, **13**, e0207718 (2018).

P-192

slipping plane shifts during the adsorption of polycations on lipid membranes: Theoretical Analysis of Electrokinetic Data

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One of the main methods for studying the adsorption of charged particles on a lipid membrane is the measurement of electrokinetic mobility and the determination of the ζ-potential. A satisfactory theoretical interpretation of these measurements exists only for ions and small charged molecules and assumes that the slip plane coincides with the surface of the membrane, which is unacceptable for large polymer at the surface of colloid particles. We developed a theoretical model describing adsorption of polycations and taking into account the shift of the slipping plane from the negatively charged surface. The model also considers other physical characteristics of the system, such as partial reversibility of adsorption and binding of counterions to the lipid membrane. We used the model to approximate electrokinetic data for polylysine molecules with varying length in liposome suspension with different ratio of negatively charged (cardiolipin, CL) and neutral (phosphocholine, PC) lipids. To substantiate the basic assumptions of the model, we performed MD simulations of polylysine-lipid systems. The model suggests two fitting parameters — thickness h of the polymer layer at the membrane surface and fraction, β_0 of the occupied area in the saturation. Numerical results well agree with the literature data. Moreover, theoretical and experimental curves show a similar dependence on the ratio CL/PC in the membranes and the length of polymer molecules. The increased amount of the charged lipid in the liposome composition results in the nonlinear increase of the polymer coverage β_0. The more extended macromolecules create the thicker layer at the membrane surface. The shift of the slipping plane explains some features the system, such as a nonmonotonous shift of the zero potential points when increasing concentration of charged lipid in the membrane and membrane overcharge during polymer adsorption.

P-193

How to measure electric dipole moment of polyelectrolyte and extract polyion electric potential from it?

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It is thought that dielectric spectroscopy[1] around MHz region can provide you the distance between polyion chains ξ either in dilute or semidilute systems. But we have shown that it can provide you much more; average dipole moment of the polyelectrolyte and from it polyion-counterion electric potential can be extracted.

We have observed that characteristic length L obtained by MHz dielectric spectroscopy depends on counterion species. We found that L for polyelectrolytes with divalent counterions is 1.3 times larger than for monovalent. Moreover, we find that L is 5 times smaller than ξ. This suggest that previous interpretation[1] was not correct.

Next, with a simple model we show that our L perfectly correspond to average dipole moment of the polyelectrolyte system.

[1]K. Ito et al, Macromolecules, **23**, 857-862 (1990)

P-194

Supramolecular organization of DNA induced by nuclear proteins HMGB1 and H1 binding

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The chromatin of the eukaryotic cells comprises of DNA and variety of nuclear proteins. The most abundant nuclear proteins are histone H1 and non-histone protein HMGB1. HMGB proteins are the family of chromatin proteins that contain the structural HMGB-motif. The histone H1 plays an essential role in the post nucleosomal level of the structural organization of the chromatin. Yet structural information about complexes of both of these proteins with DNA is still not available. We have studied structure of the H1/HMGB1/DNA complexes using AFM, MS, CD and FTIR spectroscopy. Based on the data obtained, we assume that HMGB1 interacts mostly with DNA bases while the H1 binds phosphate groups of DNA. We have also observed interactions between H1 C-terminal tail of the HMGB1, which facilitates inter protein interactions and of aggregation of the complex. Analysis of H1 mass spectrum shows the presence of phosphorylation and acetylation sites in C-terminal domain of H1 which might result in weakening protein-DNA complex.

P-195

A photosensitizing structure targeting bacterial wall through electrostatic interactions

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Antibacterial photosensitization-based treatment is a promising approach that relies on the combined action of otherwise nontoxic molecules (called photosensitizers, PS), visible light, and oxygen to induce formation of reactive oxygen species, particularly singlet oxygen, that result in cellular phototoxicity. One crucial aspect to devise effective photoactive compounds is the introduction of targeting capabilities into the delivery system, so that bacteria are selectively addressed.

The ability of the nitric oxide binding protein nitrophorin 7 (NP7) from *Rhodnius prolixus* to bind to negatively charged membranes, offers an interesting opportunity to develop a delivery system endowed with selectivity towards negatively charged bacterial walls. To establish NP7 as a selective transport structure, we have first labeled the protein with Atto647N-cadaverine and studied the interaction of the compound with model membranes and bacteria using STED nanoscopy. In the presence of negatively charged liposomes, the fluorescence emission comes from the outer layer of the spherical membrane. On the contrary, when neutral liposomes are used, only the buffer solution is fluorescent. We got similar results when Atto647N-labeled NP7 is incubated with bacteria (*E. coli* and *S. aureus*). Fluorescence Correlation Spectroscopy experiments confirm the above findings. Labeling of NP7 with a second, photosensitizing dye is in progress.

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Near-atomic resolution cryo-EM structure of a DNA translocation complex of T7 phage

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Tailed phages (Caudovirales) are the most representative and diversified class of virus group. They are traditionally divided into three families: *Myoviridae*, *Siphoviridae* and *Podoviridae*. Phage T7 belongs to the *Podoviridae* family, which presents a short and non-contractile tail, and it is an interesting system to study how these viruses puncture the double membranes of Gram-negative bacteria. It has been postulated that during infection, the tail interacts with an internal head complex, the core, to form a channel through the bacterial membrane, in order to allow DNA translocation (Hu *et al*. 2013). Here we present near-atomic resolution cryo electron microscopy (cryo-EM) structure at 3.24Å of this DNA translocation complex. Our results suggest that the interaction of two proteins of core complex show a novel type of assisted folding and assembly correlated with the proposed functional hypothesis.

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PULMONARY SURFACTANT AND NANOCARRIERS IN DRUG DELIVERY: EXPLORING THE POTENTIAL USE OF HUMAN PICOBIRNAVIRUS CAPSIDS AS A VEHICLE

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The structure and properties of both the respiratory surface and the pulmonary surfactant (PS) system have positioned lungs in the spotlight of new pathways of drug and nanoparticles administration. The respiratory surface represents the most extensive contact area of our body with the environment (75-140 m^2 in human adults). This surface is coated by a thin aqueous layer which generates an air-liquid interface. To reduce the surface tension of this interface, type II pneumocytes synthetize, assemble and secrete the pulmonary surfactant. PS is a complex mixture of lipids and proteins with unique biophysical properties to efficiently adsorb and rapidly spread along the air-liquid interface, which makes it an attractive system for delivering drugs and nanoparticles throughout the pulmonary surface. To take advantage of this PS ability, we are exploring the possibility of using virus capsids as a nanocarrier and PS as a vehicle of them. Human picobirnavirus (HPBV) capsids, formed by 120 copies of the same capsid protein (CP), could be a good candidate to encapsulate and transport molecules to the lungs.

The objective of this work was to evaluate the capability of HPBV capsids to interact with PS and be transported along the air-liquid interface. We used a biophysical model of the respiratory interface to test if PS is able to carry virus-like particles (VLPs) through the air-liquid interface. VLPs were encapsulated into PS membranes using different strategies, and its transporting capabilities were evaluated by fluorescence and electron microscopy. To improve and favour the interaction of surfactant with VLPs, we also characterized the interaction of VLPs with pulmonary surfactant proteins (the hydrophilic SP-A and SP-D, and the hydrophobic SP-B and SP-C) by different approaches.

The results of our work advance towards the development and optimization of versatile procedures to administrate PS/capsid combinations encapsulating molecules of interest inside the capsids.

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DNA SAMs on flat gold: a combined Atomic Force Microscopy and Spectroscopic Ellipsometry approach

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The increasing interest in biosensing has prompt the study of DNA films to develop reusable, highly sensitive and specific devices. To this aim, a fine control of the bio-interface is crucial: we propose a combined approach which couples Atomic Force nanolithography and Spectroscopic Ellipsometry (SE) to investigate structural and optical properties of DNA films. For ultrathin films, thickness and refractive index are highly correlated properties. The combined approach can be exploited to disentangle the correlation, as we have shown for different self-assembled monolayers (SAMs) on gold (1).

Here we exploit molecular self-assembly of a 22-base, single-stranded DNA bound through an alkyl thiol (C_6) on flat Au films. The C_6-ssDNA SAM was post-treated through exposure to mercaptohexanol (MCH), in order to replace weakly-adsorbed DNA molecules and to increase molecular order.

By AFM nanolithography, regularly defined micro-areas have been depleted from molecules under a high tip load. From the depth of the shaved area an accurate estimate of the film thickness could be obtained, showing a net increase of the SAM thickness upon MCH exposure (2).

By in situ SE dynamic scans we could monitor in real time the interaction of the C_6-ssDNA layer with MCH. SE data show a significant increase of the optical thickness of the film upon MCH exposure, a process which begins immediately after admission of MCH into the cell and goes on slowly.

The combined AFM/SE analysis provided reliable estimates of both the thickness and the refractive index of the biofilm in the Vis-NIR region. We show that the contribution of the thiol/Au interface has to be included in the optical model to obtain a reliable determination of the refractive index of the DNA SAM in liquid (2).

The careful, correlative characterization of the mixed C_6-ssDNA/MCH SAM represents a key step towards the optimization of a robust detection scheme based on helix-helix hybridization.

1) P. Parisse et al. *Ellipsometry of Functional Organic Surfaces and Films*, ed. K. Hinrichs and K. J. Eichhorn, Springer Series in Surface Sciences. **52**, 2nd edn, vol. 4, pp. 63–93 (2018).

2) G. Pinto et al. *Soft Matter*, **15**, 2463 (2019).

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Structural and functional analysis of the role of the chaperonin CCT in mTOR complex assembly

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The mechanistic target of rapamycin (mTOR) kinase forms two multi-protein signaling complexes, mTORC1 and mTORC2, which are master regulators of cell growth, metabolism, survival and autophagy. Two of the main subunits of these complexes are mLST8 and Raptor, β-propeller proteins that stabilize the mTOR kinase domain and recruit substrates, respectively. We have found that the eukaryotic chaperonin CCT plays a key role in mTORC assembly and signaling by folding both mLST8 and Raptor. A high resolution (3.97 Å) cryo-EM structure of the human mLST8-CCT intermediate obtained *in vivo* shows an almost native mLST8 bound to CCT deep within the folding chamber between the two CCT rings, and interacting mainly with the disordered N- and C-termini of specific CCT subunits of both rings. This reveals a unique binding site in CCT for this β-propeller, far away from those found for similar proteins, which are closer to the top of the folding cavity. Moreover, mLST8 associates on the side of CCT (the CCT6 hemisphere) that harbors subunits with poor nucleotide release activity, suggesting a correlation between substrate release and ATP utilization. These observations are consistent with an asymmetrical and sequential mechanism for protein folding by CCT, driven by ATP binding, hydrolysis and release, which is radically different from the rest of the chaperonins.

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Structural characterization of tyrosine hydroxylase

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The aromatic amino acid hydroxylases (AAAHs) constitute a family of enzymes that catalyse the hydroxylation of aromatic amino acids using tetrahydrobiopterin (BH_4) as cofactor and di-oxygen as additional substrate. Tyrosine hydroxylase (TH) is an AAAH that catalyses the conversion of L-tyrosine to L-DOPA, the first and rate-limiting step in the biosynthesis of catecholamine neurotransmitters (dopamine, noradrenaline and adrenaline). TH is a highly controlled enzyme, and the regulatory mechanisms include feed-back inhibition by catecholamine end products and phosphorylation at four different Ser/Thr sites. Mutations in TH are associated with a neuropsychiatric disorder characterized by a large reduction in dopamine and noradrenaline levels, and a metabolic phenotype that is also observed in the non-motor and motor symptoms of the neurodegenerative disease Parkinson's disease (PD).

TH is a 224 kDa homotetramer built by two dimers with a D2 symmetry. Each subunit consists of a regulatory ACT domain with an unstructured N-terminal tail, a catalytic domain and a C-terminal tetramerization domain. To date, only structures of truncated forms of the protein are available, such as the crystal structure of the catalytic and oligomerization domains. Improvements in the purification process have allowed to obtain an active TH with an intact N-terminus. The importance of the N-terminal region lies in its phosphorylation sites and a separated Ala-rich helical motif. These features most likely display a leading role in the regulation of TH.

In this work we have obtained a structure of the full-length human TH at 3.8 Å resolution. The data was collected in a FEI Titan Krios electron microscope equipped with a Gatan K2 Summit direct electron detector. The 3D reconstruction of the homotetramer shows a resolution range from 2.24 Å to 10 Å, corresponding to the oligomerization domains and the regulatory N-terminal domains, respectively. The lowest resolution obtained in the N-terminal tails is consistent with their high flexibility and the disordered region found between residues 1-43. This structural study shows for the first time how these regulatory domains are arranged as a dimer perpendicular to the plane formed by the four catalytic domains. This information will help complete the understanding of the hydroxylation mechanism of TH and its regulatory properties.

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Interaction of Diamminedichloroplatinum(II) with DNA and Nuclear Proteins Studied by FTIR Spectroscopy and Gel Electrophoresis.

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Cis-isomer of diamminedichloroplatinum(II) (DDP) is one of the best known and widespread anticancer drugs. The biological activity of the compound arises from its ability to interact with DNA and proteins. However, many particular aspects of these interactions are not clear yet.

We have studied complexes of core histones (H2A, H2B, H3, H4), linker histone H1 and non-histone chromosomal protein HMGB1 with *cis*- and *trans*- isomers of DDP using FTIR spectroscopy and polyacrylamide gel electrophoresis (PAGE). We have shown that both of the DDP isomers facilitate structural changes in positively charged N-terminal domain of histones and carboxyl groups of C-terminal domain of HMGB1 protein. The observed effect depends on DDP/protein ratio. Also according to electrophoretic analysis DDP causes formation of high molecular fractions of core histones and HMGB1. This effect was similar to both isomers and was not dependent on observed concentrations of DDP. For linker histone in the presence of DDP no changes were observed. Based on the data obtained we conclude that the observed interactions are due to the changes in the protein secondary structure, induced by DDP binding.

We have also investigated interactions of DNA with the DDP by FTIR spectroscopy. We processed the FTIR data using the decomposition of the spectra using second derivative. The applicability of the developed approach to the analysis of FTIR spectra of DNA-containing systems is discussed.

Part of the study was carried out using the equipment of Scientific Park of Saint Petersburg State University (the Center for Optical and Laser Materials Research and Center for Diagnostics of Functional Materials for Medicine, Pharmacology and Nanoelectronics). Financial support of the project was provided by Russian Foundation for Basic Research (grant RFBR 18-08-01500).

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Nanoparticles of PMMA-b-PDMAEMA for gene delivery

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The combination of PMMA (methyl polymethacrylate), a polymer soluble in organic solvents, and the water-soluble PDMAEMA (poly-2-dimethylamine methacrylate), creates an amphiphilic copolymer (PMMA-b-PDMAEMA) with pH-dependent physico-chemical properties. The aim of this study was to prepare and characterize nanoparticles (NPs) of PMMA-b-PDMAEMA copolymers made from different PMMA to PDMAEMA molar ratio and test their potential to delivery plasmid DNA into HEK 293T cells. In this work we demonstrated the efficiency of PMMA-b-PDMAEMA NPs in cellular transfection process instead of the conventional process based on lipofectamine. The NPs were prepared using the nanoprecipitation method with some adaptations. The NPs were characterized by measuring the hydrodynamic diameter, zeta potential (ZP), X-Ray diffraction, FTIR, DSC, and TEM. To follow the transfection process using SR GSD 3D microscopy the pIRES2-GFP plasmid was bound with Rhodamine 6G-encapsulated NPs. Bare NPs presented sizes in the range of 60-100 nm and PZ of -20 to -30 mV. The TEM and X-Ray diffraction showed spherical and solids nanoparticles, respectively. GSD microscopy showed that Rhodamine 6G-encapsulated NPs were able to pass through the HEK 293T cell plasmatic membrane and deliver the pIRES2-GFP plasmid. Phase contrast optical microscopy of POPC giant unilamellar vesicles (GUVs) showed that NPs adhered onto the lipid bilayer. The copolymer hydrophilic-hydrophobic balance was also important in GFP gene transfection since the 1:1 PMMA:PDMAEMA ratio NPs seemed to be more efficient than other copolymer studied. Furthermore, the NPs size and zeta potencial were pH sensible due to amine group present in PDMAEMA portion.

Financial support: FAPESP (2016/13368-4), CAPES, INCT-Fx

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Modelling insights into the function of respiratory complex cytochrome bc_1

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Cytochrome bc_1 is a dimeric, membranous protein complex, which plays an essential role in biological energy production as part of the electron transport chain. The enzyme catalyzes the oxidation of bound quinol (QH_2) to quinone (Q_{ox}), which is coupled to the release of two protons into the intermembrane space, leading to the formation of an electrochemical gradient which is used to drive the synthesis of ATP [1]. This oxidation involves the bifurcation of two electrons, with one reducing a cytochrome *c* molecule, and the other being used in the reduction of Q_{ox}. Currently, questions still remain about the precise mechanism of electron flow within cytochrome bc_1, which is especially relevant due to its role as one of the major ROS producers [2]. Experimental mutagenesis data is being used to provide a vital insight into the function of this enzyme [3], and to complement this ongoing research, we have used multiscale computational approaches. Classical molecular dynamics simulations have been performed on large bacterial and mitochondrial complexes in full membrane-solvent environment, which revealed large-scale conformational changes that are important for its function. In addition to this, quantum chemical DFT calculations have been used to explain the geometric and energetic effects in combination with the spectroscopic data. Our work provides novel mechanistic insights into the function of cytochrome bc_1; an enzyme at the centre stage of mitochondrial function and dysfunction.

[1] M. Wikström, V. Sharma, V. Kaila, J. Hosler, G. Hummer, *Chem. Rev.*, **115**, 2196-2221. (2015)
[2] R. Pietras, M. Sarewicz and A. Osyczka, *J. R. Soc. Interface*, **13**, 20160133. (2016)
[3] Borek A, Ekiert R., Osyczka A, *Bioch. Biophys. Acta,* **1859**, 754-761. (2018)

P-204

Determination at the molecular level of the key properties controlling bacterial flagellum interaction with plasma membrane lipids

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Animal pathogenic enterobacteria are a major source of foodborne outbreaks. Among them, Enterohaemorrhagic *Escherichia coli* (EHEC) O157:H7 is known to lead to severe diseases like haemolytic uraemic syndrome. The adhesion on tissue, which is the first step initiating the colonisation, is mediated by cell surface appendages and organelles, including flagellum. Recently, EHEC flagellum has been described to recognise the phospholipids of plant plasma membranes [1]. This finding stressed the need to investigate further the molecular mechanisms of the flagellum interaction with plasma membrane lipids.

The flagellum interaction with membrane lipids was studied using Giant Unilamellar Vesicules (GUVs). GUVs are widely used as the protein-free membrane model that renders identical the cell plasma membrane curvature. We observed by epifluorescence microscopy that EHEC O157:H7 are able to associate with and around the GUVs contrary to the aflagellated mutant. With the aim of quantifying the bacterial adhesion on liposomes, we develop a new assay specifically designed to screen in a reproducible manner several parameters such as the bacterial phenotype, the lipid vesicle diameter or their composition. Membrane curvature and lipid packing appears to be crucial for flagellum adhesion. The increase of the lipid polyunsaturation degree within the GUV lipid mixture reduces significantly the bacterial adhesion. Based on these results, the influence of the lipid packing on the bacteria interaction has been confirmed at the whole cell scale. We used HT29 cell lines incubated with different fatty acids. The incorporation of polyunsaturated lipids in HT29 cell line impacts the adhesion of WT EHEC O157:H7.

The results generated in the present study give the first elements for a comprehensive elucidation of the mechanism of the bacterial flagellum interaction with plasma membrane lipid. A precise understanding of what are the parameters that controls the interaction at the molecular level represents the first step toward a new insight on the mechanisms of pathogen persistence. These results pave the road for designing new solutions to prevent the bacteria attachment on living tissues.

[1] Rossez Y *et al.*, *Environmental microbiology* **16**, 2181-2195 (2014).

P-205

Biochemical and structural characterization of a complex involved in chaperone-assisted UPS degradation.

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Eukaryotic cells have evolved numerous strategies to maintain proteostasis, the delicate balance of synthesis, folding, trafficking and degradation of intra- and extracellular proteins. Molecular chaperones are at the heart of proteostasis as they play essential roles in both protein folding and degradation pathways. The change in the role of the chaperone is dictated by the interaction with different co-chaperones, which can either direct the chaperone:substrate complex towards the folding of the substrate or towards its degradation through two established and specific protein degradation mechanisms, the ubiquitin-proteasome system (UPS) and the autophagy system. One of this co-chaperones is BAG1 (Bcl-2-associated athanogene 1), a Nucleotide Exchange Factor of Hsp70 that contains a Ubiquitin like (UBL) domain shown to mediate the interaction with the proteasome.

In this work, we have searched for the proteasomal subunit involved in the interaction with BAG1. With this purpose, we have cloned and purified different proteasomal subunits involved in the recognition of ubiquitinated proteins or UBL domain-containing proteins. By using pull-down and gel filtration experiments we have found a strong interaction with Rpn1 (PSMD2). Binary (Rpn1:BAG1) and ternary (Rpn1:BAG1:Hsp70) complexes have been isolated and characterized using biochemical and biophysical techniques (e.g. ITC). Recently we have been able to obtain a quaternary complex formed by Rpn1, BAG1, Hsp70 and a model substrate of Hsp70, that will help to elucidate of the substrate delivery mechanism that takes place between Hsp70 and the proteasome.

Complementarily, we have performed structural studies of the different complexes by negative staining electron microscopy (EM) and obtained preliminary models of the interactions which we are currently improving using cryo-EM. The high-resolution information obtained with this technique will be key to understand the role of chaperones in the proteasome-mediated protein degradation.

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Proteins-nanoparticles interactions: influence of emerging factors affecting the protein corona formation

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Nanoparticles are ubiquitous in our environment and their presence inside our bodies is now established. In a biological medium, nanoparticles are spontaneously covered by proteins that form the so-called protein corona. Depending on the corona composition, a nanoparticle will possess a specific "biological identity" conditioning its biodistribution as well as its potential toxicity.

Despite being highly studied, many aspects of the protein adsorption mechanisms remain unknown. Here we focused on the influence of two physicochemical characteristics, which had rarely been addressed: protein size and post-translational modifications. Due to their intensive use, we worked on silica nanoparticles (SiNP).

Using yeast protein extracts and synthetic peptides, the major role of arginine asymmetric dimethylation on proteins/SiNP interaction had been established [1]. The use of experimental and simulation techniques allowed us to understand the mechanism responsible for the high affinity of proteins having this peculiar methylation. This work suggests that post-translational modifications can influence considerably the interactions between proteins and mineral surfaces.

Besides the interaction/no-interaction problematic, another aspect of the protein corona study is to know whether or not the protein adsorption will have an impact on its structure and function. A model system that we developed is the study of hemoproteins adsorbed on SiNP. Myoglobin and Hemoglobin were previously studied [2] [3]. Structural changes (relative loss of the protein secondary and tertiary structure) and modulation of the oxygen-binding activity (loss of the hemoglobin cooperativity) could be shown.

To assess the existence or not of a potential size effect we gathered several hemoproteins with a size range representing the whole protein size spectrum. The impact of their adsorption on SiNP was measured by small angle neutron scattering, circular dichroism and isothermal titration calorimetry. The bottom line of the study is that there is a size effect. The size of a protein will impact both on the way it gets adsorbed and on the structural change that is induced or not.

[1] Marichal L. *et al*. Langmuir 2018, 34, 5312−5322
[2] Devineau S. *et al*. Langmuir 2013, 29, 13465−13472.
[3] Devineau S. *et al*. Langmuir 2017, 33, 3241−3252.

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Dual interactions of bacterial division protein MatP with DNA and lipid membranes

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Cytokinesis in *E. coli* is achieved through a molecular machinery involving over 15 proteins, some of which ensure precise positioning of the division ring at mid-cell to render two daughter cells with equal genomic information and similar size. One of the mechanisms responsible for division site selection is the Ter linkage, consisting of the DNA binding protein MatP and two other proteins bridging the division ring and the chromosome. We have found that, in addition to the already known interaction of MatP with nucleic acids, the protein also binds to lipid membranes matching the lipid composition of the *E. coli* inner membrane. The interaction with lipids was studied using lipid coated microbeads, bio-layer interferometry, and biochemical reconstruction by microfluidics in microdroplets and GUVs. In addition, we have analyzed the complexes of MatP with its target DNA sequence through an orthogonal approach involving analytical ultracentrifugation, light scattering and fluorescence anisotropy methods. Our results imply that, aside of the interactions with nucleic acids, binding to lipid membranes should also be taken into account in the analysis of the function of MatP in the context of division.

This work was supported by FEDER funds, the AEI and the MINECO (BFU2014-52070-C2-2-P and BFU2016-75471-C2-1-P). M.R.-R was supported by the AEI/ESF (BES-2017-082003).

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A new solid-state NMR approach to determine 3D structures of prion amyloid fibrils at atomic resolution

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The amyloid fold is a protein fold involved in different neurodegenerative diseases and in prion propagation mechanisms. In addition, several functional amyloids have also been identified in bacteria, fungi and mammals. Thus numerous proteins can adopt this type of fold, but at the same time amyloid propensity is also highly sequence dependent. The low number of amyloid atomic structures has limited the exploration of the sequence-to-structure interplay for this important class of protein fold.

We develop a solid-state NMR-based approach (1) for atomic structure determination in the fibrillar propagative state to establish amyloid sequence-to-fold relation. Our approach aims at determining prion amyloid fibril structure at an unprecedented atomic resolution. Notably, the quality of solid-state NMR data obtained here compares favorably with the structure determination of the HET-s prion domain (2), the current benchmark in amyloid fibril structure determination.

Using this approach, we compare atomic structures of two highly divergent paralogous functional fungal prions involved in signal transduction cascades. We report a remarkable conservation of their fold, despite extreme sequence divergence. While identical backbone structures occur in these prions despite a very low level of sequence similarity, lack of cross-seeding suggests that a common amyloid fold is not sufficient to permit amyloid templating.

(1) Saupe, Habenstein, Loquet and co. to be submitted

(2) Wasmer et al., Science 2008

P-209

Controlling photosynthetic light harvesting

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Photosynthetic light-harvesting complexes have to balance two vital functions: efficient light harvesting and efficient energy dissipation. The former occurs under low solar irradiation levels, during which the absorbed photoenergy is converted into chemical energy with near-unity quantum efficiency, while the latter, finely tuned photoprotective function serves to safely remove excess absorbed energy to limit photodamage. The nature of both functions is a subject of intense research. The light-harvesting proteins commonly coordinate numerous chromophores in a dense arrangement, resulting in complex, coupled energy-transfer mechanisms. Consequently, transitions between the light-harvesting and photoprotective functions often involve only subtle protein conformational changes. Single-molecule spectroscopy allows one to monitor the functional switches in real time, to explore the protein conformational landscapes and to unravel the underlying photophysical processes. In this presentation I will first show how the functionality of individual light-harvesting proteins of various photosynthetic organisms is naturally tuned by the photon flux[1], pH[2], chromophore arrangement[3], and interaction with accessory proteins[4] by shifting the population equilibrium between states with high and low fluorescence yields. I will proceed to demonstrate how the light harvesting efficiency can be actively controlled through interactions with surface plasmons from metallic nanostructures and through quantum interference effects by applying adaptively shaped light in an iterative learning loop. Both methods allow us to dramatically enhance either inter-chromophore energy transfer or non-radiative deexcitation on demand.

References

1. Gwizdala et al. *J Am Chem Soc* 138 (36): 11616-11622 (2016).
2. Krüger et al. *Biophys J* 102: 2669–2676 (2012); Schlau-Cohen et al. *J Phys Chem Lett* 6: 860-867 (2015).
3. Krüger et al. *Proc Natl Acad Sci USA*, 108:13516-13521 (2011); Krüger et al. *Proc Natl Acad Sci USA* 114: E11063-E11071 (2017). Gwizdala et al. *J Phys Chem Lett* 9:2426-2432 (2018).

P-210

Interaction of synthetic antimicrobial peptides of the Hylin a1 family with models of eukaryotic structures: zwitterionic membranes and DNA.

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Antimicrobial peptides (AMPs) have been pointed as a possible alternative to the increasing pathogen resistance to traditional antibiotics. Particularly, Hylin a1 (IFGAILPLALGALKNLIK), an AMP extracted from the skin secretion of a South American frog *Hypsiboas albopunctatus*, presents a strong cytotoxicity against bacteria and fungus, and a considerable hemolytic action (Crusca *et al*., 2011, Biopolymers, 41). Taking into account the toxicity of these peptides in eukaryotic cells, the present work focuses on the effects of the AMPs in models of eukaryotic structures: zwitterionic vesicles and DNA. We studied the interaction of three Hylin a1 analogues, W^6Hya1 (IFGAI**W**PLALGALKNLIK), D^0W^6Hya1 (**D**IFGAI**W**PLALGALKNLIK), and K^0W^6Hya1 (**K**IFGAI**W**PLALGALKNLIK), with net charge of +3, +2, and +4, respectively, with zwitterionic vesicles of dipalmitoyl phosphatidylcholine (DPPC) and calf-thymus DNA (CT DNA). By using the Trp intrinsic fluorescence spectroscopy, we determined that the AMP affinity to DPPC membranes follows the order D^0W^6Hya1 > W^6Hya1 > K^0W^6Hya1, and showed that the increase of peptide net charge hampers the burial of Trp into the lipid bilayer. Moreover, differential scanning calorimetry profiles show that the—presence of the AMPs homogeneously disturbs the membrane transition, as already observed for the interaction of K^0W^6Hya1 and DPPC membranes, under similar conditions (Enoki *et al*, 2018, Langmuir, 34). Considering the interaction with CT DNA, Trp emission spectra show that all the AMPs interact with CT DNA, displaying a bathochromic shift in the crescent order: K^0W^6Hya1 > W^6Hya1 > D^0W^6Hya1. As for prokaryotic cells, we also investigated the interaction of these AMPs with supercoiled DNA (pDNA). DNA electrophoretic mobility experiments suggest that all the AMPs bind strongly to pDNA possibly by an intercalative manner. Furthermore, polymerization chain reactions (PCRs) of pDNA show that the presence of the peptides do not provoke double-stranded cleavage. Most of the known AMPs are cationic, and their preferential interaction with anionic prokaryotic membranes, as compared to zwitterionic eukaryotic ones, has been largely studied. The present work shows that AMPs electric charge is also relevant for their interaction with zwitterionic membranes and DNA. These findings could help the design of new therapeutic agents, balancing its antimicrobial and toxic effects.

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DESIGN AND DEVELOPMENT OF MULTI-ENZYMATIC COMPLEXES APPLIED TO CELLULOSE BREAKDOWN

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The enzymatic degradation of lignocellulose generates sugars that upon fermentation produce bioethanol. The enzymes currently used in this process are expensive and have low efficiency. It is economically relevant to increase their activity and stability. Cellulosomes are multienzymatic complexes that colocalize different cellulolytic enzymes and cellulose binding domains, increasing their degradation activity through enzymatic proximity and substrate targeting effects. However, the industrial production of natural cellulosomes has serious limitations because of the properties of their scaffolding protein. Our goal is to use the structure of an oligomeric protein that is highly stable and highly expressed in bacteria, as a scaffold for the colocalization of a consortium of cellulolytic enzymes and the development of artificial cellulosomes. For the assembly of these multienzymatic particles we use a non-covalent coupling strategy trough high affinity heterodimeric peptides complementary fused to the oligomeric scaffold and the target enzymes. For this purpose we cloned and expressed in *E.Coli* the catalytic domain of a variety of enzymes, including endoglucanases, exoglucanases, xylanases, beta glucosidases and cellulose binding domains from different organisms, fused to the coupling peptide. The amount and solubility of these fusion proteins were compared to the original isolated domains. The coupling peptide doesn't perturb the solubility of the target proteins. At least one member of each functional category is solubly expressed in significant amounts and has been able to be purified by affinity chromatography. We present advances in their functional characterization.

We have successfully assembled several of the target proteins to the oligomeric scaffold encouraging further development of the artificial cellulosomes. It is expected that these complexes will help to increase the enzymatic lignocellulose degradation, reducing the cost of bioethanol production.

P-212
Dynamics of Bio-Materials at the Nano-to Meso-Scale
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Dynamics of Bio-Materials at the Nano- to Meso-Scale (The Neutron Spin Echo Spectrometer @ SNS and its Biophysics Applications)
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The SNS-NSE instrument at the Spallation Neutron Source, Oak Ridge National Laboratory is the ultrahigh resolution neutron spin echo spectrometer for characterizing the slow dynamics of soft condensed matter. Due to its situation at a pulsating neutron source and several unique features specific only for the SNS-NSE, high data collection efficiency is achieved allowing nearly gapless coverage of a broad wave-vector-time-range with only a few scattering angle settings. The SNS-NSE instrument is particularly suitable to investigate slow dynamical processes and unravel molecular motions at nanoscopic and mesoscopic scale in biophysics. Investigation of macromolecular assemblies of great importance to human health is one of primary applications of the SNS-NSE, attracting users with interest in biophysics and medical science all over the world. Neutron Spin Echo technique can be successfully applied to access the domain dynamics of proteins and enzyme's, domain dynamics that is strongly related with folding-unfolding processes within proteins[1-2] and enzymatic/catalytic reactions in enzymes. Studies of lipid systems and biological membranes are also carried out at SNS-NSE to investigate how cell membranes organize proteins and lipids to accomplish vital physiological processes[3-4], and to observe the disruptive effects of anti-inflammatory medication on membrane cell organization and the transport process thru cell membranes.

1. *Laura R. Stingaciu et al., Scientific Reports 2016, dx.doi.org/10.1038/srep22148;*
2. *Zimei Bu et al., J. of Mol. Biol. 2017, dx.doi.org/10.1016/j.jmb.2017.03.003;*
3. *Stingaciu L. et al., Scientific Reports, 6, 2016, dx.doi.org/10.1038/srep19627;*
4. *V. K. Sharma et al., Phys. Chem. Chem. Phys. 2017, dx.doi.org/10.1039/C6CP06202D;*

Monday 22nd July
EVOLUTIONARY DYNAMICS

P-213 (O-072)
ScarTrace: CRISPR/Cas9-mediated clonal tracing in zebrafish embryonic development and regeneration
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ScarTrace: CRISPR/Cas9-mediated clonal tracing in zebrafish embryonic development and regeneration
Embryonic development is a crucial period in the life of multicellular organisms when limited sets of embryonic progenitors produce all cells in the adult body. Determining which fate these progenitors acquire in adult tissues requires simultaneously measuring clonal history and cell identity at single-cell resolution, which has been a major challenge. In this talk, I will present ScarTrace, a single-cell sequencing strategy that allows to simultaneously quantify clonal history and cell type for thousands of cells obtained from different organs of the adult zebrafish. Using ScarTrace we show that a small set of multipotent embryonic progenitors generates all hematopoietic cells in the kidney marrow. ScarTrace also reveals that epidermal and mesenchymal cells in the caudal fin arise from the same progenitors, and osteoblast-restricted precursors can produce mesenchymal cells during regeneration. Remarkably, we identify a subsetof immune cells in the fin with an epidermal and mesenchymal clonal origin, while a very similar cell type detected in the brain (microglia) shares clonality with the hematopoietic system. This suggests the existence of organ-dependent mechanisms in the role of immunity in tissue repair and maintenance in zebrafish. Similar approaches will have major applications in other experimental systems, in which matching the embryonic clonal origin to the adult cell type will ultimately allow the reconstruction of how the adult body is built from a single cell. Because ScarTrace provides a glimpse of the cellular past, it will be interesting to explore how this history is predictive of the current epigenetic and expression state.

P-214 (O-073)
Rare beneficial mutations cannot halt Muller's ratchet in spatial populations
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Muller's ratchet describes the irreversible accumulation of deleterious mutations in asexual populations. In well-mixed populations the speed of fitness decline is exponentially small in the population size, and any positive rate of beneficial mutations is sufficient to reverse the ratchet in large populations. The behavior is fundamentally different in populations with spatial structure, because the speed of the ratchet remains nonzero in the infinite size limit when the deleterious mutation rate exceeds a critical value. Based on the relation between the spatial ratchet and directed percolation, we develop a scaling theory incorporating both deleterious and beneficial mutations. The theory is verified by extensive simulations in one and two dimensions.

P-215 (O-074)
Exploring phenotypic variability of bacteria using microfluidic cell traps
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In changing environments cell-to-cell variation of phenotypic characteristics and behaviour plays a key role in the survival, adaptation and evolution of bacterial populations. Therefore, single cell level observations are fundamental in order to understand the development and functioning of bacterial populations and communities.
We are developing microfluidic devices consisting single cell traps for bacteria that are used to study long-term growth of bacterial cells and their responses to various environmental factors. We use *Escherichia coli* and *Pseudomonas aeruginosa* cells to study cell to cell variability in quorum sensing, growth, division, cell size, and response to nutrient stress and antibiotics.
In a modified mother machine we are able to trap and observe the descendants of single bacterial cells through many generations. This enables us to correlate phenotypic and behavioural variability to cell relatedness and ageing.

P-216

Phase Transitions and Phase Diagram of the Island Model with Migration

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We investigate the evolutionary dynamics and the phase transitions of the island model which consists of subdivided populations of individuals confined to two islands. In the island model, the population is subdivided so that migration acts to determine the evolutionary dynamics along with selection and genetic drift. The individuals are assumed to be haploid and to be one of two species, X or Y . They reproduce according to their fitness values, die at random, and migrate between the islands. The evolutionary dynamics of an individual based model is formulated in terms of a master equation and is approximated by using the diffusion method as the multidimensional Fokker-Planck equation (FPE) and the coupled non-linear stochastic differential equations (SDEs) with multiplicative noise. We analyze the infinite population limit to find the phase transitions from the monomorphic state of one type to the polymorphic state to the monomorphic state of the other type as we vary the ratio of the fitness values in two islands and complete the phase diagram of our island model.

P-217

Evaluation of Antibiotic Resistance Mechanisms of Dihydrofolate Reductase by Side-Chain Relaxation Times

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Proteins are complex nanomachines operating in a narrow energy range and on different time scales [1,2]. While available studies show that properties deduced from protein dynamics such as hydrogen bond networks, sulphur bridges, root mean squared deviations of backbone dynamics can shed light to this complex picture to some extent, the functioning mechanism is still an enigma for most proteins. Studies conducted in the last decade show that protein functioning is mostly driven by entropic forces. NMR and fluorescence spectroscopy are experimental techniques having unique capabilities for understanding conformational dynamics of a protein at atomic resolution, thus showing traces of these entropic forces. In this work, we have adopted the "Model-Free Approach" by Lipari and Szabo developed for the quantification of NMR data of C-H vectors [1] to the study of the relaxation behavior of side-chain $C_\alpha - C_\beta$ vectors. We analyze molecular dynamics trajectories and separate out the motions driven by side-chain/solvation dynamics and backbone dynamics by time scales by $\tau_{internal}$ and $\tau_{overall}$, respectively. The distribution of relaxation times of the former is modeled into the stretch exponent ($0 < \beta < 1$), whereby the smaller the value, the broader the distribution [2]. We have used this approach to analyze the effect of resistance conferring mutants of the E. coli DHFR towards trimethoprim on the dynamics of the enzyme. We find that our model well-describes the relaxations ($R^2 = 0.98$ for %90 of the residues). The most frequently observed mutant, L28R, slows down the internal motion of side chains with the reduction of the average stretch exponent from 0.36 to 0.26. We will report results for the 10 mutants we have traced in evolutionary experiments and we will relate the findings to the mechanism of action of the mutants at the atomistic level [3].

[1] Lipari, Giovanni, A. Szabo, *J. Am. Chem. Soc.* 104, 4546-4559 (1982).
[2] O.B. Okan, A.R. Atilgan, C. Atilgan, Biophys. J., 97, 2080-2088 (2009).
[3] Y.T. Tamer, et al., *Molecular Biology and Evolution*, under revision. https://www.biorxiv.org/content/early/2018/08/22/398065

P-218

Photodynamic processes and accessibility of singlet oxygen inside the lipid bilayers

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Photosensitizers (PS) are widely used for the photodynamic therapy of cancer. PS molecules kill tumor cells after membrane binding and oxidation of key molecules by reactive oxygen species - mainly singlet oxygen (SO). SO is generated by excitation with visible light. We studied the binding of PS to the surface of free-standing planar lipid membranes by measuring membrane boundary potentials. The same method served to evaluate the rate of SO mediated oxidation of the target molecules: the styryl dye di-4-ANEPPS or RH-421. Adsorption of both sulfonated aluminum(III) phthalocyanins and di-4-ANEPPS to the same side of the membrane resulted in a lower rate of target oxidization than adsorption to opposite membrane leaflets. The observation indicates that SO damages (i) ANEPPS' naphthalene group and (ii) ANEPPS's double bonds in the middle of the molecule. These bonds are closer to the PS on the opposite membrane leaflet and do not elicit a boundary potential change when damaged. The efficiency of target destruction increases with increasing PS penetration depth into the membrane. That is, phthalocyanins with one or two sulfo groups are much more effective than PS with 4 sulfo groups. The latter PS lie on the membrane surface. Negatively charged PS like *5,10,15,20*-tetrakis(p-sulfonatophenyl)porphyrin or positively charged PS like β-imidazolyl substituted porphyrin and it's Zn(II) and In(III) complexes did not interact with ANEPPS's double bonds. The observation suggests that differences in the adsorption depth of phthalocyanins and porphyrins govern the SO accessibility of the target molecule in the lipid bilayer.

P-219

The effect of oxysterol on cholesterol aggregation in water

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Cholesterol (Chol) is a crucial component of animal cell membranes and together with its oxidised forms is an important factor in the control of metabolism. However, an increased level of membrane Chol leads to formation of pure Chol microdomains (CMD) in the membrane. CMDs in membranes of vascular smooth muscle cells precede and contribute to atherosclerosis development [1]. In this process, Chol microcrystallisation in the aqueous environment is a key step [2]. Stereospecificity of cholesterol oxidation products has a significant impact on the development of coronary artery disease (CAD) – the level of free plasma 7β-OH-Chol in patients with CAD is significantly more elevated than that of the free α isoform [3].

In the present study we used molecular modelling methods to study the process of cholesterol aggregation in aqueous environment as a model of the initial step of microcrystallisation and to check how this process is affected by the presence of selected oxidized stereoisomers of cholesterol. We have calculated the free energy of hydration and aggregation for chosen oxysterols and analysed interactions between sterol molecules and sterol-water interactions to get a detailed picture of the initial stages of Chol self-association and self-organization.

1. Mason R. P., Jacob R. F. Circulation, 107, 2270-2273, 2003.
2. Duewell P., Kono H, Rayner K. J., Sirois C. M., Vladimer G. *et al*., Nature, 464, 1357-1362, 2010.
3. Rimner A., Al Makdessi S., Sweidan H., Wischhusen J. *et al.*, Free Radical Biology and Medicine, 38, 535-544, 2005.

Monday 22nd July

BIOPHYSICS OF MEMBRANE OXIDATION (Sponsored by IUPAB)

P-220 (O-078)

Lipids as substrates and dynamic activators: pro-apoptotic lipid peroxidation at the mitochondrial membrane surface.

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Oxidative chemical modifications of lipids change their biophysical properties and thus the behavior and structure of the surrounding membrane. An important recent realization is that the modified lipids are also employed by the cell as crucial quality control signals. The oxidation of mitochondrial lipids is an important trigger of apoptosis, in line with the common observation that pathogenic mitochondrial dysfunction generates excessive reactive oxygen species (ROS). Remarkably, it has been shown that cells do not rely merely on *incidental* oxidative events, but actually facilitate the *pro-active peroxidation of specific mitochondrial lipids* with the help the mitochondrial heme-containing protein cytochrome c.

We dissect the molecular interactions that mediate the formation and activation of the protein-lipid complex behind this pro-apoptotic peroxidation reaction [1]. By solid-state NMR, we probe the interactions between the protein and the lipid bilayer surface, interactions with solvent molecules, and the conformation and dynamics of the membrane-bound protein. Crucially, we also measure the lipid-specific peroxidase activity under these sample conditions via mass spectrometry-based lipidomics, finding a recapitulation of the type of pro-apoptotic cardiolipin peroxidation seen *in vivo*.

We observe the structural and dynamic changes affecting the protein as it associates with the negatively charged bilayer surface. While attaining a lipid-dependent activation of cardiolipin peroxidase activity, the native fold is largely preserved. This is apparent from spectral signatures of the membrane-bound protein, which are clearly distinct from those of denatured cytochrome c. Instead, specific spectral changes indicate localized dynamics that respond to the membrane fluidity. These lipid-associated dynamic changes in the protein rationalize the peroxidase activation in absence of a large-scale unfolding reaction. Upon protein-induced clustering into lipid nanodomains, the cardiolipin lipids themselves act not only as the preferred substrate but also as *dynamic regulators* in this lethal engagement between proteins and lipids at the mitochondrial inner membrane.

[1] M. Li, A. Mandal, V.A. Tyurin, M. DeLucia, J. Ahn, V.E. Kagan, & P.C.A. van der Wel (2019) Structure, **in press**, DOI: https://doi.org/10.1016/j.str.2019.02.007

P-221 (O-079)

The effect of oxidised cholesterol on model red blood cell membranes

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The human erythrocyte is responsible for delivering oxygen to all the body's tissues. To do so effectively, a red blood cell must be able to deform its 8 μm, biconcave plasma membrane to flow through blood vessels as small as 3 μm in diameter. As such, decreased deformability of a red blood cell can significantly reduce its ability to oxygenate tissues. Under oxidative stress, the red blood cell membrane becomes enriched with oxysterols, which increase membrane stiffness, decrease cell deformability and reduce the ability of the cell to transmit signals. A particularly cytotoxic oxysterol, 7-ketocholesterol, is associated with many pathologies such as type 2 diabetes, cardiovascular disease and numerous neurodegenerative diseases. It has previously been found that high levels of 7-ketocholesterol leads to more disordered and less condense lipid membranes. In a erythrocyte, a disordered inner leaflet will interact more strongly with the cytoskeleton, which leads to a stiffer, less deformable cell. We have used a series of all-atom and coarse-grain molecular dynamics simulations to probe the effects of 7-ketocholesterol on model red blood cell membranes. In doing so, we have gained a mechanistic understanding of how 7-ketocholesterol effects the order, dynamical, structural and mechanical properties of the model red blood cell membranes.

P-222 (O-080)

Lipid curvature modulates function of mitochondrial membrane proteins

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Lipid curvature plays an important role in the function of membrane proteins. Oxidative stress and formation of reactive oxygen species (ROS) in mitochondria result in significant modification of membrane lipids: (i) lysolipids are formed due to activation of mitochondrial phospholipase A2 (mt-PLA2), (ii) reactive aldehydes 4-hydroxy-2-nonenal (HNE) and 4-oxo-2-nonenal (ONE) covalently modify phosphatidylethanolamine (PE) headgroup forming HNE-PE, ONE-PE – adducts[1]. We focused on the effect of native and modified membrane lipids with different spontaneous curvatures on the activity of mitochondrial uncoupling protein 1 (UCP1) and ATP/ADP carrier (ANT). By combining measurements of total membrane conductance[2] and lipid bilayer bending moduli we revealed that (i) lysophosphatidylcholines with positive curvature, such as MPC and OPC, decrease bending modulus in neat lipid bilayers and increase protonophoric activity of UCP1 and ANT, (ii) negatively curved PE does not influence protein activity and (iii) PE modified by reactive aldehydes (HNE-PE, ONE-PE – adducts) acts similar to lipids with a positive spontaneous curvature. Molecular dynamics simulations revealed that modified PEs and lysolipids alter lateral pressure profile in lipid bilayer membrane in the same direction and range. Molecular mechanism described in this work brings new perspective in understanding (i) the transport function of mitochondrial membrane proteins and (ii) processes associated with mitochondrial dynamic, including fusion and fission, during oxidative stress.

REFERENCES

1.Jovanovic, O. *et al.* The molecular mechanism behind reactive aldehyde action on transmembrane translocations of proton and potassium ions. *Free Radic Biol Med* **89**, 1067-1076 (2015).

2.Beck, V. *et al.* A new automated technique for the reconstitution of hydrophobic proteins into planar bilayer membranes. Studies of human recombinant uncoupling protein 1. *Biochim Biophys Acta* **1757**, 474-479 (2006).

Monday 22nd July

PROTEIN STRUCTURE AND FUNCTION

P-223 (O-084)

***Campylobacter jejuni* Tlp3 dCache sensing domain specifically recognises hydrophobic amino acids**

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In *Campylobacter jejuni*, chemotaxis and motility have been identified as important virulence factors that are required for host colonisation and invasion. The chemotaxis process involves recognition of chemical cues by the ligand binding domain (LBD) of chemoreceptors also known as methyl-accepting chemotactic proteins. Recently, we determined the crystal structure of *C. jejuni* Tlp3-LBD chemoreceptor in complex with attractant isoleucine, revealing this receptor belongs to the dCache_1 family of sensing modules. In this work, we performed a high-throughput screening of potential ligands and identified additional small molecules that directly interact with Tlp3-LBD. All of the new ligands (leucine, valine, α-amino-N-valeric acid, 2-Amino-3,4-dimethylpentanoic acid, 2-Amino-3-methylhexanoic acid, amino-3,3-dimethylpentanoic acid, alanine and phenylalanine) are hydrophobic amino acids chemically and structurally similar to isoleucine. Analysis of the crystal structures of Tlp3-LBD in complex with these ligands showed that like isoleucine, they bind to the membrane-distal subdomain of the dCache Tlp3 sensing module. The Tlp3-LBD residues that interact with the main chain of isoleucine, leucine, valine, α-amino-N-valeric acid, 2-Amino-3,4-dimethylpentanoic acid, 2-Amino-3-methylhexanoic acid, amino-3,3-dimethylpentanoic acid, alanine and phenylalanine are located at equivalent positions in all complex structures, whilst residues that interact with the side chain move to accommodate the different amino acid ligands up to the length of 5 carbon chain, beyond this length the side chain is flipped from inside of the pocket towards the β3β4 loop as in the case of 2-Amino-3-methylhexanoic acid. In addition, analysis of the structure activity relationship (SAR) reveals that isoleucine possesses the most favored structure to interact with Tlp3-LBD and hence, has highest binding affinity.

P-224 (O-085)

DYNAMICS OF INTRINSICALLY DISORDERED AND UNFOLDED PROTEINS: INVESTIGATIONS USING NEUTRON SPIN-ECHO SPECTROSCOPY

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A characteristic property of intrinsically disordered and unfolded proteins is their high molecular flexibility, which enables the exploration of a large conformational space. We present neutron scattering experiments on the structure and dynamics of the intrinsically disordered myelin basic protein (MBP) and the chemically denatured bovine serum albumin (BSA) in solution (1,2,3). Small-angle neutron scattering (SANS) experiments allowed us to gain information of structural aspects of MBP and denatured BSA as response to denaturant conditions. Using neutron spin-echo (NSE) experiments, we were able to investigate collective motions of the protein chain up to several hundred nanoseconds on the nanometre length-scale. NSE results showed a high flexibility of the unfolded proteins. Internal motions of the intrinsically disordered MBP and denatured BSA were described using normal mode analysis and concepts derived from polymer theory. The contribution of residue-solvent friction was accounted for using the Zimm model including internal friction. Motions of MBP are well described by collective normal modes, while dynamics of denatured BSA show polymer-like dynamics. Disulphide bonds forming loops of amino acids of the peptide backbone have a major impact on internal dynamics of denatured BSA. We see directly in a molecular picture that topological restrictions due to disulfide bridges in denatured BSA create confinement effects: Long-wavelength Zimm modes are strongly reduced in amplitude due to loops formed by disulfide bridges. Dynamics in folded native BSA was measured by NSE as reference. The observed internal dynamic process in native BSA can be attributed to opening and closing motion of the BSA dimer. An intermolecular disulphide bridge between the BSA monomers can form a covalent cross-link establishing a molecular hinge in dimeric BSA in solution. The effect of that hinge on the observed motion of BSA in the dimer is discussed in terms of normal modes in a molecular picture.

References

[1] Stadler et al. J. Am. Chem. Soc., 2014, 136 (19), pp 6987–6994
[2] Ameseder et al. J. Phys. Chem. Lett, 2018, 9, 2469−2473
[3] Ameseder et al. Phys Chem Chem Phys, 2018, 20 (7), 5128-5139

P-225 (O-086)

Side chain to main chain hydrogen bonds stabilize a polyglutamine helix in the activation domain of a transcription factor

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Poly-glutamine (polyQ) tract expansions have been linked to nine human neurodegenerative diseases. Their biological role has been suggested to involve their organization into secondary structure elements depending on their protein sequence context[1,2,3]. For the particular case of the Androgen Receptor (AR) – linked to Kennedy's disease - we recently reported that the Leu-rich segment N-terminal to the polyQ tract acts as a helix-inducing sequence that propagates helicity into the tract itself[4]. We have collected CD, NMR and molecular simulations data on a set of recombinant, isotopically enriched peptides representing increasingly longer AR polyQ tracts up to the lengths found in the average human population (16-25 Gln residues, depending on ethnicity)[4]. Experimental data shows that the helicity of the sequence positively correlates with tract length, and that a rotameric selection affects the sidechains of the initial glutamine residues in the tract upon helicity gain. In turn, chemical shift-reweighted, MD and QM/MM simulation-derived conformational ensembles unveil that helix stabilization is achieved through bifurcate hydrogen bonds involving both the backbone and glutamine sidechains, resulting in a non-canonical helical arrangement and providing a rationale for the sidechain rotameric selection observed by NMR. Leucine to alanine mutations N-terminal to the polyQ tract result in helix destabilization, despite of Ala being intrinsically more efficient at helix propagation. We propose that Leu residues generate a hydrophobic shielding for Gln side-chain-involving hydrogen bonds, providing energetic stabilization by preventing water competition[5]. Such effect can also be observed in the polyQ tract of huntingtin, which has also been reported to populate a helical ensemble[3]. Thus, our observations provide a mechanistic basis for the link that exists between polyQ tract length and transcriptional activity in AR[6] and, more generally, between tract length and aggregation via helical oligomeric intermediates in polyQ diseases.

1. Eftekharzadeh, B. *et al.* **Biophys. J**. *(*2016) Jun 7; *110(11)*: 2361-6.
2. Warner, JB. *et al*. **JACS** (2017) *139*; 14456-15569.
3. Baias, M. *et al.* **JACS** (2017) *139*; 1168-1176.
4. Weng *et al.* **Scientific Rep.** (2017), Jan 16; *7:*40554.
5. Gao, J. *et al.* **Nat. Struct. Mol. Biol.** (2009) *16;* 684-690.
6. Beilin, J. *et al.* **J. Mol. Endocrinol.** (2000) *25*; 85-96.

P-226

The apparent pKa of D22 in the S*taphylococcus epidermis* glucose/H^+ symporter

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Staphylococcus epidermidis glucose/H^+ symporter ($GlcP_{Se}$), a membrane transporter belonging to the major facilitator superfamily (MFS), is highly specific for glucose and a homolog of the human glucose transporters. Asp22 from the proton (H^+) binding site of $GlcP_{Se}$ has been proposed to play an important role in proton translocation [1]. Surface-enhanced infrared absorption spectroscopy (SEIRA) of the immobilized $GlcP_{Se}$ on a modified gold layer [2] in an attenuated total reflectance (ATR) was used to study the pH- and substrate-dependent conformational changes of the protein. We found that Asp22 has a pKa of 8.5 ± 0.1, indifferent to the presence of glucose, similarly to the well-characterized MFS protein, Lactose Permease (LacY), for which the pKa of Glu325 determined in situ by infrared spectroscopy was insensitive to galactopyranoside [3]. Interestingly, LacY and $GlcP_{Se}$ have different locations for their proton binding sites. A neutral replacement of the negatively charged Asp22 lead to positive charge displacements over the entire pH range, suggesting that the polarity change of the *wild type (WT)* reflects the protonation state of Asp22. Mutation of further residues involved in the H^+ binding site were studied. Ile105Ser had no effect on the pKa value of the carboxylic group at position 22. Ile105Ser has a phenotype similar to *WT,* binding sugar in the protonated and deprotonated form. The effect of further variants on proton translocation will be discussed.

[1] Iancu, C. V., Zamoon J., Woo, S. B., Aleshin, A., Choe, J.Y. (2013) PNAS, vol.110, no.44:17862-17867
[2] Ataka K., Heberle J. (2006) Biopolymers 82(4) 415-419
[3] Grytsyk, N., Sugihara, J., Kaback, H. R., Hellwig, P. (2016) PNAS vol.114, no.7:1530-1535

P-227

CO Biosynthesis for the Assembly of the NiFe-dinuclear Active Site in [NiFe]-Hydrogenases

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Carbon monoxide (CO) ligands are coordinated to the metal clusters in hydrogenases that play a central role for H_2 metabolism in microorganisms. Though CO is known to be biosynthesized and assembled into the metal clusters, for which several accessory and chaperone proteins are required, detail molecular mechanisms for its biosynthesis remain elusive. Here, we have determined the crystal structures of HypX that is responsible for CO biosynthesis, based on which we elucidate the molecular mechanism of CO synthesis by HypX.

HypX consists of the N-terminal and the C-terminal domains, which are structurally homologous to the hydrolase domain of N^{10}-formyl-tetrahydrofolate dehydrogenase (FDH) and enoyl-CoA hydratase/isomerase, respectively. HypX binds CoA constitutively as a prosthetic group in the continuous cavity connecting the N- and C-terminal domains. We have also solved the structure of tetrahydrofolate (THF)-bound HypX at a resolution of 2.1 Å. Based on these crystal structures and MD simulations, we propose the molecular mechanism of CO biosynthesis by HypX as follows. The reaction starts with binding N^{10}-formyl-THF as a substrate in the N-terminal domain of HypX. The formyl-group transfer takes place from N^{10}-formyl-THF to CoA to form formyl-CoA in the N-terminal domain. The resulting formyl-CoA is converted into CO and CoA by decarbonylation of the formyl group, which is catalyzed by Tyr416 and/or Glu426 in the C-terminal domain of HypX.

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Time-lapse single-molecule atomic force microscopy investigation of fibrinogen unfolding on graphitic surfaces

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The 340 kDa fibrinogen molecule is one of the major proteins of blood plasma. Adsorption of fibrinogen molecules on a surface mediates many fundamental biological processes such as platelet adhesion, blood clotting and foreign body reaction. Understanding of fibrinogen adsorption and conformational changes on a surface is, therefore, of paramount importance for the development of biochips and biocompatible materials. The aim of this study was to characterize the dynamical behavior of fibrinogen molecules upon their adsorption on graphitic surfaces, which are believed to be promising for the use in biotechnological applications. Using time-lapse atomic force microscopy, we have investigated adsorption and conformational reorganizations of individual fibrinogen molecules onto bare and modified highly oriented pyrolytic graphite. We have visualized in real time and quantitatively characterized surface induced fibrinogen unfolding. The obtained results may be important for development of graphite based biocompatible surfaces. The work was partially supported by the Russian Science Foundation (17-75-30064).

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High resolution atomic force microscopy investigation of myeloperoxidase interaction with blood proteins

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The 140 kDa myeloperoxidase (MPO) is a globular cationic enzyme, which is a major protein of neutrophilic leukocytes. MPO participates in regulation of oxidative stress in vertebrates. It has been suggested that MPO binds with anionic proteins and negatively charged surfaces mostly due electrostatic interactions. However, myeloperoxidase interaction with other proteins of blood has not been extensively studied, especially at a single-molecule level. Such interaction may be important for regulation of essential biological processes including inflammation and blood clotting. In this work, we have characterized interaction of MPO with ceruloplasmin, lactoferrin and fibrinogen molecules using high-resolution atomic force microscopy. In particular, we have revealed the impact of MPO on ceruloplasmin and fibrinogen, which may have high biological relevance. The work was supported by the Russian Science Foundation (17-75-30064).

P-230

Production and fine characterization of Bone Morphogenetic Protein-2 and its antagonist Noggin

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Bone Morphogenetic Protein-2 (BMP-2), which belongs to the transforming growth factor β (TGF-β) superfamily, plays an important role during bone regeneration and repair, and also during various stages of embryonic development[1]. Noggin is a BMP-specific antagonist implicated in the regulation of BMP signaling pathways[2]. Both BMP-2 and Noggin are homodimeric molecules, with each subunit containing several intramolecular disulfide bonds. This complex disulfide bonds network, combined with the presence of large hydrophobic patches on the surface of these proteins, causes their low solubility in aqueous solutions[3]. As a result, BMP-2 and Noggin are commonly produced as inclusion bodies in *Escherichia coli*. The recovery of soluble and active molecules through *in vitro* refolding is difficult and the overall yield is low[4].

Here we propose a new and simple method to produce biologically active BMP-2 and Noggin. Refolding of BMP-2 and Noggin was achieved in a one-step dilution and hydrophobic or ion exchange chromatography was used to purify the refolded proteins. The best conditions yielded significant quantities of dimeric BMP-2 and Noggin, with purity greater than 95%.

Furthermore, structural and functional properties of BMP-2 and Noggin were analysed using a range of biophysical techniques and *in vitro* cell-based assays. Optical spectroscopies were performed to gain structural information, whereas biological activity was tested by measuring alkaline phosphatase activity in ATDC5 cells. These experiments demonstrated that both BMP-2 and Noggin display structural and functional properties similar to their commercial counterparts.

1 Long, Shinong et al. 2006. "Expression, Purification, and Renaturation of Bone Morphogenetic Protein-2 from Escherichia Coli." *Protein Expression and Purification* 46(2): 374–78.
2 Groppe, Jay et al. 2002. "Structural Basis of BMP Signalling Inhibition by the Cystine Knot Protein Noggin." Nature 420(6916): 636–42.
3 Pramesti, H. T. et al. 2012. "Recombinant Human Bone Morphogenetic Protein-2: Optimization of Overproduction, Solubilization, Renaturation and Its Characterization." Biotechnology 11(3): 133–43.
4 Vallejo, Luis Felipe, and Ursula Rinas. 2004. "Optimized Procedure for Renaturation of Recombinant Human Bone Morphogenetic Protein-2 at High Protein Concentration." *Biotechnology and Bioengineering* 85(6): 601–9.

P-231

Structural basis and energy landscape for the Ca^{2+}-gating and calmodulation of the Kv7.2 K^+ channel

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Kv7.2 (KCNQ2) channel is the principal molecular component of the slow voltage-gated non-inactivating K+ Mcurrent, a key controller of the neuronal excitability. To investigate the calmodulin-mediated Ca^{2+} gating of the channel, we used NMR spectroscopy, FRET and HS-AFM to structurally and dynamically describe the association of helices hA and hB of Kv7.2 with calmodulin (CaM), as a function of Ca^{2+} concentration. The structures of the CaM/Kv7.2-hAB complex at three different calcification states are here reported. In the presence of a basal cytosolic Ca^{2+} concentration (10-100 nM) only the N-lobe of calmodulin is Ca^{2+}-loaded and the complex (representative for the open channel). In response to a chemical or electrical signal, intracellular Ca^{2+} levels rise up to 1-10 μM, triggering Ca^{2+} association to the C-lobe. This, essentially the union of Ca^{2+} to EF3, causes a torsion of the helices A and B with respect to CaM of almost 18º. The associated conformational rearrangement is the key biological signal that shifts populations to the closed/inactive channel. This reorientation affects C-lobe of CaM and both helices in Kv7.2, allosterically transducing the information from the Ca^{2+} binding site to the trans-membrane region of the channel.

P-232

Effects of phosphate on photoactivity of the Orange Carotenoid Protein

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The orange carotenoid protein (OCP) is a water-soluble cyanobacterial photoactive protein which protects phycobilisomes (PBs) of these photosynthetic organisms from light-induced damage. Under intense blue–green illumination, a stable orange form turns into the active signaling red quenching form (the color is defined by ketocarotenoid chromophore).

In this study we have combined UV-VIS absorption and Raman spectroscopy to investigate the effect of high phosphate concentrations on the photoactivation and chromophore conformation of the wild type and W288A OCP mutant — a spectral and functional analogue of the red form which is capable of constitute PBs fluorescence quenching *in vitro*. However, the OCP^{W288A} does not require to be photoactivated to induce quenching. Thus, we observed the OCP^{W288A} as a model of stable active form in order to investigate the effect of phosphate stabilization of an active protein structure into the orange form.

Our experiments have demonstrated that high phosphate concentration non-specifically stabilizes the OCP into the orange state after illuminating. The OCP^{W288A}, which previously was characterized as non-photoactive mutant, less than 0.4 M phosphate gains ability to photoconvert under actinic light.

These facts demonstrate that Trp-288 is not necessary for photoswitching of the OCP itself, but rather is important for the stabilization to the orange form. High phosphate concentrations (higher than 0.8 M) effect to spectral characteristics of the chromophore including a Raman bands which are responsible for hydrogen out-of-plane distortions and increase of polyene chain conjugation length of the ketocarotenoid bound.

Our experiments have revealed that in the presence of phosphate one hydrogen bond with Tyr-201 is sufficient to preserve the OCP^{W288A} in the orange-like state. For OCP the effect of stabilization by the phosphate concentration which is higher than 0.8 M in the orange state is carried out with and without illumination.

P-233

Intrinsically disordered N-terminal domain of Ultraspiracle from *Helicoverpa armigera* influence functions of two remaining globular domains

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Ultraspiracle (Usp) belongs to the family of nuclear receptors (NRs), which are ligand-dependent transcription factors activated by lipophilic compounds. Like all other nuclear NRs it is characterized with typical molecular structure in which we can distinguish two highly conserved globular domains – DNA-binding domain (DBD) responsible for binding of proper DNA sequence and ligand-binding domain (LBD) responsible for binding of ligand compound. Besides these two globular domains there are also some regions that do not possess stable secondary structure, so called intrinsically disordered regions (IDRs). Among them we can distinguish hinge region located between DBD and LBD, as well as region F located at C-terminus. One of the most puzzling intrinsically regions is N-terminal domain (NTD). There are numerous papers describing structure and functions of DBD and LBD, but very little is known about structure-function relationship of NTD. We decided to investigate how this intrinsically disordered NTD is able to modulate function of remaining globular domains. For this purpose, we analyzed full-length Usp from *Helicoverpa armigera* (HaUsp) and HaUsp lacking NTD (HaUsp_ΔNTD). The role of NTD was examined for two well-known and easily testable NR functions, i.e., interactions with specific DNA sequence and dimerization. Electrophoretic mobility shift assay (EMSA) showed that NTD significantly changes the pattern of interaction of HaUsp with specific DNA sequence. On the other hand, size-exclusion chromatography coupled with multi angle light scattering (SEC-MALS) showed that NTD stabilizes HaUsp homodimers in solution. Finally, molecular models reconstructed based on small-angle X-ray scattering (SAXS) indicated that the intrinsically disordered NTD may exert its effects on the HaUsp functions by forming an unexpected *scorpion-like* structure, in which NTD bends towards the LBD in each subunit of the HaUsp homodimer. This structure supposedly is crucial for specific NTD-dependent regulation of the functions of globular domains in NRs.

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The folded FAD conformation in the ferredoxin-$NADP^+$ reductase from the *Brucella ovis* pathogen governs the NADPH dependent reductive-half reaction

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The *Brucella ovis* proteome codifies for a FAD containing Ferredoxin-NADP(H) reductase that belongs to the bacterial subclass 1 FPR family (BoFPR). By similarity with other FPRs, it is expected to either deliver electrons from NADPH to the redox-based metabolism and/or to oxidize NADPH for the reduction of the SoxR regulon that protects bacteria against superoxide and nitric oxide damage. Due to such potential relevant role for the pathogen survival under infection conditions, it is of interest to evaluate and act on its reaction mechanism. Here, we investigate $NADP^+$/H coenzyme interaction and reduction by hydride transfer in BoFPR. Crystal structures of BoFPR when free and when in complex with $NADP^+$ hardly differ. The BoFPR:$NADP^+$ structure allows modelling of the adenosine ring but the reactive nicotinamide portion protrudes towards the solvent without reaching the active site. Nonetheless, pre-steady-state kinetics shows how in solution binding of NADP(H) takes place with stacking of the nicotinamide and flavin reacting rings, resulting in the formation of a charge-transfer complexes prior to the hydride exchange. In addition, kinetic data identify the hydride transfer itself as the limiting step in the semi-reductive half process for the reduction of BoFPR by NADPH. On these bases, we have used all-atom molecular dynamics simulations with a thermal effect approach to visualise the transient catalytically competent interaction of the flavin and nicotinamide rings. Simulations indicate that the particular architecture of the FAD cofactor in the folded conformation (characteristic of bacterial FPRs but not observed in the more efficient photosynthetic counterparts) precisely contributes to the orientation of the N5 of the FAD isoalloxazine ring and the C4 of the coenzyme nicotinamide ring in the conformation of the catalytically competent hydride transfer complex.

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Structure of the large extracellular loop of FtsX and its interaction with the essential peptidoglycan hydrolase PcsB in *Streptococcus pneumoniae*

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Streptococcus pneumoniae is a leading killer of infants and immunocompromised adults and has become increasingly resistant to major antibiotics. Therefore, the development of new antibiotic strategies is desperately needed. Targeting bacterial cell division is one such strategy, specifically by targeting proteins that are essential for the synthesis and breakdown of peptidoglycan. One complex important to this process is FtsEX. FtsEX comprises a cell division-regulating integral membrane protein (FtsX) and a cytoplasmic ATPase (FtsE) that resembles an ATP-binding cassette (ABC) transporter.

In this work we present three different X-ray crystallographic structures of extracellular loop 1 (ECL1) of FtsX solved in the presence of different detergents (1). ECL1 present an upper extended β-hairpin and a lower α-helical lobe, each extending from a mixed α-β core. Different conformations were observed for the ß-harpin and the helical lobe amount the structures, depending of the identity of the detergent molecule bound to $FtsX_{ECL1}$ suggesting that changes in the protein backbone and side chains of the helical lobe occur when a detergent ligand is bound. The helical lobe mediates a physical interaction with the peptidoglycan hydrolase PcsB (2) via the coiled-coil domain of PcsB ($PcsB_{CC}$) according with the nuclear magnetic resonance (NMR) experiments realized in this work. Some of the amino acids present in the helical lobe that participate in the interaction with the detergents were substituted *in vivo* using the *S. pneumoniae* strain D39 and we demonstrate that helical lobe subdomain is essential for cell viability and required for proper cell division of *S. pneumoniae.*

References:

(1) Rued BE, Alcorlo M, Edmonds KA, Martínez-Caballero S, Straume D, Fu Y, Bruce KE, Wu H, Håvarstein LS, Hermoso JA, Winkler ME, Giedroc DP. 2019. **mBio** 10:e02622-18. doi.org/10.1128/mBio.02622-18.
(2) Sergio G. Bartual, Daniel Straume, Gro Anita Stamsås, Inés G. Muñoz, Carlos Alfonso, Martín Martínez-Ripoll, Leiv Sigve Håvarstein & Juan A. Hermoso. ***Nature Communications*** **(**2014); May 8, 5 :3842, (doi:10.1038/ncomms4842).

P-236

Protein-Ligand Recognition: Crystal Structures and Thermodynamics of Modified Sulfonamide Binding to Carbonic Anhydrases

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High affinity and selectivity toward target protein are usually the main challenges in the pharmaceutical sciences, aiming for the discovery of effective and safe drugs. New pharmaceutical drugs are mostly identified by scriening of the large libraries of compounds and the lead compound is subsequently optimized to bind tightly to target protein. The understanding of the structural reasons determining protein-ligand binding affinity would enable rational design of therapeutically active compounds.

As a protein-ligand recognition system we have chosen sulfonamide binding to carbonic anhydrases (CA). There are 12 catalytically active human CA isoforms. Sevaral CA isoforms are associated with diseases, for example, CA IX is overexpressed in solid tumors and is thought to be a good target for anticancer drug development. Numerous sulfonamide-based inhibitors were synthesized possesing high affinity towards target CA isoform, but due to low selectivity they usually also bound to other CA isoforms. Some of such compounds bound to CA with an extraordinary (over -70 kJ/mol) Gibbs energy change (picomolar affinity). The aim of this study is to estimate factors determining the high affinity and selectivity of sulfonamide inhibitors towards particular CA isoform. For this purpose, we modified the sulfonamide headgroup of high affinity ligands to weaken the coordination bond with Zn ion in the active site of CA. The *observed* binding affinity was determined by fluorescent thermal shift assay and *intrinsic* binding affinity representing the binding of benzenesulfonamide anion to the Zn-bound water form of CA was calculated. Our study revealed the contribution of coordination bond and hydrophobic moieties to the overall binding energetics and provided some clues into selective recognition of sulfonamide derivatives by CA isoforms.

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Coarse-grain simulations on NMR conformational ensembles highlight functional residues in proteins

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Dynamics are a key feature of protein function, and this is especially true of gating residues, which occupy cavity or tunnel lining positions in the protein structure, and will reversibly switch between open and closed conformations in order to control the diffusion of small molecules within a protein's internal matrix. Earlier work on globins and hydrogenases have shown that these gating residues can be detected using a multiscale scheme combining all atom classic molecular dynamics simulations and coarse grain calculations of the resulting conformational ensemble mechanical properties. Here we show that the structural variations observed in the conformational ensembles produced by NMR spectroscopy experiments are sufficient to induce noticeable mechanical changes in a protein, which in turn can be used to identify residues important for function and forming a *mechanical nucleus* in the protein core. This new approach, which combines experimental data and rapid coarse-grain calculations and no longer needs to resort to time-consuming all-atom simulations, was successfully applied to five different protein families.

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Monitoring conformational changes during protein adsorption on nanopatterned surfaces by in-situ ATR-IR spectroscopy

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Protein adsorption and the resulting surface-bound protein layer have long been recognized to play a significant role in cellular response, such as cell migration, proliferation etc. [1,2]. Understanding and ultimately controlling protein adsorption to artificial surfaces thus represent important milestones on the road toward optimized and highly biocompatible implants. To tailor the protein-surface affinity, the biomaterial surface can be modified through various chemical and physical surface treatments. In particular, the possibility to enhance bioactivity and osseointegration via topographic surface modifications at nanometer scales has recently received considerable interest. However, non-specific protein adsorption often results in the denaturation of the proteins, which prevents them from fulfilling their biological function and may decide over tissue integration or implant failure. Here, we thus investigate the influence of nanoscale surface topography on the adsorption and denaturation of different globular proteins.

Nanorippled silicon substrates are prepared using low-energy ion bombardment. The nanoscale ripple patterns with periodicities of about 30 nm form spontaneously by self-organization on the Si surface during off-normal irradiation with low-energy Ar^+ ions [3]. The so fabricated nanopatterned silicon surfaces and their flat counterparts are employed to investigate the effect of nanoscale surface topography on the adsorption of a selection of globular proteins with different molecular weights and isoelectric points. Adsorption dynamics are monitored in situ using ellipsometry while the morphology of the adsorbed protein films is assessed by ex-situ atomic force microscopy. Adsorption-induced conformational changes of the proteins are followed in situ by attenuated total reflection infrared (ATR-IR) spectroscopy employing nanorippled ATR crystals.

[1] I. Wittenbrink, et al., *Low-aspect ratio nanopatterns on bioinert alumina influence the response and morphology of osteoblast-like cells*, Biomaterials, 62, 58-65, **2015**.

[2] M. S. Lord, *et al*., *Influence of nanoscale surface topography on protein adsorption and cellular response*, Nano Today, 5, 66-78, **2010**.

[3] A. Keller, S. Facsko, *Ion-induced nanoscale ripple patterns on Si surfaces: Theory and experiment*, Materials, 3, 4811-4841, **2010**.

P-239

Nanosized Iron Cores in Ferritin and Its Pharmaceutical Analogue Ferrifol®: Comparison by Magnetization measurements and Mössbauer Spectroscopy

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Ferritin is synthesized for iron storage in many living systems from plant to mammals. Ferritin molecule consists of a spherical multi-subunit protein shell surrounding the nanosized iron core. The core is in the form of ferrihydrite ($5Fe_2O_3{\times}9H_2O$) with inorganic phosphates. This natural structure is reproduced in pharmaceutical products such as iron-polysaccharide complexes developed for the treatment of iron deficiency anemia. These fabricated products contain also ferric hydrous oxide iron core covered by polysaccharide shell. Thus, they can be assumed as analogues of native protein ferritin. Comparison of commercial pharmaceutical product Ferrifol® (CTS Chemical Industries Ltd., Israel) and ferritin was carried out using magnetization measurements in the temperature range 300–5 K and Mössbauer spectroscopy in the temperature range 295–20 K.

Magnetization measurements showed that ferritin and Ferrifol® iron cores are mainly in the paramagnetic state. Mössbauer spectroscopy of the iron cores confirmed the paramagnetic states down to 20 K for ferritin and to 60 K for Ferrifol®. Within the heterogeneous iron core model, the Mössbauer spectra of both samples in the range of 295–90 K demonstrated non-Lorentzian line shapes, which were better fitted using a superposition of 5 quadrupole doublets with different quadrupole splittings but with similar isomer shifts. The obtained hyperfine parameters for ferritin and Ferrifol® appeared to be slightly different with an unusual temperature-dependent line broadening. These results were explained basing on the complex nanosized iron core structure with several layers/regions which can undergo low temperature structural rearrangements.

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Replica sub-permutation molecular dynamics method for protein folding

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Molecular dynamics simulation is a powerful tool for gaining the molecular properties of biomolecules. However, obtaining sufficient conformations from the phase space remains difficult because their configurations tend to get trapped in the local-minimum free-energy state. To overcome this problem, generalized-ensemble algorithm such as replica-exchange method [1,2] and replica-permutation method [3,4] have been proposed.

In this presentation, we propose a replica sub-permutation method, which is an improvement of the replica-exchange and replica-permutation method. This method uses a new algorithm referred to as sub-permutation to perform parameter transition. The replica sub-permutation method succeeds in reducing the number of combinations between replicas and parameters without the loss of sampling efficiency. We applied this method to a β-hairpin mini protein, chignolin, to compare sampling efficiency with the replica-exchange and replica-permutation methods. The results show that among the three methods, replica sub-permutation method is the most efficient in both parameter and conformational spaces. The replica sub-permutation method is a useful technique when it is necessary to use a larger number of replicas.

Reference:

[1] K. Hukushima and K. Nemoto, *J. Phys. Soc. Jpn.* **65** (1999) 1604.
[2] Y. Sugita and Y. Okamoto, *Chem. Phys. Lett.* **314** (1999) 141.
[3] S. G. Itoh and H. Okumura, *J. Chem. Theory Comput.* **9** (2013) 570.
[4] M. Yamauchi and H. Okumura, *J. Chem. Phys.* **147** (2017) 184107.

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Comparison of the Nanosized Iron Core Structure in Some Pharmaceutical Analogues of Ferritin

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Iron-deficiency anemia is a rather wide spread disease nowadays. To compensate the low iron level in this pathological case, special pharmaceuticals were developed and used to deliver iron in non-toxic and bioavailable form as it is done in the native protein ferritin. Most effective pharmaceutical products for anemia treatment appeared to be iron-polymaltose complexes (IPC). Structure of these pharmaceuticals represents a macromolecule with nanosized ferric hydrous oxide core covered by polymaltose shell. This structure reproduces well original ferritin macromolecule where the ferryhydrite iron core covered by multi-subunit protein shell. Several samples of commercial IPC were studied using the high resolution transmission electron microscopy (HRTEM) and Mössbauer spectroscopy in the temperature range 295–20 K. HRTEM images demonstrated that the iron core size varied in the range 6–10 nm for different IPC. It was found that the core structure in the studied IPC was not homogeneous and suggested the presence of some microstructural variations. The IPC Mössbauer spectra demonstrated similar shape down to 90 K with unusual temperature dependence of the line broadening. Below 90 K the Mössbauer spectra of the studied IPC showed differences in the slowing down of magnetic relaxation in the core. Both HRTEM and Mössbauer analyses of IPC support the heterogeneous nanosized iron core model.

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Structural and thermodynamic characterization of engineered small coiled coil proteins as tools to investigate HIV-1 inhibition

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During HIV-1 infection, the envelope (ENV) subunit gp41 mediates the fusion of the host cell and virus membranes. In this process, gp41 folds into trimer of helical hairpins formed by the interaction between N-terminal (NHR) and C-terminal (CHR) heptad-repeat regions. This energetically favorable folding process brings the membranes into proximity and facilitates lipid mixing. Therefore, interfering with the NHR-CHR interaction of gp41 is a promising therapeutic approach against HIV-1.

In our previous work (1), we rationally designed single-chain protein constructs that mimic the NHR coiled-coil surface. These three-helix protein constructs (named covNHR) show high structural stability and solubility, bind the CHR region of gp41 with high affinity and possess nanomolar *in vitro* inhibitory activity for a variety of HIV-1 strains.

In this work, we designed several variants of these covNHR proteins with reduced molecular size in order to facilitate their accessibility to the target in gp41 in the context of the viral ENV. The variants were designed to include different NHR binding determinants targeting different regions in gp41 CHR. Their secondary structure, stability and aggregation state were studied using Circular Dichroism (CD) and Dynamic Light Scattering (DLS); the thermodynamic binding parameters with CHR peptides were obtained by Isothermal Titration Calorimetry (ITC). In addition, the binding to stabilized ENV spikes by ELISA and their activity as fusion inhibitors in HIV-1 with several pseudovirus strains were assayed. The results help to delineate which regions of gp41 are most susceptible to blockage of the fusion process and provide valuable information to aid in the design of improved inhibitors of HIV-1.

(1) Crespillo, S. et al., Single-chain protein mimetics of the N-terminal heptad-repeat region of gp41 with potential as anti–HIV-1 drugs, PNAS 111 (51), 18207-18212.

P-243

Pulmosaposin B1: characterization of the improved recombinant version of the N-terminal saposin of pulmonary Surfactant Protein B precursor. A new antiviral activity is suggested.

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Pulmonary surfactant is a lipid-protein complex that forms a membrane film into the alveolar air-liquid interface, essential to reduce surface tension and to avoid alveoli collapse along breathing mechanics. In this complex, the most important protein for lung homeostasis is Surfactant Protein B (SP-B). SP-B belongs to the saposin-like family (SAPLIP), a set of proteins that share several structural properties like their six conserved cysteines forming three intramolecular disulphide bonds. Despite their structural similarity, they show a low sequence identity and have different mechanism of action but always related to lipid interaction. SP-B is synthesized as a larger precursor (pro-SP-B) that contains three saposin modules. The first one (SP-B^N or Pulmosaposin B1), an anionic protein with a potential N-linked glycosylation site, was previously obtained as a recombinant form in *Escherichia coli* with important limitations like its necessary reconstitution from inclusion bodies, the contamination with endotoxin and the absence of glycosylation. On the other hand, it has been proposed that SP-B^N exhibits certain antimicrobial properties at low pH, suggesting a role in innate host defense. To validate these results, an improved system for human Pulmosaposin B1 expression in *Pichia pastoris* has been developed. Adequate yields of glycosylated and unglycosylated Pulmosaposin B1 are obtained by this procedure and both forms can be independently purified, as confirmed by N-terminal sequencing, mass spectrometry and glycosylation detection. High thermostability together with saposin-fold acquisition and dimerization ability is revealed by far-UV CD and analytical ultracentrifugation, strongly suggesting its correct conformation. Unexpectedly, Pulmosaposin B1 does not perform a stable interaction with lipid vesicles in the conditions studied. It promotes the leakage of phosphatidylglycerol vesicles at low pH but only at high protein/lipid ratios. Regarding its role in host defense, this improved version of the recombinant SP-B^N does not show remarkable antimicrobial properties. Instead, antiviral activity against several viral pseudotypes is suggested. All these results envision a different role of Pulmosaposin B1 in the lung and encourage new studies in this direction.

P-244

NMR studies on intrinsically disordered domains of Histone H1.0 and eIF4G1: Effect of phosphorylation and multivalent protein-nucleic acid recognition

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A large number of proteins with intrinsically disordered domains (IDD) are known to play key roles in many biological processes. NMR is the most suitable technique for structural and dynamics characterisation of IDDs, what is essential to understand the molecular basis of their biological roles, but experimentally difficult. Herein, we report our NMR results on IDDs involved in multivalent interactions.

H1 linker histones are thought to have an essential function for chromatin condensation and hence to perform a regulatory role in transcription. DNA-binding to the histone carboxy-terminal IDD is modulated by phosphorylation, which leads to a small decrease in DNA affinity. To get structural and dynamics information we proceeded to characterise the full-length domain in both phosphorylated and non-phosphorylated states using a novel CON-based strategy[1].

eIF4G1, which contains one N-terminal IDD, is an essential factor on translation, and also in post-transcriptional control and stress granules. We have identified the binding sites of two RNA binding proteins (Pub1[2] and Pab1), RNA oligonucleotides and characterised protein self-recognition events. We analysed these interactions individually as well as the interplay among them using chemical shift and intensity mapping, paramagnetic relaxation enhancements (PRE) and isotope discriminated spectroscopy. Finally we proposed a model in which inter- and intramolecular interaction networks are crucial for physical-chemical phase-transition observed during the nucleation of stress granules.

1. *Chaves-Arquero et al., J Biomol NMR 72* ***(2018)*** *139-148*
2. *Santiveri CM et al., PLoS One 6* ***(2011)*** *e24481*

This work has been supported by Spanish MINECO Projects CTQ2014-52633-P and CTQ2017-84371P (co-financed by European FEDER funds), and BMD-S770 RYPSE project. The NMR experiments were performed in the "Manuel Rico" NMR laboratory, LMR, CSIC, a node of the Spanish Large-Scale National Facility ICTS R-LRB.

P-245

Crucial role of conformational excitation and nonequilibrium transition in enzyme catalysis: Application to Pin1 peptidyl-prolyl isomerase

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Structural fluctuations and conformational transitions are intrinsic nature of proteins. Not only do we find these in the native state, but the character is also essential for proteins to function. Yet, the molecular link behind the dynamics and function in proteins often remain obscure.

Enzyme catalysis has been of great interest over decades, and the thermodynamic aspect has been studied extensively. In contrast, the understanding of how protein conformational dynamics contribute to catalysis remains highly controversial. To this end, here we reveal the molecular mechanism of enzyme catalysis from both static and dynamic perspectives by studying the Pin1-catalyzed peptidyl-prolyl isomerization reaction using extensive molecular dynamics simulations.

Pin1 is a member of the peptidyl-prolyl isomerase that catalyzes cis-trans isomerization reactions and is known to facilitate protein folding etc. Pin1 has been an attractive target for anti-cancer drugs, and the thermodynamic aspect of isomerization has been studied both experimentally and computationally. Nevertheless, the molecular mechanism of catalysis remains unclear. Here, we apply the replica exchange umbrella sampling and transition path sampling simulations to obtain the free energy profile and transition trajectory ensembles, respectively.

The minimum free energy path shows that the rearrangements of the hydrogen bonds within Pin1 as well as between the ligand and Pin1 occur in tight coupling with the isomerization coordinate (ζ), indicating that protein conformational changes are essential for the catalytic reaction. In sharp contrast, the transition trajectories show that the isomerization about ζ is too rapid for protein's collective rearrangements to follow, i.e. the transitions occur in a nonequilibrium manner. Instead, the key protein-protein hydrogen bonds are found to persist throughout the isomerization transition events, and can be found even up to more than 100 ns.

The current results thus show that the catalytic environment of Pin1 is prepared *a priori* in the reactant state before changes in the isomerization coordinate occurs, i.e. as the conformational excited state. This indicates that enzyme catalysis is not a simple thermal activation from equilibrium directly to the transition state, but is rather triggered by conformational excitations, implying that conformational flexibility is essential to reach these excited states.

P-246

Peroxidase activity of cytochrome c in its compact state depends on dynamics of the heme region

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Cytochrome c (cyt c), small globular hemoprotein, with main function as an electron transporter in mitochondrial respiratory chain possesses also peroxidase-like activity in the native state despite six-coordinated state of its heme iron. We studied an effect of increasing urea concentration in the range of 0 M to 6 M at pH 7 (pH value of the bulk solvent) and pH 5 (pH value close to negatively charged membrane) on peroxidase-like activity of cyt c. We show that peroxidase-like activity, measured by guaiacol oxidation and FOX2 methods, correlates with the accessibility of the heme iron, assessed from the second order rate constant of cyanide binding to cyt c, which linearly increases in the pre-denaturational urea concentrations (0-4 M) at both studied pHs without an apparent formation of pentacoordinated state of the heme iron.

Our as well as results of others suggest that dynamics equilibrium among the denaturant-induced non-native coordination states of cyt c is pre-requisite for enhanced peroxidase-like activity of cyt c in its compact state. Replacement of the native sixth coordination bond of methionine-80 by lysines (72, 73, and 79) and partially also by histidines (26 and 33) provides an efficient way how to increase peroxide-like activity of cyt c without significant conformational change at physiological conditions.

Based on the results, we hypothesize that denaturant-increased peroxidase-like activity of cyt c in the compact state is caused by increased dynamic equilibrium among different coordination states with a consequence of transient appearance of pentacoordinated state capable to bind the hydrogen peroxide and the polypeptide side chains providing suitable groups for the catalysis. The ability of cyt c in the compact state to switch between different coordination states, triggered by relatively small perturbation, is intriguing way how to shift between different functional states of the protein without undergoing of significant conformational change.

P-247

Metallochaperones assisting the Cu_A center assembly on cytochrome *c* oxidase studied by EPR and hyperfine spectroscopy

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The cellular reaction of oxygen reduction to water, essential for most eukaryotic life, is catalyzed by cytochrome *c* oxidase, a multi-subunit transmembrane metalloenzyme complex. This machine is assembled and loaded with its metal cofactors in a complex process involving a network of proteins responsible for membrane insertion and metallochaperones delivering metal ions to form the active metalloenzyme. Here we focus on the mechanism of formation of the binuclear copper Cu_A-site in subunit II of cytochrome *c* oxidase (CoxB), for which three proteins are known to be responsible in the model organism *Bradyrhizobium diazoefficiens*, the two copper chaperones ScoI and PcuC, as well as TlpA, a thioredoxin-like protein that reduces the cysteine pairs in the copper binding sites of CoxB and ScoI[1].

Using a variety of biophysical and biochemical techniques, we found that the initial step, the delivery of the copper (II) ion, is carried out by ScoI, which forms a ScoI·Cu^{2+}·CoxB complex as the first, obligatory intermediate in Cu_A center formation. Subsequently, a copper (I) ion is delivered to CoxB·Cu^{2+} by the Cu-chaperone PcuC. Using EPR spectroscopy we followed copper insertion into CoxB and the formation of the Cu_A-site. Characterization of ScoI-Cu^{2+} and the ScoI·Cu^{2+}·CoxB complex, provided well-resolved EPR as well as 1D & 2D hyperfine spectra that revealed the Cu(II)-surrounding magnetic nuclei via their hyperfine couplings to the Cu(II)-electron spin. This allowed us to study the local Cu(II) coordination with the aim to identify residues of ScoI and CoxB contributing to Cu(II) coordination.

Altogether, our results suggest a novel mechanism for the Cu_A-site formation and clarify the role of the involved copper chaperones.

[1] Abicht, H.K., *et al.* How periplasmic thioredoxin TlpA reduces bacterial copper chaperone ScoI and cytochrome oxidase subunit II (CoxB) prior to metalation. *J. Biol. Chem.* 289, 32431-32444 (**2014**).

P-248

Spectral study of catechins interaction with human serum albumin

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Catechins are plant polyphenolic compounds, they represent one of the classes of flavonoids. They are contained in higher concentrations in beans, black grapes, pears and chocolate [1]. Undoubtedly, green tea is the most important and rich natural source of catechins. Stereoisomers catechin and epicatechin, as well as epigallocatechin gallate are representatives of the catechin family. They have a beneficial effect on health in prevention and treatment of various diseases such as cancer, heart disease, diabetes, neurodegenerative diseases. These active compounds have antioxidant, anti-inflammatory, antiviral and antimicrobial effects [2]. The structure of catechins suggests the possibility of binding to enzymes and other proteins.

Human serum albumin (HSA) is the main transporter of different low-molecular ligands in blood flow. Its binding with medicaments plays asignificant role in their delivery to tissues. Understanding mechanisms of interaction of HSA with catechins can help to develop methods of more effective therapeutic usage of catechins.

We investigated conformational transitions of HSA in solutions with different concentrations of catechin, epicatechin and epigallocatechin gallate by spectral methods such as UV absorbance and fluorescent spectroscopy, FTIR, spectrophotometrical melting. As a result we observed complex formation of HSA with catechins. We can conclude there are changes in the environment of aromatic amino acids and the tendency for stabilization of the secondary structure of HSA.

A part of this work was performed using the equipment of the Centre for Optical and Laser
Materials Research (COLMR) in Research park of St Petersburg State University.

[1] Z. Durackova, S. Knasmuller, The Activity of Natural Compounds in Diseases Prevention and Therapy, Slovak Academic Press, Bratislava, 2007.
[2] L. Trnkova, I. Bousova, V. Stankova, Study on the interaction of catechins with human serum albumin using spectroscopic and electrophoretic techniques, Journal of Molecular Structure 985 (2011) 243–250.

P-249

Analysis of secondary structure of RNA polymerase II subunits Rpo4/7 by solid-state NMR spectroscopy

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The Archaeal homologues Rpo4/Rpo7 of the two subunits of the human RNA polymerase II form a stalk-like protrusion in the enzyme. They are known to bind the nascent single-stranded RNA, contribute to the efficiency of transcription initiation and termination, and increase processivity during elongation[1]. To understand these functions in molecular detail higher resolution techniques, such as NMR spectroscopy are required. Here as a first step we present the resonance assignment employing magic angle spinning (MAS) solid-state NMR experiments of the Rpo4/Rpo7 complex from the Archeon *Methanocaldococcus jannaschii* in which the Rpo7 subunit is uniformly $^{13}C/^{15}N$ labeled. We determine the secondary structure using both $^{13}C/^{15}N$ secondary chemical shifts (SCS) including the established database approach TALOS+ and compare them with the SCS data obtained from ^{1}H solid-state NMR chemical shifts. These experiments were performed at 110 kHz of MAS frequency, using only 0.5 mg of protein. In comparison to the known crystal structure, our results show that the difference of $^{13}C_{\alpha}$ and $^{13}C_{\beta}$ SCS as well as the combination of amide and alpha proton SCS are both good predictors of secondary structure elements, demonstrating the potential of ^{1}H-detected solid-state NMR for such studies.

[1] F. Werner & D. Grohmann. Nature Reviews Microbiology. V. 9, pages 85–98, 2011.

P-250

Structure and dynamics of the intrinsically disordered C-terminal tail of ErbB2, phosphorylation and interaction

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ErbB2/HER2/neu is a member of the ErbB family of receptor tyrosine kinases. These receptors are located upstream of major signaling pathways (Ras/MAPK, PI3K/Akt, Src kinases, STAT transcription factors), controlling cell proliferation, cell migration and apoptosis. ErbB2 is the only member of the family for which no ligand is required for efficient signaling. Its overexpression is correlated with the occurrence of several types of cancer, and especially poor prognosis in breast cancer. Signal transduction is triggered by homo- or hetero-dimerization with ErbB proteins, leading to phosphorylation of the C-terminal end of the protein, which we showed to be an intrinsically disordered region (IDR). This tail, which we call CtErbB2, is the hub for the interactions that will determine cell fate.

CtErbB2 is a 268-residue, proline-rich IDR. We investigated the structural and dynamic features of unphosphorylated CtErbB2 combining the atomic resolution information from high-field NMR with size and shape information from SAXS. We showed that it retains low propensities for numerous residual secondary structures, with 20% propensity for a N-terminal helix and many polyproline II (PPII) helices populated from 20 to 40%. Moreover, a N-to-C terminal contact was observed, potentially modulating the accessibility of certain sites to partners. In addition to phosphorylatable tyrosines prone to interact with SH2 or PTB domains, CtErbB2 PPII helices and PxxP motifs could favor interaction with SH3-containing proteins. Grb2, a central protein in Ras-dependent pathways and cell proliferation, contains both SH2 and SH3 domains and could therefore be subject to particular interaction mechanisms. We determined the solution organization of Grb2 domains, and then studied the interaction between unphosphorylated CtErbB2 and Grb2, as well as between Grb2 and phosphorylated peptides of ErbB2.

Strategies to obtain the phosphorylated form of CtErbB2 are also tested, to have a more global idea of the interdependence of phosphorylation events, behavior of the modified protein, and role of phosphorylation in regulating interactions.

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Depicting Conformational Ensembles of alpha-synuclein by Single-Molecule Force Spectroscopy

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Protein misfolding and toxic aggregation are very critical steps in the development of several neurodegenerative diseases, including Parkinson disease (PD), induced by the fibrillation of the intrinsically disordered protein alpha-synuclein (a-syn). Thus, elucidating properties of a-syn in misfolded states and understanding the mechanisms that drive its assembly into the disease prone aggregates are critical for the development of rational approaches to prevent protein misfolding mediated pathologies.

Although a-syn lacks a single well-defined structure under physiological conditions, it was found to assume three distinct conformational states ranging from a random coil to a highly structured conformation.

Here we afford this issue taking advantage of single molecule force spectroscopy: we stretch and unfold a polyprotein containing the human a-syn. The analysis of the different unfolding pathways gives information about the structural conformation of the protein before the mechanical denaturation.

Since ligands, such as Epigallocatechin-3-Gallate (EGCG) and Dopamine (DA), are known to affect the fibrillation process of a-syn, we used this single molecule to investigate the effect of EGCG and DA on the conformational ensemble of the WT a-syn. Moreover, knowing from several studies that the presence of point mutations, linked to familial PD, correlate with the gaining of structure and therefore with a-syn aggregation, we performed single-molecule force spectroscopy studies also on three of this a-syn point mutants (A30P, A53T and E83A).

P-252

Convective mixing of precipitants into crystallisation solutions

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The structure and function of proteins are critical for the rational design of drugs. Crystallisation is a crucial step to obtain protein structures via X-ray diffraction. Structural biologists use different crystallisation methods in order to obtain crystals, but the underlying dynamics and physical processes of the crystallisation are often neglected. We performed experiments using a crystallisation bench (Junius et al., J. Appl. Cryst. (2016). 49, 806-813) based on the dialysis crystallisation technique, that allows the control of the temperature and chemical composition of the crystallisation solution. We study the mixing of precipitant (salt, PEG) solutions with sterile water. We find that for volumes larger than a microlitre (lengthscales > 1 mm), mixing is accelerated by convection. The onset of convection when volumes are increased has consequences for crystallisers who are scaling up their systems, to grow larger crystals. Convection will affect how supersaturation varies in space and time during crystallisation, and hence both crystal nucleation and growth.

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A mechanism for coupled gain of structure and loss of function in amyotrophic lateral sclerosis

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The misfolding and aggregation of superoxide dismutase [Cu-Zn] (SOD1) is linked to inherited, or familial, amyotrophic lateral sclerosis (FALS), a progressive and fatal neurodegenerative disease. Aberrant SOD1 folding has also been strongly implicated in disease causation for sporadic ALS (SALS) which accounts for the majority of ALS cases. In developing a prion-like mechanism for the propagation of SOD1 misfolding we have previously shown how enervation of the SOD1 electrostatic loop (ESL), caused by the formation of transient non-obligate SOD1 oligomers, can lead to an experimentally observed gain of interaction (GOI) that results in the formation of SOD1 amyloid-like filaments. It has also been shown that freedom of ESL motion is essential to catalytic function. Here we investigate the possibility that restricting ESL mobility might not only compromise superoxide catalytic activity but also serve to promote the peroxidase activity of SOD1, thus implicating the formation of SOD1 oligomers in both protein misfolding and in protein oxidation. A mechanism for oxidation-induced conformation change leading to a SOD1 gain of interaction is proposed.

Eamonn F. Healy "A prion-like mechanism for the propagated misfolding of SOD1 ", PLoS ONE 2017, 12 (5), e0177284.

P-254

A Novel Chitin-Uptake Channel by Marine *Vibrio* species: Crystal Structures and Single Channel Physiology

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Marine *Vibrios* are chitinolytic bacteria that utilize chitin as a sole source of energy, and transport of chitin oligosaccharides are a major controlling step in the chitin catabolic cascade of these bacteria. This research describes the identification of a novel chitin uptake channel, designated as chitoporin or ChiP, and the structural basis of chitin translocation through *Vh*ChiP from the bioluminescent bacterium *Vibrio harveyi*. Crystal structures show that apo *Vh*ChiP consists of typical barrel-like folds like other porins. The crystal structures of *Vh*ChiP in complex the *N*-acetamido groups of the fully-stretching sugar chain interacts intensively with charged residues inside the channel lumen, while the sugar rings stack against the aromatic side chains by hydrophobic interactions. Most strikingly, the *N*-terminal segment (so called N-plug) consisted of nine amino acid residues is found to locate on the periplasmic side and it most likely acts as a mechanical gate that controls the rate of sugar entry. Single channel measurements indicated clearly that the truncated *Vh*ChiP with the *N*-plug being deleted induced the protein channel to be more widening, but the binding constant (K) towards the sugar substrate (chitohexaose) was significantly reduced by 15 fold as compared to the value observed for the intact channel. Our structural and electrophysiological data provide the first model that elucidates a stringently regulated system for the highly efficient uptake of chitin nutrients by the marine *Vibrio* species.

Supported by Thailand Research Fund and Vidyasirimedhi Institute of Science and Technology

P-255

Controlled aggregation-based molecular probes for monitoring the activity of eIF-4E, a 5'mRNA cap-binding protein.

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A nucleotide structure on 5' end of eukaryotic mRNA known as cap, partakes in many important cell functions, such as mRNA maturation and transport, initiation of translation and lastly mRNA degradation[1]. All of the above is possible due to cap-binding proteins and their in-cell activity. Abnormal activity of these proteins can lead to, or be an indicator of, many fatal diseases, including cancer.

References: [1] A. K. Banerjee et al., *Microbiological Rev.*, 1980, 175-205. [2] R. G. Rayavarapu et al., *J. Biomed. Imaging*, 2007, 1, 5-5 [3] S. Mayilo, *Nano Lett.*, 2009, 9 (12)

P-256

Targeting nucleotide-specific phosphohydrolazes in vitro and in cell extracts – inhibitor identification and evaluation

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Nucleotide-specific phosphohydrolases, including pyrophosphatases and nucleases, are enzymes that control the intracellular and extracellular levels of small nucleotides and thereby influence cellular metabolism, modulate signaling pathways, and maintain the integrity of nucleic acids. In several cases, dysregulation of the activity of these enzymes has been linked to disease development, which indicates that these enzymes form a key class of therapeutic targets. Moreover it has been shown that potent small compounds can act as therapeutic reagents in diseases induced by phosphohydrolazes dysfunction. The aim of this project is to use high throughput screening (HTS) experiment and other biophysical tools to identify and study different nucleotides analogs in terms as enzyme inhibitors and to understand role of these proteins in cells. Our studies are focused around proteins belong to HIT superfamily (Histidine triad protein superfamily). HIT is protein family containing in its structure the sequence of histidine triad motive His-α-His-α-His-α (where α is hydrophobic amino acid residue), which is an enzymatic catalytic center. These proteins demonstrate an activity of nucleotide hydrolases or transferases. Proteins of our interest are human DcpS (*Decapping Scavenger*) which is engaged in the process of mRNA degradation in eukaryotic organisms and is also recognized therapeutic in spinal muscular atrophy (SMA); human Fhit (*fragile histidine triad protein*) which acts as a cancer suppressor, contributing to induction of apoptosis, cellular cycle control and regulation of cells susceptibility to DNA damage and plant Fhit (*Arabidopsis Thaliana*) whose role is not well established yet.

P-257

A fluorescence-based high-throughput screening assay for inhibitors of viral decapping enzyme D9.

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mRNA decapping plays an important role in gene expression by controlling the stability of messenger RNA. In humans, Dcp1-Dcp2 complex plays major role in decapping of full-length mRNAs. Studies has shown that some viruses (e.g. poxviruses) possess their own decapping enzymes, but their role in viral infection has not been fully elucidated. D9 enzyme from Vaccinia virus is a Nudix hydrolase that recognizes m^7G cap structure in mRNA and cleaves its pyrophosphate bond between α and β phosphates to release m^7GDP. The cleavage triggers host mRNA degradation process which leads to the shutdown of host protein synthesis.

Here, we aimed to develop a simple fluorescence-based assay to identify small molecule compounds that could modulate the activity of D9 enzyme. In this method, m^7G nucleotide labelled with pyrene that releases fluorescence upon enzymatic cleavage was used as an activity probe. We used this approach to monitor the fluorescence intensity changes during enzymatic reactions in the presence of various potential inhibitors on a 96-well plate reader to identify the most potent compounds. Hits from the screening experiments will be verified and characterized for their binding affinity, selectivity to the target and inhibitory properties by lower-throughput but more accurate biophysical methods, e.g. microscale thermophoresis.

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7-methylguanosine 5'-monophosphate analogues as molecular tools to investigate the role of human cytosolic 5' nucleotidase IIIB (cNIIIB)

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5'-nucleotidases are enzymes responsible for hydrolysis of nucleosides 5'-monophosphates to corresponding nucleosides and inorganic phosphate. 5'-nucleotidases are involved in the regulation of cellular levels of nucleoside 5'-monophosphates. Some 5'-nucleotidases are also involved in the deactivation of certain nucleoside-derived drugs [1]. A recently identified cytosolic 5'-nucleotidase IIIB (cNIIIB) shows preference towards 7-methylguanosine monophosphate (m^7GMP) as a substrate, which suggests its potential involvement in mRNA degradation [2]. However, biological function and structure of human cNIIIB are still unknown.

Here, we synthesized a series of m^7GMP analogues that could be used to modulate processes related to cNIIIB activity. Using high-throughput screening methods a library of mono- and diphosphate 7-methylguanine nucleotide analogues was tested as for inhibition of cNIIIB. Selected compounds were applied in cell extracts to investigate the role of human cytosolic 5'- nucleotidase IIIB in mRNA degradation pathways using a mass spectrometry-based assay. Based on the crystallographic structure of cNIIIB from *Drosophila melanogaster,* two fluorescent probes were designed and synthesized, which enabled us to develop a new method to study the binding preferences of cNIIIB.

References: [1] Bianchi V. *et al*., *JBC* 2003, 278, 47. [2] Monecke T., et al. *PLOS* 2014, 9, 3; [3] Kozarski M., Kubacka D. et al. BMC 2018, 26, 1.

P-259

Nanoparticles as intermediaries in the interaction of proteins with x-ray

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Photodynamic Therapy (PDT) has gained relevance due to its applications in cancer treatments and has now stood out for its variety of applications, such as in the fight against neoplasic and inflammatory diseases, microbial infections and aging. The discovery and development of phototoxic proteins capable of producing reactive oxygen species aligned with the development of Optogenetics makes it possible to use genetically encoded photosensitizers (PSs), increasing the specificity of the treatment. However, when it comes to *in vivo* use, optical techniques are limited by the low penetration of UV-visible light into biological tissues. To overcome this limitation, the use of X-rays has been suggested as energy source of excitation of PSs, due to their high penetrability in soft tissues. Giving that most photosensitizers (PSs) have absorption coefficients that are comparatively high at visible wavelength, the X-ray-induced sensitizer (XS) usually comprises traditional PSs and scintillation nanoparticles (ScNP). Considering for the first time the use of the proteins GFP, KillerOrange and KillerRed with the scintillant nanoparticle LaF3.Tb as XS, this work presents a characterization of the system protein-ScNP and its potential use in PDT. To this end, the structure, stability and quantum yield of fluorescence and generation of reactive oxygen species were evaluated upon X-ray stimulation of the system protein-ScNP. We also tried to shed light on the mechanisms of interaction between proteins and nanoparticles, seeking for strategies to increase the transfer of energy from ionizing radiation to biomolecules in order to improve the efficiency of the techniques mentioned above.

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IMPROVING LOOP MODELING PREDICTION

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Prediction of protein loop structures is crucial for protein structure modeling, structural refinement, antibody design, or ion channels modeling. Here we present an improved version of our loop modeling online service: Random Coordinate Descent (RCD+ http://rcd.chaconlab.org/). This server combines an *ab initio* loop closure algorithm with a full-atom refinement in Rosetta. Now it includes a novel knowledge-based pairwise potential (KORP) which takes into account information of the relative position and orientation per residue. KORP and an extensive parameter optimization significantly improve the prediction accuracy of the server. Moreover, superior efficiency has been achieved by drastically reducing the number of loop candidates to be further refined, in particular in the more challenging longer loop cases (>10 residues). The approach has been successfully validated with several standard loop benchmarks. Interestingly, promising results were obtained even in one of the most challenging applications scenarios: the H3 loops prediction of the antibody complementary determining regions.

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Evaluation of PAPS analogs as inhibitors for sulfotransferases using an MST-based assay.

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Amyloids have been exploited to build up amazing bioactive materials. In most cases, short synthetic peptides constitute the functional components of such materials. The controlled assembly of globular proteins into active amyloid nanofibrils is still challenging, because the formation of amyloids implies a conformational conversion towards a β-sheet-rich structure, with a concomitant loss of the native fold and the inactivation of the protein. There is, however, a remarkable exception to this rule: the yeast prions. They are singular proteins able to switch between a soluble and an amyloid state. In both states, the structure of their globular domains remains essentially intact. The transit between these two conformations is encoded in prion domains (PrDs): long and disordered sequences to which the active globular domains are appended. PrDs are much larger than typical self-assembling peptides. This seriously limits their use for nanotechnological applications. We have recently shown that these domains contain soft amyloid cores (SACs) that suffice to nucleate their self-assembly reaction. Here we genetically fused a model SAC with different globular proteins. We demonstrate that this very short sequence act as minimalist PrDs, driving the selective and slow assembly of the initially soluble fusions into amyloid fibrils in which the globular proteins keep their native structure and display high activity. Overall, we provide here a novel, modular and straightforward strategy to build up active protein-based nanomaterials at a preparative scale.

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Amyloid proteins as synthetic devices for biotechnological purposes

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RepA-WH1 is a synthetic prion-protein that causes an amyloid proteinopathy in bacteria (1, 2). Synthetic biology seeks to implement functions of biotechnological interest through the design of minimal artificial systems. Using RepA-WH1 as a model for amyloid formation, our goal is to develop new biotools based on the properties of amyloids.

In this poster, we present results in three main aspects of research in our laboratory:

1) We have explored the conformational dynamics of RepA-WH1 through NMR $\{^1H\}$-^{15}N relaxation and H/D exchange kinetics measurements, including titration experiments with an inhibitor of amyloid formation (S4-indigo). Our objective is to define the initial stages of the conformational change that drives amyloidogenesis and the relevant interactions for amyloid remodeling.

2) We have recently achieved control of RepA-WH1 amyloidogenesis through optogenetics. For this purpose, the N-terminus of WH1-mCherry was fused to a blue light-responsive plant domain (LOV2). The expression of these chimeras under blue light illumination leads to the assembly of oligomers that are cytotoxic in *E. coli*, while in darkness large intracellular amyloid inclusion are formed which are compatible with bacterial proliferation (3). This tool provides direct control of amyloidogenesis with light.

3) Finally, we are working with cytomimetic lipid containers (GUVs) for exploring the *in vitro* formation of amyloid RepA-WH1 aggregates with a minimal set of components. We have achieved the solubilization of these aggregates by chaperones (Hsp70 + Hsp40 + NEF) in such cytomimetic containers (4, 5). This system is now being adapted for its usage with the optogenetic devices.

Together these studies constitute useful devices to explore general routes of toxicity of amyloids and to develop new tools amenable for environmental and biomedical applications.

References

(1) Fernández-Tresguerres, M. E. *et al*. Mol. Microbiol. 2010; 77: 1456-1469.
(2) Molina-García, L. *et al*. Front. Microbiol. 2017; 8: 539.
(3) Giraldo, R. J. Mol. Biol. 2019; 6: 1186-1202.
(4) Fernández, C., Giraldo, R. ACS Synth. Biol. 2018; 7: 2087-2093.
(5) Fenández, C. *et al*. Sci. Rep. 2016; 6: 23144.

P-263

Recognition and remodeling of nucleic acid conformations by cold shock proteins

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Cold shock proteins (Csps) are important in regulating several genes, particularly those involved in stress response, virulence and the regulation of flagella formation. Despite many investigations on the *in vivo* roles of Csps, particularly through knockout experiments, the biophysical mechanisms of these proteins remain poorly understood. Csps possess highly conserved structures and sequences but interestingly have different effects *in vivo*. Here, we dissect the mechanisms of RNA and DNA-targeting and remodelling by the three Csps (*Lm*CspA, *Lm*CspB and *Lm*CspD) from the foodborne pathogen *Listeria Monocytogenes in vitro,* primarily using single molecule fluorescence, magnetic tweezers and circular dichroism. Our data shows that despite the highly conserved structures, *Lm*Csps variably target different nucleic acid types and conformations (e.g. RNA or DNA hairpins, or G-quadruplexes), and display variable propensities in facilitating conformational rearrangements. The differential activities seem linked to dynamics transitions of the Csp proteins. In summary, our preliminary results have important implications on the mechanisms of cold shock proteins in the cell.

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LocalDeblur: An automatic map sharpening by restauration of local resolution

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Recent technological advances and computational developments, have allowed the reconstruction of cryo-EM maps at near-atomic resolution structures. Cryo-EM maps benefit significantly of a "sharpening" step, that tends to increase signal at medium/high resolution, and that has proven very important in the key task of structural modelling. Here, we propose a new method for local sharpening of volumes generated by cryo-EM. The algorithm, named LocalDeblur, is based on a local resolution-guided Wiener restoration approach of the original map. The method is fully automatic and parameters-free, without requiring either a starting model or introducing any additional structure factor correction or boosting. Results clearly show a significant impact on map interpretability, greatly helping modeling. In particular, this local sharpening approach is specially suitable for maps which present a broad resolution range, as it is often the case for membrane proteins or macromolecules with high flexibility, all of them otherwise very suitable and interesting specimens for cryo-EM.

P-265

Easing exhaustive rigid-body, flexible, and loops fitting in UCSF Chimera

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Building macromolecular models from cryo-electron microscopy (EM) density maps requires fitting strategies to accurately localize available atomic structures. In our lab, we have developed several approaches that are efficient enough to run smoothly as command line tools on commodity hardware. For rigid-body fitting, ADP_EM employed spherical harmonics to speed up the rotational part of the exhaustive search, whereas for flexible fitting, iMODFIT exploits Normal Model Analysis in internal coordinates to drastically reduce the explored conformational space. In addition, our ab-initio loop modeling tool (RCD) was recently improved to take into account EM electron density. Here, we present a general update and the integration of such programs into the standard visualization tool of the EM community (UCSF Chimera) to enable the interactive fitting of macromolecules (both proteins & nucleic acids) and protein loops.

P-266

Thermodynamics of interactions of human eIF4E protein with mRNA 5' cap analogues and 4EBP1 peptide fragment

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The eukaryotic translation initiation 4E factor (eIF4E) is a highly conserved small globular protein, which is responsible for recognition and selective binding of an mRNA 5' terminal regulatory structure called "cap". The interaction of eIF4E with the cap is particulary important, since this is a rate-limiting step of initiation of protein biosynthesis and therefore plays a crucial role in cell development, growth and survival[1]. The elevated level of eIF4E leads to the efficient translation of oncoproteins and is closely related to the progression of cancer. eIF4E is thus thought to be a promissing target of anticancer therapy[2]. A thermodynamic approach is still an efficient tool for searching for small molecule inhibitors of eIF4E [3].

The goal of the studies was to determine thermodynamic parameters (ΔH^o, ΔS^o, ΔG^o, ΔC_p^o) of the interactions of eIF4E with a series of new chemical mRNA 5' cap analogues containing aromatic substituents. The results obtained revealed the relationship between the cap analogues structure and thermodynamic parameters of the binding. This approach enabled us to select the most promissing cap analogue aromatic modification, and provided us with a deeper insight into the association process of mRNA 5' cap with eIF4E, in the absence or presence of the translation initiation inhibitor, 4EBP1.

[1] Siddiqui N, Sonenberg N, 2015, *Biochem Soc Trans.* 43(5):763-72.
[2] Prasad A, *et al*. 2013, *Clin Cancer Res*. 1; 19(1): 85-95.
[3] Niedzwiecka A, *et al*. 2004, *Biochemistry* ;43(42):13305-17.

P-267

Spectroscopic elucidation of the binding interaction and conformational changes of human lysozyme with an anticancer anastrozole: Involvement in in-vitro antibacterial activity

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Ligand binding to proteins is an important phenomenon. Binding of ligand essentially controls absorption and metabolism of drugs. Extent and affinity of binding of ligand to major transport proteins in blood and body fluids may strongly influence the absorption, metabolism distribution and excretion of drugs. Human lysozyme is an important constituent of mucus, tears and is body's first line of defense against gram positive bacteria. Interaction of anticancer drugs with lysozyme protein and alterations in structure and function of this protein can affect the functioning of protein in defense against microbes. Nonspecific binding to proteins other than major transport proteins in plasma can result in alterations in drug bioavailability and action. Thus, treatment outcome can be affected. It is imperative to study drug interaction with proteins like lysozyme that contribute significantly towards defense against microbes and can affect drug bioavailability because of the ability to bind ligands. To evaluate the drug binding we will employ combination of biophysical and bioinformatics techniques. Interaction of anticancer drug anastrozole with human lysozyme will be investigated using fluorescence spectroscopy, ultraviolet spectroscopy, dynamic light scattering and molecular docking techniques. Antibacterial activity of human lysozyme will be elucidated in-vitro using absorbance technique. We will explore the binding process type, whether it is dynamic quenching or static quenching. Affects on conformation and activity of human lysozyme under the influence of drug will be evaluated. Molecular topology changes and hydrodynamic behavior of protein under the influence of drug will be investigated using dynamic light scattering technique. Molecular docking will be performed to get information about the amino acid residues involved in the binding process. Present study is an attempt to gain deeper information about ligand protein interactions. Studies on this aspect deserve enquiry as they can provide information of the structural features that determine the therapeutic effectiveness of drug and binding induced alterations in some unrelated functions of proteins.

P-268

Site selective interaction of chronic obstructive bronchitis drug erdosteine with major transporter protein in human serum. Implications in drug-drug interactions and treatment outcome.

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The present study is concerned with the in vitro study of binding interaction of chronic obstructive bronchitis drug erdosteine with human serum albumin (HSA). HSA is a three-domain plasma protein and binds a variety of endogenous and exogenous ligands thereby play an essential role in their transport. Binding with HSA can essentially control drug pharmacokinetics. To know the strength of interaction of erdosteine with HSA various spectroscopic techniques like fluorescence, UV- visible (UV), and circular dichroism (CD) spectroscopy will be used. Thermodynamics of binding interaction and binding state of the drug with HSA will be calculated using fluorescence spectroscopy and molecular docking methods. The present study is an attempt to examine binding efficiency and possible clinically significant alterations in binding behavior of major transport protein in presence of erdosteine. Competitive ligand binding can give information about drug behavior and drug-drug interactions. The present research will provide important information about the interaction of erdostein with drugs that bind to major binding sites on HSA. Binding refashioning under these conditions are significant and will help to understand the changes in drug behavior, this will help in dosage design in multidrug therapy. This is imperative for achieving optimum treatment outcome

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Obtaining and characterization of a soluble recombinant precursor of Surfactant Protein SP-B.

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Surfactant protein B (SP-B), a component of pulmonary surfactant, is an essential protein required to allow for lung inflation and respiration. In alveolar cells, this lipid-associated protein is produced as a larger precursor in which the mature module is flanked by N-terminal and C-terminal propeptides. The three modules of proSP-B show a high structural stability relying on the formation of intrachain disulfide bonds typical of saposin-like family of proteins. It was previously described that the recombinant form of human proSP-B lacking the C-terminal peptide (proSP-$B_{\Delta C}$) is able to completely restore lung function in SP-B knock-out mice. The structure and lipid interacting ability of this truncated precursor were also investigated upon refolding of a denatured insoluble protein expressed in bacterial inclusion bodies. In the present work, we describe the expression and purification of a soluble proSP-$B_{\Delta C}$ protein, in two different *E. coli* strains, one of them optimized for the expression of disulfide bond-containing proteins. Both proteins have been structurally and functionally characterized in surfactant-relevant environments, including the effect of pH acidification, an important feature of SP-B maturation along the processing pathway in alveolar cells.

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STRUCTURAL ANALYSIS OF PROTEASES NS3 OF FLAVIVIRUS ZIKA AND DENGUE, AND NSP2 OF ALPHAVIRUS CHIKUNGUNYA AND MAYARO BY IN SILICO AND IN VITRO TOOLS

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The Arbovirus is a group which comprises viruses whose transmission is carried out by arthropod vectors infecting vertebrates. Among these, some arboviruses have been given great relevance as the dengue (DENV) and zika virus (ZIKV), belonging to the family Flaviviridae, genus Flavivirus, chikungunya (CHIKV) and mayaro (MAYV) of the family Togaviridae, genus Alphavirus. Among possible targets, viral proteases are recognized as proteins with considerable potential. These proteins (NS3 in Flavivirus and nsP2 in Alphavirus) have the function of cleaving certain regions of the viral polyprotein, being vital for the viral cycle. The objective of the present work is to carry out in silico and in vitro structural analyses of the proteases of arboviruses ZIKV, DENV, CHIKV and MAYV. Initially, the MAYV protease nsP2 was modeled and the structure was validated. Then, peptides containing the protease cleavage site were docked in CHIKV structure and MAYV model, and the apo and holo structures were analyzed by molecular dynamics. The substrate glycine, position P2, sits in a pocket on the protease surface and seems to assist the carbonyl group positioning, thus facilitating the nucleophilic attack. Moreover, this pocket is highly conserved in alphavirus. These results suggest that other residues with side chains bigger than glycine do not fit into that narrow pocket. In the molecular dynamics experiments of MAYV nsP2 with the peptide, the glycine phi and psi angles were majorly positioned in favoured regions, but in CHIKV dynamics, it was positioned in an outlier region. The four proteases were expressed in different E. coli strains, and flavivirus proteases were purified by immobilized metal affinity and size exclusion chromatography. The ZIKV NS2b/NS3 sedimentation coefficient indicates a molecular weight that corresponds to a homodimer. The reduction in fluorescence intensity caused by the aprotinin titration maybe explained by dimer separation and consequent exposure of Trp50 to the solvent. This is corroborated by the docking of aprotinin that shows its interaction with the protease in a region that overlaps the dimer interface, while allowing solvent access to Trp50. Finally, ZIKV and DENV crystals were obtained and conditions are under refinement.

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Remediation effect of curcumin as a natural antioxidant on damaged glyacted catalase.

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Oxidative stress is an accepted consequence of chronic hyperglycemia. It is demonstrated that catalase activity in patients with hyperglycemia is significantly lower than healthy subjects. Every factor that may undermine the activities of antioxidant enzymes can lead to accumulation of Reactive Oxygen Species (ROS) and subsequent oxidative damage to proteins. In this work the effect of pathological conditions, derived from hyperglycemia on catalase was considered by measuring enzyme activity, ROS generation, and changes in catalase conformational properties. We observed that in the presence of glucose, the catalase activity gradually decreased. The ROS content was also gradually increased at the same time. Thus, decreased catalase activity was partly considered as a result of ROS generation through glycation. However, in the presence of curcumin the amount of ROS was reduced resulting in increased activity of the glycated catalase. The effect of high glucose level and the potential inhibitory effect of curcumin on aggregation and structural changes of catalase were also investigated. Our study showed that curcumin, as a potent external antioxidant, not only can maintain the catalase against glycation by protection of its tertiary structures and its enzyme activity, but also it can induce favorable changes in the surface physicochemical properties of lysine residues. This physicochemical alterations, decreased ASA and increased pKa of lysine residues, reduced the tendency of lysine residues to take part in Maillard reaction.

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Ligand-induced conformational rearrangements regulate the switch between functions of ROCK2

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Rho-associated protein kinase 2 (ROCK2) is a membrane-anchored, long, flexible, multidomain, multifunctional protein. Its functions can be placed into two categories: membrane-proximal and membrane-distal ones. A recent study concluded that membrane–distal functions, e.g. regulatory myosin light chain (RMLC) phosphorylation requires a fully extended conformation, and this conclusion was supported by electron microscopy. The present solution small angle X-ray scattering (SAXS) study revealed that ROCK2 population is a dynamic mixture of folded and partially extended conformers. We have shown that the predicted, auto-inhibited, folded conformation of ROCK2 exists in solution, and is stabilized by weak non-covalent interactions between the N- and C-termini. Binding of RhoA to the coiled-coil domain shifts the equilibrium towards the partially extended state. The binding of natural protein substrates (e.g. LIMK1) to the kinase domain breaks up the interaction between the N-terminal kinase and C-terminal regulatory domains, , but smaller substrate analogues do not. The present studies reflect the dynamic behaviour of this long, dimeric molecule in solution, and our structural model provides a mechanistic explanation for a set of membrane-proximal functions, while allowing for the existence of an extended conformation in the case of membrane-distal functions.

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Unraveling New Binding Partners of Atg3 in Autophagy and Mitophagy

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Autophagy is a cellular process whereby damaged proteins, protein complexes and organelles are degraded and recycled. One of the key events in this process is autophagosome formation. More than 40 Atg proteins (autophagy-related proteins) are involved in the autophagic machinery, among which Atg3 plays a critical role in the initiation of the autophagosome. Biophysical characterization of Atg3 protein has indicated that it is an intrinsically disordered protein (IDP). The presence of disordered regions gives Atg3 protein the plasticity to interact with several partners. This plasticity is important in diverse signaling networks and cell processes that require prompt responses. There is also increasing evidence in the literature on the roles of Atg proteins in cellular processes other than autophagosome formation. Therefore, we set out to map the binding interactions of Atg3 in Atg3-overexpressing cells treated with and without inducers of autophagy and mitophagy.

We are currently overexpressing GFP-tagged-Atg3 in hepatoma cells treated with Torin 1 or Rapamycin and CCCP to induce autophagy and mitophagy, respectively. Potential binding partners are captured using GFP-based affinity pull down assays and identified with mass spectroscopic analysis. Our next aim is to model the link between identified protein networks using a bioinformatics approach. This will pave the way to studies directed towards defining the functional properties of interacting partners of Atg3. In the long run, these findings may enable the design of molecules targeted to correct medical disorders where autophagy and mitophagy play a vital role, such as metabolic diseases, ischemic heart disease and neurodegeneration.

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Probing the Morphology and Dynamics of Self-Assembled Lysozyme Microparticles

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Novel and more efficient biomaterials are highly required in fields as tissue engineering, bio sensing and drug delivery. In the last decades, due to their exceptional strength, elasticity and biocompatibility, protein assemblies have received a lot of attention as potential new platform for the rational design of new materials [1]. This calls for a comprehensive set of methods to unravel the connection between protein-protein interactions (PPIs) and the structure, dynamics and physico-chemical properties of the final material [2]. This will indeed allow one to rationally design protein-based materials for specific applications. In this work we focus on understanding how the protein molecules dynamics changes while protein molecules self-assemble into a specific type of microparticles, named particulates, and how this is connected to the structural feature of the final assembly. The formation of lysozyme microparticles is thermally induced at pH 10.5 and the kinetics followed by means of Thioflavin T fluorescence. The dynamics of the aggregates can be determined by measuring the changes in momentum/energy of neutrons scattered by a given sample [3]. These materials exhibit different modes of motion, like global diffusion and internal vibrations. The morphology is investigated by scanning electron microscopy and information about the secondary structure is obtained by infra-red spectroscopy. Our results indicate that particulates are readily formed after increasing the temperature. The ThT signal suggests that lysozyme undergoes aggregation instantaneously with no lag phase. The quasi-elastic neutron scattering experiment shows that the protein internal dynamics does not change during the formation of particulates, while differences occur in terms of global diffusion, as detected by a bigger elastic signal and lower quasi-elastic signal at small scattering angles. Infra-red analysis of the aggregation process is in progress, with the aim of connecting the unchanged internal dynamics with the secondary structure of the aggregates. Moreover, ongoing studies are focused on the impact of salts from the Hofmeister series on the dynamics of the protein, and thus possibly, on the properties of the final aggregates.

References

[1] Knowles TPJ et al. 2016 Adv Mater.
[2] Vetri, V. et al 2018 J Phys Chem B.
[3] Schirò G. et al. 2012 J Phys Chem B.

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Ancestral glycosidases as scaffolds for the generation of new activities

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The development of procedures to generate enzymes with new active sites capable to efficiently catalyze non-natural reactions is the most important unsolved problem in protein engineering. Current technological applications of proteins are limited to their natural activities. The availability of *de novo* enzymes would have a huge impact in biotechnology and industry.

We hypothesize that the rather limited success of most previous attempts to generate efficient *de novo* enzymes is the use of modern proteins as scaffolds. Accordingly, we propose to use resurrected ancestral proteins that may display properties (enhanced stability, catalytic promiscuity and conformational flexibility) that are convenient in scaffolds for engineering.

In this study, we report the reconstruction and characterization of glycosidases, an enzyme family belonging to the TIM-barrel fold, the most common protein fold. TIM-barrel proteins display multiple functions and could be appropriate scaffolds to test our hypothesis. In particular, glycosidases are involved in degrading oligosaccharides and glycoconjugates and therefore, play key roles in several important biological processes and industrial applications.

Ancestral glycosidases were resurrected in the lab and characterized in terms of biophysical and biochemical properties. Preliminary results show that ancestral glycosidases are more stable and show enhanced conformational flexibility than their modern counterparts, indicating that they may serve as good scaffolds for *de novo* enzyme design.

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Crystal structure of promiscuous serine ester hydrolases

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Traditionally, catalysis has been carried out by heterogenous catalysts where reactants and catalysts are in different phases, such as hydrogenation reactions, or homogeneous reactions, where both the reactants and the catalyst are in the same phase as organometallic or organocatalyst. More recent is the emergence of biocatalysis, what is the use of enzymes as catalysts. Societal benefits of nature's catalysts comprise eco-friendly process conditions and a significant reduction of toxic waste or unwanted side-products thus addressing two major concerns, namely sustainability and responsibility. Additionally, promiscuity, the ability to transform distinct reactants into the correspondent products, is another feature associated to certain enzymes. These enzymes are broadly appreciated as they can be used in more than one industrial process.

We are interested in identifying promiscuous enzymes performing better than current commercial prototypes and in identifying markers of enzyme promiscuity. The first objective was achieved by creating one of the largest collections of diverse non-redundant enzymes by applying extensive metagenomics-based screens, and testing them against a large collection of model chemicals [1]. The second objective, namely, the detailed understanding of mechanisms underlying promiscuity, was done by crystallization of top promiscuous enzymes to determine their structures in the context of their reactivity. Our work focused on the study of serine ester hydrolases from the structural superfamily of α/β-hydrolases [2]. These enzymes, which catalyze the transformation of esters into carboxylic acids and alcohols, were selected because they are widely distributed in the environment, have important physiological functions, and are among the most important industrial biocatalysts. We present the structural analysis of the first set of serine ester hydrolases with levels of substrate promiscuity higher than those of commercial prototypes. Crystal structures of the native proteins as well as complexed with different substrates have allowed us to achieve a spectacular vision of the topology of the catalytic pocket of promiscuous hydrolases, constituted by a nucleophilic serine and other residues that stabilize the intermediates, i.e. histidine/aspartic acid in family IV and lysine/tyrosine in family VIII.

[1] Martínez-Martínez, M., et al. (2018). *ACS Chem. Biol.* **13**, 225-234.
[2] Aranda, J., et al. (2004). *Biochemistry.* **53**, 5820-5829.

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Structural study of two glycoside hydrolase family 18 chitinases and their role in the production of chitooligosaccharides.

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Glycoside hydrolase (GH) family 18 chitinases catalyze the hydrolysis of β-1→4-linkages in chitin, an abundant polymer in nature and a major component of the structural fungal cell walls, the exoskeleton of insects, and the shells of crustacean. Chitin can be degraded into shorter oligosaccharides known as chitooligosaccharides (COS) which have a biotechnological interest in medicine, agriculture and food industries (1).

The catalytic domains of this family of enzymes have a characteristic $(\beta/\alpha)_8$ TIM barrel fold and based on the stereochemical outcome of products of the reaction, the general mechanism is known to retain the configuration of the substrate. Depending on the length of products formed during the degradation, the enzymes would be classified into endo- or exo- (1), hydrolysis being the favored reaction carried out by them. Alternatively, tranglycosylation takes place when a carbohydrate molecule, instead of a water molecule, performs the second substitution step leading to synthesis of a new glyosidic bond.

Recent studies have shown that decreasing the rate of hydrolysis and increasing the sugar-acceptor binding surface area results into an increase in the level of transglycosylation products (2). Furthermore, transglycosylation activity can be improved by site-directed mutagenesis and might be exploited for the synthesis of desired COS. Recent studies have described different mutations at the catalytic cleft favouring the transglycosylation reaction. Our current research is focused on the structural study of two fungal GH18 chitinases (endo/ exo). Although these enzymes follow the same catalytic mechanisms, they present significant differences in the catalytic tunnel what, consequently, provides very different specificities. We have crystallized both enzymes with different chitooligosaccharides, which illustrated the molecular determinants of the distinct enzymatic activity. The final goal is to modulate the catalytic performance, as required, for biotechnological purposes.

1. Oyeleye A, Normi Y. (2018). Chitinase: diversity, limitations, and trends in engineering for suitable applications. *Bioscience Reports*. 38(4): 1-21.
2. Madhuprakash J, Dalhus B, Rani TS, Podile AR, Eijsink VGH, Sørlie M. (2018). Key Residues Affecting Transglycosylation Activity in Family 18 Chitinases: Insights into Donor and Acceptor Subsites. *Biochemistry*. 57(29): 4325-4337.

P-278

Light-induced changes in structure and function of AsLOV2

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Algae, plants, bacteria and fungi contain LOV2 (light-oxygen-voltage) domain that function as blue light sensor to control cellular responses to light. All LOV2 domains contain non-covalently bound flavin mononucleotide (FMN) chromophore. Upon illumination FMN is excited into the singlet state, which evolves into the triplet state and form a covalent bond with the nearby photoreactive cysteine within the protein. The covalent bond decays in the process called dark state recovery in order of seconds to regenerate the ground state of FMN. Blue light illumination also induces a conformational change involving unfolding of two terminal alpha helices A'α and Jα and this change is reversible in the dark. FMN provides the LOV2 domain with yellow colour, intrinsic green fluorescence when excited with UV/blue light and ability to generate reactive oxygen species, such as singlet oxygen. Singlet oxygen has ability to oxidize many organics and bioorganics molecules and thanks to FMN LOV2 domain offers a potential use as photosensitizer for singlet oxygen generation.

Here, we present our findings regarding the study of the influence of long term illumination and reactive oxygen species on conformational and functional properties of LOV2 domain from *Avena sativa* (AsLOV2). For the purpose of this study we used 2 forms of AsLOV2 domain: wild-type form and mutant form. In the mutant, photoreactive cysteine is replaced by alanine and this form thus does not undergo a photoreaction upon blue light illumination. For the illumination we used 475 nm laser and we illuminated the sample for defined time at room temperature. We followed the structural and functional changes caused by illumination in secondary and tertiary structure by CD, fluorescence spectroscopy, UV-Vis spectroscopy and differential scanning calorimetry. In addition, we investigated the effect of long-term illumination on the triplet state lifetime and kinetic of singlet oxygen generation before and after illumination.

Our results indicate that long term illumination and singlet oxygen production lead to irreversible changes in both forms of AsLOV2 also accompanied by release of FMN to solvent.

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Fowl aviadenoviruses on the move: the structure of a highly stable complex capsid

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Adenoviruses (AdV) are studied as virotherapeutical tools for gene therapy, vaccination and oncolysis. Moreover, AdV are an advanced experimental model for cell biology and virus assembly. AdV are non-enveloped icosahedral viruses, with a T=25, 950 Å diameter capsid, packing a ~ 35 kbp ds DNA genome.

The only high resolution adenovirus structures reported so far are two human mastadenovirus species: HAdV-C5 at 3.6 Å *(1)* and recently HAdV-D26 at 3.7 Å *(2)*. There is also a 4 Å resolution study on bovine mastadenovirus incomplete particles *(3)*. Although non-human adenoviruses have been put forward as alternatives to overcome the main drawbacks of HAdV-C5 as a vector, they remain poorly characterized. Compared to HAdV-C5, fowl aviadenoviruses lack core protein V and minor coat protein IX, previously demonstrated to have an impact on virion stability maintenance *(4)*. Interestingly, fowl aviadenoviruses pack a genome of about 43-45 kbp, longer than the rest of the adenovirus genera *(5)*. It has been reported that aviadenovirus capsids are more stable than those of human mastadenoviruses *(6)*. We are using molecular, structural and biophysical analyses to understand the basis of fowl aviadenovirus capsid stability. Using extrinsic fluorescence spectroscopy we can conclude that fowl aviadenoviruses are more thermo and pH stable than Human Adenovirus type 5, despite lacking some cementing- capsid stabilizing- proteins. We collected cryo-Electron Microscopy high resolution data at the eBIC facility at Diamond (Oxford) and we have obtained a 3D map at 3.7 Å resolution of fowl aviadenovirus, whose interpretation (~14,000 expected residues in the asymmetric unit) is underway. Significant capsid proteins conformational rearrangements have been observed in the fowl aviadenovirus capsid, that might account for their enhanced physical stability.

1. Liu *et al.*, *Science* **329**, 1038 (2010).
2. Yu *et al.*, *Sci Adv* **3**, e1602670 (2017).
3. Cheng *et al.*, *Virology* **450-451**, 174 (2014).
4. Colby *et al.*, *J Virol* **39**, 977 (1981).
5. Marek *et al.*, *Veterinary microbiology* **156**, 411 (2012).
6. Swayne, *John Wiley & Sons* (2019).

P-280

The role of HCN channel helices D and E in the modulation of cAMP affinity

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Hyperpolarization-activated cyclic-nucleotide regulated (HCN) channels are non-selective cation channels controlling key physiological functions such as cardiac pacemaking and repetitive neuronal firing. They are activated by voltage and modulated by binding of cAMP to their cytoplasmic C-terminal region named cyclic nucleotide binding domain (CNBD). Altered cAMP responses caused either by single point mutations in the protein or by non-physiological levels of the cyclic nucleotide in the cell lead to pathological conditions both in the heart and in the nervous system. The recently obtained cryo-EM structure of human HCN1 confirms previous NMR studies on cAMP-induced conformational changes and reveals the presence of two additional helices at the C-terminus of the CNBD which fold in the presence of cAMP. We have previously shown, by ITC and patch clamp, that deletion of these helices (D and E) in HCN1 and in HCN2 decreases the affinity for cAMP. Here we test the role of helices D and E in HCN4 in order to extend our study to the cardiac channel isoform. We performed the experiments using an all-in-one integrated amplifier prototype (ePatch by Elements srl). The miniaturized ePatch amplifier is USB plug-n-play and integrates the analog front-end with multiple gains stage, the analog-to-digital converter and filters all into a single small headstage without any external bulky case. The results demonstrate the high-quality data acquired by the ePatch and indicate that sequences distal to the HCN channels' CNBD may control the affinity and response to cAMP.

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Control of nanopore assembly using external triggers

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Self-assembly is a ubiquitous process in many biological systems that is often tightly controlled by physical and chemical triggers. One example are pore-forming proteins, which bind to the surface of targeted cells and undergo a structural metamorphosis leading to the assembly of nanoscale pores resulting in cell death.

By genetic engineering and targeted chemical modification, we synthesized pore-forming toxins whose activity is triggered by external stimuli. Firstly, the activity of the toxin was triggered via proteolytic cleavage with furin, a protease overexpressed in many cancer cells. Then, an azobenzene photo-switchable pendant was covalently attached to the toxin, and the toxicity of the construct switched on and off by irradiation with light. Finally, the toxin was equipped with targeting molecules to refine specificity towards targeted cells.

Our engineered nanopores will be useful as component for targeted cancer therapy.

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***Flavivirus* capsid proteins and their ability to interact with host lipid systems**

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Zika (ZIKV), Dengue (DENV) and West Nile (WNV) viruses are related mosquito-borne flaviviruses. ZIKV infection was associated with severe congenital microcephaly in newborns and with Guillain-Barré syndrome. Although there is a lack of knowledge on basic aspects of the viral life cycle, much can be inferred from the closely related DENV and WNV. *Flavivirus* are viruses with a ss(+)RNA that codifies 3 structural proteins and 7 non-structural proteins. The capsid (C) protein, one of the structural proteins, mediates key steps of the viral replication. DENV and WNV C interact with host lipid droplets (LDs) and very low-density lipoproteins (VLDL), but not with low-density lipoproteins (LDL). Moreover, these interactions are dependent of potassium ions and surface proteins. Here, we investigated ZIKV C binding to host lipid systems via biophysical approaches. Zeta potential shows that ZIKV C interacts with LDs. However, zeta potential measurements show that ZIKV C-LDs interaction does not require K^+, as previously shown by us for DENV and WNV C. Atomic force microscopy (AFM)-based force spectroscopy experiments were performed in buffer with 100 mM KCl to confirm if this interaction is specific or an effect of electrostatic interactions. AFM-based force spectroscopy measurements show that there is no specific interactions between ZIKV C and LDs. The force rupture histogram shows only one peak of weak rupture forces, corresponding to unspecific interactions. The same was seen for ZIKV C-lipoproteins interaction. Strong and specific bindings do not occur for the interaction of ZIKV C with LDs, VLDL or LDL in the presence of 100 mM KCl. The understanding of ZIKV C-LDs interaction may lead to the development of new drug strategies against ZIKV and other flaviviruses.

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Biochemical and structural characterization of the propeptide of pregnancy-associated plasma protein A and its cellular effects

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Pregnancy-associated-plasma protein-A (PAPP-A) is expressed in various tissues to regulate the bioavailability of insulin like growth factors. The biological and catalytic activity of PAPP-A (aa residues 81-1627) is well characterized and it serves as a biomarker for various conditions in clinical practice. However, limited information is available about the structure and function of its N-terminal propeptide, i.e. pro-PAPP-A (aa residues 23-80).

Here we report the bacterial expression, purification, and characterization of pro-PAPP-A for the first time. We analyzed the secondary structure of propeptide by circular dichroism and found that it is primarily a random coil. An *in silico* characterization of pro-PAPP-A suggested also that propeptide is an intrinsically disordered peptide. However, a comprehensive 3D structure analysis by molecular modeling and dynamics simulations which recruited two different models led to a converged alpha helix for the middle portion of the propeptide (aa residues 51-59). Bioinformatics analysis predicted that this portion is free of any protease cleavage sites, reflecting the potential of helix to be stable in the plasma. Also, we investigated potential biological activity of pro-PAPP-A on monocytic cell lines (U937). At the cellular level, pro-PAPP-A decreased cell proliferation which was accompanied by an increase in mitochondrial superoxide level and a reduction in mitochondrial membrane potential.

Due to its small size and predicted stability off its middle portion, pro-PAPP-A may be introduced into clinical practice in future as a possible biomarker to evaluate the metabolism of PAPP-A and related disorders. In addition, our study indicates a potential biological activity of pro-PAPP-A that merits further investigation.

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Physiological Septin-Septin Interactions Prevents Amyloid Filaments Formation

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Septins are members of a conserved group of GTP-binding proteins highly conserved in eukaryotes, being linked to diverse cell process, such as cytokinesis and membrane association, in which remodeling membrane events taken place. On the other hand, the malfunction of septins is linked to several pathological processes including neurodegeneration and oncogenesis. Septins interact with each other forming heterocomplexes that polymerize in filaments. The well-known complex is a hexamer with a SEPT7-SEPT6-SEPT2-SEPT2-SEPT6-SEPT7 arrangement. Two types of interface between septins alternate along the filament: the G-interface (involving the GTP binding sites, as in SEPT6-SEPT2), and the NC-interface (involving the N- and C-terminal regions of the GTP binding domains, as in SEPT7-SEPT6 or SEPT2-SEPT2). In vitro, however, when SEPT2 does not have another septin partner, it forms dimers using a promiscuous G-interface, and have the propensity to promptly aggregate as amyloids at physiological temperatures. This work focuses on the physiological G-interface of SEPT6G-SEPT2G heterodimer, to verify the impact of this interaction on the thermostability and amyloid formation. In this context, circular dichroism and small angle X-ray scattering analysis were performed for a comparative study. Circular Dichroism spectroscopy showed the transition temperature of the secondary structure α-helical content to β-strand-like elements. And, SAXS model dependence analysis identified the percentage of the conformation at each temperature. All together, we found that the G-domain of SEPT6-SEPT2 heterodimer also moves to an irreversible state with ability to bind thioflavin-T, suggesting its amyloid-like nature. Noteworthy, this takes place at a higher temperature than observed to the single septins, showing a greater thermal and structural stability when compared to single SEPT2G or SEPT6G. The higher stability of septin partners as heterodimers in physiological temperatures, contrasting with its monomers or promiscuous homodimers, might state that a proper G-interface is an important factor for amyloid filament prevention at physiological temperatures.

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Mimicking neuroprotective Cytochrome *c* phosphorylation

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Protein phosphorylation is a well-known post-translational modification modulated by kinases and phosphatases, mediating the functionality of proteins in cell signaling, and in particular, those in redox regulation. The latter is important in mitochondria, which are the main source of reactive oxygen/nitrogen species (ROS/RNS) in the cell. Cytochrome *c* (C*c*) is one of the principal factors involved in the control of redox signaling through its post-translational modifications. These modifications alter how C*c* binds to its physiological partners, in either the mitochondria or cytosol. However, these effects are highly dependent on which residue is modified[1,2]. Tyr97 phosphorylation relates to several pathologies, including ischemia. After an insulin treatment in a brain injury, C*c* undergoes phosphorylation at position 97 and, it has been described a decrease of neuronal death to 50%. In this work, we characterize a phosphomimetic, phosphatase-resistant C*c* mutant, which was recombinantly generated by site-specific incorporation of the non-canonical *p*-carboxymethyl-L-phenylalanine amino acid at position 97 using an orthogonal amber suppressor tRNA/aminoacyltRNA synthetase pair (Y97*p*CMF C*c*)[3,4]. We found that this post-translational modification of C*c* increased the rate of C*c*O-catalyzed oxidation in comparison to WT C*c* species[4]. In fact, this correlates with a decrease in reactive oxygen ROS production[4]. Finally, the capacity of Y97*p*CMF C*c* to activate caspase-3 was reduced as well[4]. Altogether, our data suggest that the phosphorylation at Tyr97 could further contribute to the rapid adjustment of electron flow in response to cellular stress conditions, maintaining low levels of ROS/RNS[4]. This finding could lead to the use of phosphomimetic C*c* as neuroprotector with therapeutic applications, providing new opportunities to develop more efficient therapies against acute pathologies. Our findings could lead to the potential use of phosphomimetic C*c* as a neuroprotective agent, thereby providing new opportunities to develop more efficient therapies against acute pathologies.

[1]Guerra-Castellano A, *et al*. *Biochim Biophys Acta - Bioenerg* (2016) 1857, 387–395.
[2]Moreno-Beltrán B, Guerra-Castellano A, *et al*. *Proc Natl Acad Sci USA* (2017) 114, 3041–3050.
[3]Guerra-Castellano A, *et al*. *Chem Eur J* (2015) 21, 15004–15012.
[4]Guerra-Castellano A, *et al*. *Proc Natl Acad Sci USA* (2018) 115:7955–7960.

P-286

HDX MS and pull-down analysis reveal the competition between human GW182 and tristetraprolin for binding to CNOT1

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The CNOT1 protein is a central subunit of the multiprotein CCR4-NOT deadenylase complex that is a key player in miRNA-mediated gene silencing. CCR4-NOT is recruired to a targeted mRNA by the C-terminal silencing domain (SD) of the GW182 protein. The complex of the GW182 SD with CCR4-NOT can mediate both mRNA deadenylation and translational repression. However, at the submolecular level, the binding site of the GW182 SD at CNOT1 is still unknown. On the other hand, tristetraprolin (TTP) is a protein that binds to AU-rich elements (ARE) present in the 3' UTR of mRNA of cytokines. TTP interacts also with CCR4-NOT *via* its CNOT1 subunit, thus yielding repression of inflammatory processes unless it is phosphorylated. A short C-terminal helix of TTP is known to bind to the CNOT1 fragment encompassing residues 800-999. Both GW182 and TTP are intrinsically disordered proteins, which makes structural studies involving them challenging. Using hydrogen-deuterium exchange mass spectrometry (HDX MS), we identified the localization of the binding site of the GW182 silencing domain on CNOT1(800-999). In general, HDX MS experiments reveal perturbations of hydrogen bonding networks upon protein-protein or ligand binding. Surprisingly, the kinetic and equilibrium changes in the extent of the H/D exchange of the CNOT1 peptic peptides pointed to the GW182 SD binding at the same site as the TTP-binding site. To validate this suggestion, we performed pull-down assays based on affinity chromatography for a large series of CNOT1 mutants. The results confirmed that there is a competition between GW182 SD and TTP for the complex formation with CNOT1(800-999). Moreover, bioinformatic simulations done by using a coarse-grained CABS-dock software generated the best model that supports the experimental results. Together, these biophysical studies show that CNOT1 serves as the crossroads of miRNA-mediated and ARE-mediated gene silencing.

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Towards unravelling proteins' mechanism of anti-cooperative binding

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Many proteins, to perform their functions, rely on regulation of their activity via intra-protein, non-local signalling, called allostery [1]. Separate bindings of an allosteric protein communicate remotely: binding one ligand either favours or hinders the binding of a second, often identical one, at a site distant across the protein. Anti-cooperativity, denoted as negative allostery, enables a protein to limit activity by self-organisation or feedback control.

Whilst originally, allostery referred to binding-site cooperativity of proteins due to structural changes [1], allosteric signalling of structurally mostly unaltered proteins via modification of coupled collective modes is by now well established [2,3]. Successful methods to quantify this entropy-dominated allostery arise from the class of elastic network models (ENM) [4,5].

Using an ENM of small protein complexes with binding sites, based on internally elastic and harmonically interacting *allosterons* [6], we elucidate mechanisms for anti-cooperativity. The approach via a few allosterons allows us to trace single eigenmodes' contributions to the free energies of binding, and to determine how structure and weight of each mode change with successive bindings. Inhomogeneity and binding-induced re-organisation of the allosteric free energy's coupling to different modes, resulting in a re-ordering of the associated relative frequency shifts, prove vital for anti-cooperativity.

[1] J. Monod, J. Wyman, J.-P. Changeux, J. Mol. Biol. **12**, 88 (1965)
[2] A. Cooper, D. T. F. Dryden, Eur. Biophys. J. **11**, 103 (1984)
[3] T. C. B. McLeish, T. L. Rodgers, and M. R. Wilson, Phys. Biol. **10**, 056004 (2013)
[4] M. M. Tirion, Phys. Rev. Lett. **77**, 1905 (1996)
[5] I. Bahar, T. R. Lezon, A. Bakan, and I. H. Shrivastava, Chem. Rev. **110**, 1463 (2010)
[6] T. C. B. McLeish, C. Schaefer, and A. C. von der Heydt, Phil. Trans. R. Soc. B (2018)

P-288

Structural and functional studies of Salmonella phage epsilon15 tailspike

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Bacteriophages use tail fibers or tailspikes to bind to bacterial receptors in the cell wall. Hence, the host range of phages depends on these proteins. Our work is focused on the atomic interaction between epsilon15 and its host bacterium *Salmonella enterica* subspecies enterica serovar Anatum A1 (also called *Salmonella* anatum). Epsilon15 contains six tailspikes; each tailspike is composed of a trimer of the gp20 protein, which has 1070 residues. Each tailspike consists of a tail hub binding arm and a barrel-shaped main body decorated with three petals. We have determined the structures of a trimeric construct containing residues 248-1070 at 1.85 Å resolution by X-ray crystallography and of the C-terminal petal domain (amino acids 789-1069) at 1.3 Å resolution, determining the detailed fold of the whole protein, except for the N-terminal phage-binding arm. The barrel-shaped body is composed of three parallel beta-helices with a large groove on the outside. This region is structurally similar to many other bacteriophage tailspikes. The tip, at the distal end of the body, is homologous to a galactose-binding lectin. The C-terminal petal domain has structural homology to the carbohydrate esterase 2 family and residues of a putative active site are conserved.

Epsilon15 tailspikes both bind and digest O-antigen repetitions of the bacterial lipopolysaccharide. One O-antigen repetition of *Salmonella* anatum consists of →3)-α-D-Gal-(1→6)-α -D-Man-(1→4)-β-L-Rham-(1→; C6 atoms of the galactose units are acetylated. As tailspikes digest the O-antigen, the phage gets close enough to inject its genome into the bacterium. We have co-crystallized the tailspike with a hexasaccharide containing two O-antigen repeats. The hexasaccharide binds to three different sites: to a conserved groove in the beta-helical region, to the lectin domain and to the petal domain. Future research will focus on the mechanism of O-antigen cleavage and the exact role of the tailspike in phage infection.

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Conformational screening of proteins with Å-accuracy using chip-based friction measurements

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Function, activity, and interactions of proteins crucially depend on their three-dimensional structures and the relative arrangement of individual protein domains. Here, we use electro-switchable DNA nanolevers to actuate proteins on a chip surface and to analyze their conformation from a measurement of hydrodynamic friction. This allows for complex mode-of-action investigations and opens new perspectives for hit validation in drug development through in-depth information on protein conformation, oligomeric state, and stability.

We present two examples of biophysical switchSENSE assays which correlate protein structure and function. Tumor necrosis factor alpha (TNFa) is a homotrimeric cell signaling protein involved in inflammation and the regulation of immune cells, which loses its bioactivity when it decays to a monomer. We observe the monomerization kinetics of TNFa in real-time as changes in the apparent protein friction, which enables us to control the monomerization/trimerization state of the protein for antibody binding experiments. Our results show that the kinetics and affinity of antibody binding depend on the structural integrity of the homotrimer, and underline that it is important to test the structural integrity of proteins before performing binding assays. Transglutaminase 2 (TG2) is a multifunctional enzyme, involved in autoimmune and neurodegenerative diseases. Its activity is regulated allosterically by Ca^{2+} and nucleotides and involves conformational changes. Here, we combine real-time nucleotide binding kinetics with protein size measurements and observe nucleotide and ion induced conformational changes. The quick exchange of analytes provides dynamic information on TG2 conformation and yields new insights in TG2 regulation and the mode-of-action of pharmaceutically interesting peptidic inhibitors and the reversibility of structural changes.

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Structural and functional characterization of StnIII: a newly discovered actinoporin regulating *S. helianthus* venom activity

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Actinoporins are a family of pore-forming toxins produced by sea anemones as part of their venomous cocktail. This proteins remain stably folded and soluble y aqueous solution but upon interaction with lipid membranes containing sphingomyelin, they become oligomeric integral membrane structures, leading to cell death by osmotic shock. Actinoporins appear as multigene families in the sea anemone genome: there are several genes coding very similar, but not identical actinoporins within the same individual. The Caribbean sea anemone *Stichodactyla helianthus* produces two different actinoporins Sticholysin I and II (StnI and II). They are 93% identical in the amino acidic level but differs on its toxic potency: StnII is around 4 times more active than StnI when assayed against red blood cells. They can interact to each other in a synergistic way, a small amount of StnII in a mixture with StnI exerts faster cell lysis than expected if they both would interact independently. The transcriptomic assembly of S. helianthus revealed the existence of a third actinoporin not yet discovered: StnIII. Its structural and functional characterizations reveals a new step in the venom toxic activity regulation.

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Ancestral proteins as scaffolds for generation and evolution of *de novo* enzymatic activities

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Enzymes are specialised biological catalysts that perform highly efficient biochemical reactions as a result of millions of years of natural evolution. Despite all the knowledge about enzymatic catalysis, one of the main unresolved challenges in molecular biotechnology is the generation of *de novo* enzymatic activities. Directed evolution is a powerful tool to engineer enzymes based on simulating natural Darwinian evolution by randomly generating mutations in a protein sequence and selecting the target function. This method has been successful in increasing enzymatic activities in proteins. However, it is noteworthily inefficient in the generation of completely new enzymatic reactions.

Resurrected ancestral proteins display high stability and enzymatic promiscuity likely linked to an enhanced conformational flexibility/diversity, which are advantageous features that contribute to protein evolvability. Previous results in our group showed the feasibility to generate a *de novo* enzymatic activity in a resurrected ancestral β-lactamase through a minimalist active site design, assisted by global flexibility, with substantial Kemp elimination activity. This suggests that ancestral proteins might be suitable scaffolds for the design of *de novo* enzymatic functionalities better than extant proteins.

In this work, we prove that directed evolution of the engineered resurrected β-lactamase increases the Kemp activity of the enzyme by the introduction of 5 mutations while maintaining the high thermostability. In addition, we propose the use of ancestral proteins with the highly evolvable TIM-barrel fold as versatile frameworks to study the generation and evolution of *de novo* enzymatic activities. A resurrected ancestral β-glycosidase obtained in our laboratory suggests a conserved TIM-barrel like structure and displays a high thermostability, which would serve as a suitable initial scaffold for different engineering approaches in order to test the generation of *de novo* enzymatic activities.

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Androgen receptor phosphorylation at Serine 96: influence on protein structure, function and interaction network

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Androgen receptor is a transcription factor implicated in the etiopathogenesis of prostate cancer, androgen insensitivity syndrome and spinal bulbar muscular atrophy. The latter is a rare neurodegenerative disease affecting male adults with a rate of 1 on 40000. The genetic hallmark of the disease is the expansion of CAG triplet in the exon 1 of androgen receptor gene, yielding a protein with extended poly-glutamine tract (polyQ) in the N-terminal region of the protein. So far, the causes of the disease have not been identified, yet transcriptional dysregulation is known to play a role together with protein misfolding and aggregation. The aim of my work is to analyse the molecular details at the basis of protein loss of function and gain of function, at the basis of the two pathologies. In particular, I am investigating the consequences of phosphorylation at Serine 96 at molecular level. This site has been demonstrated to cause selective toxicity in case of polyQ expansion. To achieve my goal, I am undertaking a multidisciplinary approach based on NMR spectroscopy, cell based transcriptional assays and proteomics. I performed 3D-NMR on caspase fragment of androgen receptor (containing both Ser96 and poly-Q tract), demonstrating a small conformational change in the presence of phospho-mimicking mutation. In parallel, I am testing the efficiency of transcriptional activity of phospho-mimicking and phospho-defective mutants of androgen receptor, and the shift on interaction network. I am then extending the investigation on the influence of Ser96 phosphorylation of other phosphorylation sites, suggesting a more indirect and subtle role of this constitutive post-translational modification of androgen receptor. Results will increase the knowledge on basic biology of androgen receptor and will help identifying new lines for intervention in case of disease.

Laura Tosatto thanks Fondazione Caritro and Kennedy's Disease Association for support.

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Sticholysin, Sphingomyelin and Cholesterol: A Closer Look into a Tripartite Interaction

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Actinoporins are a widely studied group of toxins produced by sea anemones. Their mechanism of action consists of binding to sphingomyelin (SM)-containing membranes. Once attached to the bilayer, they oligomerize and form cation-selective pores. Although SM is usually sufficient to recruit these toxins, the presence of cholesterol in these membranes is also known to greatly facilitate the process. Despite years of research, there are still many unknown details about the actinoporin-membrane interaction. What is the most favorable phase state of the membrane for their action? What happens to SM distribution in the membrane after the toxins bind to the bilayer? What is the exact role of cholesterol and how do these proteins interact with it?

To shed some light on these questions we have tested the effect of sticholysins, the actinoporins produced by the Caribbean sea anemone *Stichodactyla helianthus*, on bilayers of varied compositions. To study the behavior of SMs in the events subsequent to toxin binding, we took advantage of the properties of fluorescent SM analogs labelled with pyrene. By measuring the extent of the excimer to monomer ratio from the emission of pyrene we were able to assess the degree of SM-SM interaction before and after pore formation. Similarly, we used a fluorescent analog of cholesterol, cholestatrienol, to gain further knowledge on the interaction between sticholysins and sterols. To do this, we used a RET approach in which the tryptophan residues of sticholysins acted as donors.

Monday 22nd July

GENE NETWORK DYNAMICS AND SIGNALLING

P-294 (O-090)

A nonequilibrium phase transition theory for the formation of diverse homeostasis and the emergence of systematic aging in multicellular systems

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The breakdown of homeostasis in tissues is fatal to living beings. Numerous factors across multiple scales from subcellular to extracellular levels could account for the deregulation of homeostasis. Here we present a prototypical multicellular homeostasis model in the form of a 2D stochastic cellular automaton with three cellular states, proliferative, dead and quiescent, of which the state-updating rules based on fundamental cell biology regarding self-replication, apoptosis and cell cycle arrest. This simple model can reproduce the formation of diverse homeostatic patterns with distinct morphologies, turnover rates and lifespans without considering genetic, metabolic or other exogenous variations. Besides, through mean-field analysis and Monte-Carlo simulations, those homeostatic states are found to be classified into extinctive, proliferative and degenerative phases, whereas healthy multicellular organizations (characterized by a large proportion of quiescent cells with few proliferative cells) could evolve from proliferative to degenerative phases over long time, undergoing a systematic aging. From statistical analyses, this aging process is found to be deeply related to the phase transitions into an absorbing state in some nonequilibrium physical systems featuring generalized epidemic process. A theory in the language of nonequilibrium statistical physics is thence proposed to attribute the collapse of homeostasis (at the multicellular level) to an isomorphism between a generalized epidemic process and the fundamental nature of biology regarding cell proliferation, death and cell cycle arrest.

P-295 (O-091)

Post-transcriptional regulation, noise and spatial transcript localization of small RNA-controlled genes in an *Escherichia coli* stress response network

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Post-transcriptional regulatory processes may change transcript levels and affect cell-to-cell variability or noise. We study small-RNA regulation to elucidate its effects on noise in the iron homeostasis network *of Escherichia coli*. In this network, the small-RNA RyhB undergoes stoichiometric degradation with the transcripts of several target genes, as well as upregulates the translation of other target genes, in response to iron stress. Using single-molecule fluorescence *in situ* hybridization (smFISH), we measured transcript numbers of RyhB-regulated genes in individual cells, as a function of iron deprivation. We observed a monotonic increase of noise with iron stress, but no evidence of theoretically predicted, enhanced stoichiometric fluctuations in transcript numbers, nor of bistable behavior in transcript distributions. Direct detection of RyhB in individual cells shows that its noise is much smaller than that of these two targets, when RyhB production is significant. A generalized stochastic, two-state model of bursty transcription that neglects RyhB fluctuations describes quantitatively the dependence of noise and transcript distributions on iron deprivation, enabling extraction of *in vivo* parameters such as RyhB-mediated transcript degradation rates. The transcripts' threshold-linear behavior indicates that the effective *in vivo* interaction strength between RyhB and its two target transcripts is comparable. Visualization of various transcripts by smFISH and super-resolution microscopy indicates that their subcellular localization is not significantly affected by iron stress. The results do not support predictions of a theoretical model that claims that excluded volume effects favor transcript localization at the cellular poles.

P-296 (O-092)

Modeling cytoneme guidance for Hedgehog signaling.

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Cell-cell communication is crucial during the development of an organism and is mediated through signal molecules called morphogens, which are distributed in a morphogenetic field in graded form, activating different target genes in a concentration-dependent manner. Classical models assume that the gradient distribution of those signaling is through a diffusion mechanism. However, new experimental results challenge this idea since most of the morphogens cannot diffuse freely through the extracellular matrix (ECM) due to their biochemical properties. A new mechanism for morphogen transport has been proposed that solves this issue and is based on the idea of distribution of the signal molecules through filopodia-like structures, also called signaling filopodia or cytonemes. These actin-based structures seem to play a critical role in the process of cell communication in several biological systems during development. Cytonemes protrude from the cell membranes of both producing and receiving cells and extend through the ECM to reach distant cells to deliver and collect the signaling molecules. Therefore, knowing the mechanism that control their dynamics, elongation, polarization and orientation is key to understand the correct signaling pattern. We are studying those cytonemes implicated in the Hedgehog (Hh) signaling pathway in *Drosophila* epithelia. We have analyzed in this system the possible role of certain adhesion and ECM proteins of this pathway in the cytoneme guiding process. We have used a set of genetic tools to modify the local levels of those proteins and then quantify biophysical parameters, such as length and orientation of cytonemes, under these conditions. Due to the non-linear dynamics of the process, we have developed a mathematical model using a novel theoretical approach where cytoneme guiding can be explained as an emergent biophysical property of potentials generated by concentration-dependent sources of several proteins.

Monday 22nd July

NEW FRONTIERS IN BIOIMAGING

P-297 (O-096)

Wavefront shaping for low backgound, high resolution STED-FCS in three dimensions

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Fluorescence correlation spectroscopy (FCS) is a powerful tool to study molecular diffusion. However, the spatial resolution of FCS experiments is restrained by the diffraction limit and many diffusion processes happening at the nanoscale cannot be resolved by conventional FCS. To overcome this limitation, FCS can be used together with stimulated emission depletion (STED) microscopy, and this STED-FCS approach has already found multiple applications, particularly when studying the cellular plasma membrane. STED-FCS has however rarely been used to study 3-dimensional diffusion, mainly because with the most widely used STED depletion pattern, a ring-shaped focus ("doughnut", 2D STED), the non-depleted out-of-focus volumes contribute to high background noise and deteriorate the effective spatial resolution. A solution to this problem consists in using a bottle-shaped pattern as the depletion beam (3D STED); but this pattern suffers from an exacerbated sensitivity to optical aberrations.

We present here an adaptive optics method that corrects aberrations affecting 3D STED-FCS experiments. Using a spatial light modulator (SLM) as a wavefront shaping device in the depletion path of a STED microscope, we corrected aberrations affecting STED-FCS measurements in the cytoplasm of living cells, leading to increased resolution, improved signal quality, and larger accessible depth range for measurements.

P-298 (O-097)

High density single particle tracking reveals nano- and meso-scale dynamic organization of plasma membrane receptors in living cells

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Transmembrane adhesion receptors at the cell surface, such as CD44, are often equipped with modules to interact with the extracellular matrix (ECM) and the intracellular cytoskeletal machinery. CD44 has been recently shown to compartmentalize the membrane into domains by acting as membrane pickets, facilitating the function of signalling receptors. While spatial organization and diffusion studies of membrane receptors are usually conducted separately, here we combine observations of the organization and diffusion of CD44 using high spatial and temporal resolution imaging on living cells to reveal a hierarchical organization of the receptor. We used high-density single particle tracking (HD-SPT) to generate cartography maps, with nanometre localization accuracy, of regions explored by the receptor as it diffuses in the cell membrane. Using this approach, we found that CD44 dynamically organizes along a meshwork-like pattern at the mesoscale. Interestingly, these patterns are enriched by actin-dependent CD44 nanoclusters. We characterized the properties of these clusters in terms of size and time evolution for different CD44 mutants and reconstructed an in-silico meshwork to further quantify to strength of the interaction of CD44 with the underlying network. Overall, our methodology provides valuable insight into the hierarchical organization of CD44 at the cell surface and should be equally applicable to the study of other membrane receptors.

P-299 (O-098)

Photophysics and engineering of transgene labels for optoacoustics

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Photo- or Optoacoustic (OA) imaging combines optical contrast with ultrasound resolution enabling high-resolution real time *in vivo* imaging well-beyond the 1 mm penetration depth typical of purely optical methods. While OA already successfully employs endogenous contrast like blood or lipids to inform on tumor states, vascularization, inflammation or metabolic processes, targeted labels similar to those used in fluorescence imaging are few. This scarcity, particularly of labels that can be expressed as transgenes, limits the full exploitation of OA in life science applications. Since both modalities rely on optical absorption, OA labels can be developed from fluorescent ones, but their photophysical characteristics have to be engineered specifically for OA.

Our group studies the photophysics of OA signal generation from major classes of chromophore-bearing proteins (GFP-like, Bacteriophytochromes and Phycobiliproteins) using OA, absorption and fluorescence spectroscopy under ns-pulsed laser excitation as in OA imaging [1]. We use the insights from these analyses to engineer novel OA labels through screening- and structure-based rational design [2]. A focus of our group is on photo-controllable labels to overcome the abundant signal from natural absorbers during OA imaging *in vivo* [1,3-4]. In parallel, we explore ways to develop molecular sensors for OA imaging (e.g. Ca^{2+}-responsive) as well as cell-based sensors, e.g. our recent work in tracking macrophage activity within tumors using changes in the spectra of bacteriochlorophyll [5].

Our long-term goal is to provide a varied toolbox of OA labels and sensors that will allow researchers to exploit the penetration depth and non-invasiveness of this powerful imaging technique for their basic and preclinical studies of neural signaling, metabolism, cell physiology and tumor biology in entire organisms. Our conference contribution will introduce to this novel area of label development and its underlying photophysics.

Further reading:

1) Vetschera, *Analytical Chemistry*, 2018
2) Fuenzalida-Werner, *JSB*, 2018
3) Stiel, *Optics Letter*, 2015
4) Deán-Ben XL, *Optics Letters*, 2015
5) Peters, *Nat. Comm.*, 2019

P-300

Combining optical tweezers with IRM, TIRF, Widefield and STED: a platform for dynamic, single molecule analysis

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Biological processes performed by proteins interacting with DNA/RNA, cell membranes or cytoskeletal protofilaments are key to cell metabolism and life. Detailed insights into these processes provide essential information for understanding the molecular basis of life and the pathological conditions that develop when such processes go awry. The next scientific breakthrough consists in the actual, direct, real-time observations and measurements of the individual mechanisms involved, in order to validate and complete the current biological models. Single-molecule technologies offer an exciting opportunity to meet these challenges and to study protein function and activity in real-time and at the single-molecule level. Here, we present our efforts for further enabling discoveries in the field of biology and biophysics using both the combination of optical tweezers with correlative fluorescence microscopy (widefield, TIRF, confocal and STED) and label-free Interference Reflection Microscopy (IRM). We present several examples in which our technologies enhanced the understanding of protein folding, cytoskeletal mechanics, protein-membrane interactions and genome structure and organization. Furthermore, we show that advances in hybrid single-molecule methods can be turned into an easy-to-use and stable instrument that has the ability to open up new venues in many research areas.

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Super-resolved and confocal spectroscopy reveal heterogeneity in live cell plasma membrane dynamics

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The cellular plasma membrane is a highly heterogeneous structure organised on nano-scales and displays a crucial interaction platform for proteins, lipids and soluble ligands. Investigating the molecular membrane organisation by measuring diffusion dynamics offers a better understanding of its biological function.

Fluorescence correlation spectroscopy (FCS) is one of the prominent tools to elucidate these dynamics in living cells but can only report on the dynamics at one given spatial position at a time. Using scanning fluorescence correlation spectroscopy (sFCS), a multitude of FCS measurements at different spatial locations can be obtained. Here, we combine sFCS with stimulated emission depletion (STED) microscopy and by alternating conventional and super-resolved excitation we introduce line interleaved excitation scanning STED-FCS (LIESS-FCS). Moreover, we suggest a novel statistical analysis pipeline for sFCS data. By measuring dynamics in confocal and STED, we are able to distinguish free (Brownian) from hindered (non-Brownian) diffusion in great detail and obtain the so called diffusion modes of molecules. With LIESS-FCS these diffusion modes can be directly determined at multiple spots within the cellular plasma, thus providing comprehensive insights into the spatiotemporal organisation and function of plasma membrane components. Interestingly, by statistically analysing large confocal sFCS data-sets with an advanced fitting approach, hindered diffusion dynamics can already be inferred. Using both approaches we show free diffusion for phospholipids in model membranes and cells but reveal hindered diffusion of sphingolipids and GPI-anchored proteins in cells.

Overall we present a novel toolkit to investigate nano-scale molecular diffusion dynamics for shedding a new light on membrane organisation and heterogeneity.

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Nanoscale imaging of light-induced damage in amyloid fibers

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The misfolding and aggregation of proteins into amyloid fibers is generally toxic and is involved in many neurodegenerative disorders. Photochemical strategies are becoming an interesting alternative to degrade amyloid aggregates and fibers. In our studies we use a thioflavin T derivative, which is able to target pathogenic aggregates in the presence of non-pathogenic proteins [1]. In addition to fluorescence, this compound produces reactive oxygen species upon blue light exposure, affecting amyloid structures through oxidation [1]. We investigate the photodamage induced by this compound on model amyloid fibers using a combination of spectroscopic tools and correlative fluorescence and atomic force microscopy. Our results provide a nanoscale view of light-induced amyloid breakage, and are relevant to improve phototherapeutic strategies for amyloid-related disorders.

References

1. Taniguchi, A., Taniguchi, A., Shimizu, Y., Oisaki, K., Sohma, Y., & Kanai, M. *Nature Chemistry*, **2016**. *8:* p. 9

P-303

Plasmonic fluorescent assay for molecular lipid membrane binding, permeation and dynamics of permeation.

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Membrane permeability is a key property of any prospective drug and the interaction of a drug with the cellular membrane influences its pharmokinetics as many drug targets are intracellular, Herein we describe a new concept based on plasmonics for gaining new insights into the determination as to whether a molecule is membrane permeable, mechanic insights into membrane interactions and the kinetics of permeability. This is achieved using metal cavity supported lipid bilayers. In this approach lipid bilayers spanned over gold microcavities are exploited as surface enhanced fluorescence (SEF) substrates. Through the use of Raman and Fluorescence spectroscopy, we can detect if and how a drug interacts with the lipid membrane, while also obtaining an insight of the dynamics of the interaction and the rate of permeation through the bilayer and arrival into the plasmonic field of the gold cavity. It was found that gold microcavity substrates gave a significant increase in fluorescence intensity over planar gold substrates, regardless of modifications or bilayer presence. Through Electrochemical Impedance spectroscopy (EIS), DOPC symmetric bilayers are shown to be stable for an average of 7 hours, which increases with membrane complexity, where our ternary bilayer composition (DOPC/SM/CH) (40/40/20%) shown an average stability for 8 hours. Through SEF measurements, the bilayer residential time and diffusion time were found for the drugs Doxorubicin and Daunorubicin over a range of concentrations and membrane compositions. Interestingly, the method could easily distinguish aggregation of drug above certain concentrations that lead to a reduction in permeation rate. Through Raman spectroscopy we can clearly identify that drug-membrane interactions occur. This work demonstrates a new versatile and useful paradigm for drug- membrane permeability assay.

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How many molecular species are required to create membrane domains inside which proteins can diffuse? A molecular interactome question answered by High-Speed Atomic-Force-Microscope

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The cell membrane is a place where spatial organization and segregation is essential for proteins to fulfil their functions. Optimal microscopy (OM) techniques observe constant dynamic restructuring of the partitioning of cell membranes. In cells OM observes at specific moments some membrane proteins to transiently localize and diffuse in membrane domains. These domains are believed to promote the protein-protein interactions that are needed for a specific cell functioning tasks. Once the task accomplished, the proteins move out of the domain and diffuse freely in the membrane[1].

Both, the lipids and the proteins in the membrane are active players of the membrane-protein recruitment. Both the lipids and the proteins can create lateral heterogeneities by altering the local lipid stoichiometry, local lipid phase structure, or local membrane curvature.

We ask the following question: How many molecular species are required to create membrane domains inside which proteins can localize and diffuse transiently?

The answer to the minimal number of molecular species required to create membrane domains inside which proteins can localize and diffuse transiently is important as constrain parameter for the elucidation of the molecular interactomes of cells.

Surprisingly, by High Speed Atomic Force Microsocope we found that membrane domains can be induced by the same protein that diffuses in the domain. This is the case for the of bacterial membrane-bound glycosyltransferase (MurG) that forms at 37°C domains on membranes composed of one lipid species (DPPG, POPG or DOPG), all in fluid phase. The mechanism of formation of MurG-induced membrane domains is at this point unclear. It seems to depend on protein-lipid interactions as the domains change with the alkyl chain of the lipid species. Models are provided. The addition of the MurG substrate UDP-GlcNAc stops the diffusion in the MurG-domains.

A. Kusumi et al. 2005 Annu. Rev. Biophys Biomol Struc. 34, 351-U54

P-305

Observation of motility of Cyclotella meneghiniana by using the self-made inverted microscope

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Diatoms are important photosynthetic organisms that create about 25% of the oxygen of the earth. Observation of floating phenomenon of diatoms is interesting to understand the ecological mechanisms of diatoms since it is known that the floating of the diatoms is believed to be related to the efficiency of photosynthesis. However, it is difficult to observe the floating direction perpendicular to the ground surface by using conventional microscopes because the specimen stand is horizontal to the ground surface. Thus, we developed the microscopy system rotating the inverted microscope 90 degrees and observed the floating behavior of the diatom *Cyclotella meneghiniana* using a developed one. The observation sample was the diatom *Cyclotella meneghiniana.* We continuously cultured it every 3 weeks by using the BBM medium. The observation sample was cultured for about 10 days. The sample diluted 40-fold by BBM medium. The measurement was used petri dishes (52Φ×12 mm) and microchamber (1 × 1 × 1 mm). All measurements were observed for 1 hour. We have fixed rotated 90 degrees inverted microscope (Olympus CKX53) with an L-shaped aluminum jig in order to observe the floating phenomenon of diatom. The fixing brackets were installed in three places on both sides of the specimen stand and near the bottom of the microscope so as not to affect the microscope optics. In addition, the microscope was supported by using a lab jack so as not to bend by the own weight of the camera used for photographing. By using the developed microscope system, we observed the different and similar motion for of diatoms for the petri dish and the microchamber in each measurement, respectively. The developed microscope is suitable for observing the behavior of floating diatoms and for evaluating the behavior differences of diatoms.

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Protein-stabilized metal nanoclusters as tools for sensing and imaging

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Protein-stabilized metal nanoclusters as tools for sensing and imaging

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Metal nanoclusters have been considered ideal nanomaterials for biological applications due to their strong photoluminescence, excellent photostability, and good biocompatibility. Protein-stabilized AuNCs offer unique properties, however up to now protein-based nanocluster synthesis has been done using commercially available globular proteins, which properties are not easily tuned, therefore limiting their applicability in different research areas. We have shown that designed repeat proteins can be used for the fabrication of multiple protein-based hybrid functional nanostructures.[1,2]

Here, we explore the potential of designed repeat proteins for nanocluster synthesis and stabilization. We present simple strategies to use proteins to encapsulate metal nanoclusters[3] and to design proteins, via incorporation of specific metal binding sites into a protein structure, for the sustainable synthesis and stabilization of different metal NCs.[4,5]

The resulting protein stabilized metal nanoclusters of different metallic compostions showed good photoluminescence, strong photostability and excellent biocompatibility.

Finally, the combination of cluster stabilizing protein modules with protein modules with specific binding capabilities has been successfully used to stabilize nanoclusters and applied as a sensor. In addition, we evaluated the potential application of the protein stabilized metal NCs in the field of cell labeling as fluorescent intra-cellular markers and as markers for synchrotron radiation X-ray fluorescence spectrometry.

References

1. Mejías, S. H., et al., Chem. Sci. **2016**, 7, 4842.
2. López-Andarias, J., et al., Adv. Funct. Materials **2018**, *28*, 1704031.
3. Couleaud, P., et al., Biomacromolecules **2015**, 16, 3836.
4. Aires et al. Patent EP17382451, **2017**.
5. Aires et al. Angew. Chem. **2019**, DOI: 10.1002/anie.201813576.

Web Page: http://personal.cicbiomagune.es/alcortajarena/

P-307

Measuring Protein Insertion Areas in Lipid Monolayers by Fluorescence Correlation Spectroscopy

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The insertion of proteins into lipid membranes is an important step in many cellular processes, ranging from signaling over enzyme catalysis to vesicular transport. The increase in membrane area that accompanies the insertion influences the stress induced in the membrane and thereby its curvature. This in turn may change the affinity of protein binding. Insertion areas in lipid monolayers are measured mainly on a Langmuir film balance. Obtaining exact values however proves challenging for two reasons: The number of inserted molecules must be determined without disturbing the binding equilibrium and the change in the film area can be very small, especially at high surface pressures. Here we address both of these problems using Fluorescence Correlation Spectroscopy (FCS): By labeling a fraction of the protein molecules and performing FCS experiments directly on the monolayer, we are able to extract the density of inserted proteins *in situ* without relying on invasive techniques. We finally remove the need for macroscopic measurements altogether by determining the area increase upon protein insertion with FCS as well, utilizing a second color channel for the monolayer. We monitor the relative change of lipid density before and after insertion by employing a small fraction of fluorescently labeled lipid molecules and relate this quantity to the number of proteins in the focus. Thus, our method determines the insertion area solely from local measurements on a system in equilibrium, which enables the user to perform experiments on a large range of possible membrane systems and has the potential to achieve a high spatial and temporal resolution for these measurements. In this work, we apply our method to Sar1p, a protein of the COPII complex. We find an insertion area in the low nm^2 range for 'major-minor-mix' monolayers, in good agreement with the expected insertion area for its amphipathic helix.

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Lipid composition-triggered transition from a vesicle to a sponge self-assembled nanostructure revealed by cryo-TEM and SAXS

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The intermediate states upon a vesicle-to-sponge nanoparticle transition in self-assembled monoolein (MO)/eicosapentaenoic acid (EPA, 20:5) mixtures are investigated by cryogenic transmission electron microscopy (cryo-TEM) microscopy and synchrotron small-angle X-ray scattering (SAXS). Mixed membrane assemblies involving the ω-3 polyunsaturated fatty acid EPA are characterized by non-trivial porous topologies. Cryo-TEM imaging revealed the transformation pathway of the vesicular bilayer membranes into sponge nanoparticles (spongosomes). Experimental evidence is obtained that the topological transition, which is governed by the amphiphilic composition, occurs through the proliferation of membrane-linking pores (MLP). Synchrotron SAXS patterns characterized the overall structural transition from vesicles to sponge membranes in the studied lipid systems. The obtain results suggest that the incorporation of the ω-3 polyunsaturated fatty acid at increasing concentration causes swelling inhibition (dehydration) of the host liquid crystalline nanoarchitectures. This structural knowledge is valuable for the design of stimuli-responsive nanomaterials and drug delivery carriers.

1. Angelova, A.; Drechsler, M.; Garamus, V.M.; Angelov, B., *ACS Omega*, 2018, *3*, 3235-3247.
2. Rakotoarisoa, M.; Angelov B., Garamus, V. M.; Angelova, A. *ACS Omega*, 2019, *4*, 3061-3073.
3. Angelova, A.; Angelov, B., Garamus, V.M.; Drechsler, M., *J. Mol. Liq.*, 2019, *279*, 518-523.
4. Angelov, B.; Garamus, V. M.; Drechsler, M.; Angelova, A., *J. Mol. Liq.*, 2017, *235*, 83-89.

Angelov, B.; Angelova, A.; Filippov, S.; Drechsler, M. *et al*. *ACS Nano*, 2014, 8, 5216-5226.

P-309

Exploring the potential of unconventional fluorophores in super-resolution imaging

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Super-resolution fluorescence microscopy techniques improve by an order of magnitude the spatial resolution in light microscopy and have become a powerful tool to study nanosized structures with unprecedented detail. Several super-resolution methodologies are currently available, which combine clever illumination schemes, and crucially, special fluorophores with specific photophysical properties such as fluorescence photoswitching. While the quest for novel fluorophores with tailored properties is indeed being actively pursued, there is currently a very rich palette of molecules already available for other fluorescence applications. The development of many of these fluorophores has required compromises in brightness and/or photostability, in exchange for other interesting spectroscopic or biochemical properties.

In this work, we aim to evaluate the potential of "unconventional" fluorophores for super-resolution imaging techniques. The models selected have inherently moderate brightness and photostability and produce reactive oxygen species upon light illumination, which is typically undesired and detrimental for fluorescence imaging applications. We optimize imaging protocols and quantitatively assess the performance of the fluorophores in different super-resolution imaging techniques, such as Airyscan, stimulated emission depletion microscopy and single-molecule localization microscopy. This work may contribute to expanding the palette of photoactive compounds suitable for super-resolution fluorescence imaging.

P-310

A fluorescence-based method to measure ATP/ADP exchange of recombinant mitochondrial adenine nucleotide translocase (ANT)

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Several mitochondrial proteins, such as the adenine nucleotide translocase (ANT)[1], aspartate/glutamate carrier (AGC)[2], dicarboxylate carrier (DIC)[3] and uncoupling proteins 2 and 3 (UCP2, 3)[4,5] are suggested to have dual transport functions. While the transport of charges (protons, anions) can be characterized by the alteration of membrane conductance, the investigation of the (neutral) substrate transport is challenging. Up-to-date mainly radioactively labeled substrates are used that is very expensive and require demanding precautions for measurement preparation and conduction.

We describe a fluorescence-based method[6] using magnesium green (MgGr™), a Mg-sensitive dye, for measurements in liposomes. Given the different binding affinities of magnesium to ATP and ADP, changes in ATP and ADP concentrations are detectable. We obtained ATP/ADP exchange rate of recombinant ANT reconstituted into unilamellar proteoliposomes with 3.92 ± 1.98 µmol ATP per minute and mg ANT that is comparable to values measured in radioactivity assays in mitochondria and in isolated protein. Our results show that ATP/ADP exchange calculated from MgGr™ fluorescence solely depends on ANT content in liposomes and is inhibited by ANT specific inhibitors bongkrekic acid and carboxyatractyloside. We revealed that the presence of fatty acids in the membrane significantly affects ATP/ADP exchange rate of ANT.

The application of MgGr™ to investigate ATP/ADP exchange rates of ANT contributes to the understanding of the function of ANT in mitochondria.

References:

1. Andreyev, A., et al. (1989), Eur J Biochem **182**(3): 585-592
2. Samartsev, V. N., et al. (1997), Biochim Biophys Acta **1319**(2-3): 251-257
3. Wieckowski, M. R. and L. Wojtczak (1997), Biochem Biophys Res Commun **232**(2): 414-417
4. Rupprecht, A., et al. (2014), PLoS One **9**(2): e88474
5. Hilse, K. E., et al. (2018), Front Physiol **9**: 747
6. Chinopoulos, C., et al. (2009), Biophys J **96**(6): 2490-2504

P-311

Reconstruction of 3D information from limited set of projections: mesoscopic biological objects and destructive X-ray imaging

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X-ray imaging of microscopic non-reproducible biological particles by short intense laser pulses, such as provided by Free electron lasers, is limited by radiational damage to 2D projections only. In order to achieve the full potential of diffract-before-destroy technique, several simultaneous projections of the same system must be achieved. However, the amount of information, obtained using full tomographic scan, as known in both classical and microtomography is not achievable due to experimental and technical limitations.

Due to Nyquist sampling theorem, the few projections of the system lack the complete information for full 3D reconstruction. However, decreasing spatial resolution and restricting the number of classes of recognizable objects, the pattern recognition techniques can be used to assign the resulting set of projections to the most likely class of objects, in an ideal case, the class of single object.

In my contribution I will discuss the suitability of the mesoscopic objects for such approach in light of current experimental capabilities.

P-312

Arrays of high-aspect ratio nanostructures for biological applications

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The endeavour of exploiting arrays of vertical high-aspect-ratio nanostructures for biological applications is experiencing a pronounced surge of activity. The interest is primarily rooted in their dimensions (diameter 100- 500 nm and height of several microns), which make them relevant for a very broad range of applications with biological samples due to their size compatibility with proteins and cells. Furthermore, their intrinsic optical properties are also relevant for enhanced detection of biological events.

The combination of theoretical and experimental studies on high-aspect ratio nanostructures provided a detailed understanding of their intrinsic optical properties as well as their interface with proteins and living cells. We will review a series of examples of new biological applications with perspectives in diagnostics and drug discovery.

References

T. Berthing *et al.* Small, **7(5),** 640-7 (2011,)
T. Berthing *et al.* Nanotechnology, **23**, 415102 (2012)
S. Bonde *et al.* ACS Appl. Mater. Interf., **5(21):**10510-9 (2013)
S. Bonde *et al.* Nanotechnology, **25,** 362001 (2014)
N. Buch Månson *et al.* Adv. Funct. Mater. **(21);** 3246–55 (2015)
N. Buch-Månson *et al.* Sci Rep **7**:9247 (2017)
N. Buch-Månson *et al.* Nanoscale **9(17)**; 5517-27, (2017)
R. Frederiksen *et al.* Nanoletters **15 (1),** 176–81 (2015)
R. Frederiksen *et al.* ACS Photonics 3 (7), pp 1208–16 (2016)
S. Vutti *et al.* Chemistry-A European journal 22, (2) p496-500 (2016)

P-313

Investigation of intracellular thermal properties that depend on polymers

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Previous studies suggested that there is temperature fluctuation by 1K in living cells (Okabe et al, 2018) and this raised an argument about the discrepancy from the simulated temperature change based on the thermal diffusion equation assuming that the thermal properties of cytoplasm is water (Baffou et al, 2014). Regards to the significance of intracellular temperature on cell functions, our study suggested that intracellular local temperature increase initiates stress granule (SG) formation and this phenomenon is called thermal signaling (Shi unpublished). In addition, it also suggested that an excess amount of fluorescent polymer thermometer (FPT) inhibited thermal signaling. However, physical mechanisms which explain these characteristic thermodynamic phenomena in living cells have not yet been revealed. In this study, to analyze intracellular temperature, we used FPT and artificial local heating by infrared (IR) laser. At first, we introduced FPT into COS7 cells by microinjection and locally heated the cytoplasm by IR laser to quantify thermal diffusivity and thermal conductivity. As a result, FPT increased thermal diffusivity and thermal conductivity in a concentration dependent manner. In addition, we showed that FPT accumulated to heat source and this phenomenon promoted heat transfer by FPT. From these results, we confirmed that living cells had characteristic thermal environment distinct from water, and revealed that polymers inhibited thermal signaling by relaxing intracellular temperature gradient. Furthermore, to investigate the relationship between biopolymers and intracellular temperature, we focused on RNA. When we treated cells with RNase A which digest single strand RNA and locally heated the cytoplasm, we found that temperature increase in the cytoplasm which was treated with RNase A was higher upon heating compared to normal cells. This result indicates that RNA has a large heat capacity in living cells. This is the first study to show that characteristic thermal environment distinct from water produced by biopolymer contributes to cellular functions.

P-314

Innovative light-sheet fluorescence microscopy to investigate receptor recruitment in receptor-mediated virus entry

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The cell membrane is the primary physical barrier that the Human Immunodeficiency Virus (HIV) needs to overcome to penetrate cells and cause disease. HIV virions initially interact with cell-membrane receptors CD4 and CCR5/CXCR4 on the surface of cells of the immune system. Upon virus binding, these HIV receptors redistribute in the plasma membrane and cluster at the site of virus attachment. This facilitates the molecular interactions that ultimately lead to HIV penetration and release of the viral genetic material into the cell. Little is known about how HIV receptors are recruited to virus-binding sites. Recruitment and clustering require receptor mobility in the membrane, which can be influenced by receptor interactions with the cellular cortical cytoskeleton. Recent studies suggest that HIV receptor-cytoskeleton links may be established upon HIV engagement via certain intracellular linker molecules, and that these links, together with dynamic cytoskeleton rearrangements, may mediate receptor clustering. However, these events have not been observed at the single molecule level and our knowledge of HIV-receptor diffusive mobility is very limited.

We present measurements of CD4 mobility in the plasma membrane of living cells (NIH 3T3 mouse fibroblasts) in the presence and absence of one of the possible CD4-cytoskeleton linker molecules, the intracellular tyrosine kinase Lck. Data is acquired with our unique, bespoke light-sheet fluorescence microscope with a temporal resolution of tens of ms and analysed via single particle tracking. Crucially, single molecule mobility is measured on the top surface of cells, away from glass (to avoid artefacts due to cell-glass interactions) with the aid of a specially designed sample holder. CD4 diffusion is measured in near-physiological conditions at 37°C and with cells on a hydrogel of controlled stiffness integrated into the sample holder.

P-315

Nanoscale Bioimaging with Scanning Ion Conductance Microscopy

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The ability to precisely move the nanopipette and to measure simultaneously an ion current allows an unprecedented level of nanoscale imaging of living cells – scanning ion conductance microscopy (SICM) [1]. SICM is a type of non-contact scanning microscopy technique creating topographical image of sample surface by means of glass nanopipette filled with electrolyte scanning over the sample. We have introduced a novel imaging mode of SICM, referred to as the "hopping mode", which for the first time resolved topography of convoluted biological structures such as stereocilia bundles of inner ear hair cells or multi layered neuronal networks in their full complexity at nanoscale resolution. When it comes to imaging topography of soft cells with complex morphology under physiological conditions, the capabilities of hopping mode SICM remain largely unmatched to this day.

The speed of data acquisition positions this as a technology which may be suited to relatively highspeed scanning of cell membrane during various biological processes in real time. SICM can be used in combination with other techniques such as confocal and fluorescence microscopy [2], electrochemical measurement [3], and patch clamp recording [4]. This has the potential to open new horizons in medicine and biology and could be of particular value to the pharmaceutical industry.

We have demonstrated developed semiautomated technique for spatially resolved recording of single channel and whole terminal activity in small synaptic terminals in neuronal culture. The technology was used to investigate the effect of subthreshold somatic potentials on the broadening of action potential recorded at the small synaptic bouton of the same neuron and also for mapping of ligand gated receptors in and sensory neurons.

[1] P. Novak et al. Nature Methods 6, 279–281 (2009)

[2] Gorelik et al. PNAS December 10, 99 (25) 16018-16023 (2002)

[3] A. Erofeev et al. Scientific Reports 8, 7462 (2018)

[4] P. Novak et al. Neuron 79 (6), 1067-1077 (2013)

P-316

Lipid Tracking through iSCAT microscopy reveals novel details into plasma membrane dynamics

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Understanding the dynamics and organization of lipid membranes is a central topic in modern Biophysics. In fact, the molecular mobility of the lipid fraction is a very important factor in processes such as cellular signalling and immune cell activation. Imaging studies of these phenomena, however, require simultaneously high spatial and temporal resolution, which makes them particularly challenging. To fulfil these requirements, we adopted Interferometric Scattering Microscopy (iSCAT) to detect and interpret the motion of individual gold nanoparticle-tagged lipids on the plasma membrane of living cells at high sampling rates (2 kHz). We also developed a statistics-driven analysis scheme for Single Particle Tracking, to better evaluate the effect of localization error on fast tracking experiments and the diffusion mode of the target particle. Our results reveal that a large fraction of the lipids shows a compartmentalized, or hopping, diffusion mode. This study therefore not only provides a novel approach for the analysis of SPT data, but also highlights the viability and potential of iSCAT microscopy for lipid tracking on live cell membranes.

Monday 22nd July

CELL MEMBRANE BIOPHYSICS Sponsorized by CPL, Elsevier

P-317 (O-102)

Linking Structure, Stability and Function in the Anti-Measles Virus Action of Tocopherol-Derivatized Peptide Nanoparticles

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Amyloids have been exploited to build up amazing bioactive materials. In most cases, short synthetic peptides constitute the functional components of such materials. The controlled assembly of globular proteins into active amyloid nanofibrils is still challenging, because the formation of amyloids implies a conformational conversion towards a β-sheet-rich structure, with a concomitant loss of the native fold and the inactivation of the protein. There is, however, a remarkable exception to this rule: the yeast prions. They are singular proteins able to switch between a soluble and an amyloid state. In both states, the structure of their globular domains remains essentially intact. The transit between these two conformations is encoded in prion domains (PrDs): long and disordered sequences to which the active globular domains are appended. PrDs are much larger than typical self-assembling peptides. This seriously limits their use for nanotechnological applications. We have recently shown that these domains contain soft amyloid cores (SACs) that suffice to nucleate their self-assembly reaction. Here we genetically fused a model SAC with different globular proteins. We demonstrate that this very short sequence act as minimalist PrDs, driving the selective and slow assembly of the initially soluble fusions into amyloid fibrils in which the globular proteins keep their native structure and display high activity. Overall, we provide here a novel, modular and straightforward strategy to build up active protein-based nanomaterials at a preparative scale.

P-318 (O-103)

Antimicrobial Peptides Impair Bacteria Cell Structure Within Seconds

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antibiotics based on membrane active antimicrobial peptides (AMPs) are promising candidates for defending the spread of diseases caused by multi-resistant pathogenic bacteria [1]. Notwithstanding the number of works that explore the relationship between AMP activity and membrane architecture [2,3], the dynamics and full mechanism that lead to cell death are currently not clear. This prompted us to investigate the cinematographic effects of human lactoferricin derivatives on live *E. coli* using high-resolution (ultra) small-angle X-ray scattering (USAXS/SAXS) measurements and complementary techniques.

In order to discriminate scattering signals originating from peptides and bacteria, SAXS measurements of AMP suspensions were firstly analysed by means of colloidal and polymer models. Secondly, dependencies of AMP Minimum-Inhibitory-Concentration on bacterial number density were modelled via a phenomenological Hill equation in order to extract effective association constants and the minimum number of AMPs required for inhibiting cell growth. Finally, time-resolved USAXS/SAXS combined with stopped-flow mixing allowed us to probe AMP activity on the submicron to nanometre length (cell body and cell envelope, respectively) and time scales using a recently reported multi-scale model [4]. First results suggest that part of the cell function is lost due to a general disruption of the cell wall, involving a decrease of correlations between inner and outer membranes, as well as a loss of cytoplasm. These key events of killing occur just within a few seconds after exposure to AMPs, i.e. much faster than anticipated from previous reports.

[1] K. Lohner, editor, *Development of novel antimicrobial agents: Emerging strategies.* Horzon Scientific Press, Norfolk, UK (2001).
[2] K. Lohner, E. Sevcsik and G. Pabst, *Liposome-based biomembrane mimetic systems: implications for lipid-peptide interactions*. In *Advances in Planar Lipid and Liposomes* (Vol. 6). A. Leitmannova-liu, editor. Elsevier, Amsterdam, pp. 103-137 (2008).
[3] D. Zweytick et al., *J. Biol. Chem.* **286**(24), 21266 (2011).
[4] E. F. Semeraro et al., *IUCrJ* **4**(6), 751 (2017).

P-319 (O-104)

Elucidating the nanoscale architecture of the plasma membrane with super-resolution spectroscopy

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Cellular homeostasis is usually maintained via complex signalling mechanisms. Although these signalling events involve a multitude of signal transduction molecules, they are usually triggered by interactions between a ligand and a receptor on the cell membrane. The investigation of spatiotemporal interactions between signalling components in the membrane has been hampered by **the complex structure** of the cell membrane: signalling in the plasma membrane involve not only the core protein components, but also membrane lipids, the actin cytoskeleton and the glycocalyx. Moreover, early phases of signalling occur at **very fast temporal (milliseconds) and small spatial (nanometre) scales**, which further hampers thorough elucidation of these processes. Therefore, studies aiming at a complete understanding of signalling require both advanced imaging techniques with high spatiotemporal resolution and well-defined reconstituted systems that can pinpoint the role of each functional component. Here, I will explain how we utilize super-resolution STED microscopy combined with fluorescence correlation spectroscopy (STED-FCS) to access the diffusion characteristics of fluorescently labelled lipids and proteins in the live cell plasma membrane. Our data showed that nanoscale mobility of lipids and proteins in the plasma membrane is highly heterogeneous and this heterogeneity gives invaluable information on the molecular bioactivity. We also developed simplified biomimetic of cell membrane to clarify the principles underlying cell signalling at the molecular level and to dissect the essential drivers of these processes.

P-320

Studies of the interaction of Alyteserin 1c with membrane models imitating Gram negative and erythrocytes membranes

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Antimicrobial peptides (AMPs) are effector molecules of the innate immune system, which represent the first line of defense against infectious agents. They act mainly on the cell membranes of pathogens, disrrupting it, releasing the cytoplasmic content toward the outside of the cell, dissipating the membrane potential and leading to the rapid death of the pathogen. In this work, the peptide constituted by 23 amino acids with a net charge= +2, Alyteserin 1c, was synthesized by the method of solid phase synthesis (SPPS). The interaction of the AMP with membrane models (MLVs) constituted by phosphatidylcholine (PC, mimicking the membrane of erythrocytes) and by the phosphatidylcholine/phosphatidylglycerol (1:3) mixture (PC/PG, mimicking the Gram-negative membrane), was evaluated applying differential scanning calorimetry (DSC) and infrared spectroscopy (FT-IR). The results showed that the peptide was able to decreases the phase transition temperature from 22.9 to 21.2°C and the transition enthalpy from 1.14 to 0.62 J/g as the concentration of peptide was increased on PC-MLV. On the other hand, the frequency of $_{vs}(CH_2)$ from PC-MLV vesicles was slightly increased under peptide effect, while the frequency of the carbonyl vibrations was slihjtly increased in both membrane models, PC-MLVs and PC/PG-MLVs. Finally, the secondary structure of the peptide was determinated by deconvulonization of the amide I band, and it was exhibited that the disordered structure decreased after the interaction of the peptide with membrane models, whereas a higher helix propensity (59%) was acquired after the interaction with PC-MLV.

P-321

Unveiling Red Blood Cell death induced by Pb(II) toxicity: the role of ions, ceramide and scramblases

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The mechanisms of Pb(II) toxicity have been studied in human red blood cells using a variety of techniques such as confocal microscopy, immunolabeling, fluorescence-activated cell sorting and atomic force microscopy. The process follows a specific sequence of events, starting with calcium entry, followed by potassium release, morphological change, generation of ceramide, lipid flip-flop and finally cell lysis. Clotrimazole blocks potassium channels and the whole process is inhibited. Immunolabeling reveals the generation of ceramide-enriched domains linked to a cell morphological change, while the use of a neutral sphingomyelinase inhibitor greatly delays the process after the morphological change, and lipid flip-flop is significantly reduced. These facts point to three major checkpoints in the process: first the upstream exchange of calcium and potassium, then ceramide domain formation, and finally the downstream scramblase activation necessary for cell lysis. In addition, partial non-cytotoxic cholesterol depletion of red blood cells accelerates the process as the morphological change occurs faster. Cholesterol could have a role in modulating the properties of the ceramide-enriched domains. This work is relevant in the context of cell death, heavy metal toxicity and sphingolipid signaling.

Reference:

Ahyayauch*, H.; García-Arribas*, A. B.; Sot, J.; González-Ramírez, E. J.; Busto, J. V.; Monasterio, B. G.; Jiménez-Rojo, N.; Contreras, F. X.; Rendón-Ramírez, A.; Martin, C.; Alonso, A.; Goñi, F. M. Pb(II) Induces Scramblase Activation and Ceramide-Domain Generation in Red Blood Cells. *Scientific Reports* **2018**, 8(1),7456

P-322

Quantification of protein dynamics in nanotubes pulled from transfected cell-derived vesicles

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The ability of proteins to sense and/or generate membrane curvature is crucial for many biological processes inside the cell [1]. The biophysics underlying the effect of these proteins has been explored extensively in synthetic membrane vesicles or in living cells [2], [3]. However, both synthetic membrane vesicles with simple lipid compositions and living cells with an underlying actin skeleton might not adequately reflect the dynamics of the plasma membrane. Here, we examine how different types of membrane proteins respond to changes in membrane curvature and to lipid phases found in plasma membranes. Vesicles derived from transfected cells (GPMVs) enable the analysis to be performed in a biological membrane with properly folded proteins that possess the correct membrane topology. Moreover, we show how lipid phases and nanoscale membrane curvature control the lateral organization of membrane proteins and that protein shape and oligomerization critically affect protein recruitment to curved membranes [4].

[1] H. T. McMahon and J. L. Gallop, "Membrane curvature and mechanisms of dynamic cell membrane remodelling," *Nature*, vol. 438, no. 7068, pp. 590–596, Dec. 2005.
[2] K. R. Rosholm, N. Leijnse, A. Mantsiou, V. Tkach, S. L. Pedersen, V. F. Wirth, L. B. Oddershede, K. J. Jensen, K. L. Martinez, N. S. Hatzakis, P. M. Bendix, A. Callan-Jones, and D. Stamou, "Membrane curvature regulates ligand-specific membrane sorting of GPCRs in living cells," *Nature Publishing Group*, vol. 13, no. 7, pp. 724–729, Jul. 2017.
[3] C. Prévost, H. Zhao, J. Manzi, E. Lemichez, P. Lappalainen, A. Callan-Jones, and P. Bassereau, "IRSp53 senses negative membrane curvature and phase separates along membrane tubules," *Nature Communications*, vol. 6, p. 8529, 2015.
[4] G. Moreno-Pescador, C. D. Florentsen, H. Østbye, S. L. Sønder, T. L. Boye, E. L. Veje, S. Semsey, J. Nylandsted, R. Daniels, and P. M. Bendix, "Curvature and phase induced protein sorting quantified in transfected cell-derived giant vesicles," *ACS Nano*, pp. 1–23, Feb. 2019.

P-323

Emerging factors for improved cell membrane permeabilization with membrane active peptides

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In the last two decades, numerous studies on membrane active peptides have been reported. These molecules interact with the cell membrane either by disrupting the lipid bilayer integrity through different lytic mechanisms like antimicrobial peptides (AMPs), or by direct translocation without compromising its integrity, like cell-penetrating peptides (CPPs). The interest for membrane active peptides increased exponentially in the last years since they offer an alternative to currently used pharmaceuticals.

Here, we focus on AMPs, CPPs and cell penetrating homing peptides (CPHPs) in order to understand structural factors controlling the interaction between peptides and lipid membranes and their penetration activity. CPPs are peptides that have the property to penetrate the cellular membrane and function as cargo-carriers for different molecules: fluorophores, drugs, proteins, radiotherapeutic agents, DNA, magnetic nanoparticles and liposomes. Antimicrobial peptides represent an alternative to the classical antibiotics and kill bacteria, virus, and fungi. CPHPs are cell penetrating peptides that bind specifically to either normal tissues or receptors overexpressed in cancer cells.

In this study, we show using fluorescence confocal microscopy that internalization of membrane active peptides in giant unilamellar vesicles (GUVs) occurs as a sum of different factors: length and secondary structure of the peptide, charge distribution of aminoacids, lipid composition, membrane curvature, and anionic charge of lipids.

P-324

Understanding the role of motifs in a series of anticancer peptides acting on tumor cells

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The study of the mechanisms of action of anticancer peptides (ACPs) interacting with membranes is a challenging field of research. Thanks to their ability to induce apoptosis in tumor cells, ACPs are considered as promising drugs to treat cancer. A significant number of ACPs are positively charged and are thought to be drawn to their target by the net charge, considering the fact that external cancer cell membranes tends to contain negatively charged molecules such as phosphatidylserine (PS), and glycoproteins rich in sialic acid, or heparan sulfate. In spite of a remarkable similarity in amino-acid composition, many ACPs display significant differences in terms of activity. This strongly suggests that particular relative disposition of amino acids (motifs) has a role in the recognition process. In order to better understand this effect, we decided to study an interesting anticancer peptide with sequence FAKLLAKLAKKLL-NH2. This peptide is reported to be active on skin, lung, cervical, breast, colon and prostate cancer, according to cancerPPD database. Sequence alignment of similar peptides obtained by our in-house developed web-server ADAPTABLE (http://gec.u-picardie.fr/adaptable), highlighted conserved motifs which might be at the origin of the activity.

In this study, we changed the order of some important residues in the sequence by solid-phase synthesis and measured activities towards breast cancer cells (MDA-MB 231). The results confirmed our hypothesis, showing that very light modifications in the order of amino-acids result in significant changes in the anticancer activity. This prompted us to investigate the interaction of the peptides with membrane models of cancer cells. The study of such systems is challenging, due to the difficulty to characterize the features of peptide-lipid assemblies in membranes, an essential interaction for the action of membrane-targeting ACPs. Well-established models based on DHPC/DPMC/DMPS isotropic bicelles, allowed the study of the interaction by liquid-state NMR.

P-325

Bacterial inactivation enables the study of endotoxin neutralization within the bacterial cell envelope by synthetic anti-lipopolysaccharide peptides.

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The rise of bacterial resistance against therapeutic drugs is leading to a concomitant reduction of treatment options [1]. Therefore, the development of bioactive molecules with a novel mechanism of action has a critical priority. This need is particularly urgent in the case of drugs targeting Gram-negative bacteria [1]. In this context, antimicrobial peptides (AMP) are among the most promising candidates. To evaluate their mechanism of action, there is a growing need for methods that allow studying molecular mechanisms in microbial systems, particularly bacterial membrane systems in all their complexity. We use ionizing radiation at low doses and sodium azide to generate bacterial ghosts (non-metabolically active bacteria), in which the metabolic activity of the bacteria and cell division processes cease. These procedures allow the direct analysis of the binding process of AMP on the bacterial outer membrane by isothermal titration calorimetry (ITC) to get valuable thermodynamic information. Our approach gives new insights to disclose the role of the bacterial membrane potential ($\Delta\Psi$) as a possible modulating parameter of the antimicrobial peptide binding and activity.

We applied this method to evaluate the anti-inflammatory activity of anti-lipopolysaccharides peptides (SALP). The suppression of inflammation signals in human in vitro systems (isolated human mononuclear cells and whole blood assays) is connected to an extremely high covering of the bacterial cell envelope (peptide/bacteria surface area ~ 7). This peptide accumulation on the outer membrane causes a strong reorganization of the lipid packing of the inner membrane with the loss of the phase transition [2]. We show a direct proof of the biological relevance of peptide binding to LPS not only in free form but as a constituent of the bacterial outer membrane to avoid the hyperproduction of pro-inflammatory cytokines.

This way, we provide an approach to bridge the gap between molecular and cellular investigations. Other biophysical studies can benefit from this strategy by minimizing biosafety problems while reducing damage and modification to bacterial envelopes.

References.

1 Tacconelli, E. *et al. The Lancet Infectious Diseases* **18**, 318-327, doi:10.1016/s1473-3099(17)30753-3 (2018).

2 Correa, W. *et al. The FEBS journal*, doi:10.1111/febs.14805 (2019).

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Antibacterial activity of photosensitizers with different charge and structure

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The number of bacteria resistant to antibiotics is growing sharply that makes it necessary to create new alternative antibacterial agents. Possible approach to the destruction of bacteria lies in the action of so-called photosensitizers (porphyrins and phthalocyanines), which adsorb on the bacterial cell walls and membranes and produce singlet oxygen upon illumination oxidizing cell proteins and lipids and leading to the cell death. Due to the high reactivity of singlet oxygen, its action is limited to the immediate environment. Thus, such activity is selective and depends on the interaction of the photosensitizer with the target. We investigated various types of photosensitizers based on porphyrins and phthalocyanines with different charge and structure in order to clarify their antimicrobial properties, using the model of *Escherichia coli*. We have demonstrated that these compounds have antibacterial activity and we have selected among them the best candidates for the creation of antimicrobial agents. Further research in this area can expand the possibilities of using photosensitization to create antibiotics or antimicrobial agents to which bacteria cannot develop resistance.

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Antimicrobial activity of peptide-conjugated silver nanoparticles

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The challenge of bacterial resistance and the problems caused by the limitations of common drugs has led to the search new therapeutic strategies. Antimicrobial peptides (AMPs) are pointed out as a future alternative to conventional antibiotics, due their special properties. Silver nanoparticles (AgNPs) have shown antimicrobial properties and, additionally, have been used in the design of new antibiotics by using them as carriers for some biomolecules. In the present work, we conjugated the antimicrobial peptide PaMAP 1.9 with silver nanoparticles. Three conjugated nanoparticle shapes were evaluated in terms of stability and antimicrobial activity. Preliminary results showed that the peptide-AgNP conjugate has a higher antimicrobial activity against *Escherichia coli* when compared to the nanoparticles alone. Regardless the antimicrobial effect, the binding of the peptide to the nanoparticle needs to be optimized to confer more stability to the system. Through biophysical techniques, our attractive preliminary results suggest a promising antimicrobial agent that could be an alternative to treat microbial infections.

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Antimicrobal peptides forms lipid pores aligning on the membrane surface via its elastic deformations

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The use of antibiotics leads to the emergence of resistant strains of pathogenic bacteria. Antimicrobial peptides are a promising class of antimicrobial agents that are active against antibiotic-resistant strains. These peptides, binding to the bacterial membrane, form through pores in it. Currently, mechanisms of their adsorption on the membrane surface of bacteria and subsequent formation of conductive defects in it are not fully understood.

Using the method of compensation of the intramembrane field, we investigated the processes of pore formation by amphipathic alpha-helical peptides in bilayer lipid membranes. The obtained adsorption isotherm allowed approximation by the Hill model. The results agreed with the theoretical model, according to which the deformation of the lipid bilayer leads to parallel alignment of the peptides relative to each other at the distance of 6 nm. Amphipathic alpha-helical peptides have two possible configurations in which they are able to exist for a long time: the I-configuration, perpendicular to the membrane plane, in which peptides can form a through-pore, and S-configuration, parallel to the membrane plane. According to our results, reaching the threshold concentration and the transition of peptides from the S-configuration to the I-configuration lead to the formation of stable pores lined with peptide molecules.

Thus, this study refines the mechanisms of pore formation with antimicrobial peptides — with an increase in the concentration of adsorbed peptides and membrane deformation, a transition occurs to a parallel configuration of molecules, between which lipid pores lined by peptide molecules can form.

The work was supported by the Russian Foundation for Basic Research (grant #17-04-02070).

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Plasma membrane damage and calcium fluxes activate ESCRT-III mediated repair mechanism upon ferroptosis

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Ferroptosis is a non-apoptotic, caspase-independent form of regulated cell death characterized by the generation of lethal amounts of specific lipid peroxides through iron-dependent enzymatic reactions. It has attracted considerable attention due to its implication in several pathophysiological processes such as: cancer, neurodegeneration, acute renal failure, hepatic and heart ischemia-reperfusion injury, and antiviral immunity. A key step in ferroptosis execution is the final disruption of the plasma membrane integrity, which mediates the release of intracellular factors. However, the molecular mechanism causing the loss of plasma membrane impermeability during ferroptosis remains completely unsettled. Here we characterized the mechanism of plasma membrane permeabilization that happens during ferroptosis triggered by erastin-1 and RSL3. Using live-cell imaging and flow cytometry, we tracked in parallel different hallmarks of ferroptosis. We show that the progression of this type of cell death involves the increase of cytosolic calcium, a process that takes place prior to cell rounding and detachment, and final plasma membrane breakdown. We identified the formation of pores in the range of 1 nm in diameter as a core mechanism that triggers plasma membrane permeabilization and the increase in cytosolic calcium in ferroptosis, and correlated these processes with the activation of ESCRT-III-mediated membrane repair mechanism. Our findings reinforce the notion that the activation of calcium fluxes across the plasma membrane is a common feature of different lytic forms of regulated cell death and position membrane repair mechanisms as a central machinery to protect cells from death upon the activation of these killing pathways.

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Interaction Between Tethered Bilayer Membranes and s100A9 aggregates: Implification of neurodegeneration

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A central event in pathogenesis of Alzhaimer's diseases are thought to be intracellular and extracellular accumulation, aggregation and misfolding of low molecular mass peptides such as β-amyloid ($A\beta_{1-42}$), tau protein (Tau) and s100A9 [1,2, 6]. Small size aggregates-oligomers were found to be extremely neurotoxic *in vitro* and *in vivo* with the ability to disrupt the major neuron membranes [3,4] and lead to synaptic dysfunction, mithochondrial dysfunction, neuronal apoptosis and brain damage [5].

In this work different sizes of soluble recombinant s100A9 aggregates were used to investigate their interaction with tethered phospholipid membranes (tBLM). The morphology and size of misfolded protein aggregates were monitored by dynamic light scattering (DLS) and atomic force microscopy (AFM). These protein aggregates exhibited the different membrane damaging properties as probed by the electrochemical impedance spectroscopy (EIS). The function and morphology of s100A9 aggregates were depending on different oligomerisation conditions: temperature and time. The interaction between s100A9 and tBLM was monitored by EIS time series measurements. The observed lag phase of this interaction were significantly decreased at s100A9 aggregates concentration level. Membrane composition was found to be one of the important factors affecting the interaction of the s100A9 oligomers to phospholipid membranes.

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Exploring the effect of medium composition on the interaction mechanisms between engineered nanoparticles and artificial model membranes

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ABSTACT

With the development of nanotechnology, an enormous interest in the industrial and biomedical applications of nanoparticles (NPs) has arisen. The nanometre scale confers novel physicochemical properties and the ability to enter individual cells and gain access to their inner biochemistry. Nonetheless, to do so NPs need to penetrate cell membranes without compromise the membrane integrity and the cell homeostasis. The wide variety of NP designs, their sensitivity to environmental conditions and the complexity of biomembranes lead to a high variability and complexity of NP-biomembrane interaction mechanisms. The present work is a biophysical approach to investigate the effect of the medium composition on the interaction of inorganic nanoparticles with model biomembranes by understanding the physical perturbations they cause to the structural, mechanical and dynamical properties of lipid membranes. This investigation will eventually provide valuable information for the development of nanotoxicity screening platforms and the interpretations of their outputs as well as for generating safer nanomaterials for biomedical applications.

We investigated the effect of silver NPs (AgNPs) and silica NPs (SiO_2NPs) on the membrane permeability of Large Unilamellar Vesicles (LUVs) using advanced fluorescence spectroscopy techniques. These experiments were complemented with studies on Giant Unilamellar Vesicles (GUVs) using confocal microscopy for direct observation of structural/morphological changes of the membrane, monitoring transmembrane dye transport, detecting changes in membrane fluidity by fluorescence recovery after photobleaching (FRAP) and quantifying the rigidity and tension of the membrane using flicker spectroscopy.

Our data show that AgNPs have a greater impact in the physicochemical properties of the membrane at physiological conditions (high ionic strength) than when suspended in glucose solution (low ionic strength). We also observed that a protein corona around SiO_2NPs modulate the effect that these NPs produce to GUVs. Therefore, we conclude that the NP-biomembrane interactions do not only depend on the composition and physicochemical properties of the NPs and the membrane but are also highly influenced by the environmental conditions, such as ionic strength and presence of biomolecules in the medium.

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***Caiman crocodilus* Blood-derived Peptides as Potential Anticancer and Antimicrobial Therapeutic Agents**

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Antimicrobial peptides (AMPs) are small cationic molecules present in the innate immune system of all organisms which exhibit activity against several pathogens due to their negatively charged membrane. Some of these molecules have also demonstrated an anticancer activity and are called anticancer peptides (ACPs). The mechanism of action of AMPs on pathogenic cells through membrane destabilization reveals their potential use as novel therapeutics against antibiotic-resistant bacteria and other pathogens, as well against cancer. Alligators are species known to live in unsanitary environments and to suffer several injuries from territorial fights without getting infections and also for having high healing rates. These characteristics lead us to believe that they have some kind of protective molecules circulating in their blood. Preliminary data demonstrated that the serum of Caiman crocodilus blood, an alligator species from Delta do Parnaíba, Brazil, has an antimicrobial effect on bacterial growth, analyzed through minimum inhibitory concentration and minimum bactericidal concentration assays, and an anticancer effect when tested against breast cancer cells in viability and cytotoxicity assays. Both of these effects were also determined by Atomic Force Microscopy (AFM) imaging. Therefore, in this project we aim to isolate, characterize, and test the effect of alligator blood-derived peptides as antimicrobial and anticancer molecules, evaluating their biophysical proprieties, their interaction with pathogenic biomembranes through biophysical techniques, their effects on cell and bacteria viability, their mechanism of action and eventually, the most relevant models will be tested against tumours in in vivo assays.

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Spectrally-resolved lifetime imaging of erythrocyte plasma membrane fluidity for a quantitative biological evaluation of Type 2 Diabetes-associated cardiovascular risk

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Over the past three decades, the global incidence of type 2 diabetes mellitus (T2DM) has risen significantly, making it one of the most important public health challenges. Although long-term weighted mean glycosylated hemoglobin (HbA1c) is closely associated with the development of severe cardiovascular complications in T2DM, it is not representative of all risk factors contributing to the cardiovascular risk score in T2DM. Beyond the average amount of plasma glucose, also plasma lipids levels and oxidative stress could indeed affect cellular membrane composition, its packing and lipid distribution, leading to physical and chemical alterations of membranes in T2DM patients. The membrane fluidity of erythrocytes, reflecting this complex network of metabolic and homeostatic processes activated in the disease, can constitute a more valuable tool to monitor the progression of diabetes and the onset of cardiovascular complications. To this aim we present a spectrally-resolved lifetime imaging of erythrocyte plasma membrane fluidity in order to contextually evaluate quantitative variations in membrane composition and lipid distribution. Using a global phasor representation to analyse spectrally-resolved changes in Laurdan's fluorescence lifetime we obtain different phasor signature in a metabolic plane allowing to define an indicator of cardiovascular risk in type 2 diabetes subjects. This technique, providing a powerful bio-imaging system to measure erythrocyte plasma membrane heterogeneity with high spatial resolution, can be applied to complement HbA1c in defining the quality of long-term management of diabetes.

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Studies of the non-porogenic activity of the antimicrobial peptide *Ec*AMP1R2 against *E. coli*

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The current drought in the pipeline of antibiotic drugs and the spread of antimicrobial resistance are a major threat to public health. Antimicrobial peptides (AMPs) are membrane-active peptides that have drawn the attention of the scientific community due to their potential as alternative antibiotics. We have studied the membrane activity of *Ec*AMP1R2, a designed AMP highly specific towards *Escherichia coli*. Concentrations above the minimum inhibitory concentration of *Ec*AMP1R2 cause hyperpolarization in *E. coli*, ruling out the paradigmatic 'pore formation mechanism of action' for this peptide. Studies with large unilamellar vesicles that mimic the lipid composition of the membranes of *E. coli* show that *Ec*AMP1R2 promotes fusion of vesicles containing cardiolipin (CL), a phospholipid that modulates the protein complexes of energy transducing membranes. We hypothesize that the observed hyperpolarization might be caused by some sort of malfunctioning of proteins involved in the dissipation of the electrochemical potential. Coarse-grained molecular dynamic simulations suggest that *Ec*AMP1R2 hampers the availability of CL molecules to interact with the ATP synthase, an interaction extensively reported to be crucial for its activity.

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Simultaneous blocking of pulmonary surfactant permeability and breathing-like interfacial dynamics by anti SP-B antibodies

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Pulmonary surfactant is an essential lipid-protein complex which coats lung alveoli, reducing surface tension at the air-liquid interface to avoid alveolar collapse during expiration. Among specific surfactant proteins, hydrophobic SP-B and SP-C are involved, together with the lipids, in the surface active role of the system. In particular, SP-B is crucial for respiration and life, facilitating the formation and proper performance of the surfactant interfacial film.

We had previously described the existence of supramolecular complexes of SP-B in native surfactant consisting in ring-shaped oligomers, which could be mediating membrane contacts among surfactant structures, and allowing a fast flow of lipids towards the alveolar interface. In this work, by blocking assays with antibodies against surfactant proteins, we have confirmed that SP-B is required for the generation of pores in surfactant membranes, defining permeability towards polar solutes smaller than a well-defined threshold. We have demonstrated that permeability of surfactant giant vesicles to polar fluorescent dextrans of different molecular sizes depends on the protein and lipid composition of the membranes, indicating that SP-C and anionic lipids are also important to modulate the proper architecture of SP-B-based pores. Blocking effects of membrane permeability by SP-B antibodies correlate with a significant impairment of the formation and dynamics of interfacial surfactant films, as studied in a captive bubble surfactometer, indicating that SP-B oligomers are responsible for both permeability properties and lipid transfer between surfactant membranes. The data have been integrated in a model in which SP-B would be a key player of surfactant function.

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Exploring electrical signalling alterations of *Nitellopsis obtusa* cells in response to modulators of ion channels

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The *Nitellopsis obtusa* cell provides a model system for biophysical investigation of instantaneous effects of various biologically active compounds on the generation of plant bioelectrical signals *in vivo* at individual ion transport systems and single cell levels. It was documented that calcium transport systems modulator tetrandrine, exhibits positive therapeutic effects in treatment of several tumor types. Dimethyl sulfoxide (DMSO) used as a solvent of tetrandrine was shown to have some effects on plasma membrane. Our model system allows the examination of the effects of these substances on generation of Action potentials (AP) separately. High discretization frequency allows precise, high time resolution analysis of real-time processes such as changes in excitation threshold, AP amplitude and velocity of repolarization values indicating the effect of investigated substances on ion channels involved in AP generation. We found that tetrandrine, but not DMSO depolarises membrane potential. Both substances affect AP amplitude, but in different ways. Tetrandrine depolarises excitation threshold which is related to inhibition of Ca^{2+} channels, while DMSO decreases peak of AP, and this can be attributed to the effect on Cl^- channels. Application of voltage clamp confirmed those propositions. The cytoplasmic droplets made from internodal cells served for the investigation of the effects on single tonoplast ion channels by patch clamping in cell attached mode. It is known that tetrandrine blocks Ca^{2+}- dependent K^+ channels in animal tissues. However, large-conductance K^+ channels in the tonoplast of *N. obtusa* were not affected neither by tetrandrine, nor by DMSO. We suggest that *Nitellopsis obtusa* cells can be used as simple and low cost plant model system to examine the effects of different ion channel inhibitors.

The research was funded by DAINA – Polish-Lithuanian joint project S-LL-18-1 „Long-distance electrical signaling systems in plants – adaptaptation to the change from water to terrestrial environment".

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PaMAP's family: antimicrobial peptides efficient against multi-resistant show evidence of anticancer activity

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Multi-resistant pathogens have increased the number of severe infections on patients that current available drugs cannot attend, especially on those that their immune system is compromised. The World Health Organization has already pointed out the urgency in finding new molecules against different multi-resistant bacteria. To make the scenario worse, the number of drug resistance cases in cancer patients has also increased in recent years, associated with the occurrence of hospital infections that debilitate patients' health. Antimicrobial peptides (AMPs) are pointed out as a potential alternative to conventional drugs in these contexts. AMPs are usually small, hydrophobic and with a global positive charge, which promotes the interaction with the pathogens or cancer cells membranes (with negative surface charges). Despite of their mode of action not being yet fully understood, their mechanism is independent of intermediators, transporters or protein channels, being active mostly by peptide-membrane interaction, against bacteria, viruses or cancer cells (anticancer peptides, ACPs). In our work, we focused in two AMPs (PaMAP 1.9 and 2), synthetically designed using as precursor a natural AMP as template. After initial promising results against bacteria, complemented by biocomputational studies, peptide-membrane interactions were extensively studied, using lipid vesicles and bacterial cells (including clinical multi-resistant strains). Different biophysical techniques, including fluorescence spectroscopy and microscopy, flow cytometry, dynamic light scattering, zeta-potential, circular dichroism and atomic force microscopy allowed to infer about the mechanism and efficiency in promoting bacteria cell death. Recently, we hypothesized if any of these AMPs could be good candidates for cancer therapies. Using the same approach, we demonstrated that PaMAP 1.9 has relevant anticancer activity, but not PaMAP 2.

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Membrane fission by Drosophila reticulon is coupled to membrane motility

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The endoplasmic reticulum (ER) is a continuous membrane organelle composed by structural elements of different curvatures and topologies. Formation and maintenance of dynamic tubular ER network spanning the whole cytoplasm relies on membrane fusion. However, it remains unclear whether membrane fission, the process expected to balance the fusion in the context of the ER network maintenance, occurs in the ER. Fission is generally associated with proteins producing high membrane curvature. Reticulons, the protein family supporting the curvature of the ER tubules, have long been implicated in ER fragmentation, though by an unknown mechanism. Here we demonstrate that fission indeed operates in tubular ER and is mediated by reticulons. Using *Drosophila* reticulon (Rtnl1) as a model, we found that it produced ER fragmentation in the flies, acting antagonistically to atlastin, the major fusogen in *Drosophila* ER. Ectopic overexpression of Rtnl1 in Cos7 cells identified the dynamic membrane constriction and fission as the cause of the ER fragmentation. Reconstitution of purified Rtnl1 into biomimetic lipid nanotubes further revealed that fission is produced by a combination of static and dynamic membrane constriction, the latter associated with the increase of effective membrane viscosity by Rtnl1. Hence, while Rtnl1 acts as stabilizers of membrane curvature of static ER tubules, it can also produce scission of the tubules upon their movement, thus linking the balance of membrane fusion and fission in the tubular ER network to membrane motility.

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Chitosan changes the barrier properties of the outer membrane of Gram-negative bacteria and increases its permeability; A computational study.

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The role of the outer membrane of Gram-negative bacteria is to act as a protective permeability barrier. The outer membrane is almost impermeable to large molecules and hydrophobic compounds from the environment. Unfortunately, the complicated double layer structure of the outer membrane of this type of bacteria makes its outer layer low permeable for antibiotics as well, so it's challenging to destroy this kind of bacteria cells. Generally in Gram-negative bacteria, Lipopolysaccharide (LPS) is essential to the function of the outer membrane protection.

About 20 years ago, experimentalist claimed that Chitosan, the deacylated derivative of chitin, damages different kinds of Gram-negative bacteria membrane and kills the cell.

We performed various molecular dynamics (MD) simulations to study the effect of the presence of Chitosan on the molecular insight of the Gram-negative bacterial outer membrane especially interactions of LPS, lipids, and ions with each other inside of the bacteria.

In our computational model, we benefit the full structure of the LPS molecule that makes us able to investigate the role of the LPS molecule in the permeability of bacteria and molecular interactions inside it. Also, this model is flexible, and we are able to study fifteen different types of gram-negative bacteria including Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica, and Chlamydia trachomatis.

By this computational model, we numerically proved the experimentalists hypothesis that Chitosan damages the membrane of gram-negative bacteria as we see a significant increase of bacteria permeability in the presence of Chitosan. As well, we realized in the presence of Chitosan interactions of solutes inside of bacteria with LPS molecules highly
increased.

As every specific type of Gram-negative can take many different types of lipid A (it's part of LPS), we studied bacteria of the same kind with different lipid A. We found out in every type of bacteria, the value of outer membrane permeability strongly depends on its type of lipid A. In other words, the permeability of every kind of Gram-negative bacteria is a function of its lipid A type.

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Comprehensive biophysical assays: From single channel electrophysiology to overall cell behavior

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Integral membrane proteins, predominantly ion channels and transporters, have been the focus of basic biophysical research, as well as drug discovery and safety projects for decades. Electrophysiological experiments on fully functional artificial lipid bilayers enable the investigation of basically any membrane-affecting agent. In combination with automated patch clamp and impedance/electrical field potential (EFP)-like recordings of relevant targets expressed in heterologous systems, as well as of human iPSC-derived cardiomyocytes and neurons, we demonstrate broad biophysical application assays, connecting single channel electrophysiology with overall single cell and cell population behavior.

Here, we present the temperature dependent activation or deactivation of different Transient Receptor Potential (TRP) channels by means of planar patch clamping on our medium and high throughput screening (HTS) platforms Patchliner and SyncroPatch 384PE, as well as with highest resolution on a single channel level on our recently introduced Orbit mini setup. Additionally, the effect of drugs on action potentials as recorded in iPSC-cardiomyocytes is important for assessing the interaction of the cardiac ion channel ensemble. We present our advances in development of iPSC-cardiomyocytes "ready-to-use" assays for automated patch clamp. We also show, short and long-term impedance/EFP-like recordings of diverse cell-types, such as drug safety experiments on iPS cardiomyocytes and cancer tox-assays. In summary, medium and high throughput screening (HTS) assays such as automated electrophysiological patch clamp and impedance-based assays allow for the determination of drug effects on a whole cell level whereas artificial bilayers provide a robust environment for the assessment of single ion channel molecules.

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Effects of respiratory sensitizers on the biophysical performance of pulmonary surfactant

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Effects of respiratory sensitizers on the biophysical performance of pulmonary surfactant

Compounds such as 4,4'-methylenediphenyl diisocyanate (MDI) and trimellitic anhydride (TMA) are extensively used in the industry, mainly for the synthesis of rigid polyurethane foams and epoxy and alkyd resins, respectively. MDI is not soluble in water and is highly toxic by ingestion, inhalation, and skin absorption. Conversely, TMA is very reactive, readily hydrolysed in water to trimellitic acid, which is moderately soluble in water. Although their contribution to lung damage and asthma has been studied, their effects on pulmonary surfactant (PS) activity have not been investigated yet. Films formed by PS at the respiratory air-liquid interface are crucial in order to stabilize the lungs and minimize the work required to maintain the lungs open along continuous breathing dynamics. In this study we used PS purified from pig lungs to assess how the partition of MDI and TMA into surfactant membranes may affect PS functional, structural and thermotropic properties by captive bubble surfactometry (CBS), Langmuir Blodgett isotherms and differential scanning calorimetry (DSC), respectively. In order to assess the dependence of the time of exposure on the effect of the reactive chemicals, these experiments were carried out at two different incubation times: 1 and 4 hours. Comparing the two compounds, the more hydrophobic one, MDI, exhibited the strongest impact on PS, including impairment of surfactant biophysical activity even after 1 hour of exposure. MDI affected both the interfacial adsorption of surfactant and its behaviour during breathing-like expansion-compression cycling. Conversely, TMA does not affect the *in vitro* biophysical activity of PS.

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Extracellular Vesicles interaction with model membranes

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Extracellular vesicles (EVs) are a potent intercellular communication system. Within a lipidic bilayer, such small vesicles (diameter ranging from 30 to few hundreds nanometers) transport biomolecules between cells and throughout the body, strongly influencing the fate of recipient cells. They have been proposed as biomarkers for several diseases and as optimal candidates for therapeutic applications, due to their small size and specific biological functions. Nonetheless, since their isolation, quantification and biophysical and biochemical characterization are challenging tasks, the understanding of the complex network of EVs/cell interaction is still incomplete. Here we propose a combination of Atomic Force Microscopy and Small Angle X-Ray and Neutron Scattering (SAXS and SANS)) for the careful characterization of isolated vesicles and for the analysis of their interaction with model membranes, in form of liposomes and supported lipid bilayers. Our analysis reveals a strong interaction of EVs with model membranes mimicking lipid rafts, pointing out the importance of rafts-like structure in the uptake processes.

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Mechanism of synergistic action of membrane-active peptides SP-A and SP-B^N on multidrug-resistant *Klebsiella pneumoniae*

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As the key components of innate immunity, human host defense antimicrobial peptides and proteins play a critical role in warding off invading microbial pathogens. We have previously shown that pulmonary surfactant protein (SP-A) exerts a synergistic antimicrobial activity with the lung antimicrobial peptide SP-B^N against the respiratory pathogen *Klebsiella pneumoniae* K2 *in vivo.* However, the factors that govern SP-A/SP-B^N antimicrobial activity are still unclear. The aim of this work was to characterize the mechanism by which SP-A and SP-B^N act synergistically against capsulated *K. pneumoniae*, which is otherwise resistant to either protein alone. Our results indicate that the SP-A/SP-B^N complex, but not the individual proteins, alters the bacterial ultrastructure, forming pores in the membrane that favor the translocation of both proteins to the periplasmic space, where they interact with the inner membrane causing its permeabilization and depolarization. *In vitro* studies with model membranes, which mimicked the internal and external bacterial membranes, showed that both SP-A and SP-B^N bound to lipopolysaccharide molecules present in the outer membrane, inducing lipid phase separation and disrupting membrane packing. This effect was stronger for the SP-A/SP-B^N complex, which rendered both the outer and inner bacterial membranes leaky as determined by permeabilization studies. On the other hand, SP-A/SP-B^N induced a positive curvature in the inner bacterial membrane observed by differential scanning calorimetry that may lead to the formation of toroidal pores. Taken together, our results indicate that the antimicrobial activity of SP-A/SP-B^N complex is related to its capability to alter the integrity of outer and inner bacterial membranes. This membrane activity could facilitates and potentiate the action of antibiotics against capsulated *K. pneumoniae* K2.

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The effect of adding an N-terminal tryptophan on the antibacterial action of the cecropin-melittin inspired antimicrobial peptide BP100

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Bacterial infections remain a major public health concern due to an increase of bacterial resistance to antibiotics and dissemination of multidrug-resistant strains. In an era of stagnation in the field of antibiotic development, antimicrobial peptides (AMPs) present attractive properties to overcome antibiotic resistance. Most AMPs are short cationic and amphipathic molecules, having a partial or total membrane-dependent mechanism of action, which makes them less prone to elicit bacterial resistance. Among the plethora of AMPs available, BP100 (KKLFKKILKYL-NH_2) is a cecropin A-melittin hybrid derivative that displays high antibacterial activity against both susceptible and clinically relevant Gram-negative bacteria, while having low cytotoxicity and susceptibility to proteolytic degradation. Herein, we aim at studying the antibacterial activity and membrane interactions of a new derivative of BP100 – W-BP100 – which includes an additional tryptophan residue at the peptide's N-terminus.

Peptides were synthesized by solid-phase peptide synthesis using the standard Fmoc/tBu orthogonal protection scheme and characterized by HPLC and mass spectrometry. The antibacterial activity was assessed by determining the minimum inhibitory concentration (MIC) using the broth microdilution method against susceptible reference bacterial strains. Absorbance and fluorescence properties of W-BP100 were assessed in aqueous media. Membrane partition constants and quantum yield measurements were determined in the presence of anionic large unilamellar vesicles (LUVs), using steady-state fluorescence.

As compared to BP100, W-BP100 showed a substantial high antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212), and a similar antibacterial activity against Gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853).

Peptide titration with anionic LUVs revealed distinct fluorescence spectra for both peptides. Thus, while the fluorescence profile of BP100 was in accordance with literature, W-BP100 presented both an enhancement and hypsochromic shift of fluorescence, with fluorescence quenching occurring at higher lipid concentrations. Atypical partition curves were obtained for W-BP100, suggesting a different mechanism of action for this peptide. Partition constants are being determined by a partition fitting, alternative to the simple partition model, as described elsewhere [1]. Membrane localization and vesicle leakage studies are currently being assessed to further elucidate the mechanism of action of W-BP100.

1. Melo, MN, Castanho, MARB. BBA, 2007. **1768**(5): p. 1277 1290.

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Cryo-EM imaging of neuronal membrane interface

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Cell adhesion and membrane interactions at biosensor interface are very important for neuronal microelectrode recordings. In this study, we investigate morphological structure of the interface between intact rat neuron cells and surface of biosensor electrode by applying cryo-EM (electron microscopy) preparation technique. Investigations of neuronal interface using conventional FIB/SEM sectioning techniques sometimes deliver inconsistent results because of disruptive structural changes during the preparation processing. In comparison to chemical fixation, which may take 1÷10 seconds, cryo-preparation is extremely fast, which provides freezing/fixation within milliseconds any molecular or morphological changes at the cellular interface. Fast fixation technique is especially important for preservation of highly dynamic plasma membrane, which displacement flow rate between soma and axon growth cone is up to 50μm^2/min /J.Dai et al., Cell, v.83, p.693, 1995/. We developed a technique, which allows rapid freeze-fixation of 10÷ 20μm neuron cells grown at surface of our solid biosensor substrate. We have investigated the neuronal membrane interface at flat and micro-structurally modified electrode by cryo-FIB sectioning. Direct scanning microscope micrographs of FIB cryo-sections showed tight physical contact between cell body and electrode surface. In order to provide the details of membrane interface we applied a gentle sublimation of cell cryo-sections at high vacuum at -100°C. The flat electrode areas reveal good adhesion contact at some places and partly decoupled membrane with interface cavities of size about 40 nm. At structurally modified surface cells adhere very well to microstructures engulfing the caps of gold mushroom shape structures. At contact interface, the plasma membrane follows the shape of microstructure with membrane cleft width evaluated, within space resolution, to be less than 5nm. Bright traces around micro pillars in the intracellular space probably relate to cytoskeletal elements responsible for the membrane deformation. Some cell cryo-sections after sublimation surprisingly

reveal the shapes of subcellular organelles (nucleus) without heavy metal contrasting.

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The dependence of small molecule electrotransfer into the cell independence of medium conductivity and presence of extracellular plasmid DNA

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Electroporation is a widely established method that uses electric fields to enhance molecular transport across plasma membrane. Nowadays, it is used for drug and gene delivery into the cells and tissues for therapeutic purposes and for the extraction of bioactive compounds from tissues during food processing. In our study, we examined the dependence between the conductivity of electroporation medium and the efficiency of small molecule electrotransfer. We found the inverse dependence and propose that this can be caused by the differential electrodeformation of the cells in the external media that have higher or lower conductivity than the cell cytoplasm: in the external conductivity lower than that of the cytoplasm, cells would elongate to the form of a prolate spheroid, and in the external conductivity higher than that of cytoplasm, the cells would compress to the form of an oblate spheroid. Further we investigated the efficiency of small molecule electrotransfer in dependence of extracellular plasmid DNA added before delivery of electric pulses. We found that the presence of plasmid DNA in electroporation medium increases the extraction of fluorescent dye calcein from calcein-AM loaded cells, as well as the electrotransfer of small-molecules, such as anticancer drug bleomycin, into the cells. Possible mechanisms of this effect are discussed.

Tuesday 23rd July

MECHANISMS OF MEMBRANE PROTEINS

P-347 (O-108)

Structural insight into TRPV5 channel function and modulation

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TRPV5 (transient receptor potential vanilloid) is a uniquely calcium-selective TRP ion channel that plays a crucial role in the maintenance of calcium homeostasis. TRPV5 and its close homologue TRPV6 do not exhibit thermosensitivity or ligand-dependent activation, unlike other TRPV channels. They are constitutively opened at physiological membrane potentials. Both are tightly regulated by calcium and the calcium-sensing protein calmodulin (CaM). However, little was known on CaM binding and stoichiometry, or how it arranges TRPV5/6 channel inactivation. Full length and truncated TRPV5 proteins, expressed in human embryonic kidney (HEK293) cells, were affinity-purified and reconstituted into lipid nanodiscs (disc-shaped membrane mimics) or detergent micelles. After assessing the reconstitution efficiency with size-exclusion chromatography, SDS-PAGE and negative stain EM, pure TRPV5 protein fractions were pooled, concentrated and used for cryo-EM analysis. Data collection took place on a Titan Krios electron microscope, operated at 300kV. Data analysis was carried out in CryoSPARC and RELION software. We report high resolution cryo-EM structures of full length and truncated TRPV5 in lipid nanodiscs, a TRPV5 W583A mutant structure, and a complex structure of TRPV5 with CaM. Overall, TRPV5 closely resembles previously reported TRPV channel structures but we highlight some new features, which include an extended S1-S2 linker that forms tight interactions with the upper pore region, as well as an essential tryptophan residue (W583) at the bottom pore. While the W583A mutation does not affect the conformation at the upper pore, the lower pore region is clearly open for ion permeation. Our TRPV5-CaM complex structure demonstrates interaction of CaM with specific carboxy-terminal regions of TRPV5. Interestingly, residue K115 of CaM inserts deeply into the lower pore surrounded by W583, thereby blocking the pore. Furthermore, 3D classification suggests a flexible stoichiometry of 1:1 or 2:1 CaM binding to TRPV5. Our structures highlight interesting channel features divergent from the thermosensitive TRPV channels and extend our understanding from what is currently known for TRPV5 and its homolog TRPV6. Most notable, we provide insight into TRPV5 channel gating and propose a model for CaM-dependent channel regulation.

P-348 (O-109)

Biophysical insights into membrane fission mediated by ESCRT-III

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The endosomal sorting complex required for transport (ESCRT) engages in processes of membrane remodelling and fission, such as formation of multivesicular bodies, plasma membrane repair, neuron pruning, virus budding and autophagy as reviewed in [1]. The ESCRT machinery contains more than 15 proteins organized in four sub-complexes (ESCRT-0, ESCRT-I, ESCRT-II and ESCRT-III), among which the ESCRT-III (composed by Vps2, Vps20, Vps24 and Vps32) is highly conserved across the eukaryotic lineage and mediates the processes required for membrane deformation and fission [2]. Membrane models such as giant unilamellar vesicles (GUVs) [3] can be employed to unravel the ESCRT action in vitro, given the complexity and the number of proteins involved. It has been recently shown that solely Vps20, Vps32 and Vps24 from the phagocytic parasite *E. histolytica* are required to generate intraluminal vesicles (ILVs) in GUVs [4]. However, the current models do not provide a complete picture of the biophysical mechanisms by which the ESCRT-III components reshape the membrane. Moreover, the role of the membrane material properties in tuning the ESCRT-III activity is unrevealed. In this study, we observed for first time the consecutive action of the ESCRT-III proteins on a single-vesicle level, combining GUVs and microfluidics. We characterized several mechanisms involved in the membrane remodelling by the ESCRT-III complex and the regulation of the protein activity. Namely, (i) increase in the membrane tension results in distortion of the ESCRT-III scaffold in the intraluminal buds; (ii) the ESCRT-III proteins influence both the membrane stiffness and the spontaneous curvature, and thus control the size of the ILVs; (iii) a membrane fluid-fluid phase separation was induced in the presence of the ESCRT-III machinery, whereby the ILVs formed from the liquid-ordered phase.

References

[1] L. Christ et al., Trends Biochem. Sci., 2017, vol. 42, p. 42

[2] J Schöneber et al., Nature Rev., 2017, vol. 18, p. 5

[3] R. Dimova, Annu. Rev. Biophys., 2019, DOI: 10.1146/annurev-biophys-052118-115342.

[4] Y Avalos-Padilla et al., Front. Cell. Infect. Microbiol., 2018, vol. 8, p. 53

P-349 (O-110)

Ion transport, interfacial effects and scaling behavior in protein channels

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Many protein channels have in common the importance of electrostatic interactions between the permeating ions and the nanochannel. Since ion transport occurs under confinement conditions, interfacial effects such as access resistance (AR) may play a significant role. We measure AR in a large ion channel, the bacterial porin OmpF, by means of single channel conductance measurements in electrolyte solutions containing varying concentrations of high molecular weight PEG, sterically excluded from the pore. We found that AR might reach up to 80% of the total channel conductance in diluted solutions, where electrophysiological recordings register essentially the AR of the system and depend marginally on the pore characteristics. On the other hand, charged polar groups of the lipid may have a strong influence on the electric potential and the ionic concentration near the membrane-solution interface. Charged residues within the protein located near the pore mouth can also play a role, although to a lesser extent than AR and membrane surface charges. These three factors are obviously coupled and are strongly dependent on the channel aperture size, 3D structure and channel-lipid assembling. Comparison of experiments performed in charged and neutral planar membranes shows that lipid surface charges modify the ion distribution and determine the value of AR, indicating that lipid molecules are more than passive scaffolds even in the case of large transmembrane proteins. These findings are relevant to the fact that ionic conductance in membrane channels exhibits a power-law dependence on electrolyte concentration ($G \sim c^{\alpha}$). We critically evaluate the predictive power of scaling exponents by analyzing conductance measurements in four biological channels with contrasting architectures. We show that scaling behavior depends on several interconnected effects whose contributions change with concentration so that the use of oversimplified models missing critical factors could be misleading. In fact, the presence of interfacial effects could give rise to an apparent universal scaling that hides the channel distinctive features. We complement our study with 3D structure-based Poisson−Nernst−Planck calculations, giving results in line with experiments and validating scaling arguments.

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The role of individual cardiolipin species on stability and activity of Magnesium transporter A (MgtA)

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Membrane proteins are defined by their environment, the lipid bilayer, and are in many cases actually present as protein-lipid complexes. For several cases, the importance of specific phospholipids or sterols for optimal protein activity has been documented [1]. However, in many cases, specific lipid interactions with membrane proteins have not been studied or the role of the lipid in activity, stability or the folding process of the protein is not well understood. We aim to study membrane protein - lipid interaction using the bacterial magnesium transporter A (MgtA) as a model system. MgtA belongs to the P-type ATPases family, believed to transport Mg^{2+} from the periplasm into the cytoplasm [2]. The enzymatic function of MgtA is highly dependent on anionic phospholipids, especially cardiolipin, and co-localization of MgtA with cardiolipin in *E. coli* cells has been documented [3]. We have shown that MgtA selectively retrieves cardiolipin from a diverse lipid mixture and further, exhibits high selectivity on specific cardiolipin species for its activity. However, the molecular basis for cardiolipin activation and interaction with MgtA, remains unknown. We aim to further study the membrane-protein interaction with techniques, such as native mass spectrometry and enzymatic studies, to understand how MgtA and cardiolipin interact.

References

1. Opekarová, M.; Tanner, W. (2003) *Biochim Biophys Acta*, 1610(1):11-22

2. Maguire, M.E. (1992) *J Bioenerg Biomembr* 24, 319-28

3. Subramani, S.; Perdreau-Dahl H.; Morth J.P. (2016) *Elife*, 5

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Preparation and biophysical characterization of nAChR for high-resolution studies

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The nicotinic acetylcholine receptor (nAChR) has long been the holy grail of membrane protein research. These receptors are important as a biological target for the therapeutic development of a large number of diseases such as Alzheimer's Syndrome, Myasthenia Gravis Disease, Parkinson's Syndrome, Schizophrenia and nicotine addiction. Over the past 5 years we have developed a multi-attribute analytical platform that has allowed us to prepare milligram amounts of a functional, high-pure, and stable *Torpedo* (muscle-type) nAChR detergent complex (Tc-nAChR-LFC16) to achieve a high-resolution structure. We have developed a purification process, which yields crystallization-quality preparations of functional nAChRs for structural studies. Native nAChRs was extracted from the electric organ of *Torpedo californica* (Tc), using LFC-16 as a detergent, followed by multiples step of chromatography purification. We use MALDI TOF TOF and Nano-LC-MS/MS to identify various important peptides form Tc-nAChR, to demonstrate its purity. A total of 105 target peaks were detected, resulting in confident protein identification by Proteome Discoverer software version 2.1. Also, we characterized the glycan profile of the Tc-nAChR. Furthermore, we used circular dichroism spectroscopy, dynamic light scattering, zeta potential, and a two-electrode voltage-clamp assay to examine the stability and functionality of the Tc-nAChR-LFC16. Finally, we performed fluorescence recovery after photobleaching (FRAP) assays displayed a lower mobile fraction with cholesteryl hemisuccinate (CHS) incorporation compared to the implementation of cyclodextrin, suggesting that the removal of the CHS increase the mobile fraction significantly, in comparison with CHS. Our next goal is used theses protein preparation to obtain high-resolution X-ray data of the Tc-nAChR.

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Nicotine-induced conformational changes in the α4β2 nicotinic receptor

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Cigarette smoking is considered, nowadays, to be a major public health problem. Recent estimates indicate that approximately 1/4th of the world's population smokes and that smoking is the second most prevalent cause of death in the world. Currently, the FDA-approved smoking cessation drugs, such as varenicline, are only moderately effective in reducing the symptoms of nicotine withdrawal and may cause undesirable side effects. Consequently, there is a growing need to develop new smoking cessation agents with improved effectiveness and tolerability.

Nicotine is the major biologically psychoactive agent in tobacco, and it binds to the nicotinic acetylcholine receptors (nAChRs). These receptors are members of the pentameric ligand-gated ion channel family, and they mediate synaptic transmission in the nervous system. The α4β2 nAChR is one of the most widely expressed subtypes in brain, and it has attracted considerable attention due to its involvement in nicotine addiction. Over the last decades, this receptor has been widely explored, and our understanding of its molecular mechanisms has made extensive progress. However, despite a plethora of available structural and biochemical data, it is still not clear how ligand binding induces the conformational changes necessary to modulate the receptor's dynamics.

In this work, we use a combination of equilibrium and nonequilibrium molecular dynamics simulations to map the dynamic and structural changes induced by nicotine in the human α4β2 nAChR. These simulations reveal a striking pattern of communication between the extracellular binding pockets and the transmembrane domains and show the sequence of conformational changes associated with the initial steps of signal propagation.

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Molecular mechanism strong cell-cell adhesion mediated by Cadherin-23.

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Molecular mechanism strong cell-cell adhesion mediated by Cadherin-23.

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Cadherin-23(Cdh23) is a remarkable member of the cadherin family with 27 extracellular domains, a single-pass transmembrane domain and one cytoplasmic domain known to mediate heterophilic and homophilic interactions as well. Its role as a mechanoreceptor protein is comparatively well studied, it forms heteromeric interactions at the apical N-terminus with Protocadherin-15(Pcdh15) as shown by the crystal structure studies. The expression of Cdh23 was observed at the cell-boundaries of various human and mice tissues including kidney, muscle, testes, heart. Recent studies showed that this protein mediates homotypic cell adhesions in MCF-7 cells and heterotypic contacts between MCF and fibroblasts. The homodimers play an essential role in cell-cell adhesion, as the downregulation of Cdh23 in cancers loosen the intercellular junction resulting in faster-migration of cancer cells which is also explained by the significant drop in patient survival data. *In-vitro* studies have measured a stronger aggregation-propensity of Cdh23 than typical E-cadherins. While the structure of the heterodimer-interface is well-understood, the homodimer structure is not yet resolved. We deciphered the unique trans-homodimer structure of Cdh23 in solution which consists of two electrostatic-based interfaces extended up to two terminal domains. As opposed to most classical cadherins, Cdh23 interacts with its two apical domains in both homo and hetero trans interactions. This unique interface is robust with low off-rate of ~8x10-4 s^{-1} which supports its strong aggregation-propensity. Out of 27 extracellular domains, only the apical two domains are known to mediate homo interactions. We deciphered the role of these domains on the binding affinity of trans interactions using cell-aggregation studies and single molecule force measurements. *In-vitro* aggregation studies showed that cell-lines expressing longer extracellular domains showed faster aggregation.

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Identification of a putative region critical for cold sensitivity of TRPA1

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Transient receptor potential (TRP) channel subtype A1 (TRPA1) is considered to function as a cold receptor in mammals. This channel can be also activated by various pain-producing agents, cooling chemicals or membrane depolarization. The TRPA1 sensitivity to cold differs between primate and rodent species and a single residue within the fifth transmembrane domain S5 (V875 in human but G878 in mouse) seems to underlie the observed differences. Importantly, the same S5 region also determines the sensitivity of TRPA1 to several agonists and antagonists , and contributes to an intracellular vestibule that forms a side fenestration through which hydrophobic modulators and membrane phospholipids may affect channel functioning. To gain insights into the cold-sensing mechanism of TRPA1, we used a structure-guided mutagenesis screen for non-conserved residues within the region that connects the TRP-like domain with the C-terminal coiled-coil (Y1006-Q1031) and is predicted to affect the channel's interaction with membrane phospholipids. Our quantitative analysis of the effects of cooling on the mouse and human TRPA1 combined with molecular modeling suggests that this subunit-joining region may be responsible for species-dependent cold regulation due to different interaction with membrane phosphoinositides.

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Electrocatalytic studies on bd oxidases: identification of the distinct reaction mechanism from enzymes originating from extremophiles

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Cytochrome *bd* oxidases catalyse the reduction of oxygen coupled with the oxidation of quinones. They are part of the respiratory chains of bacteria, including several pathogens. Cytochrome *bd* oxidases play a crucial role in protection against oxidative stress, in virulence, adaptability and antibiotics resistance. No homologues are found in eukaryotes. A comprehensive understanding of the structure, assembly and catalytic mechanism of bd oxidase requires knowledge of their unique structure, the specific reaction mechanism, the interaction with the membrane and the knowledge about specific inhibition of the reaction.(1)

Here, we present the electrochemical and vibrational spectroscopic study on two *bd* oxidases originating from different organisms. Despite the significant difference in the length of the so-called Q-loop, both enzymes include the same hemes in the core of the enzyme: two b-type hemes and one d-type heme. Previous studies on the *E. coli* enzyme revealed that oxygen is reduced at the d-heme center and that the electron transfer starts at heme b-558. From the recently published structure of the *bd* oxidase from *Geobacillus thermodenitrificans* an electron transfer pathway different from the proposed one would be expected based on the inverted redox potentials of the hemes. Interestingly, the electrocatalytic study(for the method see 2) of both bd oxidases points towards a protonation reaction in *G. thermodenitrificans,* that is not detectable in *E coli.* FTIR spectroscopies reveal the involvement of different acidic residues in a highly hydrophobic environment, including Glu 107 in *E. coli*, that seems to show a pK value above 9. The crucial role for these residues in the catalytic mechanism is discussed.

(1). Fournier, E.; Nikolaev, A.; Nasiri, H.; Hoeser, J.; Friedrich, T.; Hellwig, P.; Melin, F. 'Creation of a gold nanoparticle based electrochemical assay for the detection of inhibitors of bacterial cytochrome bd oxidases' (2016) *Bioelectrochemistry, 111, 109-114.*

(2).

Meyer, T.; Melin, F.; Xie, H.; von der Hocht, I.; Choi, S. K.; Noor, M. R.; Michel, H.; Gennis, R. B.: Soulimane, T.; Hellwig, P. 'Evidence for distinct electron transfer processes in terminal oxidases from different origin by means of protein film voltammetry' (2014) *J. Am. Chem. Soc.,* 136, 10854-10857.

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IMPACT OF LIPID POLYUNSATURATION ON DOPAMINE D2 RECEPTOR ACTIVATION AND SIGNALING

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The dopamine D2 receptor (D2R) – which belongs to the GPCR family – has been implicated in the etiology of several psychiatric disorders such as schizophrenia, depression or bipolar disorders, and is a main target of most antipsychotics. Interestingly, a "whole-body" decrease in long-chain polyunsaturated fatty acids (PUFA) levels – n-3 PUFAs such as docosahexaenoic acid (DHA) in particular - has been consistently described in these psychiatric disorders [1]. However, the mechanisms by which alteration in PUFA levels may contribute to pathogeneses and could alter the functionality and pharmacology of the D2R are unknown. Our project aims at unraveling the impact of membrane PUFAs on D2R pharmacological properties and conformation through biochemical, biophysical and modeling studies in both PUFA enriched cells and membrane model systems of controlled lipid composition. To this aim, we have investigated the impact of membrane PUFAs in the first stages of receptor activation, that is in the receptor/ligand interaction using fluorescence anisotropy and plasmon waveguide resonance (PWR). Moreover, PUFAs impact on the recruitment and activity of D2R signaling effectors was investigated by BRET approaches. Overall the data indicate that membrane PUFA composition impacts both agonist and antagonist affinity for D2R. This effect could be related to a preferential interaction of PUFA-containing phospholipids with the D2R leading to the formation of microdomains around the receptor as revealed by molecular modeling. Regarding the signaling cascades, preliminary data indicates an absence of influence of PUFAs on cAMP production but an impact on β-arrestin recruitment. The results could have a significant impact in the development of adjuvant therapeutic strategies for psychiatric disorders implicating the D2R.

References:

Erik Messamore and Robert K. McNamara, Lipids in Health and disease. **2016**, *15*, 5.

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Solubilisation and characterisation of Pma1 in SMA lipid nanoparticles

C. Cavalier, S. Heit, J.F. Bada Juarez, A. Watts, M. Bublitz.
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Fungal infections are a major threat for immunocompromised patients. A potential target for the treatment of such invasive infections is Pma1, a P-Type ATPase that pumps protons across the plasma membrane of fungi to create an electrochemical gradient essential for the secondary transport of nutrients. Studies to determine the structure of Pma1 have led to a low resolution cryo-EM map (8 Å) [1] revealing a hexameric ring assembly. As yet, efforts to enhance protein expression and purification have been focused on detergent solubilization and purification, which does not necessarily preserve the adjacent lipids and can potentially alter the native oligomeric assembly and ATPase activity. In addition, Pma1 resides within detergent-resistant lipid-rafts of the fungal plasma membrane, making solubilisation ineffective. Recently, a copolymer of styrene-maleic anhydride (SMA), has demonstrated some potential as a new tool to solubilise and purify membrane proteins for structural analysis directly from their native environment, preserving the lipid and oligomeric state of the protein intact [2]. Here we have been able to solubilise Pma1 from its native source (*Neurospora crassa*) using SMA copolymer. We expect to perform structural analysis of the protein using electron microscopy and to apply mass spectrometry for the analysis of the copurified lipids. Using SMA to purify Pma1 directly from the native membrane will enable us to analyse its native lipid environment and to reveal essential protein-lipid or protein-protein interactions for hexamer formation and ATPase activity. Furthermore, Pma1 incorporated into SMA nanoparticles provides us with a new approach to obtain a high-resolution cryo-EM structure.

[1] Kühlbrandt*, et al* (2002) 'Structure, mechanism, and regulation of the Neurospora plasma membrane H+-ATpase', *Science*, 297(5587)
[2] Sun, C. *et al.* (2018) 'Structure of the alternative complex III in a supercomplex with cytochrome oxidase', *Nature*, 557(7703)

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Dimerization of surfactant protein C studied by molecular dynamics simulations

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Pulmonary surfactant (PSurf) is a multilayered lipid-protein complex located in the alveoli of mammalian lungs. It reduces the surface tension during breathing and acts as a part of the immune defense system. A healthy PSurf is essential for breathing and normal functioning of the lungs. Abnormalities in the structure and function of the PSurf are linked to several major diseases such as the infant respiratory distress syndrome (IRDS) and the acute respiratory distress syndrome (ARDS). Despite this, there is a significant lack of knowledge in our understanding of the structure of the PSurf and its mechanisms of action.

Surfactant proteins play a major role in the functioning of the PSurf. One of the key proteins is surfactant protein C (SP-C), which enhances the adsorption and spreading of phospholipids at the air-water interface and participates in maintaining the integrity of the multilayered PSurf structure. Deficiency of SP-C leads to severe chronic respiratory pathologies, such as idiopathic pulmonary fibrosis (IPF) and interstitial lung disease (ILD). It has been suggested that SP-C contains an AxxxG dimerization motif, and that the oligomerization of SP-C may be important in SP-C sorting and trafficking along cellular pathways.

In this work, we explored the dimerization of SP-C in model membranes that describe the key features of realistic PSurf membranes. We used both coarse-grained and atomistic molecular dynamics simulations to determine the SP-C dimerization interface and the dependence of dimer formation on the lipid distribution next to the dimer complex. The results predict a number of features that could be used to modulate the stability of SP-C dimers.

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The histidine-hydroxide exchange at iron of heme *a* in bovine cytochrome c oxidase induced by alkaline pH

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The membrane bound respiratory cytochrome c oxidase (CcO) participates in the energy conversion in cells of aerobic organisms. In CcO the electron transfer to O_2, facilitated by four redox centers, is associated with the transmembrane proton translocation, the proton pumping. The key component of this pumping is the redox-dependent and reversible structural change associated with some specific acid-base group(s) transferring the proton. However, the identity of the redox center(s) and the acid-base group(s) crucial for the pumping is not completely elucidated. Here, the pH-dependence of the exchange of ligands at one of the redox centers, iron of heme *a* (Fe_a^{3+}), is established in the oxidized bovine CcO ligated by cyanide. Using the electron paramagnetic resonance and UV-Vis spectroscopy we have found that the bis-histidine coordination of heme *a* (His61-Fe_a^{3+}-His378) at neutral pH is changed to the monohydroxide derivative at the pH values above 9,0. We present reasons that His378 is most likely displaced by hydroxide anion to produce His61-Fe_a^{3+}-OH^- coordination and this exchange is suppressed by the reduction of the catalytic center. The ligand switch at Fe_a^{3+} correlates with a strong decrease of the redox potential of Fe_a (E_m(a)) at pH above 10,5. However, E_m(a), determined when the other redox centers of CcO are oxidized, exhibits in the pH range 5,4 – 10,0 a shallow linear dependence with a slope of –13 mV per pH unit. The relevance of these findings for the proton pumping of CcO is discussed.

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Atomistic Insights into the Dynamics of γ-Secretase Activity: Aβn Processing, Inhibition and the Role of Active-Site Protonation States.

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Due to its role in the degradation of the amyloid precursor protein (APP), the membrane spanning hetero-tetramer γ-Secretase is implicated in the development of early-onset Alzheimer's disease.

The sequential cleaving of the C-terminal APP fragment, C99 results among other products, mostly in a 38 to 42 amino acid long peptide. These amyloid beta (Aβ) peptides are known to form the amyloid plaques, found in the brain tissue of Alzheimer's disease patients. Usually, C99 processing results in the 40 amino acid long Aβ40 peptide. Several mutations, however, are known to shift the production line towards the more aggregation-prone Aβ42. This is considered to be the main cause of early-onset Alzheimer's disease.

In order to gain a deeper understanding for APP processing and to investigate new routes for γ-Secretase inhibition and modulation, we performed a number of long time scale atomistic molecular dynamics (MD) simulations of various Aβn - γ-Secretase complexes.

Based on our simulations that also include the cleaving intermediates of C99, we postulate the following mechanism of APP processing: The scissile bond of the substrate is relocated towards the active center of the enzyme by a combined sliding and unwinding mechanism. A combination of helical strain and energetically unfavorable lipid penetration of polar residues, provides an explanation for the termination of Aβn processing after the cleaving to Aβ40.

Even though large parts of the substrate are relocated towards the active site of the enzyme during processing, simulations also predicted the formation of a very stable N-terminal anchoring region where interactions between nicastrin and Aβn seem to play an important role in the stabilization of the enzyme-substrate complex.

Starting from the recently published Cryo-EM structure of the C83-γ-Secretase complex (Zhou et al., Science 2019), we used MD and free energy calculations to study the effects of different active-site protonation states on the formation of potentially reactive active-site conformations. In addition, we also discuss the influence of active-site protonation on the stability of beta-sheets formed between substrate and enzyme as well as the binding affinity of γ-Secretase transition state inhibitors.

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Specific Lipid–Protein Interactions Determine the Biophysical Activity of Pulmonary Surfactant Protein SP-B

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The lungs are formed of small air-sacks, the alveoli, which provide a large surface area for efficient oxygen exchange. The epithelial surface of the alveoli is covered by a thin aqueous layer, where the air–liquid interface is coated with a surface-active lipid–protein complex called the pulmonary surfactant (PS). PS prevents the collapse of the alveoli during the expansion and compression cycles of breathing by reducing the surface tension at the air–water interface. The surface activity of the PS is highly dependent on the cooperative interactions of the proteins and lipids at the interface. Impaired activity of the PS leads to fatal conditions, such as the respiratory distress syndrome (RDS).

The function of the surface-active film is highly dependent on a surfactant protein called SP-B. SP-B is in charge of transferring surface-active phospholipids from membrane-based surfactant complexes into the air-liquid interface. Once there, it also participates in the formation of a highly cohesive multilayered film able to provide maximal mechanical stability under the high pressures achieved at the end of expiration. Despite its importance, the structure of SP-B as well as its function at the molecular scale have remained poorly characterized. In this work, we used multi-scale *in silico* modeling and molecular dynamics simulations to characterize how SP-B might define PS functional determinants. In particular, we explored how various oligomeric states of SP-B interact with specific surfactant lipids, and how SP-B reorganizes its membrane environment to modulate PS structure and function. The results show specific lipid–protein interactions that give rise to functionally relevant membrane reorganization. The results further indicate that SP-B oligomers catalyze lipid transfer between adjacent surfactant layers, driven by changes in the lateral pressure of the interfacial monomolecular lipid films.

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Structure and dynamics of GPCR-bound ghrelin reveal the critical role of the octanoyl chain

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For a decade, a number of G-protein coupled receptor (GPCR) structures have been elucidated by X-ray crystallography and cryo-electron microscopy. However, only few complexes between receptors and their physiological agonists are known, particularly with peptide ligands. NMR emerged as a method of choice to determine the conformation and dynamics of GPCR-bound peptide ligands [1]. Here, we report our work on ghrelin, a neuropeptide hormone that activates the growth hormone secretagogue receptor (GHSR) to control growth hormone and insulin secretion, food intake, reward and stress. We used NMR and molecular mechanics simulations to study GHSR-bound ghrelin with a particular focus on the role of its post-translational octanoylation, which is a unique feature of this neuropeptide.

Transferred ^{1}H NOE experiments with GHSR reconstituted into lipid nanodiscs [2] allowed us to determine ghrelin bound-state structure. We also used ^{15}N transverse relaxation and STD measurements to decipher the neuropeptide conformational dynamics and interaction area. It revealed that ghrelin N-terminal part folds with its octanoyl chain upon receptor binding and form a hydrophobic core in strong and persistent interaction with GHSR. In contrast, ghrelin C-terminal region remains disordered in the bound state. We then combined our NMR data with all-atom and coarse-grained (CG) molecular dynamics to delineate the neuropeptide possible binding pathways and model the ghrelin-GHSR complex. NMR experiments and CG simulations with unacylated ghrelin further showed that the octanoyl chain is essential for the formation of ghrelin hydrophobic core and promote the access to the receptor ligand-binding site. It indicates that this essential post-translational modification acts as a dynamic structural hub that stabilizes ghrelin in a specific receptor-bound conformation.

Our results provide a novel insight into the molecular mechanisms responsible for ghrelin interaction with GHSR and illustrates the complex conformational space GPCR peptide ligands explore during their binding process. Considering the pharmacological relevance of ghrelin and its receptor, our results may lead to the design of new drugs with applications in the treatment of obesity, diabetes and addiction.

[1] O'Connor *et al.*, PNAS USA, 2015, **112**, 11852-11857.
[2] Damian *et al.*, PNAS USA, 2015, **112**, 1601-1606.

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Proton transfer pathways in the antiporter-like subunits of complex I revealed by multiscale simulations

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Respiratory complex I (NADH:ubiquinone oxidoreductase), one of the largest protein assemblies (500 kDa in bacteria to 1 MDa in mammals), is responsible for ca. 40 % of ATP synthesis in mitochondria [1]. It efficiently couples the reduction of ubiquinone from NADH to proton pumping at a distance of ca. 200 Å, which is remarkable at biological length scales. Several mitochondrial and neurodegenerative diseases are associated with complex I dysfunction. Despite its central importance in biology and available structural data from cryo EM and X ray crystallography [1], the molecular mechanism of complex I remains unknown. Here, we adopt multiscale computational approach, combining atomistic molecular dynamics simulations and hybrid QM/MM calculations to identify and characterize the proton transfer pathways in the membrane-bound subunits of complex I. Our results shed novel insights in to the proton pumping mechanism of complex I involving conformationally dynamic amino acid residues and water molecules that allow rapid proton transfer without the loss of energy, and provide blueprints to design future experiments.

[1] O. Haapanen, V. Sharma. A modeling and simulation perspective on the mechanism and function of respiratory complex I. Biochimica Et Biophysica Acta (BBA) - Bioenergetics, 7 (2018), pp. 510-523.

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Putative interaction site for membrane phospholipids controls activation of TRPA1 channel at physiological membrane potentials

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The transient receptor potential ankyrin 1 (TRPA1) channel is a polymodal sensor of environmental irritant compounds, endogenous proalgesic agents and cold. Upon activation, TRPA1 channels increase cellular calcium levels *via* direct permeation and trigger signaling pathways that hydrolyze phosphatidylinositol-4,5-bisphosphate (PIP2) in the inner membrane leaflet. Our objective was to determine the extent to which a putative PIP2-interaction site(Y1006-Q1031) is involved in TRPA1 regulation. The interactions of two specific peptides(L992-N1008 and T1003-P1034) with model lipid membranes were characterized bybiophysical approaches to obtain information about affinity, peptide secondary structure andpeptide effect in the lipid organization. The results indicate that the two peptides interact withlipid membranes only if PIP2 is present and their affinities depend on the presence of calcium..Using whole-cell electrophysiology, we demonstrate that mutation at F1020 producedchannels with faster activation kinetics and with a rightward shifted voltage-dependentactivation curve by altering the allosteric constant that couples voltage sensing to poreopening. We assert that the presence of PIP2 is essential for the interaction of the two peptidesequences with the lipid membrane. The putative phosphoinositide-interacting domaincomprising the highly conserved F1020 contributes to the stabilization of the TRPA1 channelgate.

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Mechanism of K^+ binding and selectivity in the sodium potassium pump

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The Sodium-potassium pump (Na^+/K^+-ATPase) transfers Na^+ and K^+ opposed to their concentration gradient across the cell membrane [1]. Ion binding is guided by glutamates and aspartates located in the binding site in the transmembrane region. However, the exact mechanism of preferential binding of K^+ despite the presence of a higher concentration of Na^+ in the extracellular region, is still not clearly understood. Previous studies [2-3] suggest that protonation of specific glutamate residues in the ion binding site is essential for the ion selectivity of the pump. Taking the combination of protonated glutamates as a reference, here we investigate ion binding site to the Na^+/K^+-ATPase in the outside open E_2P state to deduce the mechanism of K^+ binding and selectivity using MD simulations and free-energy methods. We observe that the transient protonation/deprotonation of D808 is key to the binding of the two K^+ ions while maintaining selectivity over Na^+. Our results are consistent with pKa calculations and across two different methods for the estimation of relative ion-binding free energies. Our data offers new hypotheses into the order in which ions bind to the outside open state of the Na^+/K^+-ATPase.

References:

1) Morth JP, Pedersen BP, Buch-Pedersen MJ, Andersen JP, Vilsen B, Palmgren MG, Nissen P. A structural overview of the plasma membrane Na^+, K^+-ATPase and H^+-ATPase ion pumps. Nature reviews Molecular cell biology. 2011 Jan;12(1):60.
2) Yu H, Ratheal IM, Artigas P, Roux B. Protonation of key acidic residues is critical for the K^+-selectivity of the Na/K pump. Nature structural & molecular biology. 2011 Oct;18(10):1159.
3) Han M, Kopec W, Solov'yov IA, Khandelia H. Glutamate water gates in the ion binding pocket of Na^+ bound Na^+, K^+-ATPase. Scientific reports. 2017 Jan 13;7:39829.

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Oxidative stress as a modulator of BAX apoptotic activity

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Apoptosis is a regulated process of cell death that plays an important role in embryonic development, regulation of tissue homeostasis or removal of harmful cells in multicellular organisms. A failure in apoptosis can lead to severe pathological disorders including cancer, autoimmunity or neurodegenerative diseases. Mitochondria play a key role in the intrinsic apoptotic pathway, which is tightly regulated by Bcl-2 protein family. In response to cytotoxic stress, these proteins initiate cell death by permeabilization of the mitochondrial outer membrane (MOM) followed by the release of apoptotic factors, such as cytochrome c, which represents a point of no return in apoptotic progression. BAX protein, a member of the pro-apoptotic Bcl-2 family with a pore forming activity, is a critical effector of the mitochondrial cell death pathway. Upon interaction with BH3-only proteins, cytosolic BAX undergoes conformational activation and translocation resulting in MOM permeabilization. However, the underlying mechanism controlling this membrane-associated BAX action is still poorly understood. Recent findings suggest that the activity of BAX protein is significantly regulated by the lipid composition of the MOM. In particular, oxidized phospholipids (OxPL) that are generated under oxidative stress conditions seem to directly influence BAX membrane penetration and its activity [1,2].

By employing single molecule fluorescence spectroscopy techniques and various dye leakage assays we study the BAX membrane interactions and its activity in *in vitro* system that makes use of synthetic giant unilamellar vesicles (GUVs) and large unilamellar vesicles (LUVs) doped with varying amounts of OxPL species, which mimics oxidative stress conditions. Dye leakage assays revealed that BAX activity is sensitive to the type and concentration of different OxPL species in the MOM-mimicking vesicles. Higher levels of oxidized phospholipids in MOM mimicking model lipid bilayer significantly enhance membrane affinity and partial penetration of full length BAX. In addition, solid state NMR studies and calorimetric experiments on the lipid vesicles revealed that the presence of OxPL disrupts the membrane organization enabling BAX to penetrate into the membrane.

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In search of lost tail- study on the KATP channel dynamics

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ATP-sensitive potassium channels (KATP) are transmembrane proteins that play a key role in insulin secretion from pancreatic beta-cells. They close in response to a change in the ATP to ADP ratio and stop the K^- outflow, leading to the whole sequence of steps which finally results in insulin secretion. The KATP channels have become one of the main targets of type 2 diabetes treatment. Drugs from sulfonylurea (SU) group are known to exert inhibitory function on these channels. Nevertheless, the exact molecular mechanism of their action as well as the mechanical background of closing/opening of KATP are still not known. The publication of a number of KATP channel structures[1-4], has open the door to gaining detailed insight into the mechanism of action of channel inhibitors. The overall architecture of the channel consists of two different types of protein subunits: Kir6.2 (inward rectifier potassium channel) and SUR1 (sulfonylurea receptor). Nevertheless, several important regions of the KATP channel have remained unresolved in all of the structures. One of such regions was the N-terminus of Kir6.2, which has been unresolved up to position 32, suggesting that this region is highly flexible. A special role of N-terminus of Kir6.2 in the modulation of channel gating by SUR1 has been reported since late 90s. Here, we use docking modelling and molecular dynamics simulations of realistic all-atom model which, along with recently confirmed position of the "tail" in the system, bring us one step towards understanding of the role of the N-terminus of Kir6.2 in the KATP channel activity.

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Nanoscale Lipid models to study Membrane Proteins

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Developments in the field of X-ray diffraction, such as XFEL (X-ray Free Electron Laser) has become advantageous in studying Membrane Protein's structure and dynamics. For in-vitro studies, membrane protein needs to be reconstituted in a more native like hydrophobic environment. A novel membrane model system, Nanodisc due to its defined size and low mass-ratio of lipid to protein, proves to be a suitable candidate for XFEL studies. Nanodiscs are nano-sized lipid bilayer patches held intact with the help of a scaffold protein, MSP (Membrane Scaffold Protein). Engineering this protein will help in creating higher order structures, such as disuphide linked oligomers of Nanodiscs in the case of cysteine mutagenesis. This approach of engineering the protein to create well-ordered oligomeric structures will help in avoiding the normal crystallisation process. Another recent development in the area of membrane model system is the DEBs (DNA Encircled Lipid Bilayer), where the MSP scaffold in Nanodisc is replaced by alkylated DNA, the alkylation provides enough hydrophobicity to accommodate a lipid patch. DNA being a versatile tool for nanotechnological application, DEB system with further development and characterization will help in enhancing the studies in Membrane protein.

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Photoswitchable hydrophobic helical peptide shows slow and multiexponential folding kinetics in POPC membranes

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While the folding/unfolding kinetics of helical peptides in solution has been studied in detail, the kinetics of helical hydrophobic peptides in lipidic membranes has received much less attention, largely because conventional experimental procedures to induce folding/unfolding in peptides are not suitable when working with lipidic membranes. An alternative approach is the use of a photoswitch crosslinked to a peptide. The photoswitch, an organic molecule that isomerizes between cis and trans conformations when irradiated with light, can induce reversible changes in the structure of the peptide, as shown previously for soluble peptides (1). Here, we studied for the first time to our knowledge the folding/unfolding of a photoswitchable hydrophobic helical peptide in lipidic membranes. A synthetic model peptide for transmembrane helices with alanines and leucines flanked by two lysines, was covalently crosslinked through two cysteine residues to an azobenzene photoswitch. The photoswitchable peptide (KCALP-azo), was characterized by UV-Vis and FTIR spectroscopies and shown to retain normal photo-isomerization and a highly helical structure in POPC membranes. Time-resolved studies by FTIR spectroscopy showed the unfolding process of KCALP-azo was faster than our time-resolution (100ms). However, the folding process was extremely slow (minutes) and multiexponential. This contrasts with previous studies using photoswitchable helical soluble peptides, with folding/unfolding events completed in few microseconds (2). Interestingly, the folding of KCALP-azo was accelerated using SDS micelles instead of POPC membranes. Our results indicate that the folding of helical structures in lipidic membranes is more constrained than in detergent micelles or in solution, with multiple free energy barriers, as deduced by the much slower and complex folding kinetics in POPC membranes.

References: (1).Flint DG et al. Chem. Biol. 2002, 9, 391–397.
(2). Bredenbeck J et al. Proc Natl Acad Sci U S A. 2005, 102(7), 2379-84.

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HIV-1 Gag proteins interaction with lipid membranes

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Gag polyprotein is an essential component for human immunodeficiency viruses (HIV-1) reproduction. Many Gag copies assemble laterally, either in the cytoplasm or on the plasma membrane of the host cell, to produce new viruses. Despite multiple attempts to clarify the basic principles of Gag interactions with cell membranes, there are still open questions about the main partners of such interactions among lipids and cell and viral proteins, as well as about the mechanism of viral protein envelope self-organization during budding of progeny virions from the infected cell. In this work we focused on the effect of membrane lipids, its curvature and the pH of the environment of on Gag self-assembly. For this purpose we used high-resolution atomic force microscopy, allowing studying biological objects in non-destructive, close to native conditions. Using super-sharp soft cantilevers we characterized the morphology of recombinant Gag polyproteins, adsorbed on mica and on supported lipid bilayers. We estimated protein distribution between aqueous solution and supported lipid membranes, evaluated the surface-volume concentration ratio and investigated the effect of membrane curvature on the Gag self-organization under physiological conditions. Finally, we carried out experiments in acidic conditions of late endosomes (at pH 4) and compared results.

This work was supported by the Russian Foundation of Basic Research (grant #17-54-30022).

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In situ spectroscopic studies of the dual chromophore proton pump Archaerhodopsin4 with and without the second chromophore

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Archaerhodopsin 4 (aR4), an unknown structured proton pump found in the claret membrane of *Halobacterium species* (*H. sp.*) XZ515, is one of the photoreceptors containing not only the retinal chromophore, but also a noncovalent bound carotenoid molecule as the second chromophore. aR4 performs light collection and proton translocation similar to bacteriorhodopsin(bR) that only possesses a single Ret chromophore, but shows a different proton pumping behavior with an opposite temporal order of the proton release and uptake under physiological conditions. To address the photocycle kinetics and function of aR4, *in situ* light-induced transient absorption change spectroscopy, low temperature FTIR difference spectroscopy and 2D solid-state NMR, together with molecular dynamic simulation and mutagenesis, were employed to study the photocycle, photo-intermediates and function of the key residues of aR4 with and without the second chromophore bacterioruberin. Our results revealed for the first time that the retinal thermal equilibrium in the dark-adapted state is modulated by bacterioruberin binding via a cluster of aromatic residues. Bacterioruberin not only stabilizes the protein trimeric structure but also affects the photocycle kinetics and the function of the key residues, and further the ATP formation rate. Furthermore, a constitutively activated aR4 was also observed in the bacterioruberin-excluded aR4 which can be characterized by the structural instability and conformational response to the deletion of the second chromophore. These new insights may be generalized to other receptors and proteins in which functions are perturbed by ligand binding.

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Effects of a SGLT2 inhibitor on intracellular ion levels and mitochondrial membrane potential in ventricular H9c2 cell line

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Introduction and Aim: It has been suggested that sodium glucose co-transporter 2 (SGLT2) inhibitors has some cardioprotective effects independent of their blood glucose lowering actions in diabetic patients. Although it has not been clearly identified the underlying mechanism of their cardioprotective effect, these inhibitors can act as an inhibitors of Na^+/H^+-exchanger (NHE). Therefore, in this study, we aimed to examine the molecular mechanisms of a SGLT2-inhibitor, dapagliflozin (DAPA), on the altered electrophysiological parameters of hyperglycemic embryonic rat ventricular cells (H9c2 cell line).

Material and Methods: One group of H9c2 cells were incubated with high glucose (25 mM, HG group) medium for either 24-h or 48-h at 37°C while second group has only 5 mM glucose. Half of high glucose incubated cells also were treated with DAPA (100 nM or 1 μM; D185360, Research Chemicals) while second half was kept under control condition. Intracellular ion concentrations, ($[X]_{in}$ such as Na^+, Ca^{2+}, Zn^{2+} and H^+), reactive oxygen speices, ($[ROS]_{in}$) and mitochondria membrane potential, (MMP) were monitored via specific fluorescent dye loaded cells (DCFDA, FluoZinc, JC-1, SNARF, FURA-2AM ve SBFI) using confocal microscope and microspectrofluorometer.

Results: Since 1 μM DAPA incubation presented some toxicity, the cells were incubated with 100 nM DAPA and all experiments were conducted with these cells. Although $[Na^+]_{in}$ levels in all group of cells were not significantly different from each other, $[H^+]_{in}$ level in HG group cells were significantly high comparison to those of controls, DAPA treatment (both 24-h and 48-h) induced marked augmentation in that level. However, $[Zn^{+2}]_{in}$ level in HG cells was significantly increased while DAPA treatment has no further benefits. In addition, increased $[Ca^{+2}]_{in}$ in HG cells was reduced with DAPA treatment to those of control levels. More importantly, DAPA treatment reduced elevated $[ROS]_{in}$ in these HG cells, however, DAPA did not improve the depolarized MMP in HG cells.

Conclusion: As summary, our present data indicate that DAPA treatment of HG cells can protect the increases in cellular ion levels including $[H^+]_{in}$ and $[ROS]_{in}$ and therefore provide important cardioprotective action and improve altered heart function in hyperglycemia.

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Tethered Bilayer Lipid Membranes – a Comprehensive Tool for Electrochemical Studies of Pore-Forming Toxins

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Formation of plasma membrane is considered as a crucial event during evolution and life, as known today, would not be possible without them. To investigate this complex membrane environment of lipids and membrane proteins significant progress has been made to model native membranes. The most common artificial systems include lipid monolayers, lipid vesicles, and supported lipid bilayers (SLBs) [1]. Distinct group of solid supported bilayers, tethered bilayers (tBLMs), are considered as perspective experimental platforms for membrane biosensors [2]. In particular, the modulation of the ionic conductance of tBLMs may be utilized to develop biosensors of the membrane damaging agents such as pore-forming toxins (PFTs) [3].

We have used the tBLM platform for detection, visualization and investigation of the mechanism of action of cholesterol dependent cytolysins (CDCs) – a class of PFT produced by a large number of Gram-positive pathogens and considered as virulence factors promoting bacterial invasion and infection. Most of the experimental data was obtained by particularly applying electrochemical impedance spectroscopy (EIS) and atomic force microscopy (AFM) techniques. tBLM modification in aqueous environment has been performed, using Methyl-β-cyclodextrin (MβCD) and cholesterol complex, to achieve picomolar sensitivity of CDC detection. Moreover, the additional receptor – human CD59, essential for some of the CDCs to reconstitute, was successfully attached to tBLM, enabling a better insight into different mechanisms of how the same toxin can operate.

[1] I. Köper, "Insulating tethered bilayer lipid membranes to study membrane proteins.," *Mol. Biosyst.*, vol. 3, no. 10, pp. 651–7, Oct. 2007.
[2] D. J. McGillivray *et al.*, "Molecular-scale structural and functional characterization of sparsely tethered bilayer lipid membranes.," *Biointerphases*, vol. 2, no. 1, pp. 21–33, 2007.
[3] R. Budvytyte, M. Pleckaityte, A. Zvirbliene, D. J. Vanderah, and G. Valincius, "Reconstitution of cholesterol-dependent vaginolysin into tethered phospholipid bilayers: implications for bioanalysis.," *PLoS One*, vol. 8, no. 12, p. e82536, Jan. 2013.

P-374

SGLT1: kinetics revealed by solid-supported membrane (SSM) based electrophysiology

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SLGT1 is responsible for the uptake of glucose from the intestine. Thus it has drawn interest as a pharmacological target for the treatment of diabetes. The energy source for glucose uptake is the Na+ gradient which is maintained by the Na+/K+ ATPase. The mechanism involves alternating access of the Na+ and sugar binding sites to both sites of the membrane - a commonly used kinetic scheme is the 8-state model.

Here we used inside-out vesicles containing SGLT1 to characterize its reverse transport mode using SSM-based electrophysiology. Our aim is to compare the results with cell-based assays to conclude differences in the kinetic properties of the intracellular and extracellular site of the transporter and reveal details about transporter asymmetry.

Compared to conventional electrophysiology, SSM-based electrophysiology allows usage of cell-free samples and enhances the signal-to-noise ratio due to a large sensor surface. Patch clamp is used to measure voltage induced transporter activity. SSM-based electrophysiology on the other hand only relies on substrate concentration gradients to activate the transporter. Due to the high resolution of this technology, SSM-based electrophysiology allows detection of pre steady-state currents, e.g. sugar binding or conformational transitions of the transporter. Beside steady-state parameters, we measured pre steady-state kinetics of SGLT1. We investigated various mechanistic properties, e.g. (1) binding kinetics of various sugar substrates and inhibitors, (2) transport kinetics under different conditions, (3) cooperativity between Na+ and sugar binding sites and (4) competitive Inhibition.

Our data is in agreement with kinetic parameters obtained with other techniques. In addition SSM-based electrophysiology revealed information about the kinetics of the sugar translocation pathway and the initial state of the transporter helping to evaluate the 8-state kinetic model of sugar transport in SGLT1.]

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LOV-TRIP8bnano as a new tool for the regulation of HCN channels by blue light

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Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels are main players in controlling rhythmic activity in cardiac pacemaker cells and spontaneously firing neurons. HCN channels are activated by voltage and modulated by the direct binding of cAMP to their cytoplasmic cyclic nucleotide binding domain (1). HCN channels activity is further regulated by modulatory proteins. One of them, TRIP8b, is a brain-specific auxiliary subunit which antagonizes the facilitatory effect of cAMP on channel opening (2). We have identified by rational design a 40 aa long peptide (TRIP8bnano) that recapitulates affinity and gating effects of full length TRIP8b. We have then fused TRIP8bnano to LOV2 (Light Oxygen Voltage domain 2) of the Avena sativa blue-light receptor phototropin to obtain a light-regulated inhibitor of HCN channels (3). Our results show that the synthetic LOVTRIP8bnano protein prevents cAMP regulation in HCN2 and HCN4 channels after bluelight irradiation.

(1) Biel, M. et al., Phys Rev 89: 847–885 (2009)
(2) Saponaro, A. et al., eLife. 7:35753 (2018)
Lungu, OI et al., Chem Biol. 19(4):507-17 (2012).

P-376

Distinct allosteric networks underlie mechanistic speciation of ABC transporters

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ATP-binding cassette (ABC) transporters couple the energy of ATP hydrolysis to the trans- membrane transport of biomolecules. We combine a novel molecular simulations approach with experiments to study long-range allostery in three representative ABC transporters, and find that each transporter employs a different allosteric network. In the vitamin B_{12} importer BtuCD, ATP binding is the driver of allosteric connectivity, and docking/undocking of the substrate binding protein (SBP) is the driven event. In BtuCD, the allosteric signal originates at the cytoplasmic side of the membrane before propagating to its extracellular side. The opposite causality and directionality is observed in the maltose transporter MalFGK, where the SBP is the main driver of allostery, binding of ATP is the driven event, and the allosteric signal originates at the extracellular side of the membrane and then propagates to its cytoplasmic side. A third variation is revealed in the lipid flippase PglK, where a cyclic cross-talk between ATP and substrate binding underlies the allosteric communication. The distinct allosteric network of each system explains their different mechanisms and demonstrates how speciation of biological functions may arise from variations in allosteric connectivity.

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Implications of the oligomerization of pulmonary surfactant protein SP-C in membrane fragmentation and macrophage activity.

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Pulmonary surfactant (PS) is a lipid-protein complex located at the bronchoalveolar air-liquid interface of lungs. Due to its ability to reduce surface tension, PS has a major role in breathing dynamics. Moreover, as it is the first barrier between inhaled air and systemic circulation, PS is also involved in lung defense against pathogens, especially through the interaction with alveolar macrophages.

Surfactant protein C (SP-C) is a small, very hydrophobic membrane protein essential for the biophysical function of PS. Moreover, since SP-C can interact with lipopolysaccharide (LPS) from Gram-negative bacteria and with CD14 (the co-receptor of LPS in macrophages), SP-C may also play a relevant role in lung homeostasis and immunity.

The link between both functions could rely on the recently described ability of SP-C to induce fragmentation in biological membranes. Furthermore, it has been proposed that this activity may be the consequence of the increase in membrane curvature, presumably through the generation of SP-C oligomers because of the interaction of putative N- and C-terminal oligomerization motifs within the protein.

To test this hypothesis, we have carried out bimolecular fluorescence complementation assays to define the contribution of the suggested oligomerization motifs in protein-protein interaction. Besides, we have described various parameters involved in membrane fragmentation using a tunable resistive pulse sensing system. Finally, we have conducted several flow cytometry and fluorimetry experiments to link different features in SP-C-loaded large unilamellar vesicles, such as the oligomerization state of the protein and the presence of LPS, with its role in macrophage phagocytosis, cytokine production and phenotype.

P-378

Photo-activation of mechanosensitive ion channels

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Mechanosensitive ion channels are present in all three domains of life, archaea, bacteria and eukaryota, and are responsible for a wide variety of functions, from osmotic pressure control in bacteria and cell turgor in plants, to touch and pain sensation in humans. The mechanosensitive ion channel of large conductance MscL from E. coli is a model system for studying ion channels that react to the lateral pressure exerted in the lipid membrane.

To gain information at a molecular level on structural changes, lipids-protein interaction and the gating mechanism of MscL, we are using FT-IR spectroscopy. To induce a change in lateral pressure in the membrane, synthetic photoswitchable lipids are inserted in the lipid bilayer. Indeed, upon illumination, these azobenzene-containing phospholipids isomerize and occupy a different volume inducing a pressure, allowing for a light control of the lateral pressure in the membrane and therefore of the ion channel activity. Preliminary results on the photoswitchable lipids show the reproducibility and reversibility of the light switching mechanism in the lipids. Polarized attenuated total reflection IR spectra give an insight on the orientation and reorientation of photoswitchable lipids in a multilayer structure. The induced pressure change is measured in the monolayer with a Langmuir trough. The insertion of the protein in the photoswitchable lipid bilayer is our next step. This work, other than elucidating a particular mechanism of ion channel gating, could contribute to opening the way to new applications in the field of photopharmacology.

P-379

Characterisation of the light-activatable proton pump Archearhodopsin-3 in a droplet interface bilayer

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Most cell bioenergetics in nature are initiated by the generation of an electrochemical proton gradient. In archaea, proteins from the rhodopsin family are responsible for generating this gradient and the resulting membrane potential. The best characterised of these rhodopsins is the proton pump bacteriorhodopsin, bR. Upon illumination, the retinal chromophore, embedded within the rhodopsin, undergoes photoisomerization. Consequently, the rhodopsin protein facilitates the translocation of protons across the bilayer acting as a light-activated proton pump. Previously it has been shown that bacteriorhodopsin can be vectorially incorporated into an artificial lipid bilayer in its native membrane[1], to generate a bio-pixel. Unfortunately, the currents obtained using bR are too close to the baseline noise which hampers its electrical characterisation thus limiting its use. Using a droplet interface bilayer system, we have explored the proton pumping behaviour of a second rhodopsin, archearhodopsin-3, which can generate up to five times more current[2] than bR. We have characterised the proton pumping behaviour of archearhodopsin-3 within an artificial bilayer in a range of conditions including pH and detergent type. We have also explored the effect of trimerisation and studied protein insertion behaviour into the droplet interface bilayer. Using archearhodopsin-3, it is now possible to perform a comprehensive characterisation of proton pumps in membranes without having to resort to noise analysis. In the future, these bio-pixels could be incorporated into synthetic tissues or used to build biodevices and medical implants.

[1] Restrepo-Schild, V., Booth, M.J., Box, S.J., Olof, S.N., Mahendran, K.R. and Bayley, H., (2017) Light-patterned current generation in a droplet bilayer array. Sci.Rep. 7, 46585

[2]Chow, B.Y, et al. (2010) High-Performance Genetically Targetable Optical Neural Silencing via Light-Driven Proton Pumps. Nature 7;463(7277): 98-102. doi:10.1038/nature08652

P-380

Protein evolution approach to increasing solubility of GPCR

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G-protein coupled receptors (GPCRs) are cell surface receptors that mediate the cellular responses to many endogenous signalling molecules. They are a major target for the pharmaceutical industry - more than 40% of all drugs available today act on a GPCR. Effective drug design and functional characterization of these receptors strongly depend on high-resolution structural information. The main problem in effort of crystallization and solving structure of GPCRs is associated with low solubility of membrane proteins. In principle, this obstacle can be overcome by improving solubility of the receptors, or in the extreme case by converting the receptors from “membrane-soluble” into water-soluble form. In the present research, we attempt to convert κ-opioid receptor, member of GPCR family, to a water-soluble form. The main strategy to reach the goal was redesigning the membrane surface of a GPCR by substituting the hydrophobic amino acids of the protein/lipid interface with suitable polar or charged residues, to produce a molecule that is able to fold and function in aqueous solution. It was highly improbable that the desired result can be reached in a single step by rational design. Instead, we have chosen a highly interdisciplinary approach of in vitro protein evolution. We were able successfully perform ribosome display for the κ-opioid receptor library and select binders, that specifically bind to proper ligands for this library. The selected binders are variants of κ-opioid receptor and were purified without using a detergent. However, so far selected receptors form water-soluble oligomers (from penta- to octamers). Despite the robustness of this selection method, we were not able to evolve water-soluble functional κ-opioid receptor, which would form monomeric or dimeric functional unit. We believe that improvement of selection step and involvement of rational design in final steps of increasing solubility of the receptor might lead to preparation of water-soluble analogue of monomeric and/or dimeric structure of the receptor.

P-381

The MemBlob framework: determination of transmembrane region using cryo-EM maps

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Membrane proteins play an important function in many cellular processes and are highly significant drug targets. To understand their folding, maturation and function, as well as to develop new therapies, high resolution protein structures and the knowledge of their embedment into a membrane bilayer would be required. Experiments with membrane proteins tagged around putative TM helices provided low resolution data on the membrane boundaries. In the case of X-ray crystallography lipids can be identified infrequently. NMR has been applied mostly for small regions of transmembrane (TM) proteins, but it has not been exploited to systematically map membrane interaction sites. Due to the limitations of experimental approaches, various *in silico* methods are widely used to determine TM regions (e.g. TMDET, PPM, MEMPROTMD). Nowadays, transmembrane protein structures are determined in the presence of membrane mimetics, using cryo-EM microscopy. Importantly, the resulting electron density maps contain densities not only for the target protein, but also for the membrane environment. To extract the TM boundaries form the densities, we developed a computational pipeline called MemBlob. The MemBlob input includes a cryo-EM map, the corresponding atomistic structure, and the potential bilayer orientation determined by the TMDET algorithm. The results contain residue assignments to the bulk water phase, lipid interface, and the lipid hydrophobic core. They present the membrane region as a volume with boundaries that follows the shape of the lipid environment. Our MemBlob pipeline will be useful for researchers working on structure determination of membrane proteins using cryo-EM and also for developers of membrane region predictors, who can apply MemBlob results as a true positive experimental set. We also created a server and a database, which includes the MemBlob results of protein structures calculated from cryo-EM maps with a resolution better than 4 Å, available at **http://memblob.hegelab.org**

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Destabilisation of internal water networks underpins the sensitisation of a membrane-embedded photoreceptor protein: Structures of dark- and light-adapted Archaerhodopsin-3 H^+transporter to atomic resolution

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Archaerhodopsin 3 (AR3), expressed in the light-harvesting membrane of *Halorubrum sodomense* (from a distinct archaeal genus to *Halobacterium*), is a retinal-containing photoreceptor that transports H^+ ions to generate a transmembrane electrochemical gradient for ATP synthesis (Ihara *et al.* 1999). Its absorption spectrum is the most red-shifted of all known archaerhodopsins and AR3 has been shown to generate a 7- to 8-fold higher photocurrent than bacteriorhodopsin (bR, Chow et al. 2010). Mutants of AR3 are now routinely used for optogenetic studies, as a genetically targetable neuron silencer in mammals (Chow *et al.* 2010) and as a fluorescent sensor of transmembrane potential (Kralj *et al.* 2011; McIsaac *et al.* 2014).

Wild-type AR3 was crystallised in LCP (in the absence of detergent) and the structures of the light- and dark-adapted states were solved to 1.07 Å and 1.3 Å resolution. The high-resolution of these structures enables internal water molecules within the protein to be resolved and demonstrates how remodelling of the hydrogen bonding networks is induced by isomerisation of the retinal chromophore. Consistent with previous FTIR data (Saint Clair *et al.* 2012), the structures confirm that key water molecules, linking aspartate and arginine residues implicated in H^+ translocation, are more disordered in AR3 than bR and that this disorder increases as the protein transitions from the dark-adapted to the light-adapted state.

These very high-resolution structures will inform both our mechanistic understanding of proton translocation by archaerhodopsins, but also the design of future mutants for applications in biotechnology and optogenetics. These insights underscore the role of non-covalent bond formation and breaking in the sensitisation and de-sensitisation mechanisms of this important class of photoreceptor proteins.

References

- Ihara et al. (1999) J. Mol. Biol. 285 163-174
- Chow et al., (2010) Nature, 463(7277):98-102
- Kralj et al. (2011) Nature Methods 9(1):90-5
- McIsaac et al. (2014) PNAS 111:13034-13039
- Saint Clair *et al.* (2012) *J. Biol. Phys.* 38:153-168

P-383

Clenbuterol-mediated modulation of ion currents in a cellular model of spinal and bulbar muscular atrophy

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Spinal and bulbar muscular atrophy (SBMA) is a neurodegenerative disease characterized by the selective loss of motoneurons from the brainstem and spinal cord. Motoneurons are sensitive to changes in the generation and release of neurotrophins, such as brain-derived neurotrophic factor (BDNF). In this sense, BDNF levels are decreased in SBMA muscles. BDNF promotes local protein synthesis to improve synaptic function (through ion channels activity) and ameliorates axonal transport defects in SBMA. Recently, has been demonstrated that β-agonists delay the disease onset, ameliorates motor function and extend life span in models of neurodegenerative disease. Clenbuterol (Cbl) is a β-agonist that increases the phosphorylation of cyclic AMP response element binding (CREB), in a time-dependent manner, in cultured SBMA myotubes. CREB protein can activate and upregulate downstream targets such as the BDNF. Therefore, activation of the AC/PKA/CREB/BDNF pathway might support the beneficial effects of clenbuterol in SBMA. In this work, we evaluate this hypothesis by whole-cell voltage-clamp applied to motoneuron-derived cell line (MN-1) expressing polyQ repeats in the gene coding for the androgen receptor, which is the mechanism causing the development of SBMA. We observe that a brief application (1 min) of Cbl to SBMA cells produce a transient hyperpolarization in the resting membrane potential; while the incubation (24 h) with Cbl lead to modifications in the total currents compared with untreated cells. These findings suggest that treatment with Cbl ameliorated motor function and extended survival in SBMA cells through a modulation of the ion channel activities and the membrane excitability. *[Work funded with the contribution of Fondazione CARITRO Trento, Italy].*

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Inhibition mechanism of UCP2´s protonophoric function

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UCP2 is a membrane uncoupling protein belonging to the family of mitochondrial anion carriers. It is abundant in tissues relaying mainly on the glycolysis, such as stem, cancer and activated immunological cells[1]. UCP2 has been implicated in a wide range of pathological states, such as malignant neoplasms, ischemia, neuroimmunological and neurodegenerative diseases, based on its ability to act as a mild uncoupler and to reduce reactive oxygen species (ROS). UCP2-mediated proton transport is activated by fatty acids (FA) and inhibited by purine nucleotides (PN). The mechanism of proton transport inhibition is not known for UCP2, but was proposed for UCP1 and recently for UCP3[2].

We combined atomic force microscopy[3] and electrophysiological measurements of recombinant UCP2 reconstituted in planar bilayer membranes to measure UCP2-PN binding force and inhibition of UCP2´s activity. The latter was obtained by measurements of the total membrane conductance in the presence and absence of PN. Our data revealed that, in contrast to UCP1 and similar to UCP3, all PNs (ATP, ADP and AMP) were able to fully inhibit UCP2. However, increasingly higher PN concentrations was required with decreasing phosphorylation. Bond lifetimes of UCP2-PN interactions correlated with PN-mediated inhibition, both in the presence and absence of FA. The mutation of amino acid residues, known to be involved in UCP1/UCP3-PN interactions (R279, R185 and R88), revealed that only the mutation of R279 is crucial in UCP2: it rendered UCP2 immune to inhibition by all PNs. The results demonstrated important differences in the molecular mechanism of UCP2 inhibition in comparison to UCP1 and UCP3. It supports the hypothesis of different adaptation of the proteins of uncoupling subfamily to the various biological and transport functions.

REFERENCES

1. Rupprecht, A. *et al.* Uncoupling protein 2 and 4 expression pattern during stem cell differentiation provides new insight into their putative function. *PLoS One* **9**, e88474 (2014).
2. Macher, G. *et al.* Inhibition of mitochondrial UCP1 and UCP3 by purine nucleotides and phosphate. *Biochim Biophys Acta* **1860**, 664-672 (2018).
3. Koehler, M. *et al.* Combined Recognition Imaging and Force Spectroscopy: A New Mode for Mapping and Studying Interaction Sites at Low Lateral Density. *Sci Adv Mater* **9**, 128-134 (2017).

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Development of an *in vitro* pre-clinical nociceptor model for studying paclitaxel-induced neuropathic pain

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Paclitaxel (Taxol) is one of the most effective drugs used for the treatment of solid tumors. However, between 50-90% of patients receiving this therapy develop a chemotherapy-induced peripheral neuropathy (CIPN) that can be eventually translated into acute and chronic pain, limiting its clinical use. Although it is well-known that the neurotoxic effect of paclitaxel is produced by the alteration of the sensory neurons, the mechanisms underlying the pain symptoms remain unclear. To address this issue, we have established a primary culture of murine sensory neurons that can survive up to 10 days *in vitro*. Then, using electrophysiological techniques, we studied separately the biophysical properties of two main groups of nociceptive neurons, or nociceptors: IB4- (peptidergic) and IB4+ (non-peptidergic). The spontaneously and electrically evoked action potential firings were analyzed in the absence or after direct exposure (24h) to paclitaxel throughout time. As a result, we observed that this compound was able to alter the excitability of both IB4- and IB4+ small DRG neurons. The two main effects observed were: (i) cells with wider action potentials and fast adapting firing; and, (ii) cells with higher AP frequency and spontaneous activity. Furthermore, functional changes produced on the ion channel TRPV1 were assessed measuring the current evoked after application of capsaicin. We observed that subsets of IB4- neurons exposed to paclitaxel also showed increased TRPV1 activity, indicating the crucial role of the channel on this pathology. In summary, using a preclinical nociceptor model it has been possible to find different cellular mechanisms that could explain paclitaxel-induced painful CIPN. Our results highlight the suitability of *in vitro* systems as models of neuropathic pain.

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Protein-lipid interactions regulating entry and budding of enveloped viruses

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Enveloped viruses represents large group of pathogens including such dangerous species as HIV, Zika virus, Ebola virus, Hepatitis, Herpes, and Influenza viruses. Despite the differences in their target host cells and the clinical pattern of the infection, all these pathogens share common principles of cell entry and multiplication of the infection. Enveloped viruses wrap their genome into the scaffold from by so-called matrix proteins and the outer lipid shell, which the part of the host cell plasma membrane captured during the virion production. Matrix proteins of every enveloped virus serve several purposes: protecting the integrity of the virus, enabling controlled release of the genetic material, and assembly of new virions in the infected cell. In all these processes, matrix proteins interact with lipid membranes, and the molecular mechanisms underlying these interactions are far from being fully understood. We have studied the role of matrix proteins of Influenza A virus and Newcastle disease virus and their lipid interactions both at the stage of release of the genetic material and during assembly and budding of progeny virions. We have shown that matrix proteins of these two viruses utilize rather simple but completely different mechanisms to perform membrane remodeling during viral entry and budding of progeny virions.

This work was supported by the Russian Foundation for Basic Researches (project # 17-54-30022).

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Contribution of hyperpolarizing ion channels to acute cold hypersensitivity induced by oxaliplatin administration

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Oxaliplatin (OHP), a platinum-based chemotherapeutic agent, causes peripheral neurotoxicity (OIPN) characterized by an acute cold-induced syndrome accompanied by cramps, paresthesias/dysesthesias in the distal limbs and perioral region. No therapies are available to treat OIPN and patients often suffer from pain and sensory symptoms long after the end of the therapy. The molecular and cellular mechanisms that produce OIPN are not fully understood; however, one common mechanism appears to be changes in ion channels expression and/or function in primary afferent sensory neurons.

Here we have combined different electrophysiological approaches in order to investigate, in mice dorsal root ganglia (DRG) neurons, the impact of OHP treatment on the molecular targets that are known to participate in the processing of noxious stimuli as well as in the tuning of cold perception. We found that therapeutically-relevant OHP concentrations (0.1 µg/mL) lead to an acidification of the DRG cytosol neurons. It is likely that different classes of channels could be affected by this alteration in pH homeostasis. In particular, single channels recordings suggest the involvement of the two pore domain K^+ (K2P) channel family members. These are background channels that contribute to the regulation of neuronal excitability hyperpolarizing the membrane potential of peripheral nerve terminals and that are tightly regulated by different stimuli, including variations of internal pH.

In light of this, the hyperexcitability underlying the OINP can be explained as the resultant effect of multiple regulations on different targets in response to the OHP alteration of pH homeostasis. This provides useful insights for the understanding of the pathogenesis behind this neuropathy in order to propose effective therapeutic pain management.

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Activation and inactivation properties of the TRPV family and their structural origin

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Introduction

The transient receptor potential vanilloid (TRPV) family is a class of cation channels that belongs to the TRP superfamily. Recently, 3D structures of all TRPV family members have been elucidated, demonstrating that the channels have a similar tetrameric architecture. However, the family consists of two subgroups: TRPV1-4 are non-selective cation channels that are ligand-activated and play a role in thermosensation and nociception, while TRPV5-6 are constitutively active channels with a high calcium selectivity that have specialized transport function in kidney and intestine to maintain the body's calcium balance. Current TRPV1 and TRPV5 structures will be used to investigate the (structural) differences and similarities in the (in)activation mechanisms of the two groups of the TRPV family. There will be a focus on lipid-like ligand-based activation and calmodulin-dependent inactivation.

By using radioactive $^{45}Ca^{2+}$ uptake assays and Fura-2 AM-mediated calcium imaging, the response of TRPV5 and TRPV1 is assessed upon addition of a concentration range of various established TRPV1 agonists. Structural analysis of the TRPV1 and TRPV5 structures is performed to examine lipid and agonist binding pockets, as well as compare calmodulin binding sites, with a specific focus on the recent TRPV5-calmodulin complex structure. Several CaM mutants are generated based on the structural TRPV5-CaM interaction and their role in TRPV1 function will be assessed using Fura-2 calcium imaging, followed by immunoprecipitation assays and fluorescence life time imaging microscopy (FLIM)-based FRET imaging. Preliminary results indicate that TRPV5 can be stimulated by a few established TRPV1 agonists; i.e. PPAHV (phorbol-12-phenylacetate-13-acetate-20-homovanillate), PDDHV (phorbol-12,13-didecanoate-20-homovanillate) and NADA (N-Arachidonyldopamine). Moreover, we confirm the interaction of TRPV5 and TRPV1 with wildtype calmodulin, and demonstrate differences in binding to a Ca^{2+}-deficient CaM mutant (apoCaM). Our Fura-2-AM data also suggests a link between different CaM binding mutants and TRPV1/TRPV5 channel function.

This study challenges the idea that TRPV5 is not ligand-activated by showing that TRPV1 agonists can stimulate TRPV5 channel gating. Future work should characterize the structural binding pockets and mechanism of action. Moreover, we provide preliminary insights into the functional differences of the TRPV channels by demonstrating that calmodulin regulation differs for the two TRPV subgroups.

P-389

Membrane activity of the bacterial virulence factor VapA provides insights into the survival strategy of the intracellular *Rhodococcus equi*

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Gram-positive *R. equi* is a pulmonary and tuberculosis-like pathogen of young horses and immunocompromised patients that can cause pneumonia. It succeeds in surviving intracellularly by avoiding phagosome maturation. Recently, it has been shown that virulence-associated protein A (VapA) is a decisive virulence factor transported from bacteria-containing phagosomes to lysosomes, preventing acidification of these intracellular compartments and thus allowing survival and replication of *R. equi* [1]. We have characterized the secondary structure of VapA, its binding to lipid membranes and the pH-dependent permeabilization of lipid membranes.

With decreasing pH, permeabilization of free-standing membranes is upregulated by VapA. This results in larger lesion diameters and a qualitative shift to more defined transient lesions. In addition to regulating permeabilization, VapA is also involved in the exclusion of the proton pumping ATPase from the phagosome membrane and thus provides a pH neutral milieu for *R. equi*.

Meanwhile, the β barrel structure of VapA undergoes only minor changes with respect to secondary structure constituents as observed in circular dichroism experiments. The insertion of VapA into model membranes is surprisingly low at bilayer-equivalent lateral pressures - independent of pH changes. We conclude from this the necessity of local membrane pressure defects as initiation locus for the establishment of VapA.

With time-resolved atomic force microscopy analyses we could follow the formation of locally accumulated interaction sites with nearly molecular resolution. At low pH, the interaction becomes more intense and the present cholesterol-rich domains are perforated.

In summary, VapA counteracts several essential mechanisms of immune defense, including modification of acidification by pH-dependent permeabilization, and enables *R. equi* to survive the degradation in the phagosome of immune cells.

References

[1] von Bargen, K. et al., Cell Microbiol 21.1 (2019): e12958.

P-390

Molecular mechanism for membrane targeting of plant Calcineurin-B Like protein

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In plants, the coordinated action of a family of ten calcium sensors (CBLs), twenty-six protein kinases (CIPKs) and phosphatases (PP2Cs), produces transient phosphorylation and the activation of several signaling pathways in response to environmental stresses. Available data suggest a general mechanism in which stable CBL-CIPK-PP2C complexes may be regulating the activity of various ion transporters involved in abiotic stress response.

A characteristic of CBL proteins is the presence of N-terminal sequences with membrane targeting motifs. The presence of these membrane-localization signatures is important since it is believed they determine the localization of the CBL-CIPK complexes as was first shown for myristoylated CBL4/SOS3. SOS3, CBL1, CBL9 and CBL5 contain both palmitoylation and myristoylation sites and are located at the plasma membrane. Another mechanism for membrane localization involves a single transmembrane helix at the N-terminal of CBL10, to target the sensor to the vacuole and to the plasma membrane. Additionally, some CBLs contain a region rich in lysines that could interact with phospholipids in a process called snorkeling. The involvement of these motives in protein function is unclear, for instance, in vivo assays showed that myristoylation of CBL4/SOS3 is required for salt tolerance in plants, however, no significant difference in membrane association was observed between the myristoylated and non myristoylated protein.

We have examined the myristoylation and the calcium and membrane binding properties of CBLs. Our results showed that non-myristoylated CBLs are targeted to model membranes in a Calcium independent manner. However, we observed a strong correlation between the Calcium and membrane binding properties of myristoylated proteins. These data provide the basis for a model coupling calcium sensing and membrane targeting of CBL-CIPKs complexes.

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Response of the glucose transporter due to differential stimulation

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Cellular glucose uptake via Glucose Transporter 4 (GLUT4) in adipose tissue and muscle cells is initialized by binding of extracellular insulin to its receptors in the plasma membrane. GLUT4 translocates from intracellular vesicles to the cell surface and facilitates glucose internalization. Insulin signaling is altered by a variety of diseases (e.g., diabetes mellitus type 2) and results in impaired glucose clearance of the blood. We visualized the translocation of GLUT4 to the plasma membrane after addition of soluble insulin using single-molecule-sensitive fluorescence microscopy. GLUT4 is genetically modified with eGFP. In order to distinguish between to the membrane translocated GLUT4 and vesicular GLUT4, we excite the cell membrane with an evanescent wave produced (total internal reflection configuration). Another possibility is formation of Giant Plasma Membrane Vesicles (GPMVs) of stimulated cells. The last approach reduces the influence of free GLUT4-eGFP near the membrane further. In contrast, we tested the localized stimulation with insulin using the functionalized tip of an atomic force microscope (AFM) cantilever. Thereby, insulin-coated beads were brought into contact with the cell. A localized significant increase of the translocated GLUT4-bound eGFP signal was found. Obviously, binding affinity is increased via surface immobilization in comparison to soluble insulin. The next step will be the functionalization of bionanoparticles like lipoproteins as carrier system. We speculate, that their intrinsic biocompatibility will enhance the viability of its cargo and should yield a prolonged steady basal insulin level in the body thus stabilizing the blood glucose level.

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Investigation of Transient receptor potential ankyrin 1 (TRPA1) expression and function in a pancreatic adenocarcinoma cell line

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Investigation of Transient receptor potential ankyrin 1 (TRPA1) expression and function in a pancreatic adenocarcinoma cell line

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Transient receptor potential ankyrin 1 (TRPA1) is a member of the TRP channel family which is overexpressed in several tumors such as breast or small cell lung carcinoma. In this study we investigated the expression and function of TRPA1 in pancreatic adenocarcinoma (PDAC) cells.

Using confocal microscopy, we found that TRPA1 ion channels were present in the plasma membrane of PDAC cells and exhibit also cytoplasmic localisation. We also recorded and characterized AITC (allyl isothiocyanate, a selective TRPA1 agonist) -triggered Ca^{2+} signals in PDAC cells. These signals were abolished under Ca^{2+}-free conditions and blocked by A967079, a specific TRPA1 antagonist. Moreover, using the patch clamp technique in the whole-cell mode, we recorded AITC-elicited currents with a reversal potential close to 0 mV that were reversibly inhibited by A967079.

Preliminary studies also revealed that activating the channel with selective agonists significantly changed cell migration. Our results suggest that TRPA1 may be a marker of pancreatic cancer and a putative target for molecular therapies.

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The bacterial insertase YidC forms an ion conducting pore in the membrane upon ribosome binding

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Bacterial YidC belongs to the family of insertases also comprised of mitochondrial Oxa1 and plant Alb3. YidC facilitates co-translational insertion of single- or multi-spanning alpha helical transmembrane proteins by allowing the hydrophobic nascent chains to slide along its interface with the lipid bilayer. YidC's half of the membrane spanning water-filled groove (1) is believed to be important for the process. The exact molecular mechanism is not known. It is also not known how YidC may facilitate the insertion of large proteins while maintaining the membrane barrier to small molecules. Here we measured the electrical conductance of purified and reconstituted YidC. In its resting state YidC is impermeable to ions. However, upon ribosome binding YidC forms ion channels in planar lipid bilayers with a single channel conductance of about 330 pS. We performed single molecule fluorescence microscopy experiments to clarify whether the channel is formed by YidC dimers or monomers. About 80 % of the fluorescence sports bleached in a single step, while 20 % underwent two bleaching steps. The result indicated that the membrane contained both monomers and dimers. Since the crystal structure does not show the presence of a pore, ion permeation across the dimer appears to be more likely. The dimer has also been observed in vivo (2). Yet, more experiments are required to clarify whether the channel provides a pathway for the nascent chain.

References

1. Tanaka Y *et al. (*2018) 2.8-Å crystal structure of Escherichia coli YidC revealing all core regions, including flexible C2 loop. *Biochemical and Biophysical Research Communications* 505:141–145.
2. Boy D, Koch H-G (2009) Visualization of distinct entities of the SecYEG translocon during translocation and integration of bacterial proteins. *Molecular Biology of the Cell* 20:1804–1815.

P-394

How does the multidrug transporter ABCB1 convert the energy of ATP to energise cargo transport?

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The human genome codes for 42 ABC membrane transporters and 4 ABC proteins, grouped into subfamilies A-G. ABC transporters, which use ATP binding and hydrolysis to energize cargo transport, have been intensively studied. The appearing of crystal and cryo-EM structures in the last 13 years have provided much needed structural insight to understand the function of these molecular machines, but progress remained limited. The static structures could not unambiguously reveal the mechanism of how the energy of ATP is harvested and converted into conformational changes that lead to directional substrate transport across the membrane. We used molecular dynamic simulations to investigate the conversion from chemical energy stored in the phosphate bonds of ATP to mechanical energy that energizes the transport cycle of the multidrug transporter ABCB1 (P-glycoprotein). Potential of Mean Force profiles showed that ATP hydrolysis is the power stroke that creates a high energy state, which is essential for the transporter to propagate through the transport cycle.

All ABC transporters have two ATP binding sites, but a significant number of ABC exporters carry a degenerate nucleotide binding site (NBS), which does not show a sufficient ATP hydrolysis rate to sustain the physiological transport function. The hallmark of the degenerate NBS is the substitution of the catalytic glutamate in the Walker B motif. When the corresponding glutamate is mutated in canonical NBSs of ABC transporters, they become transport incompetent. We used the highly homologuous multidrug transporters ABCB1 and the bile salt export pump ABCB11, which share 49% sequence identity, but importantly, ABCB1 has two canonical NBSs, while in ABCB11 one is degenerate, to elucidate the enigmatic function of degenerate NBSs. We could for the first time create a seemingly fully functional ABCB1 transporter that carries a degenerate NBS. Complementation of these experimental data with simulations allowed us to identify the molecular details, which allow ABC transporters with a degenerate NBS to remain functional and sustain substrate transport, despite the eponymous catalytic glutamate is missing.

P-395

Relationships between the structure and membrane-disruptive activity of antimicrobial peptoids

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Peptoids are a class of peptide mimetic molecules where the side chain is appended to the backbone amide nitrogen atom rather than the alpha carbon. As a result peptoids are not able to form the hydrogen bond stabilized helices which are typical of peptides. However, incorporation of charged and aromatic side chains in small linear peptoids can result in helical structures which possess the key characteristics for membrane-disruptive antimicrobial activity, namely cationicity and amphipathicity. Peptoids also display good resistance to in vivo proteolytic activity, making them promising candidates for future antimicrobials. Using experimental and computational methods we demonstrate that the biophysical and biochemical properties of peptoids, including secondary structure, hydrophobicity and antibacterial activity can be altered by rearrangements of the primary structure. We investigate a novel library of short, linear peptoids with combinations of different charged and aromatic side chains arranged in both regular repeating motifs and scrambled sequences. Using circular dichroism (CD) spectroscopy we demonstrate that a regular repeating motif is not required for peptoids to form stable helical structures in aqueous and organic environments. In addition, these peptoids undergo distinct secondary structural changes upon binding to lipid vesicles, to different extents depending on their primary structure and the lipid composition of the vesicles, demonstrating some selectivity towards bacterial-type membranes.

In order to investigate the observed relationships between primary and secondary structure computationally we have adapted the General Amber Force Field to accurately capture the backbone torsional preferences of peptoids. Using these new parameters we have performed Hamiltonian replica exchange molecular dynamics of peptoids from our library in water and octanol solvents. We are then able test methods for calculating the electronic CD spectra of these peptoids from the molecular dynamics trajectories. Further work into developing a coarse-grained model for peptoids compatible with the Martini lipid model will allow us to gain insight into the membrane disruption mechanism of the peptoids. Developing these techniques will aid in the rational design of peptoids for antimicrobial applications.

P-396

Towards and understanding of the role of Bax on bile acid-mediated cytoprotection

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Towards and understanding of the role of Bax on bile acid-mediated cytoprotection

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Cytoprotective bile acids such as ursodeoxycholic acid (UDCA) and tauroursodeoxycholic acid (TUDCA) are known for their ability to inhibit apoptosis at submicellar concentrations in both hepatic and nonhepatic cells. The exact mechanism by which they exert this cytoprotection is not yet entirely clear, but their effect seems to be related with the blockage of processes that converge on mitochondrial damage. Bcl-2-associated X protein (Bax) plays a key role in apoptosis, which is achieved through translocation of the protein to the mitochondria from the cytosol after an apoptotic stimulus and formation of pores in mitochondrial membranes. We show here that both UDCA and TUDCA inhibit the interaction of Bax with activator molecules such as the Bid-BH3 peptide and decrease the affinity of Bax for liposomes mimicking outer mitochondrial membrane composition. Importantly, UDCA and TUDCA are shown to dramatically inhibit Bax-induced permeabilization of model membranes. The direct impact of apoptotic and cytoprotective bile acids on Bax translocation is monitored in HCT116 Bax/Bak DKO cells expressing Bax-GFP. The findings presented here clearly show that at physiologically active submicellar concentrations, bile acids have the ability to inhibit Bax pore-forming activity and suggest that the cytoprotective activity of UDCA and TUDCA could be the result of this process.

Tuesday 23rd July

SINGLE MOLECULE BIOPHYSICS

P-397 (O-114)

ParB dynamics and the critical role of the C-terminal domain in DNA condensation unveiled by combined Magnetic Tweezers and TIRF Microscopy

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ParB is a central component of partition systems responsible for the faithful segregation of chromosomes and low-copy number plasmids in bacteria. *Bacillus subtilis* ParB forms multimeric networks involving non-specific DNA binding leading to DNA condensation. In previous work [1], it was found that the C-terminal domain (CTD) of ParB is essential for the formation of those higher-order structures, and that an excess of the free CTD impeded DNA condensation or promoted decondensation of pre-assembled networks. However, interpretation of the molecular basis for this phenomenon was complicated by the inability to uncouple protein binding from DNA condensation or to correlate both measurements in parallel. Here, we have combined lateral magnetic tweezers with total internal reflection fluorescence microscopy to simultaneously control the restrictive force against condensation and to visualize ParB protein binding by fluorescence. At non-permissive forces for condensation, ParB binds non-specifically and highly dynamically to DNA, whereas at low forces ParB condenses DNA as shown previously [2]. Our new approach allowed direct tests of different models to explain CTD-dependent condensation inhibition. We conclude that the free CTD blocks the formation of ParB networks by heterodimerization with full length ParB which remains bound to the DNA. This strongly supports a model in which the CTD acts as a key bridging interface between distal DNA binding loci within ParB networks [3].

[1] Fisher, G.L.M., *et al.*, Elife, 2017. 6: e28086
[2] Taylor, J.A., *et al.*, NAR, 2015. 43(2): p. 719-31
[3] Madariaga-Marcos, J., *et al.*, Elife, 2019 (*accepted*)

P-398 (O-115)

A folding nucleus and minimal ATP binding domain of Hsp70 identified by single-molecule force spectroscopy

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The folding of large proteins can be a very complex process; many large proteins critically rely on the aid of molecular chaperones while some equally large proteins fold spontaneously. Along the folding pathways, partially folded intermediate states are frequently populated and can accelerate or even decelerate efficient folding process. The structures of these intermediates are generally unknown because they are often very short-lived. In our recent work [1], single-molecule force measurements were used to scrutinize the hierarchy of intermediate states along the folding pathway of the nucleotide binding domain (NBD) of Escherichia coli Hsp70 DnaK. DnaK-NBD is a member of the sugar kinase superfamily that includes Hsp70s and the cytoskeletal protein actin. Using optical tweezers, a stable nucleotide-binding competent en route folding intermediate comprising lobe II residues (183-383) was identified as a checkpoint for productive folding. We have obtained a structural snapshot of this folding intermediate that shows native-like conformation. To assess the fundamental role of folded lobe II for efficient folding, we turned our attention to yeast mitochondrial NBD, which does not fold without a dedicated chaperone. After replacing the yeast lobe II residues with stable E. coli lobe II, the obtained chimeric protein showed native-like ATPase activity and robust folding into the native state, even in the absence of chaperone. In summary, lobe II is a stable nucleotide-binding competent folding nucleus that is the key to time-efficient folding and possibly resembles a common ancestor domain.

[1] Bauer D, Meinhold S, Jakob RP, Stigler J, Merkel U, Maier T, Rief M, Žoldák G. A folding nucleus and minimal ATP binding domain of Hsp70 identified by single-molecule force spectroscopy. Proc Natl Acad Sci U S A. 2018 May 1;115(18):4666-4671.

P-399 (O-116)

Single-molecule dissection of the dihydrofolate reductase reaction revealed multiple conformers leading to a catalytic product release

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It is generally accepted that enzymes stabilise the transition-state of a catalysed reaction. Here using a nanopore as a single-molecule nano-reactor we provide experimental evidence for a more sophisticated narrative. The sampling of hundreds of consecutive reactions from single enzymes revealed that dihydrofolate reductase (DHFR) populates frequent non-productive transition-state conformations and undergoes second-long catalytic pauses. We also found that the free-energy landscape of the enzyme is sculpted with multiple ground-state conformers with different affinity for substrate, cofactor and product that undergo hierarchical changes during the catalytic cycle. As in a two-stroke engine, the chemical step provides the power stroke to switch the enzyme to the product-bound conformer, promoting the simultaneous release of the oxidised cofactor. The subsequent binding of a reduced cofactor to the vacated site provides the free energy for the recovery stroke, which induce the allosteric release of the product and resets the initial configuration. This catalytic remodeling of the affinity landscape of DHFR is likely to be a general feature for complex enzymatic reaction where the release of the products must be facilitated.

P-400

Protein folding inside the ribosomal tunnel

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Isolated α-helices and partially structured protein folding intermediates were previously reported to be able to fold even before exiting the ribosomal tunnel [1]. Increasingly new evidence appears showing that also small protein domains can fold within the ribosomal tunnel [2]. Nevertheless, it is far from being adequately studied mainly due to a lack of appropriate methods.

Single-molecule techniques are essential for the study of protein synthesis and folding since these are asynchronous processes that are difficult to be observed using ensemble methods. Especially optical tweezers were used in the past to show full synthesis and cotranslational protein folding outside the ribosomal tunnel in real time [3].

Here we combine confocal fluorescence with optical tweezers to study the folding of the small zinc-finger domain ADR1a that takes place while still in the ribosomal tunnel. We use continuous dual-trap optical tweezers featuring two-color confocal scanning fluorescence with single-photon sensitivity (C-trap, Lumicks, Amsterdam), allowing correlated high-resolution force spectroscopy and smFRET measurements [4]. This allows for the observation of folding states, transitions, as well as kinetics and at the same time for the determination of the role of the interactions between the nascent chain and the tunnel on the entire process.

Studying such a domain inside the tunnel and how the rate of translation and the folding are influenced by the spatial constraints and the interactions within the tunnel will help elucidating the role of the ribosome in initial protein folding events.

(1) Bhushan, S.; Gartmann, M.; Halic, M.; Armache, J.-P.; Jarasch, A.; Mielke, T.; Berninghausen, O.; Wilson, D. N.; Beckmann, R. *Nat. Struct. Mol. Biol.* **2010**, *17*, 313-317.
(2) Nilsson, O. B.; Hedman, R.; Marino, J.; Wickles, S.; Bischoff, L.; Johansson, M.; Müller-Lucks, A.; Trovato, F.; Puglisi, J. D.; O'Brien, E. P.; Beckmann, R.; von Heijne, G. *Cell Rep.* **2015**, *12*, 1533-1540.
(3) Wruck, F.; Katranidis, A.; Nierhaus, K. H.; Büldt, G.; Hegner, M. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, E4399-E4407.
(4) Hashemi Shabestari, M.; Meijering, A. E. C.; Roos, W. H.; Wuite, G. J. L.; Peterman, E. J. G. *Methods Enzymol.* **2017**, *582*, 85-119.

P-401

Force sensing with nanoscale DNA tethers

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Force measurement at the single-molecule level provides insight into intra-molecular dynamics and inter-molecular interactions. Traditionally, researchers used atomic force microscopy and magnetic or optical tweezers to investigate the force-dependent biological interactions and mechanical properties of biomolecules. The popularity of these techniques, however, suffers from drawbacks including slow image acquisition rate and/or low data throughput, as well as connections to macro-beads that introduce drift and prevent the investigation of minor conformational changes. Here, we present a force sensor consisting of a single nanoparticle tethered to double stranded DNA (dsDNA) on an ultrasensitive dark-field microscope, enabling the simultaneous characterisation of multiple tethers. We show that upon application of an electric field, the charged nanoparticles are driven from their equilibrium positions, and the stretched dsDNA acts as a polymer nanospring, exerting a restoring force on the piconewton scale. We monitor the nanoparticle displacement by tracking the scattering signal with a micromirror total internal reflection dark-field microscope with angstrom precision. As a result, we can quantify the charge dependent displacement of the tether, and how it depends on surface charge and applied potential. Our approach opens up new avenues to study force- and charge-relevant dynamics and the interactions of biological macromolecules.

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Sidedness-Dependence of Current Fluctuations Caused by Serine-Containing Peptides Interacting with the α-HL Nanopore

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In this work we investigated the interactions between a macro-dipole-like peptide and the α-hemolysin (α-HL) nanopore via single-molecule electrical recordings, to gain insights into the conditions ensuring optimal throughput of peptide readout by the nanopore. For this purpose, we used a 30-amino-acid-long peptide containing a central group of six serine residues, flanked by segments of oppositely charged amino acids. We probed the sidedness-dependence of peptide association to and dissociation from the electrically and geometrically asymmetric α-hemolysin nanopore by adding the peptides solution to the trans- or cis-side of the nanopore. We observed that the peptide capture by the nanopore and its residence inside it depend strongly on the transmembrane potential polarity and addition sidedness of the peptides. The synergistic couplings between the electroosmotic, entropic and electrostatic interaction contributions alter distinctly the capture rate of entry of analytes on either side of the nanopore and the dwelling time inside it. The presented approach has direct implications to the efficient capture of peptide from solution, intended to provide increased temporal and spatial resolution and pave the way for peptide sequencing with single amino acid sensitivity.

1. A. Asandei, I.S. Dragomir, G. Di Muccio, M. Chinappi, Y. Park, T. Luchian, *"Single-Molecule Dynamics and Discrimination between Hydrophilic and Hydrophobic Amino Acids in Peptides, through Controllable, Stepwise Translocation across Nanopores"*, **Polymers 2018**, 10(8), 885;

P-403

Multiplex probing of the anaphylatoxin C5a binding to C5aR reveals a two-site binding mechanism

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A current challenge in the field of life sciences is to decipher the functional activation of cell surface receptors with a complex binding mechanism towards specific ligands in their native environment. Lack of suitable nanoscopic methods has hampered our ability to meet this challenge in an experimental manner. During the past decade, force-distance based atomic force microscopy (FD-based AFM) has established itself as a powerful method that combines imaging biological samples at high resolution (≈5 nm) and mapping dynamic receptor-ligand interactions [1,2]. In this work, we used the interplay between FD-based AFM and steered molecular dynamics (SMD) to elucidate the two-site binding mechanism of the C5a anaphylatoxin to its receptor (C5aR)[3]. Receptor-ligand interactions were probed at the sub-site level for the first time and we developed a new theoretical framework to extract kinetic and thermodynamic parameters of complex ligand binding to intramolecular multiple sites. Our results, obtained in physiologically relevant conditions, corroborate the previously suggested two-site binding model and clearly identify a cooperative effect between two binding sites within the C5aR. While previous body of work has shown that the interaction between the C-terminal region of C5a and the receptor effector site is essential to trigger full receptor activation, our study provides a deep molecular understanding of the interplay between both effector and binding sites leading to a fine-tuning in the C5aR activation process. We anticipate that our methodology could be used for development and design of new therapeutic agents to negatively modulate C5aR activity.

1 Alsteens, D. *et al.* Imaging G protein-coupled receptors while quantifying their ligand-binding free-energy landscape. *Nat Meth* **12**, 845-851, (2015).

2 Alsteens, D. *et al.* Nanomechanical mapping of first binding steps of a virus to animal cells. *Nature Nanotechnology* **12**, 177, (2016).

3 Dumitru, A.C et al., Multiplex probing of the anaphylatoxin C5a binding to C5aR reveals a two-site binding mechanism (submitted)

P-404

Real-time observation of superstructure-dependent DNA origami digestion by DNase I using high-speed atomic force microscopy

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DNA origami nanostructures are promising tools for numerous biomedical applications, ranging from diagnostics to drug delivery and targeted therapy. They are not only intrinsically biocompatible, biodegradable, and noncytotoxic, but can be synthesized in a wide variety of different shapes and sizes, and further functionalized in a precisely controlled manner with various organic and inorganic species. This enables their defined loading with therapeutic cargos and may be further exploited to facilitate cell targeting, cellular uptake, and cargo release. The performance of such DNA origami vehicles strongly depends on their structural and shape integrity. Unfortunately, previous studies have observed that DNA origami nanostructures are rapidly degraded in biological media, which poses serious limitations for their application in such environments. In particular the presence of nucleases has been identified as a major factor contributing to the limited stability of DNA origami nanostructures in biological media.

In this work, we study the degradation of four well established and structurally distinct 2D DNA origami designs (different lattice types, different edge types, and different flexibility) under the attack of DNase I using high-speed atomic force microscopy (HS-AFM). The temporal resolution in our experiments ranged from 5 to 10 seconds per frame, thus allowing a real-time observation of the digestion process. Our results reveal that digestion of the different DNA origami exhibits a superstructure dependence. Furthermore, we could identify structural features of each DNA origami design that are most susceptible and most resistant to DNase I digestion, respectively. The results acquired for DNA origami nanostructures immobilized at a solid surface are compared to digestion profiles obtained under identical conditions in bulk solution. It is found that DNA origami designed on the square lattice without twist-correction show remarkably different digestion profiles in bulk solution and at the solid-liquid interface, which is attributed to adsorption-induced shape distortions and strain build-up. Our findings may thus not only help in creating more resilient DNA origami nanostructures, but could also be applied in designing structures with building blocks possessing distinct susceptibilities to nucleases.

P-405

Protein sensors screened from a genetically-engineered nanopore library

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Tools for the detection and quantification of proteins in biological samples are essential for diagnostics and fundamental biological studies. Traditional methods include ELISA, Western blots, and mass spectrometry. However, these assays are limited in throughput, multiplexing capabilities, specificity, and sensitivity. Nanopore stochastic sensing, which notably has been used to sequence DNA, is a single-molecule approach to protein detection which can overcome these limitations.[1] This technique relies on pore-forming membrane proteins that allow for ionic flow between two buffer-filled compartments; the ionic current changes upon binding of the target protein and this change can be used to identify the protein and deduce binding parameters. A library of nanopore sensors would allow for tailored detection of any protein of interest, including low concentrations of disease markers in a biological sample.

To demonstrate label-free stochastic protein sensing, known binding epitopes were engineered into the peripheral loops of a protein nanopore. Single-molecule binding of the corresponding targets—an antibody and a kinase—was observed as a change in the electrical read-out. To expand this sensing platform to any protein of interest, nanopore libraries were constructed and screened by bacterial display. In this technique, a nanopore with a different loop modification is expressed to the surface of each bacterium, and flow cytometry is used to sort bacteria which bind a target protein that has been fluorescently labelled. Initial library screens against an antibody have identified candidates with low nanomolar affinity.

We are currently developing a method to test screened sensors based on droplet interface bilayer electrophysiology, which use small reagent volumes and can be automated.[2] The optimized technology will then be applied to identify novel, label-free nanopore sensors for disease markers from the libraries. Incorporation of the platform into a miniaturized device for parallel protein detection would be a valuable tool for precision medicine.

[1]Bayley H, Cremer PS. Stochastic sensors inspired by biology. *Nature.* 413, 226–230 (2001).
[2]Bayley H et al. Droplet interface bilayers. *Mol Biosyst.* 4, 1191–1208 (2008).

P-406

Linker effects in single molecule protein folding from molecular simulations

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Single molecule force spectroscopy (smFS) experiments of protein mechanics have been extremely useful to study folding and unfolding behaviours and, more recently, even to resolve protein folding transition paths [1]. However, these experiments are inherently affected by the instrumental apparatus used to modulate the extension of the protein by the application of a pulling force. Recent analytical methods have been used to explain these effects both in the thermodynamics and kinetics. This is

essential to understand the relevance of experimental results from these techniques. However, these theoretical methods are often validated against trajectory data from simulations on very simple onedimensional models and then directly applied to the analysis and interpretation of smFS experimental results. Here I cover the gap between simple models and experiments using molecular simulations with an explicit chain model that recapitulates the most fundamental aspects of protein folding. The focus is on the simplest case of force lamp experiments, which allow monitoring reversible folding/unfolding transitions. I study a simple twostate protein where the effects of the linker turn to be dramatically important to understand the measured kinetics [2].

References:

[1] The Power of Force: Insights into the Protein Folding Process Using Single-Molecule Force Spectroscopy. J Schöenfelder, D De Sancho & R Pérez-Jiménez, J.
Mol. Biol. 428, 4245-4257 (2016).
[2] Instrumental Effects in the Dynamics of an Ultrafast Folding Protein Under Mechanical Force, D. De Sancho*, J. Schönfelder, R. B. Best, R. Perez-Jimenez, V.
Munoz. J. Phys. Chem. B, in press (2018).

P-407

Electrophoretic migration of PAMAM G1 dendrimers into confined nano-spaces: a single-molecule assay

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Dendrimers are tree branch-like macromolecules developed around a core, which often adopt a spherical three dimensional architecture. These nanostructured molecules have various applications due to their ability to form multifunctional therapeutic systems (e.g., drug-delivery systems, vector-systems in gene therapy or target-systems for imaging agents). Despite their versatility, dendrimers still need further investigations in order to improve their physical and chemical properties. Polyamidoamine (PAMAM) dendrimers are composed of three distinct structural components: a central ethylenediamine core, repetitive amidoamine concentric units and the terminal amino functional groups. Herein, we investigate the cationic PAMAM dendrimer of low generation (G1) by temporarily trapping it in the confined space of a γ-cyclodextrin (γ-CD) molecular adapter lodged by an alpha-hemolysine (α-HL) protein nanopore. The single-molecule studies employed here regard the interactions of PAMAM G1 dendrimer with the α-HL nanopore embedded in a lipid membrane that separates two chambers filled with a salt solution, and with the γ-CD adapter which narrows down the constrictions domain inside the nanopore. The applied voltage across the membrane induces an electric current and drives the PAMAM G1 dendrimer through the nanopore due to the electrophoretic force. We observed three types of transient reductions in the electric current with particular signatures: (i) the first blockade represents the translocation of the dendrimer through the pore; (ii) the second blockade is the γ-CD temporarily lodged inside the pore and (iii) the third blockade is given by the dendrimer ─ γ-CD interactions, without any translocation of the dendrimer to the other side of the nanopore. The statistical analysis of the blockades amplitude, the dwell times and the interaction events-frequency of the dendrimers with either the nanopore or the molecular adapter allowed for the dendrimer to be detected and characterized in confined nano-spaces. Further research on this topic is needed in order to improve the transport of nanoscale drug carriers across biological membranes.

P-408

Mechanoregulation of *Staphylococcus aureus* protein A binding to von Willebrand factor

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Staphylococcus aureus is the main pathogen causing endovascular infections, such as infective endorcarditis or heart valve prosthetic infection.[1] During infective process, S.aureus needs to adhere and withstand the shear stress of flowing blood. Under hydrodynamic condition *S. aureus* cell surface protein A (SpA) can bind to the large plasma glycoprotein von Willebrand factor (vWF).[2] While vWF binding to SpA plays a role in *S. aureus* adherence to platelets and endothelial cells under shear stress,[3] the molecular basis of this stress-dependent interaction has not yet been elucidated.

Using single-molecule atomic force microscopy, we demonstrate that vWF binds to the S. aureus surface protein A (SpA) via a previously undescribed force-sensitive mechanism. We identify an extremely strong SpA-vWF interaction, capable to withstand forces of ~2 nN, both in laboratory and in clinically-relevant MRSA strains. Strong bonds are activated by mechanical tension, consistent with flow experiments revealing that bacteria adhere in larger amounts to vWF surfaces at high shear rate. Our results support a mechanism relying on the remarkable mechanosensitivity of vWF: under force, elongation of vWF leads to the exposure of a high affinity cryptic SpA-binding site, to which bacteria firmly attach. This force-regulated interaction is of medical importance as it may play an important role in bacterial adherence to platelets and to damaged blood vessels.

1. Moreillon Pet al. 2004. Lancet 363:139-149.
2. Fowler VG et al. 2005. JAMA 293:3012-3021.
3. Claes J et al. 2014 Blood:blood-2014-02-558890.

P-409

Sequence-dependent bending in nucleic acids studied by atomic force microscopy and molecular dynamics

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DNA conformation and flexibility are highly dependent on the sequence. Probably the most striking examples of such modulations are A-tracts: short runs of consecutive adenines that, when in phase with the helical pitch, induce a global bending in the DNA. This effect is known as intrinsic bending and has implications in processes such as nucleosome positioning or supercoil localization (1). Here we characterize the mechanical properties of intrinsically bent DNA molecules and predict the existence of this phenomenon in dsRNA.

Using atomic force microscopy (AFM), we studied DNA molecules with phased A-tracts. When fitted to the worm-like chain (WLC) model these molecules showed an anomalously small persistence length and remarkable deviations from the fit (2). We rationalized these results by developing an extension of the WLC that accounts for the intrinsic bending. This intrinsically-bent WLC (IBWLC) provides an excellent fit to our AFM measurements and allows decoupling intrinsic from entropic bending in DNA molecules.

We have previously used all-atom molecular dynamics (MD) to study the structure and mechanical properties of dsDNA and dsRNA (3, 4). Here, we found that sequence-induced bending also occurs in dsRNA. A systematic study of dsRNA sequences revealed a singular structure for the poly-AU molecule, with a particularly narrow major groove. When placed inside random sequences, short AU-tracts induced an abrupt and localized compression of the major groove, ultimately leading to bent structures. A comparison of A-tract dependent bending in dsDNA and dsRNA shows that the structural mechanism behind this phenomenon is different for both molecules. The compact structure of dsRNA presents limited access to the bases, and thus, it is likely that such sequence induced deformation plays a role in dsRNA sequence recognition.

[1] Haran T & Mohanty U (2009) *Q. Rev. Biophys.* 42
[2] Moreno-Herrero F, *et al* (2006) *Nucleic Acids Res.* 34(10):3057-3066.
[3] A Marin-Gonzalez, J G Vilhena, R Perez & F Moreno-Herrero (2017) *Proc Natl Acad Sci* 114 (27) 7049-7054
[4] A Marin-Gonzalez, J G Vilhena, F Moreno-Herrero & R Perez (2019) *Phys Rev Lett* 122, 048102

P-410

AFM shows that human CtIP forms a tetrameric dumbbell-shaped particle which bridges DNA ends

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Homologous recombination (HR) is one of the main DNA damage-repair mechanisms. Because it uses a homologous gene as a template it is a potentially error-free repair mechanism. The process of repair involves the concerted action of multiple proteins; therefore the handling and maintenance of the DNA ends in close proximity is critical. A key early factor is CtIP. Little is known about this protein apart from its role in the initial steps of HR and its tetrameric state in solution(Davies, Forment et al. 2015). Here we have studied the oligomeric state of human CtIP (hCtIP) and its interaction with DNA using AFM. Imaging of hCtIP shows five classes of particles classified according to their morphology and volume analysis. The largest class is consistent with a tetrameric dumbbell structure formed by a central N-terminal domain connected to peripheral C-terminal domains via flexible coils. Electron Microscopy data support the dumbbell-shape arrangement observed in AFM micrographs. In addition, AFM images of CtIP-DNA binding reactions show the formation of long molecules as a consequence of association of shorter DNAs by CtIP acting as a bridge. Bridging events are dependent on [hCtIP] and on the oligomeric state of hCtIP. Interestingly, in vitro fluorescence anisotropy studies indicate a higher CtIP avidity for complex DNA ends. All together, we propose a model where CtIP binds DNA at internal positions and slides along the duplex before being captured at DNA non-canonical ends (Wilkinson, Martin-Gonzalez et al. 2018).

Davies, O. R., J. V. Forment, M. Sun, R. Belotserkovskaya, J. Coates, Y. Galanty, M. Demir, C. R. Morton, N. J. Rzechorzek, S. P. Jackson and L. Pellegrini (2015). "CtIP tetramer assembly is required for DNA-end resection and repair." Nat Struct Mol Biol **22**(2): 150-157.
Wilkinson, O. J., A. Martin-Gonzalez, H. J. Kang, S. J. Northall, D. B. Wigley, F. Moreno-Herrero and M. S. Dillingham (2018). "Human CtIP forms a tetrameric dumbbell-shaped particle which binds and bridges complex DNA end structures for double-strand break repair." bioRxiv.

P-411

Single-molecule force measurements show that ribosomal proteins uL4 and uL24 mechanically stabilize a fragment of 23S rRNA essential for ribosome assembly.

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The ribosome is the large molecular machine that synthetizes the proteins in all living cells. It is remarkably conserved among species and a major target of antibiotics. Ribosome assembly is a highly efficient and cooperative process, but is still poorly understood in spite of many efforts. Using single-molecule force measurements, we have investigated the initial step of the *Escherichia coli* large ribosomal subunit assembly - involving an essential 23S rRNA fragment and two ribosomal (r-) proteins, uL4 and uL24.

The 79-nucleotides-long RNA fragment was incorporated approximately in the middle of a ~5 kilobase-pairs RNA/DNA hybrid duplex molecule, this in order to be easily manipulated with a dual-beam optical trap. The resulting molecular construction was attached to two micrometer-sized beads and each bead was separately captured by one of the two optical traps. Mechanical unfolding of the RNA fragment, either in the absence or in the presence of the r-proteins (uL4, uL24 or both), was obtained by increasing the distance between the traps, one of them being slowly moved while the other was held fixed and used to measure the force. In order to accurately analyze the data collected in these single-molecule force experiments, we implemented a new method which uses Hidden Markov Modeling (HMM). From the raw data obtained by manipulating single RNA/DNA molecules, this HMM procedure allowed us to reproducibly map the intermediate states visited by the RNA fragment when it was unfolded in the presence or absence of interacting r-proteins.

We show that each one of the two r-proteins, uL4 and uL24, individually stabilizes the rRNA fragment. Moreover, this mechanical stabilization is strongly enhanced when both proteins are bound simultaneously. We also find that the two proteins bind cooperatively to the rRNA fragment. Overall, we show that uL4 and uL24 act together as cooperative RNA-clamps to mechanically stabilize their target 23S rRNA fragment, and we argue that the combined action of the two r-proteins is of general importance to initiate large ribosomal subunit assembly (Geffroy et al. (2019) **RNA**, 25, 472-480).

P-412

Quantitative assessment of tip effects in single-molecule high-speed atomic force microscopy

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High-speed atomic force microscopy (HS-AFM) has become a powerful tool for investigating the dynamics of biomolecular processes at a single-molecule level [1,2]. Because of its time resolution down to less than 1 sec per frame, HS-AFM enables the real-time visualization of numerous biological phenomena, such as the motion of molecular motors, the self-assembly of protein complexes, and the activity of enzymes. However, it remains an open and somewhat controversial question, how the recorded biomolecular dynamics are affected by the rapidly scanned AFM tip [3]. While tip effects are commonly believed to be of minor importance when investigating systems governed by comparatively strong interactions with dissociation constants (K_d) in the nM range, weaker interactions may significantly be disturbed by the forces exerted on the sample during scanning [3].

Here, we quantitatively assess the role of tip effects during HS-AFM in strongly binding biomolecular systems by employing a DNA origami-based single-molecule assay. DNA origami substrates were decorated with molecularly defined arrangements of biotin modifications to facilitate the mono- and bidentate binding of streptavidin. HS-AFM is used to determine mono- and bidentate binding yields in dependence of various scan parameters, such as scan rate and amplitude setpoint. Despite the fact that monodentate streptavidin-biotin binding has a K_d in the fM range is thus among the strongest non-covalent interactions known, our results show a clear dependence of both mono- and bidentate binding yields on the scan parameters. In particular, a continuous decrease of the equilibrium binding yield with increasing scan rate is observed. While the yields obtained at low scan rates are consistent with results obtained from conventional AFM measurements under dry conditions, scan rates of 50 Hz and higher result in the rapid reduction of both mono- and bidentate binding events, even at high amplitude set points around 90 %. Our results thus demonstrate that even for strongly interacting biomolecular systems such as streptavidin-biotin, tip interactions during HS-AFM imaging may induce sizeable and even dramatic effects.

[1] T. Ando *et al.* Annu. Rev. Biophys.;2013;42;393–414
[2] A. Rajendran *et al.* Chem. Rev.;2014;114 (2);1493–1520
[3] A. Nievergelt *et al.* Nat. Nanotech.;2018;13;696–701

P-413

Sensing of nanoparticles and biomolecules using an AFM controlled, cantilever embedded nanopore

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Scanning ion-conductance microscopy (SICM) is a well-established technique for the measurement of topographical properties or surface charges on the nanoscale [1]. Those measurements are based on the ionic current through a nanopore, caused by an externally applied electrical potential, superimposed by other effects, such as ion rectification and/or surface charges. Prior we reported a SICM setup that is built on an AFM, together with a micro-channeled cantilever with an opening in the nanometer range at the apex of the cantilever tip [2].

Here we use a SICM setup, in order to measure the translocation of nanoparticles and DNA through a, modified nanopore. The translocation is measured by monitoring the ionic current through the tip of the AFM cantilever, which is measured by two electrodes; one is placed inside the microchannel of the cantilever and a reference electrode in aqueous solution. We expect to obtain spatially localized information about the size of nanoparticles and maybe even the sequence of DNA molecules similarly to the specifically designed solid state and biological nanopores that already have been used for the measurement of DNA translocation [3]. Preliminary results indicate that the translocation of single 20 kbp DNA through the tip opening can be measured..

This technique is a new approach for DNA sensing and later on also could be extended to examine other biomolecules.

[1] D. Perry, R. Al Botros, D. Momotenko, S. L. Kinnear, and P. R. Unwin, *ACS Nano*, vol. 9, no. 7, pp. 7266–7276, 2015.
[2] D. Ossola, L. Dorwling-Carter, H. Dermutz, P. Behr, J. Vörös, and T. Zambelli, *Phys. Rev. Lett.*, vol. 115, no. 23, pp. 1–5, 2015.
[3] B. M. Venkatesan and R. Bashir, *Nat. Nanotechnol.*, vol. 6, no. 10, pp. 615–624, 2011.

P-414

Mechanical stiffening of human rhinovirus by capsid-binding antiviral drugs

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Human rhinovirus (HRV) is the major causative agent of common colds leading to important economic losses each year, and is also associated with more severe diseases. There is a renewed interest in developing effective anti-HRV drugs, but no such drug has been approved so far. We have investigated the suggestion that the antiviral action of drugs that specifically bind into capsid hydrophobic cavities (pockets) may be ultimately related to changes in virus stiffness. Mechanical analysis using atomic force microscopy shows that filling the pockets with specific chemical compounds dramatically stiffen the virus. Drug-mediated stiffening occurs at regions distant from the pockets and involved in genome uncoating, possibly through a subtle overall structural rearrangement. The results suggest a linkage between increased mechanical stiffness of HRV by pocket-filling antiviral drugs, and reduced viral equilibrium dynamics (breathing) leading to impaired infectivity. From a fundamental physics perspective, these drugs may exert their biological effect by decreasing the deformability of the virion. This conclusion encourages the design of novel drugs against different viruses that may inhibit infection by mechanically stiffening the viral particle.

P-415

Durability analysis of the cryopreservation of DNA origami nanostructures

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DNA origami nanostructures as first presented by Rothemund [1] have demonstrated great potential as functional platforms for various biomedical applications. Due to their broad applicability in technology and science, stability analysis of DNA origami structures is of high importance [2]. While in the academic setting, experiments are typically performed using freshly prepared DNA origami samples, technological applications will require the storage of structurally intact DNA origami for extended periods of time. Among preservation processes, lyophilization has been discussed as a promising method for long term preservation of DNA origami structures [3] at ambient temperatures. One drawback of this method is that it requires temperatures of down to -80 °C which may not be reached using typical lab equipment. In another work, freezing has been shown to reduce the lifetime of DNA molecules under tension [4]. Here, we thus investigate the durability of triangular DNA origami structures under repeated freezing and thawing using single-molecule atomic force microscopy. The DNA origami triangles maintain their structural integrity over many freezing and thawing cycles, up until a certain threshold after which the fraction of intact DNA origami is rapidly decreasing. We also evaluate the possibility to further increase this threshold by addition of cryoprotectants.

[1] P. W. K. Rothemund, Nature. 2016, **440**, 297-302.
[2] S. Ramakrishnan et al., Comp. Struct. Biotechnol. J. 2018, **16**, 342-249.
[3] B. Zhu et al., ACS Applied Materials & Interfaces 2017, **9**(22), 18434-18439.
[4] W. Chung et. al., J Biol Phys. 2017, **43**(4), 511–524

P-416

Concurrent Atomic Force Spectroscopy

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Force-spectroscopy by Atomic Force Microscopy (AFM) is the technique of choice to measure mechanical properties of molecules, cells, tissues and materials at the nano and micro scales. However, unavoidable calibration errors of AFM probes make it cumbersome to quantify modulation of mechanics. Here, we show that concurrent AFM force measurements enable relative mechanical characterization with an accuracy that is independent of calibration uncertainty, even when averaging data from multiple, independent experiments. Compared to traditional AFM, we estimate that concurrent strategies can measure differences in protein mechanical unfolding forces with a 6-fold improvement in accuracy or a 30-fold increase in throughput. Prompted by our results, we demonstrate widely applicable orthogonal fingerprinting strategies for concurrent single-molecule nanomechanical profiling of proteins.

P-417

Quantifying protein oligomerization directly in living cells: a systematic comparison of fluorescent proteins and application to Influenza A virus infection

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Fluorescence fluctuation spectroscopy has become a popular toolbox for minimally invasive studies of molecular interactions and dynamics in living cells. The quantification of e.g. protein oligomerization and absolute concentrations in the native cellular environment is highly relevant for a detailed understanding of complex signaling pathways and biochemical reaction networks. Of particular interest in this context is the molecular brightness, which serves as a direct measure of oligomerization and can be easily extracted from temporal or spatial fluorescence fluctuations. However, fluorescent proteins (FPs) typically used in such studies suffer from complex photophysical transitions and limited maturation, potentially inducing non-fluorescent states, which strongly affect molecular brightness measurements. Although these processes have been occasionally reported, a comprehensive study addressing this issue is missing.

Here, we investigate the suitability of widely-used FPs (mEGFP, mEYFP and mCherry), and novel red FPs (mCherry2, mRuby3, mCardinal, mScarlet and mScarlet-I) for the quantification of oligomerization based on the molecular brightness, as obtained by Fluorescence Correlation Spectroscopy (FCS) and Number&Brightness measurements in living cells. For all FPs, we measured a lower than expected brightness of FP homo-dimers, allowing us to estimate, for each fluorescent label, the probability of fluorescence emission in a simple two-state model. By analyzing higher FP homo-oligomers, we show that the oligomeric state of protein complexes can only be accurately quantified if this probability is taken into account. Further, we provide strong evidence that mCherry2, an mCherry variant, possesses a superior apparent fluorescence probability, presumably due to its fast maturation. We conclude that this property leads to an improved quantification in fluorescence cross-correlation spectroscopy measurements and propose to use mEGFP and mCherry2 as the novel standard pair for studying biomolecular hetero-interactions.

Finally, we apply brightness and cross-correlation analysis to quantify protein-protein interactions involved in the nuclear export of progeny Influenza A virus genome segments (vRNPs). For the first time, we were able to demonstrate the ability of the viral nuclear export protein (NEP), a key-regulator in the nuclear export of vRNPs, to dimerize in the cytoplasm of an infection relevant lung epithelial cell-line.

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Using Fluorescence Correlation Spectroscopy (FCS) to measure protein stabilization by PNIPAm nanoparticles under mechanical stress

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Protein aggregation presents a key challenge in the development of biological formulations as it can have an impact on product quality of the final therapeutic protein in terms of efficacy and immunogenicity. In the context of biopharmaceutical manufacturing, some steps during downstream processing (DSP) and storage of therapeutic proteins have been shown to cause unwanted protein aggregation.[1-2] This issue can sometimes be solved by the use of appropriate stabilizing additives. PNIPAm (poly(*N*-isopropylacrylamide)) is a well-known biocompatible, thermoresponsive polymer that can form nanoparticles (NP) in solution and could be used as a potential thermally modulated stabilizing agent. Here we investigated the use of Fluorescence Correlation Spectroscopy (FCS) for the discrimination of protein aggregates from stable protein-PNIPAm complexes, where the protein was Alexa Fluor 405 labelled human serum albumin (HSA). 20 nM labelled HSA solutions were analysed by FCS in the absence of stirring, and under high-speed stirring (approx. 1,000 rpm) with and without PNIPAm. HSA:PNIPAm molar ratios varied from 1:10 to 1:10,000, the latter corresponding to 0.8% w/v PNIPAm ($M_n \approx 40$ kDa). For the control sample (HSA stirred in the absence of PNIPAm) no clean autocorrelation functions (ACF) were recorded due to extensive aggregate formation and thus reliable diffusion parameters couldn't be retrieved. However, the ACF of the HSA-PNIPAm solutions resembled that of free, unstirred HSA in solution, indicating some degree of interaction and stabilization. The 1:10,000 HSA-PNIPAm sample diffusion coefficient was shorter than that of free, unstirred HSA: 30±3 $\mu m^2/s$ versus 48±2 $\mu m^2/s$, indicating a very significant protein-PNIPAm interaction. As a consequence, the 1:10,000 HSA-PNIPAm sample had a significantly larger hydrodynamic radius R_h of 7.2 ± 0.2 nm compared to 4.5 ± 0.3 nm for free HSA. This indicated that even under extreme mechanical stress, HSA did not aggregate in solution when PNIPAm was present. Our current estimate is that the stable HSA-PNIPAm NP complexes, contain 1 to 2 PNIPAm coils acting as stabilizers.

References

1. Sharma,B., *Biotechnology Advances* **2007,** *25*, 310-317.
2. JeffreyL. Cleland,M.F.P., Steven J. Shire, *Critical Reviews in therapeutic Drug Carries Systems* **1993,** *10* (4), 307-377.

P-419

Single Molecule Dynamics of an Hsp70 Chaperone

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Huge machinery of specialized proteins, the molecular chaperones, has evolved to assist protein folding in the cell. One major cellular system to prevent aggregation and premature folding of proteins is constituted by molecular chaperones of the conserved Hsp70 family. Hsp70s are highly dynamic nanomachines that modulate the conformations of their substrate polypeptides by transiently binding to short, mostly hydrophobic stretches. To overcome the obstacle to conformational investigations of the Hsp70-substrate interaction, the transient nature of this interaction, we fused the fragment of the sigma-32 peptide, via a 30-residue long linker to the C-terminus of DnaK. We employ high-precision dual-beam optical tweezers setup to observe the kinetics of the tethered peptide in the presence of nucleotides and other co-chaperones to understand the DnaK/DnaJ/GrpE foldase machinery. Under the constant mechanical load, the rapid kinetics of tethered peptide and peptide-induced ATP-hydrolyzed conformation of DnaK was observed. Our concentration series experiment of GrpE also shows that GrpE binding to this complex stimulates the exchange of ADP to ATP and DnaK remain in its low-affinity conformation. Furthermore, non-hydrolysed mutant of DnaK (T199A) measurements in the presence of DnaJ shows that in high concentrations, DnaJ act as a substrate. Our results provide the basis for the Hsp70 chaperone cycle and its regulation in the presence of the substrates and co-chaperones.

P-420

Nano-scale topoplogy of voltage gated Ca2+ channels: an in vivo single molecule analysis

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Information processing in our brains depends on precisely controlled Ca^{2+}-activated exocytosis of neurotransmitter (NT) containing synaptic vesicles (SV) from release sites within synaptic active zones (AZs). These AZs are covered by a complex array of scaffolding proteins which couple Ca^{2+}-channels to SV-release site generating molecules. In order to simulate Ca^{2+} dynamics and levels at release sites, knowledge about Ca^{2+}channel numbers and localizations within AZs and their relation to release sites is essential. Using step-wise photobleaching of GFP tagged Ca^{2+} channels at the larval *Drosophila* neuromuscular junction (NMJ), we count the number of Ca^{2+} channels at the single AZ level. Preliminary data suggest that Ca^{2+}-channel numbers of single AZ vary from 8 to 20 with on average 11 Ca^{2+}-channels per AZ. Based on this result we could also predict the spatial position of each Ca^{2+} -channel at the active zone. Thus, we have begun to quantify and link live imaging of synaptic Ca^{2+}-channels in a systematic way to exploit the genetic possibilities of the Drosophila system to quantify this number in wild type and mutated animals.

P-421

A relaxase-DNA complex is translocated through a nanopore in a multi-step process

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The dissemination of antibiotic resistance genes by bacterial conjugation requires a sophisticated machinery, named Type IV secretion system (T4SS) (1). In our model, conjugative plasmid R388, DNA is transferred through the secretion channel in complex with TrwC, a protein that cleaves one of the DNA strands and it is then transferred to the recipient cell through the secretion channel (2). Given the large size of TrwC (over 100 kDa in R388 model), it is unlikely that this protein-DNA complex can pass through the channel in a native state. In this work, our main goal has been to reproduce the secretion of this nucleoprotein complex by studying the co-translocational unfolding of a TrwCR–DNA complex through a nanopore. These covalent protein-DNA complexes correspond to TrwCR bound to the DNA through residues Tyr18 or Tyr26, respectively. After the cleavage reaction, these nucleoprotein complexes were transferred across a membrane by pulling the protein-DNA complexes through an α-haemolysin pore (3). The results showed that the nucleoprotein complexes pass through the nanopore in an unfolded state, allowing the analysis of this process at a single-molecule level. Close inspection of the current traces suggests that the co-translocational protein unfolding of TrwCR is a multi-step process. Moreover, TrwCR Y18-DNA and TrwCR Y26-DNA complexes present different unfolding kinetics. TrwCR Y18-DNA complex is translocated more effectively through the pore, which perfectly correlates with the accepted idea that TrwC is translocated to the recipient cell bound to the DNA through Tyr18. Therefore, the structure presented to the pore is essential to determine the effectiveness in the translocation process. Our results provide a new tool for understanding the mechanism of translocation in many different secretion systems.

REFERENCES

1. Cabezón, E., Ripoll-Rozada, J., Peña, A., de la Cruz, F., and Arechaga, I. (2014). Towards an integrated model of bacterial conjugation. FEMS microbiology reviews *39*, 81-95.
2. Guasch, A. et al. Recognition and processing of the origin of transfer DNA by conjugative relaxase TrwC. *Nat Struct Biol* **10**, 1002-1010 (2003).
3. Rodriguez-Larrea, D. & Bayley, H. Protein co-translocational unfolding depends on the direction of pulling. *Nat Commun* **5**, 4841 (2014).

P-422

CAN TIGHT BINDING AND STRUCTURAL DISORDER COEXIST IN PROTEINS?

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Cap-dependent initiation of translation is regulated by the interaction of the eukaryotic translation initiation factor 4E (eIF4E) with the 120-residue disordered eIF4E binding protein 2 (4E-BP2) in a phosphorylation-dependent manner. Previous NMR studies have shown that 4E-BP2 interacts with eIF4E at two distinct sites, via a transient α-helical structure at the canonical binding site and a disordered secondary binding site ~20 residues away. Phosphorylation of 4E-BP2 at 5 distinct sites decreases its binding affinity for eIF4E by a factor of ~4000, partially due to the formation of a 4-stranded β-sheet motif that sequesters the canonical binding sequence. Single-molecule Förster resonance energy transfer (smFRET) data acquired for 4E-BP2 between residues 32 and 91 show an increase of FRET efficiency binding to eIF4E. Multiparameter data analysis indicates that the eIF4E-bound protein adopts extended and, quite surprisingly, highly dynamic conformations that wrap around eIF4E. Intermolecular smFRET experiments with evanescent-field excitation were designed to study the dynamics of the interaction between donor-labelled, surface-immobilized eIF4E and acceptor-labelled 4E-BP2 in solution. FRET efficiency-time trajectories from thousands of individual molecules were used to derive the distributions of ON and OFF binding times and derive the rates of binding and release. Our single-molecule study constitutes new and stronger evidence for the dynamic bipartite interface between 4E-BP2 and eIF4E and provides a mechanistic/thermodynamic model for high affinity in the presence of structural disorder in protein.

P-423

Propanol isomers cause markedly different changes in the higher-order structure of DNA: Unveiling the reason on the protocol to isolate DNA

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In the present study, we measured the changes in the higher-order structure of genomic DNA molecules in the presence of alcohols by using single-DNA observation with fluorescence microscopy, with particular focus on the different effects of 1-propanol and 2-propanol. The results showed that, with an increasing concentration 1-propanol, DNA exhibits reentrant conformational transitions from an elongated coil to a folded globule, and then to an unfolded state. On the other hand, with 2-propanol, exhibits monotonous shrinkage into a compact state. The propanol isomers also had different effects on the changes in the secondary structure of DNA, as revealed by circular dichroism (CD) measurements. With 1-propanol, DNA maintains a B-form secondary structure. An A-like conformation appears with the addition of 2-propanol. Thus, DNA molecules are more effectively condensed/precipitated with 1-propanol than with 2-propanol.

P-424

Solid state and biological nanopore sensing using a portable mini reader and flow cell

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Nanopores are interesting sensors receiving a wide attention in the past two decades thanks to the high sensitivity and versatility of their sensing technique. This led to a rapidly growing field of different applications, from water quality monitoring, environmental samples analysis, agriculture and disease markers in medicine. However electronics capable of read out small signal (pA to nA range) are bulky and not scalable for portable device applications.

We present a compact, portable and easy to use Nanopore Reader (NPR) device, intended for laboratory experiments and development of sensors for the detection of single molecules and biomarkers in solutions. The NPR platform enables to perform experiments ranging from resistive pulsing, nanopores conductance and stability, to the more complex analysis of translocations of DNA and other analytes. The NPR can hold two different miniaturized flow-cell with chambers for fluidic handling:

- one flow-cell for solid state nanopore chip;
- one BLM chip for artificial Bilayer Lipid Membranes formation using a polyimide septum as substrate.

NPR has dimensions of 101 x 44 x 18 mm, it is USB powered and can perform real-time analysis using the easy to use control software, enabling the fast characterization of nanopores conductance by I/V curves plot, as well as the event detection and generation of histograms for blockade sizes and dwell times.

The Nanopore reader was tested under different conditions:

- with solid state nanopores used to detect and characterize DNA fragments
- with biological nanopores (such as alpha-hemolysin) hosted into BLM created into the BLM chip.

We report ultra low noise features suitable for quick and easy characterizations of nanopores and resistive pulse sensing devices, for both educational and research purposes and the development of portable sensor devices.

P-425

Dynamics of a Mechano-stable Protein with High Force Magnetic Tweezers

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Gram-positive pathogens use micrometer-long single-protein structures to adhere to target tissues. These organelles, termed pili, provide the bacteria with a reliable anchoring which prevents their detachment under mechanical perturbations. Pili proteins contain internal isopeptide bonds, which increase their mechanical stability and limit their extension. Under force, only a small polypeptide region—termed isopeptide-delimited loop (IDL)—can be extended, acting as a shock absorber, which protects the protein and hence the pilus integrity. Atomic Force Microscopy (AFM) has been the only single-molecule force spectroscopy technique able to explore these highly stable proteins. Our previous studies on the pilus protein FimA from the oral pathogen *Actinomyces oris*, revealed that forces >600 pN were required to extend its IDL, placing this protein among the most mechanically stable ever studied. Nevertheless, further delving into the IDL properties remained elusive due to the instability and low force sensitivity of the AFM. On this matter, magnetic tweezers (MT) technique offers nm and pN resolution, and days-long stability, but it is limited to forces <120 pN. To overcome this limitation, we implement HaloTag and split-protein chemistries for the covalent anchoring of pilus proteins between a superparamagnetic bead (M450) and a glass surface. Employing the unfolding extensions of protein L as a molecular ruler, we measured the accessible range of forces to be from 2 pN to 300 pN. With this advance, we submit a FimA polyprotein to long stretching-relaxation cycles, resembling the perturbations experienced by a pilus during mastication or teeth brushing. Applying 200-250 pN pulses, we can unfold the IDLs in <50 s, revealing two populations of IDLs with different stabilities, suggesting a time-dependent maturation which confirms our previous observations. Previously unattainable with AFM, we are now able to observe the IDLs contractions at forces <15 pN, enabling us now to characterize the shock absorbing properties of the bacterial pilus. Our work demonstrates that MT can now be used for applications previously only reachable by AFM, and facilitates future research oriented to address the mechanics of bacterial adhesion and to develop antibacterial compounds targeting the pilus mechanical features.

P-426

DNA nicking and unwinding by the RepC-PcrA complex in Rolling-Circle Replication

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The rolling-circle replication mechanism is the most common mode of replication of small bacterial plasmids carrying antibiotic resistance. It is initiated by the binding and nicking of a replication initiator (Rep) protein to the double-stranded origin of replication (*dso*). After the nicking, duplex unwinding is then performed by the PcrA helicase, which processivity is critically promoted by its interaction with Rep [1]. How Rep and PrcA proteins interact to nick and unwind the duplex in not fully understood. We have previously shown that nicking activity of *Staphylococcus aureus* RepC depends on the negative supercoiling degree of the DNA [2]. Here, we use Magnetic Tweezers to monitor the real-time dynamics of DNA unwinding by *Staphylococcus aureus* PcrA helicase and its relation with the nicking activity of RepC at the single-molecule level. We find that PcrA is prone to stochastic pausing resulting in average translocation rates of 30 bp s^{-1} and a mean pause-free unwinding velocity of 50 bp s^{-1}. Pauses duration follows a single exponential distribution with a pause lifetime of 8.9 s. Addition of SSB proteins, did not affect PcrA translocation velocity but slightly increased its processivity. Analysis of the supercoiling degree of DNA required for RepC nicking and the delay time between RepC nicking and DNA unwinding, suggests that RepC and PcrA can form a protein complex on the DNA binding site before nicking. A comprehensive model that rationalizes these findings is proposed.

1. Soultanas, P., et al., *Plasmid replication initiator protein RepD increases the processivity of PcrA DNA helicase.* Nucleic Acids Res, 1999. **27**(6): p. 1421-8.
2. Pastrana, C.L., et al., *Force and twist dependence of RepC nicking activity on torsionally-constrained DNA molecules*. Nucleic Acids Res, 2016.

P-427

Single molecule FRET studies on nucleosomes

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To understand how DNA compaction into nucleosomes controls genome accessibility, one needs information about the dynamics. This requires experimental methods that probe the structure and its fluctuations not only in one static, frozen state but in solution with as little constraints as possible. Far from a simple concept of DNA unrolling and/or histones dissociating from nucleosomes, we are investigating individual steps of the opening of the chromatin structure, characterizing their dependence on DNA sequence, histone variants, mutations and modifications.

We summarize our findings on mono and small oligonucleosomes. FRET measured in bulk or on single molecules enables us to determine distances between well selected parts of the nucleosome and follow their changes. We found that the opening angle and distance between the linker DNA arms decreases with salt in the physiological regime and also upon the addition of linker histone H1. No crossing of linker DNAs has been observed in solution contrary to presumptions. To understand the structure of nucleosomes we disassembly them by salt induced destabilization and use alternative structures to find the key interactions. We have shown that acetylation, an important epigenetical marker influences the structure and stability of nucleosomes on a histone specific way. Analyzes of multiparameter fluorescence signals allowed us to propose a detailed pathway of nucleosome disassembly characterized with dynamical parameters. We could demonstrate the effect of the asymmetric DNA sequence on the disassembly path. We could demonstrate allosteric effects on the histone tails due to a point mutation in the core octamere.

P-428

Stoichiometry and mechanism of action of the minimal fission machinery assembled by Dynamin 1

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Dynamin 1 (Dyn1) is a large GTPase orchestrating membrane fission in endocytosis. Dyn1 forms a tight collar around a neck of an endocytic vesicle and then uses energy of GTP hydrolysis to constrict and severe the neck. The stoichiometry and mechanism of action of Dyn1 fission machinery remains a subject of debate. To quantify how many Dyn1 molecules are needed for membrane fission we reconstituted the reaction in a minimal system containing lipid membrane nanotubes and Dyn1-eGFP. We verified that eGFP addition had minor effect of on membrane-mediated oligomerization and GTPase activities of Dyn1. We then resolved the real time compactization and deformations of metastable Dyn1-eGFP oligomers assembling on the nanotubes under constant GTP turnover. At low bulk concentration of the protein, the oligomers formed on the nanotubes were generally smaller than a single helical rung, yet they produced stochastic membrane scission. Thus, fission does not require uniform radial constriction, as our results implicate the local torque created by small Dyn1 oligomers as a major driver of membrane fission.

P-429

Mechanopharmacology: High-throughput search of mechanoactive molecules against HIV-1 entry

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Cell-surface receptors are essentials in the communication between cells and their extracellular space. CD4 is a coreceptor present in T lymphocytes membrane and it is required in the activation process of these cells, but also, is the primary receptor of HIV-1. It has been suggested that mechanical forces can play an important role in this infection mechanism, allowing conformational and chemical changes on CD4 during viral attachment, but the exact mechanism of how these mechanical forces affect at the dynamic of CD4 is not fully understood. Here we show how three different mechano-active molecules enhance mechanical stability of CD4 and prevent these structural and chemical changes needed for a successful virus infection. We use high-throughput virtual screening and docking to search in a chemical library of more than a million compounds that interact with mechanically relevant parts of human HIV-1 receptor CD4. We have identified three molecules capable of increasing the mechanical stability of CD4 and prevent HIV-1 entry into CD4+ cells. We have used single-molecule force spectroscopy and analysis on HIV-1 infectivity to probe it. We propose these molecules as an example of mechanodrug opening the possibility of investigating diseases and disorders through the intervention in the mechanical properties of the proteins involved.

P-430
Single Molecule FRET studies of Cellulosomal Cohesin-Dockerin interaction: a story of two exclusive binding modes, a cohesin-dockerin code and allosteric control.
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Cellulosomes are huge multienzyme complexes produced by some anaerobic bacteria for the degradation of cellulose. Spatial and temporal coordination of different enzymatic activities makes the cellulosomes extremely efficient machineries for the degradation of highly recalcitrant lignocellulose. Cohesin-dockerin interaction between scaffolding and catalytic units plays a key role in the auto-assembly of theses complexes, with the particularity that the high degree of similarity in the dockerin helices suggests the presence of two different binding modes. Using single-molecule FRET measurements, we show for the first time the existence of both binding modes in different cohesin-dockerin pairs without any doubts. Surprisingly and in contrast to what was previously thought, different cohesin and dockerin pairs are not equivalent, displaying preferential binding to alternative binding modes. This implies that a cohesin-dockerin code exists and therefore the finding has important implications on the final 3D assembly of cellulosomes. Besides, binding mode selection is allosterically controlled by a dockerin terminal clasp, formed by two residues far away from the interaction surface. Using single molecule kinetics measurements and molecular dynamics simulations, we show that this clasp thermodynamically stabilizes the binding by dockerin Helix 1.

P-431
Single vesicle detection to study protein-lipid interactions
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The pre-synaptic protein α-synuclein (α-syn) can be found in amyloid plaques in patients with Parkinson's Disease (PD). However, the mechanisms behind how α-syn is involved in the course of PD is still unknown. An important event seems to be the interaction between α-syn and lipids, which are the main building block of the membrane surrounding each cell. In this project, single, fluorescently-labelled lipid vesicles are used to obtain information about vesicle fusion and exchange of lipids between different vesicles, both due to interactions with α-syn. This is done using vesicles containing lipids labelled with two dyes forming a fluorescence resonance energy transfer (fret) pair. Lipid exchange or fusion between unlabeled vesicles and vesicles labeled with a fret pair results in a change in the fret signal due to an increase in distance between the fret pair. This makes it possible to distinguish between lipid exchange and vesicle fusion events, which are quantified using a calibrated relation between fret signal and lipid mixing, obtained from samples with known dye concentrations.
During the project, several vesicle systems have been used to confirm the validity of the method; (i) mixtures of vesicle samples containing different dye concentration, (ii) synthetic vesicles with incorporated native material and (iii) mixtures of labeled and unlabeled synthetic vesicles (either sonicated or in presence of α-syn.) The samples were imaged with a two-color total internal reflection fluorescence microscope to optimize the detection conditions for the studies. An interactive MATLAB program that detects the single vesicles and measures the fluorescence intensities was also developed in order to analyze the data.
Our preliminary data shows that the experimental assay can obtain information that it is not possible to obtain using traditional bulk-based techniques, which measures an average value over many vesicles. We believe that the presented assay is a general tool to study protein-lipid interactions and lipid exchange, which can be implemented on most fluorescence microscopes and analyzed using the developed MATLAB program.

P-432 **Scattering labels for ultrafast tracking of biomolecular machinery**
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The study of the function of biological structures has attracted a great deal of attention in the last years. In that respect, tracking single-protein behavior by means of labels and localization microscopy has been established as the method of choice, improving our understanding of life at the single-molecule level.
One of the promising tools for this purpose is the interferometric scattering microscopy (iSCAT), which relies on scattering labels for creating strong contrasts from the background signal, via elastic scattering processes [1,2].
Among all the possible scatterers, gold nanoparticles (GNPs) have shown a great potential thanks to their ease of synthesis and well-known surface chemistry, especially relevant for the conjugation of the GNP with a certain biomolecule (e.g. proteins or DNA strands) [3]. These surface functionalization processes typically rely on the chemisorption of highly-specific molecules, known as a linker, to further anchor the target biomolecule selectively on the GNP surface through covalent bonds.
However, some applications may require a strict control in the amount of linkers on the GNP surface to avoid cross-linking effects or other possible sources of uncertainty in the measurement. Optimization of this process is, thus, a critical part to obtain reliable results.
In this work, we focus on the preparation and optimization of GNPs for tracking different fast biological events in order to achieve a nanometer precision with a microsecond time resolutions. The applicability of these GNPs is demonstrated by tracking the microtubule disassembly and the movement of microtubule-associated proteins, such as PCR1.

References

1- M. Piliarik and V. Sandoghdar, Nature Commun., 5, 4495 (2014).
2- K. Holanová, M. Vala and M. Piliarik, Optics & Laser Technology, 109, 323-327 (2019).
3- Y.-C. Yeh, B. Creran and V. M. Rotello, Gold nanoparticles: preparation, properties, and applications in Bionanotechnology, Nanoscale, 4, 1871, (2012).

P-433
Single Influenza virus particle detection for diagnosis
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Influenza is epidemic each year, including hundreds of deaths related to it, with hundreds of thousands of deaths worldwide. Therefore, although measures against influenza are being considered worldwide, no effective measures have been established. One of the causes is the low sensitivity of influenza diagnosis. A common diagnostic method is detection of the virus by immunochromatography, but accurate diagnosis can not be made until about 24 hours have passed after the fever. Therefore, we have developed a digital influenza detection method as a highly sensitive virus detection method that is simple to replace the immunochromatography method. The principle of this method is that the influenza virus and the fluorescent substrate are traped in a micro-reactor and detected using the neuraminidase activity possessed by the influenza virus. As a result of observing the micro-reactor, a reactor emitting fluorescence was confirmed. In addition, the number of reactors emitting fluorescence changed depending on the concentration of virus, and when oseltamivir, which is an inhibitor of neuraminidase, was added, the fluorescence disappeared. This indicates that the observed fluorescence is from influenza virus. In addition, since a reactor that does not emit fluorescence and a reactor that emits fluorescence are observed simultaneously, it is strongly suggested that single virus particles are trapped in the container that emits fluorescence. This indicates that digital detection of influenza virus is possible. Next, determination of detection limits was attempted by serial dilution of samples of known virus concentration. Then, in this experiment, it was found to be 10^3 PFU / ml. This is 10,000 times more sensitive than the detection limit by the existing immunochromatography method. In addition, when compared with the RT-PCR method for the same sample, the detection sensitivity was comparable. Moreover, when the time to detection was also examined, that it can detect in 5 minutes or less. From these things, it turned out that it can become a virus detection method far exceeding existing methods. In addition, we also developed a detection device based on a smartphone, so we would like to report it together.

P-434

The Organization of the BCL-2 Family Member BOK in Lipid Environment

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The BCL-2 family of proteins are the key mediators of the intrinsic pathway of apoptosis by triggering mitochondrial outer membrane (MOM) permeabilization. Dysregulation of this sophisticated protein interaction network has been implicated in various diseases, including cancer. The BCL-2 ovarian killer (BOK) protein has sequence and structure similarity to both pro-apoptotic effectors and anti-apoptotic proteins. However, the role of BOK in apoptosis regulation is less understood. Recently, BOK core structure has been solved by X-ray crystallography and NMR, both reflecting to the protein structure in aqueous environments. MOM is regarded as the "playground" of the BCL-2 family proteins, as the key interactions take place there. Therefore, Studying the structure and function of these proteins is more relevant in the membrane environment. Here, we have examined the organization of BOK in membrane using single-molecule brightness experiments. To enable this method, expression and purification of a truncated form of BOK lacking the C-terminal tail were optimized to yield the protein in a monomeric form with high purity. Different model membrane systems were formulated and used to assess the function and binding preference of BOK. Sortase-mediated labelling was performed for the coupling of a fluorescent dye to the protein N-terminus with very high labelling efficiency and controlled stoichiometry. In addition, BOK-containing nanodiscs were produced using Styrene maleic anhydride polymer (SMA). The process were optimized for efficient reconstitution, purity and homogeneity. Nanodiscs provide a soluble bilayer system that mimic the native environment of the protein, and can be employed for functional and structural studies of membrane proteins. The combined results will pave the way to harvest structural information on BOK at different levels of detail and in a near-native environment.

P-435

Spatiotemporal Dynamic of Assembly and Activation of Class II Cytokine Receptors

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Class II Cytokines play a key role in activation and regulation of innate and adaptive immunity with particular implications for viral infections. While the downstream signaling pathways and negative feedback regulators of cytokine receptors are well characterized, the role of spatiotemporal dynamics in signal propagation remains obscure. Most prominently debated are the questions: (i) Are receptor subunits spatially (pre-)organized in the plasma membrane and how does ligand binding alter receptor organization; (ii) how is signal activation regulated by receptor organization in the plasma membrane as well as during endocytosis and endosomal trafficking? To tackle these questions, we are comparing several class II cytokine receptors, namely the type I, type II and type III interferon (IFN) receptors as well as the interleukin-10 (IL-10) receptor. With their partially overlapping, partially exclusive use of receptor subunits as well as JAKs and STAT effector proteins, these receptors are well suitable for systematically address these questions. Moreover, marked differences in plasma membrane dynamics and endocytic trafficking have been reported for these receptors. Using highly effective in situ labeling for single molecule fluorescence imaging, we have characterized diffusion and interaction in the plasma membrane of living cells for all four receptors. Co-localization and co-tracking analysis based on multi-color TIRF-imaging allowed to quantify receptor dimerization and oligomerization. These studies unambiguously reveal ligand-induced receptor dimerization in all cases, yet identified surprising differences in the dynamics of receptor diffusion and endocytosis, providing the future basis to correlate spatiotemporal receptor dynamics with signaling activity.

P-436

Mesoscopic Model for DNA G-quadruplex unfolding.

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The G-quadruplexes (G4) are non-canonical secondary DNA and RNA structures composed of four guanine basis bonded each other in quartets forming planes eventually piled in two, three or four layers. They are present both in vivo and in vitro cultures, and have important role in telomere end-protection, chromosome stability, aging control. Their folding patterns and structures are also found in eukaryotic promoter regions of oncogenes, making them increasingly recognized among chemists and biologists due to their potential applications in Nanomedicine as therapeutic targets in cancer treatments.

Optical and magnetic tweezers, as well as Atomic Force Microscopies, are employed to characterize not only the mechanical stability and unfolding dynamics of G-quadruplexes, but also to unveil structural intermediates not accessible to ensemble-average techniques due to their relatively low occurrence. The stability of the G-quadruplex structure is related, among the others, to the specific G-quadruplex conformation, and the presence of a cation between each of the G4 planes.

Although an increasing number experiments have been conducted with the purpose to finely analyze rupture profiles in single force-extension curves, the theoretical predictions remain difficult, due essentially to the long computational time required by atomistic simulations, which, moreover, use parameter values -- specifically the velocity at which one extreme of the quadruplex is pulled to induce the rupture -- orders of magnitude far away from the experimental values. With the aim to bridge the gap between experiment and theoretical expectations, we build a mesoscopic physical model of the G-quadruplex structure with a reduced number of degrees of freedom and a few effective potentials that permits to study the mechanical unfolding in a wider interval of time scales than those allowed in all-atom simulations, in particular under different pulling velocities. The subsequent analysis on the light of the most recent stochastic theories for rupture force -- as those of Bell-Evans-Richie, Evans-Hummer-Szabo, and Friddle-Noy-DeYoreo -- permit the estimations of the potential barriers and positions that characterize the energy landscape of the unfolding process.

In this communication the model will be presented together with its validation against the results of an unfolding experiment on RNA G-quadruplex pulled by an optical tweezer.

Tuesday 23rd July

BIOPHYSICS OF THE IMMUNE RESPONSE

P-437 (O-120)

Insights into the Molecular Mechanism of Lipids/Toll-Like Receptors Interaction

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Toll-like Receptors (TLRs) are the main protagonists of the innate immune system. Among them TLR2 and TLR4/MD2 recognize lipid patterns located in bacterial membrane and alert the immune system of pathogen invasion through stimulating secretion of pro-inflammatory cytokines. We showed that TLR4 and 2 are able to recognize also synthetic lipids, used as nucleic acid nanocarriers, and the mitochondrial lipid cardiolipin.

In silico analysis coupled with in vitro and in vivo experiments brought us to understand the molecular parameters for which a lipid would be inert or recognized by TLR2 or TLR4/MD2. More specifically, we demonstrated that short saturated di-acyl cationic lipids lipopolyamines (LPAs) activate TLR2 and 4, whereas longer saturated cationic lipids activate only TLR2(Lonez et al., 2015 CMLS; Pizzuto et al., 2017, 2018 J Control Release). Moreover the tetra-acyl mitochondrial lipid cardiolipin (CL) acts as a TLR4/MD2 agonist or antagonist depending on its unsaturation degree (Pizzuto et al. 2019 CMLS).

Although we found that all CL docked to the hydrophobic cavity of MD2, unfortunately, molecular docking failed to predict physiologically meaningful conformations or the pharmacology of CLs, revealing the limitations of such an approach with this family of molecules.

By contrast, docking of di-acyl LPAs in TLR2/TLR1 and TLR2/TLR6 was able to predict their activity, suggesting potential TLR2 binding modes reminiscent of bacterial lipopeptide sensing (natural TLR2/1/6 ligands). The saturated or mono-unsaturated LPAs share the ability of burying their lipid chains in the hydrophobic cavity of TLR2 and, in some cases, TLR1, at the vicinity of the dimerization interface. The cationic headgroups form multiple hydrogen bonds, thus crosslinking TLRs into functional complexes. According to our in silico analysis and in vitro data, TLR2 activation could no longer be achieved if both LPA chains were unsaturated. (Pizzuto et al., 2017 J Control Release)

We therefore recommend the use of unsaturated C18 chains for the synthesis of inert transfection agents or TLR4 antagonists. On the other hand, we recommend saturated LPAs in vaccine formulation as their immunostimulatory activity coupled to their carrier properties conferred good adjuvant properties as demonstrated by our in vivo studies(Pizzuto et al., 2018 J Control Release).

P-438 (O-121)

Super-resolution microscopy analysis of molecular interactions between epithelial cells and tissue-resident T cells in mouse epidermis

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Epithelial tissues, such as skin, gut or lung, are the first line of animal defence against external factors. Mammal epidermis is populated by a large number of immune-cells. In mouse these include immune monitoring γδ T cells, also known as dendritic epidermal T cells (DETC), which form a dense network in parallel to the network of Langerhans cells. Previously, a transmembrane-protein Skint1 expressed by keratinocytes was suggested to play a pivotal role in molecular interactions between T cells and surrounding epithelial cells. Here we applied a super-resolution microscopy approach to investigate the role of Skint1 in defining tissue homeostasis and cell functionality. Using Structured Illumination Microscopy, we were able to visualize Skint1 clustering in vicinity of T cell receptor (TCR) clusters at dendrite contact points of DETC, suggesting their direct interaction in tissue. The clustering of Skint1 on epithelial cell surface was further characterised in Skint1 mutant, Skint1 knockout, Skint1 transgenic and γδ TCR knockout mouse lines. The role of dimerization between Skint1 and Skint2 molecules was investigated by analysing Skint1 in Skint2 knockout line and by quantitating Skint2 clustering in Skint1 knockout and transgenic lines. Moreover, we applied two-colour Single Molecule Localization Microscopy approach revealing Skint1-TCR interactions in tissue at 15 nm isotropic resolution. These super-resolution imaging data provide a direct support for Skint family protein -mediated immune sensing in epithelial tissues.

P-439 (O-122)

Shape matters: Towards a molecular understanding of the innate immune response to microbial lipids

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Bacterial membrane components such as lipopolysaccharides (LPS) and lipopeptides are highly effective activators of the innate immune response and trigger inflammation and antimicrobial defense mechanisms by activation of Toll-like receptors (TLR). Dysregulation of this immune response can lead to severe diseases like pneumonia, acute respiratory distress syndrome, and sepsis. We characterize the structural and physical prerequisites for the inflammatory activity of bacterial lipids to elucidate how lipids are recognized by the immune system as danger signals. To this aim we investigate natural and reconstituted membrane systems and use biophysical methods such as fluorescence and infrared spectroscopy to characterize membrane properties. Small angle X-ray diffraction (SAXS) at the electron synchrotron PETRA III at DESY is employed to determine the membrane organization in solution. We have analyzed a variety of isolated natural bacterial lipids, synthetic analogs, and synthetic microbial-lipid-mimetics. These studies reveal that the three-dimensional organization of lipid aggregates correlates with their ability to activate or antagonize cell activation. Accordingly, only cubic inverted aggregate structures exhibit high inflammatory activity, whereas cylindrical molecules, forming lamellar aggregate structures, exhibit low or no activity. Interestingly, we found several examples showing that this behaviour is not correlated with a specific degree of acylation, but depends on the respective packing of the acyl chains in the hydrophobic moiety. Thus, inflammation inducing structures can be adopted by di- and triacyl-as well as highly acylated hexa-acyl lipids of different molecular origin. Comparing reconstituted lipid aggregates from purified lipopolysaccharide (LPS) of Gram-negative bacteria with purified outer membrane vesicles (OMV) isolated from bacterial cultures we can demonstrate that the interaction mechanisms of both membrane systems with the host cell are quite different. Whereas LPS requires protein mediated transport and activates cell surface receptors, LPS as part of OMVs can enter the host cell by membrane interaction without transporter and activates intracellular signalling pathways. Our data suggest that physico-chemical characteristics represent a key to an understanding of the biological activity of bacterial lipids and determine the molecular mode of action of activators and inhibitors. This knowledge is essential for the further development of lipid-based drugs as adjuvants and therapeutics.

P-440

Aquaporin-3 is involved in NLRP3-inflammasome priming and activation

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Aquaporins (AQPs) are protein channels that facilitate the transport of water and/or small solutes through cell membranes, essential for cell volume regulation and water and glycerol homeostasis. Variations in cell osmolarity and consequent cell volume regulation precede NLRP3 inflammasome activation and AQP blockage was shown to limit IL-1β release from NLRP3-activated macrophages. However, the role of AQPs in inflammation is still unclear. THP-1 macrophages cultured under stimuli for cell priming and inflammasome activation were shown to be a valuable model to study inflammation.

Human peripheral blood monocytes (HPBMs) and THP-1 macrophages cultured under stimuli for cell priming and inflammasome activation are valuable models to study inflammation. Here, we used HPBMs to evaluate AQPs expression in a healthy or pro-inflammatory phenotype. Then, we used THP-1 macrophages to uncover the mechanism where AQPs are players and potential new targets. Our results show that AQP9 and AQP3 are the most representative and are upregulated by LPS-priming in primary monocytes and THP-1 cells, respectively. PMA-differentiated macrophages-like cells and LPS-primed macrophages-like cells incubated with and without Auphen, a selective AQP3 inhibitor, showed similar water and glycerol permeability values, and glycerol permeability was affected by Auphen. Therefore, we investigated the role of AQPs during cell priming and inflammasome activation using the AQP3 inhibitor Auphen. LPS-priming was partially blocked by Auphen, decreasing mRNA expression and protein release levels of IL-6 and IL-1β. This suggests an involvement of AQP3 in macrophage priming by Toll-like receptor 4 engagement. NLRP3 inflammasome priming and activation was also blocked by Auphen, decreasing mRNA expression and protein release of IL-1β after NLRP3 activation with nigericin and ATP. Moreover, challenging LPS-primed cells with hyperosmotic solutions of glycerol increased IL-1β release. Altogether these data evidence AQPs as candidate players in the setting of the inflammatory response.

P-441

Anti-HIV antibody-lipid interactions enhance affinity for the native Env glycoprotein as revealed by single virion STED microscopy

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The Env glycoprotein is the only viral protein exposed on the HIV-1 surface and thus, constitutes a prime target in the antiviral immune response. Most antibodies targeting Env offer protection against a limited number of HIV strains, but a few that recognise the conserved MPER region in Env show a nearly pan-neutralising capacity. The exceptional antiviral activity of anti-MPER antibodies heavily relies on their ability to interact with the viral membrane, although the contribution of this process to the neutralisation breadth and potency is poorly understood. In this work we have investigated the nature of antibody-Env and antibody-membrane interactions using super-resolution STED microscopy and fluorescence correlation spectroscopy. We have found that anti-MPER antibodies 10E8 and 4E10 do not spontaneously bind the viral membrane, but use lipid-interacting regions as secondary docking sites to increase affinity for the Env glycoprotein. MD simulations support the conclusion that bulk biophysical membrane properties govern antibody dynamics. Notably, we have engineered lipid interaction sites in antibodies to enhance their neutralising activity. Overall, this work shows how biophysical characterisations of antibody-pathogen interactions can contribute to the development of innovative antiviral strategies.

P-442

Partial Dissociation of Antigenic Peptides from MHC I -- Linking NMR Data to Microstates Observed in MD Simulations

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Major Histocompatibility Complex I (MHC I) is one of the key players in adaptive immunity. Expressed on the surface of all nucleated cells, it displays sample antigenic peptides from the cytosol to patrolling cytotoxic T-cells that can thus identify and kill malignantly transformed and virally infected cells.

Although several crystal structures of MHC I in complex with an antigenic peptide (pMHC I) have been solved, the structural dynamics of pMHC I at the cell surface remain largely elusive. According to a recent NMR study on HLA-B*35:01 (Yanaka et al., J. Biol. Chem., 2014), pMHC I complexes can adopt a minor state in which the antigenic peptide is bound tightly to MHC I and a major state in which the peptide is bound more loosely. In unbiased MD simulations of the pMHC I studied by Yanaka et al., the peptide N-terminus dissociated from the MHC I binding groove during a few hundred nanoseconds. This finding suggests that pMHC I with completely bound peptide may correspond to the proposed minor state, whereas pMHC I with partially dissociated peptide may constitute the major state.

To underpin this, the potential of mean force (PMF) along the distance between the anchor residue of the peptide N-terminus and its binding partner in the MHC I binding groove has been calculated. The resulting free energy differences can be compared to values derived from the published NMR data. Preliminary results from umbrella sampling (1 microsecond of MD simulation per umbrella window) suggest that pMHC I in which the peptide N-terminus has dissociated from MHC I are lowest in free energy. Contrarily, Hamiltonian replica exchange simulations (2 x 2 microseconds), in which the effective temperature of the MHC I binding groove and the antigenic peptide is increased, predict pMHC I with completely bound peptide to be the global minimum of the free energy. This seemingly contradicting difference is discussed and further elucidated.

P-443

Single-molecule and super-resolution investigation of chemokine receptor behaviour within the T cell immunological synapse

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The immunological synapse (IS) serves as a highly dynamic yet tightly organised platform for the transfer of information between T cells and a number of different antigen-presenting cell (APC) types. It is required for the correct activation of T cells in response to infection, as well as the prevention of autoimmunity, and licensing of APCs for subsequent anti-microbial processes. Chemokine receptors are G-protein coupled receptors that recognise inflammatory and homeostatic chemokines, mediating leukocyte migration towards sites of infection or within specialised tissues of the immune system. In recent years it has become apparent that a number of chemokine receptors, particularly CXCR4, also contribute to the stability and longevity of the IS, and do so through signalling processes distinct from those involved in cell migration. Nonetheless, the spatiotemporal organisation of this behaviour within the complex architecture of the IS is unstudied, and hence its integration with the known signalling events of T cell activation is not understood. Here we use total-internal reflection fluorescence (TIRF) microscopy to examine CXCR4 and other chemokine receptors within primary human $CD4^+$ T cells during the process of IS formation on supported lipid bilayers. Single-molecule tracking demonstrated substantial changes in receptor dynamics over the lifetime of the synapse, both in terms of spatial location and nature of diffusion. Combining TIRF with super-resolution structured illumination microscopy (SIM) also revealed the segregation of some chemokine receptors from other signalling domains, most significantly those of the T cell receptor. These observations shed light on the mechanisms by which T cell activation is influenced by chemokine receptors, and hence how such processes are regulated during a coordinated immune response.

P-444

HIV-1 dynamically trap specific lipids during assembly in living T cells.

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HIV-1 Gag protein self-assembles at the plasma membrane of infected cells for viral particle formation. Gag targets lipids, mainly the phosphatidylinositol (4,5) bisphosphate, at the inner leaflet of this membrane. We recently shown that Gag alone was sufficient to trap PI(4,5)P2 in biomimetic membranes[1]. In this study, we address the question whether Gag is able to trap PI(4,5)P2 and/or other lipids during HIV-1 assembly in the living host CD4+ T lymphocytes. Here, we determine lipid dynamics within and away from HIV-1 assembly sites using super-resolution STED microscopy coupled with scanning Fluorescence Correlation Spectroscopy. Analysis of HIV-1 infected cells revealed that, upon virus assembly, HIV-1 is able to specifically trap PI(4,5)P2, and cholesterol, but not phosphatidylethanolamine nor sphingomyelin. Furthermore, our data show that Gag is the main driving force to restrict mobility of PI(4,5)P2 and cholesterol at the cell plasma membrane. This is first direct evidence showing that HIV-1 creates its own specific lipid environment by selectively recruiting PI(4,5)P2 and cholesterol, instead of targeting pre-existing ones as it was previously suggested.

[1] Yandrapalli N, Lubart Q, Tanwar HS, Picart C, Mak J, Muriaux D, Favard C. Sci Rep. (2016) ;6:39332.

[2] A. Honigmann, V. Mueller, H. Ta, A. Schoenle, E. Sezgin, S. W. Hell, C. Eggeling Nat. Commun. (2014), 5 5 5412

[3] Chojnacki, J.; Waithe, D.; Carravilla, P.; Huarte, N.; Galiani, S.; Enderlein, J.; Eggeling, C. Nat Commun (2017), 8, 545.

P-445

Failures and success in particle retraction by macrophage filopodia

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Macrophages are immune cells, which take up pathogens such as viruses and bacteria in a process called phagocytosis. On their surface, macrophages express abundant ruffles and filopodia that they use to catch and retract pathogens, which are later engulfed. Filopodia are thin, needle-like protrusions of the cell that contain filamentous actin bundles and are connected to the cell membrane via linker molecules. Macrophages' success in phagocytosis depends crucially on filopodia function.

We investigate the adaptive mechanics of filopodia and analyze the biophysical principles governing the attachment and retraction of particles. To this end, we use a Photonic Force Microscope in which we combine DIC microscopy, fluorescence microscopy, optical tweezers and interferometric particle tracking. Filopodia retractions are induced by presenting optically trapped polystyrene beads to macrophage cells. The motion of the bead in the trap can be tracked in 3D with nanometer precision at a micro-second timescale using back focal plane interferometry.

We present novel results, which reveal binding, pulling and unbinding events of macrophage filopodia on a molecular scale. The information gained from interferometric particle tracking is used to analyze the stiffness of the bond between cell and bead, the viscosity of the surroundings, the velocity of the retraction and the force-dependency of all these parameters. We show that the strength of the attachment between cell and bead evolves dynamically during pulling. Experimental evidence indicates that characteristic failure mechanisms limit filopodia pulling and that macrophage filopodia can adapt to high counteracting loads.

The experiments are complemented by fluorescence microscopy of live cells with labeled actin. The characteristics of the bead movement along filopodia are compared to the dynamics of the underlying actin retrograde flow. The results indicate that the bead transport to the cell is based on a force-dependent coupling to the actin cytoskeleton.

P-446

Modelling the action of Mycobacterium tuberculosis virulence factors on macrophage membrane: one lipid at a time

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Modelling the action of *Mycobacterium tuberculosis* virulence factors on macrophage membrane: one lipid at a time

Mycobacterium tuberculosis (*Mtb*) is the main causative agent of the disease Tuberculosis. Among the various factors associated with the *Mtb* virulence, lipids constituting the bacterial cell wall have recently gained attention. These lipids can be used as virulence effectors acting at the macrophage membrane to damage it and modulate immune response. Phthiocerol dimycocerosate (DIM) is one of these main virulence factors. Multi-scale molecular modeling combined with 31P-NMR experiments revealed that DIM shape induces negative membrane curvature. Infection of macrophages pre-treated with lipids of various shapes, underpined the role of membrane curvature in the process of phagocytosis. Taken together, these results show how the molecular structure of DIM leads to the modulation of macrophage biological functions, such as phagocytosis.

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Single cell measurements of protein affinity in contacting cells

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There has been substantial controversy about the mechanism of T cell receptor activation and downstream signaling. This activation mechanism is the first step followed by a signaling cascade leading to an adaptive immune response. The debate in T cell activation indicates the necessity of developing a new biophysical model to better understand how our immune system discriminates between self and foreign peptides and ultimately decompose the physicochemical mechanisms of how an immune response is initiated. Key to this understanding is the binding affinities between membrane proteins on the T cell´s surface and receptors on the surface of antigen presenting cells. However, the existing methods of measuring these interactions are time-consuming and technically challenging for weakly binding protein pairs.

In this project, we are developing a new method of measuring interactions between proteins on contacting cells in order to reduce the measurement time and increase the sensitivity, while additionally obtaining single cell affinities. This is achieved by replacing the T cell with a supported lipid bilayer (SLB) containing different T cell proteins. This method extends the Zhu-Golan method [1] to single cells and can be extended to measure very weak interactions [2]. To validate the method, we study the interaction of fluorescently labeled CD2 anchored on SLBs with CD58 expressed on B cells. By varying the concentration of CD2 under each cell and by evaluating the amount of bound CD2 for each condition, the affinity of CD2/CD58 is determined for each cell. Furthermore, we assess our new method by examining the interaction of CD4 in the SLB with MHC class II on B cells, with CD4 being one of the weakest binding proteins ever reported [2]. We believe that this novel method will reduce the measurement time significantly compared to traditional 2D affinity measurements, as well as pave the way for weak protein interactions to be monitored in cell-cell contacts.

1. Zhu et al. *Biophys. J.* **92**:1022 (2007).
2. Jönsson et al. *PNAS* **113**:5682 (2016).

P-448

Investigation into how T cells trigger on various surfaces

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The first steps in initiating an adaptive immune response, after antigen presentation, is the triggering of molecular complexes on the T cell's surface called T cell receptors (TCRs). However, how this is achieved is not fully understood on a molecular level. In fact, recent studies have shown that T cells can also trigger when binding to surfaces coated with different adhesion molecules, even without any antigen, as well as PLL-coated surfaces.[1,2] Care must therefore be taken when studying T cell triggering, to distinguish between surface- and antigen-based triggering.

In this work we looked at how the fraction of triggering cells differs depending on the conditions of different cell membrane model systems. A common model system is the supported lipid bilayer (SLB). Nickel-chelating lipids are frequently incorporated, which allows for binding of histidine-tagged membrane proteins. It was investigated how many Jurkat T cells that trigger on an SLB containing different amounts of nickel-chelating lipids, which was compared with the triggering fraction when the adhesion molecule CD2 was linked to the SLB.

The triggering events are marked by the cells' release of Ca2+-ions and were observable with fluorescence microscopy. Preliminary results indicate only small changes (<5%) in the fraction of triggered cells as a function of the amount of nickel-chelating lipids in the SLB in the range of 2-10 wt%. Triggering on 200 CD2 molecules/μm^2 was similar to triggering on SLB without nickel-chelating lipids, while the triggering fraction was found to be substantially higher when having 800 CD2 molecules/μm^2. It was observed that a higher density of CD2 facilitates more easily cell contact formation. This could potentially lead to more exclusion of the tyrosine phosphatase CD45 and prevent the TCRs from undergoing dephosphorylation, according to the kinetic segregation model.

This work highlights how the presence of nickel-chelating lipids in the SLB and the protein density can affect TCR triggering of T cells. The choice of model membrane system must therefore be considered when studying TCR triggering.

References

1. Chang, V. T. *Nature Immunology* **17**, 574–582 (2016).
2. Santos, A. M. *et al. Nature Immunology* **19**, 203–205 (2018).

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Role of pulmonary surfactant protein SP-C in pulmonary surfactant homeostasis and lung defense.

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Pulmonary surfactant (PS) is a complex consisting of proteins and lipids that is located at the air-liquid interface of alveoli. PS is essential for a proper breathing process since it reduces surface tension to a minimum and therefore minimizes the work required to maintain lung patency. Besides, PS has a key function in lung defense against inhaled pathogens and potentially harmful particles. In this sense, PS is closely related with alveolar macrophages, the main cell type involved in the removal of wasted or dangerous material in the lungs.

Among PS proteins, surfactant protein C (SP-C) has been classically related to the biophysical function of the system, because it is a small, very hydrophobic membrane protein that contributes to phospholipid membrane stacking, transfer and interfacial adsorption. Moreover, SP-C can bind to lipopolysaccharide (LPS), the major component of outer membrane in Gram negative bacteria, and to CD14, which is a macrophage receptor protein that also interacts with LPS by itself to induce inflammatory responses against pathogens. Therefore, SP-C may also have a relevant role in immunomodulation and pulmonary homeostasis.

In this context, the present work aimed to analyze the effect of SP-C in the uptake of lipid vesicles by alveolar macrophages. Our approach consisted in flow cytometry analysis for the evaluation of fluorescence as the result of the phagocytosis of fluorochrome-labelled vesicles in the presence or absence of SP-C. In addition, because of the ability of SP-C to induce membrane fragmentation, we have also determined the contribution of the size of vesicles to their uptake by macrophages. Using confocal microscopy, we have verified the phagocytosis of fluorescent particles containing SP-C.

Finally, we have delved into the cytokine production pattern and phenotype of these macrophages after the interaction with vesicles loaded with SP-C, LPS or both molecules. We have used ELISA and flow cytometry approaches to define an emerging role of SP-C in lung physiology.

P-450

Can a phospholipid intermediate rescue monocytes from mitochondrial damage?

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Mitophagy is the selective degradation of mitochondria by autophagy. Damaged and dysfunctional mitochondria can cause cellular degeneration, which may lead to neurodegenerative, metabolic and cardiovascular diseases. CDP-Ch, an intermediate in the Kennedy pathway for production of phosphatidylcholine (PC), has potential neuroprotective properties. During mitophagy, the level of PC, one of the most abundant phospholipids of cellular membranes, decreases.

In this study, we investigated the effect of CDP-Ch on mitophagy in U937 monocytes. Mitophagy was induced by CCCP in the presence and absence of CDP-Ch. Mitochondrial dynamics and mitophagy-related proteins were investigated by western blotting. We determined that the levels of Pink1, Drp1, Mfn2, CoxIV and LC3B changed in response to CDP-Ch treatment. We further measured mitochondrial membrane potential, mitochondrial mass and mitochondrial superoxide production by flow cytometry and confocal microscopy using mitochondria-specific dyes. The decrease in mitochondrial mass upon mitophagy induction was reversed by CDP-Ch treatment. These changes in mitochondrial fusion or fission and mitochondrial mass in mitophagy-induced U937 cells suggest a modulatory role of CDP-Ch in mitochondrial dynamics.

We propose that CDP-Ch may contribute to the production of mitochondrial membranes in mitophagy-induced cells. New mitochondria can then rescue cells quickly from the stress and maintain cell survival.

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The Interplay of Immune Cell Proteins Affects the Orientation, Organisation and Affinity within a Cell-Cell Contact

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During the contact formation between immune cells different proteins play an important role and are highly organized. One key event is the interaction between the T cell receptor (TCR) and a peptide presenting major histocompatibility complex (pMHC). Each TCR has a specific affinity for their counter pMHC, which varies from very weak to strong depending on the presented peptide. Strong affinities correspond to binding agonist peptides. However, measuring affinities in a cellular system is complicated due to various protein interactions taking place. I will in this presentation show some of our current work on how these interactions affect both the orientation, organisation and affinity of proteins in cell-cell contacts. We have recently shown using a technique called hydrodynamic trapping (HDT) that protein organization can be affected by intermolecular interactions.[1,2] These interactions are influenced both by protein glycosylation and flexibility and I will show how HDT can be used to quantify these effects. We have also measured the 2D affinity of TCR binding to gliadin-MHC class II, an agonist system, and how this affinity is affected by the presence of the adhesion molecule CD2. TCR and CD2 were fluorescently labelled and anchored to a model cell membrane, a supported lipid bilayer. Jurkat T cells expressing the counter molecules gliadin-MHC II and CD48, respectively, were added afterwards. Our preliminary data suggest that the TCR/MHC affinity decreased by 20% when having 100 molecules/ μm^2 of the adhesion molecule CD2 present. In addition, TCR was found to deplete CD2 from the contact area, with the CD2 concentration decreasing 80% when increasing the TCR concentration from 100 TCR molecules/μm^2 to 1000 TCR molecules/μm^2. High amounts of TCR also seemed to affect the cells' cytoskeletal reorganisation observed as large membrane skirts forming, which was not seen for high CD2 concentrations alone.

Thus, HDT and affinity measurements on a multi-protein SLBs opens for new information about membrane protein behavior and shows how protein flexibility, glycosylation and density can dominate this.

References:

1. Junghans et al. *Sci Rep* **8**: 12479 (2018)
2. Junghans et al. *Front Immunol* **9**:2215 (2018)

P-452

Subcellular macromolecular changes induced in rat glial cells by ALS IgGs as evidenced by synchrotron radiation-based Fourier Transform Infrared micro-spectroscopy

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Amyotrophic lateral sclerosis (ALS) is an adult onset fatal neurodegenerative disease characterized mainly by the progressive loss of upper and lower motor neurons. The importance of immune mechanisms has been shown in the disease. Purified immunoglobulins G from ALS patients (ALS IgGs) increase intracellular Ca2+ in motor neurons, and enhance the release from synapses onto motor neurons and at the neuromuscular junction. Our previous studies have shown that ALS IgGs may also affect glial cells (Ca2+ homeostasis, vesicle trafficking, and intracellular oxidative status).

Cell cultures of rat astrocytes and microglia treated with ALS and control IgGs, and ATP (as physiological control) were grown on CaF2 slides. After fixing cells were scanned by high resolution synchrotron radiation-based Fourier Transform Infrared (FTIR) micro-spectroscopy with the aperture 10x10 μm, to achieve single cell resolution. Prinicipal Componenat Analysis (PCA) was used to investigate biomacromolecular expression of glial cells treated with ALS IgGs from 2 patients (ALS 1 & 2) compared to healthy and disease (polydiscopathy) control IgGs and 0.2 mM ATP action. All the treatments were performed for 20 min or 24 h.

PCA revealed the following prominent observations: 1) in astrocytes only the 20 min treatment gave apparent differences for the lipid (3100 - 2800 cm-1) and protein and esters (1800-1480 cm-1) bands between the two ALS patients, and between ALS1 and healthy control and disease control as well, 2) the difference between the two ALS patients was also retained for the carbohydrate and nucleic acids band (1480-900 cm-1), 3) in microglia with 20 min treatment more apparent effects were observed as compared to 24 h, most apparent differences being in the lipids band with ALS1 IgG compared to the healthy control while 4) the effect of ALS1 and 2 IgGs differed in the protein and ester band.

In conclusion, basic changes in glial cell metabolism were revealed as induced by immune humoral factors, that may serve for better stratification of patients for personalized medicine.

Tuesday 23rd July

BIOMOLECULAR SIMULATION II

P-456 (O-129)

The catalytic mechanism of MB-COMT: new drug design paradigm?

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We used computational molecular modelling in synergistic combination with a wide range of experimental methodologies, to study a pharmaceutically important enzyme: catechol-O-methyltransferase (COMT) [1]. The enzyme has both water soluble (S-COMT) and membrane bound (MB-COMT) isoforms; MB-COMT is a drug target in the treatment of Parkinson's disease: it is desirable to selective target this isoform. This should also be possible since the substrate profiles of S-COMT and MB-COMT differ, in spite of the fact that they possess identical catalytic domains [2]. While our study culminates in the determination of the catalytic mechanism of MB-COMT [3] that differentiates it from S-COMT, and opens the door to the possibility of selective targeting, this presentation describes a ten-year arc of research that preceded this publication.

We hypothesized that the mechanism of substrate differentiation was the interaction with the membrane of both potential inhibitors or substrates and the catalytic domain of MB-COMT; we then proceeded to determine the catalytic mechanism specific to MB-COMT. We ascertained that (1) substrates with a preferred affinity for MB-COMT over S-COMT had an orientation in the membrane conducive to catalysis from the membrane surface and (2) binding of COMT to its cofactor ADOMET induced a conformational change that causes the catalytic surface of the protein to adhere to the membrane surface, where substrates and Mg^{2+} ions, required for catalysis, are found. Through bioinformatics analysis we found evidence of this mechanism in other proteins, including several existing drug targets.

References

[1] P. T. Männistö and S. Kaakkola, Pharmacol. Rev., **51**, 593–628, (1999).
[2] R. G. Robinson *et al.* ACS Chem. Neurosci., **3**, 129–140, (2012).
[3] A. Magarkar *et al.* Chem. Commun., **54**, 3440, (2018).

P-457 (O-130)

Combining theoretical and experimental approaches to understand the mechanism of antibiotic permeation: implications on the fight against bacterial resistance

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Bacterial resistance is a critical public health threat whose importance has been increasing over the years. Fluoroquinolones (FQ) are a group of antibacterial drugs that are classified as "highest priority critically important antimicrobials" by the World Health Organization. The extensive use of FQs led to an exponential increase in bacterial resistance to these drugs.

Known mechanisms of bacterial resistance to FQs, often encompass a reduction of the antibiotics' permeation through the Omp porin channels present at the bacterial cell membrane. Since FQs have intracellular targets, the study of their permeation is crucial to comprehend bacterial resistance and develop alternative drugs.

With the perspective of finding alternatives to the use of FQs, copper complexes of FQs have been studied, as they are expected to have an increase permeation through the membrane. In the present study the translocation of Ciprofloxacin (Cpx) and its copper ternary complex (CuCpxPhen) are compared. Model membranes mimicking the bacterial membrane in the presence or absence of the OmpF porin were used. Spectroscopic methods were employed to determine drugs' partition coefficient to the model membranes and drugs' binding constant to the porin.

To further explore structural and dynamical aspects of the translocation process, a theoretical approach was considered, using molecular dynamics simulations. Using umbrella sampling we were able to describe the thermodynamics of the translocation process for each pathway.

The results show that CuCpxPhen has a greater partition in lipid membranes, with theoretical results pointing for a greater ability of this complex to cross the lipid membrane, as it shows lower energy barriers at bilayer's centre. Additionally, and despite experimental results showing similar binding constants for Cpx and CuCpxPhen with OmpF, theoretical results show a more favourable energy profile for Cpx translocation across this channel.

Overall, our results indicate that CuCpxPhen's permeation in the bacterial membrane should be porin independent. Copper complexes are then expected to be good candidates to bypass the bacterial resistance related with reduction of antibiotic permeation by porin mutation. The findings of this work are important to proceed with the study of CuFQPhen complexes as an alternative to free FQs in resistant bacteria.

P-458 (O-131)

Exploring Conformational Transitions and Free Energy Profiles of Proton Coupled oligopeptide Transporters

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Proteins involved in peptide uptake and transport belong to the proton-coupled oligopeptide transporter (POT) family. Crystal structures of POT family members reveal a common fold consisting of two domains of six transmembrane α helices that come together to form a "V" shaped transporter with a central substrate binding site. Proton coupled oligopeptide transporters operate through an alternate access mechanism, where the membrane transporter undergo global conformational changes, alternating between inward-facing (IF), outward-facing (OF) and occluded (OC) states. Conformational transitions are promoted by proton and ligand binding, however, due to the absence of crystallographic models of outward-open state, the role of H^+ and ligands are still incomplete. To provide a comprehensive picture of the POT conformational equilibrium, conventional and enhanced sampling molecular dynamics simulations of $PepT_{st}$ in the presence or absence of ligand and protonation were performed. Free energy profiles of the conformational variability of $PepT_{st}$ were obtained from microseconds of adaptive biasing force (ABF) simulations. Our results reveal that both, proton and ligand, change significantly the conformational free energy landscape. In the absence of ligand and protonation, only transitions involving IF and OC states are allowed. After protonation, the wider free energy well for E300 protonated $PepT_{st}$ indicates a greater conformational variability relative to the apo system, and OF conformations became accessible. For the E300 Holo-$PepT_{st}$, the presence of a second free energy minimum suggests that OF conformations are not only accessible, but also, stable. The differences in the free energy profiles demonstrate that transitions toward outward facing conformation occur only after protonation and, probably, this should be the first step in the mechanism of peptide transport. Our extensive ABF simulations provide a fully atomic description of all states of the transport process, offering a model for the alternating access mechanism and how protonation and ligand binding control the conformational changes.

P-459

Density Based Protein Docking

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Predicting the assembly of multiple proteins into specific complexes is critical to the understanding of their biological function in an organism, and thus the design of drugs to address their malfunction. Proteins are flexible molecules, and this inherently poses a problem to any protein docking computational method, where even a simple rearrangement of the side chain and backbone atoms at the interface of binding partners complicates the successful determination of the correct docked pose. We present STID maps, a structural descriptor capable of simultaneously representing protein surface, electrostatics and local dynamics within a single volumetric descriptor. We then demonstrate that the application of STID maps into a protein-protein docking scenario bypasses the need to accommodate for specific side chain packing and small conformational changes at the interface of binding partners. This representation is leveraged in our *de novo* protein docking software package, JabberDock, which we show can accurately predict complexes with success rates comparable to or greater than currently available methods.

P-460

Mechanical Vibration Characteristics of DNA-wrapped Single-walled Carbon Nanotube Composites Studied by Finite Element Simulation

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Single-walled carbon nanotubes (SWCNTs) have superior mechanical characteristics. Although SWCNTs aggregating in water have a limited applications in biology, the technology for providing affinity in SWCNTs through wrapping with single-stranded DNA (ssDNA) was developed in 2003 [1]. The ssDNA-SWCNTs have been used as a biosensor because it has properties of the light emission in the near-infrared and the absorption of light in the visible region. Previous studies have used SWCNTs as a sensor for detecting the antioxidant activity of catechin in Japanese tea [2]. Meanwhile, many interesting phenomena addressed by SWCNTs have been reported such as ignition, intense motion by microwaves [3] and the change of band gap modulation due to radial deformation [4]. Thus, vibration characteristics of SWCNTs are important for the safely application. Although this composites have been studied by molecular dynamics (MD), we investigated the vibration characteristics of ssDNA-SWCNTs using a finite element method (FEM) that is suitable for calculation of the large structure compared to MD.

We developed the cylindrical ring of SWCNTs and helically-shaped ssDNA using FEM and adopted the modal analysis method to calculate the motion equation of the ssDNA-SWCNTs with free boundary conditions. The π-π stacking interaction between the ssDNA and the SWCNTs was represented using a linear spring. The modal analysis method was simulated by the Lanczos algorithm in the commercial MSC Nastran.

We analyzed the mechanical vibration characteristic of ssDNA-SWCNTs by using the FEM. First, the SWCNTs was calculated from the 1st-bending mode to the radial breathing mode (RBM). The natural frequency of the SWCNTs was consistent with the well-known Bernoulli-Euler beam theory. Additionally, we obtained the vibration modes and conditions to be coupled with the 1st-bending mode of the ssDNA-SWCNTs under vacuum and water conditions. Thus, the FEM makes it possible to understand the behaviour of the composites and to design the vibration characteristics for the bio-nanodevices.

[5] Zheng M, et al., Nat. Mater 2003, 2,338–342.
[6] Matsukawa Y, et al., Colloids Surf B: Bio. 2018, 172,684–689.
[7] Imholt TJ, et al., Chem. Mater 2003, 15, 3969-70.
[8] Taira H, et al., Coup. Sys. Mech. 2013, 2, 147-157.

P-461

Studying beta-lactoglobulin dimerization using molecular dynamics

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Milk and its derivatives are an important worldwide food source, particularly for infant nutrition, but their use faces a major health complication: some of their proteins are allergens, especially β-lactoglobulin (BLG), a major component of the bovine milk.

The fate of BLG upon human ingestion remains unsettled, being unclear how extensive BLG proteolysis is and how it relates to allergenicity. The fact that its proteolytic resistance and antigenic response remain related even in the case of non-oral administration [1] suggests that they are not causally related but rather reflect an underlying common feature.

This feature may be the formation of dimers, which can hinder proteolysis and seems to facilitate the binding of protein allergens to IgE antibodies; indeed, BLG is dimeric when complexed with IgE Fab fragments [2] and shows lower antigenicity when in the monomeric form [3]. As shown in experimental studies, this form is predominant at pH below 3 and above 8 and between these there's the formation of a reversible dimer at a moderate ionic strength [4]. The changes in pH are also associated to the Tanford transition, that is, a change in the conformation in a loop near the binding site, allowing or inhibiting the binding of ligands, regulated by the protonation of Glu89 [5]. Previous studies have shown that the dimerization involves electrostatic interactions, for which a better understanding at a molecular-level is essential. In this study, we intended to analyse the effect of the pH in conformational alterations on the monomer and dimer and its dissociation process. For that, Constant pH molecular dynamics (CpHMD) simulations were performed for the monomer and dimer, which allows us to treat pH as an explicit parameter and couples the MM/MD and Poisson-Boltzmann/Monte Carlo (PB/MC) algorithms.

[1] Huby et al. (2000) Toxicol. Sci. 55: 235; [2] Niemi et al. (2007) Structure 15: 1413; [3] Rouvinen et al. (2010) PLOS ONE, 5: e9037; [4] Mercadante et al. (2012) Biophysical J. 103: 303; [5] Kontopidis et al. (2004) J. Dairy Sci. 87: 785.

P-462

Theoretical Study on DNA Binding Mechanism for Blue-Light Photoreceptors

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Photolyases and cryptochromes are blue-light photoreceptors, exerting various functions, and they also are flavoprotein which conserve the flavin adenine dinucleotide (FAD). Photolyases has function repair UV-induced DNA lesions, while cryptochromes serve as the magnetic compass in birds, the growth and development in plants, and the regulation of circadian rhythm in animals. Cryptochromes are high sequence homology with photolyases, however, cryptochromes do not exert the DNA repair reaction. A reason is to FAD of typical cryptochrome (Cry1 and Cry2) does not become fully reduced form, $FADH^-$. Recently, new type cryptochrome, cryptochrome-DASH, is identified, indicating that FAD of that can become $FADH^-$. However, cryptochrome-DASH did not observe the DNA repair activity in vitro and in vivo. Why cryptochrome-DASH does not exert the DNA repair reaction is not clarified.

To elucidate a behavior to DNA of cryptochrome-DASH and photolyases, we compared those behaviors in terms of the DNA binding mechanism. We performed the molecular dynamics simulation, the umbrella sampling method, and the fragment molecular orbital method for double strand DNA and photolyase complex or double strand DNA and cryptochrome-DASH complex, respectively. In the results, photolyse indicated the high interaction between a loop part (Pro400 - Arg 404) and DNA. Photolyase will recognize DNA using a loop part, might induce UV-induced lesions to active site. On the other hand, the interaction of cryptochrome-DASH was lower than that of photolyase. Therefore, cryptochrome-DASH will not exert the DNA repair reaction, because of cryptochrome-DASH cannot recognize double strand DNA.

P-463

Polysaccharide-lipid interactions: Theoretical and experimental study using molecular dynamics simulations, quantum chemical ^{13}C NMR spectra computation and solid-state ^{13}C NMR

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Lipids and polysaccharides are essential components of living order and understanding their interactions is of great importance. Among the different experimental approaches used to analyze such molecular complexes, solid-state NMR is a method of choice. However, realistic models at the atomic scale are sometimes necessary to understand the NMR spectral signatures obtained experimentally and to reveal the detailed three dimension structure of such complexes.

Theoretical approaches such as classical molecular dynamics (MD) or quantum chemical methods are suitable to bring support to these studies. Before using these computational method in a routine manner, several validation steps are necessary, based on the quantum chemical calculations of ^{13}C NMR spectra on one hand and on molecular dynamics simulations of complexation process on the other.

For this purpose, several calculations have been carried out on amylose-palmitic acid complexes. First, molecular dynamic simulations using modified GLYCAM force field have been undergone using increasing degree of polymerization in order to study the complexation process as a function of amylose size. This study has revealed the minimal amylose size necessary to undertake a stable complex with characteristic V-helix structure in presence of lipids. On a second time, based on structures extracted from MD simulations, Density Functional Theory (DFT) B3LYP using GIAO method has been employed in order to simulate ^{13}C NMR spectra of these amylose-lipid complexes. Then, this process has been applied to POPC-amylose complexes. This study revealed the ability of the protocol to explain ^{13}C CP-MAS spectra recorded on samples containing amylose complexes with different lipid concentrations. These spectra present self-complexed amylose and/or POPC-amylose V-helix signals at different intensity ratios. This strategy leading to comparison between simulated and experimental NMR spectra can safely be employed to study the interactions of different polysaccharides with others lipids or with others components (DNA, proteins …) and help to decipher the structuration of heterogeneous systems.

P-464

Monitoring the formate-NAD interaction during formate oxidation in the active site of Candida boidinii formate dehydrogenase

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NAD-dependent formate dehydrogenase (FDH) uses NAD^+ as cofactor to catalyze the oxidation of formate to CO_2. The initial interaction between formate and NAD^+ is a textbook example of an anion-π interaction. It has expectedly a strong electrostatic component, however, the low-lying empty π orbitals of NAD^+ make this oxidant also a potential acceptor for donor-acceptor covalent bonding. In this work, we used two energy decomposition schemes, EDA[1] and NEDA[2] to monitor the nature of the substrate-cofactor interaction during the reaction ($H\text{-}COO^- + NAD^+ \rightarrow CO_2 + NADH$). The coordinates of the substrate-cofactor pair were taken from a QM/MM simulation of the reaction inside the Candida boidinii FDH by Guo et al.;[3] these coordinates enabled us to study the interaction in the reactants state, transition state, and products state in the conformation and orientation the reacting partners have in the active site of the enzyme.

[1] G. te Velde et al., *J. Comput. Chem.* 2001, *22*, 9-31.
[2] E. D. Glendening, *J. Phys. Chem. A* 2005, *109*, 11936-11940.
[3] Q. Guo et al., *Biochemistry* 2016, 55,: 2760-2771.

We thank professor Dan Major for kindly providing us with the coordinates of snapshot ensembles from a QM/MM simulation of Candida boidinii FDH and for valuable comments. Access to computing facilities of the MetaCentrum of the of the Czech National Grid Organization and to the CERIT Scientific Cloud is gratefully acknowledged.

P-465

Particle-based model of the glucose-stimulated insulin secretion in human beta cells

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Glucose-stimulated insulin secretion from pancreatic β-cells is a key factor for the adequate regulation of glucose levels in the human body. At the cellular level, insulin is secreted by a well-established process involving an electrical activity pattern as a response to an increased metabolic activity following glucose stimulation, resulting in the increase in the intracellular Ca^{2+} concentration, which is the signal that ultimately promotes the release of insulin granules to the cell exterior. While in traditional models of the pancreatic β-cell these processes are incorporated neglecting important aspects such as the localization, number, and properties of the single ionic channels and insulin granules, in this work we use a particle-based approach in which not only all the cellular processes involved are considered, but also Ca^{2+} channels, Ca^{2+} ions and insulin granules are modelled explicitly in a three-dimensional geometry, thus allowing us to track their position throughout the whole simulations. By using this particle-based approach, we were able to evaluate how the localization of Ca^{2+} channels, relative to each other as well as to insulin granules located in the submembrane domain, could be relevant for an adequate secretory response. In addition, we estimated the number of insulin granules reaching a neighbor cell depending on the distance between the two simulated cells in different scenarios of interest, aiming to determine the potential effects of the localization of Ca^{2+} channels and insulin granules on the paracrine signals within the islets of Langerhans in the human pancreas.

P-466

The role of electrostatics in monoamine oxidase A catalysis rationalized by interaction between dipole moment and electric field.

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The origin of enzyme catalysis has long been a hotly debated topic in chemical biology, with the main issue being whether dynamical effects or preorganized electrostatics are the driving force behind catalysis. In our study we built a simple multiscale model that allows us to elucidate the effects of electrostatics on the rate-limiting step of the reaction between phenylethylamine (PEA) and monoamine oxidase A (MAO A), an enzyme that catalyses the oxidative deamination of biogenic monoamines. As such it plays a key role in the metabolism of several neurotransmitters and is an important factor in the development of some psychiatric disorders.

In our multiscale model, the reacting moieties (PEA and truncated cofactor lumiflavin) comprised the quantum part, while the protein environment was represented by atomic point charges. Single point quantum calculations were performed at the M06-2X/6-31G+(d,p) level of theory on the snapshot structures obtained by our previous classical simulation of this reaction, representing both the state of reactants as well as the transition state. Atomic charges of the enzyme were also extracted from that simulation. Several parameters were taken under consideration, including the energy barrier, the HOMO-LUMO gap, the dipole moment and the charge transfer within the reactive subsystem. This simple representation of the enzymatic environment allowed us to manipulate with the charges (scale them by various factors, randomize them, place large point charges close to the reacting moiety, etc.) and to investigate the effect of these manipulations on the computed parameters. At the same time, the electric field exerted by the enzyme on the reacting moiety can be evaluated trivially from the aforementioned point charges. This allowed us to rationalize the effect of electrostatics on catalysis through the interplay between the dipole moment of the reacting moiety and the electric field of the surroundings. The results present convincing evidence that the electrostatic environment enhances the reaction by all the considered criteria and confirm that the catalytic function of MAO A derives from preorganized electrostatics.

P-467

Structural difference of Aß between at a solution surface and in bulk water using molecular dynamics simulations

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The amyloid-β peptide (Aβ) is composed of 39–43 amino-acid residues. Aβ tends to form amyloid fibrils, which are associated with the Alzheimer's disease. It was reported that formation of amyloids is accelerated at a hydrophobic/hydrophilic interface such as an air/water surface or an interface between sugar-head groups and hydrocarbon chains of glycolipids. It is necessary to clarify the amyloid formation process at the interface in order to find a remedy for Alzheimer's disease. To investigate amyloid formation process at the hydrophobic/hydrophilic interface, we performed molecular dynamics (MD) simulations for a full-length Aβ molecule, Aβ40, in the presence of the solution surface. For comparison, MD simulations of Aβ in a bulk water were also performed.

We found that residues in the β1 region (residues 10–22) and the β2 region (residues 30–40) existed in the vicinity of the solution surface. Furthermore, these residues tended to form helix structures as reported in experiments. In my presentation, other simulation results will be shown, and solution surface effects on the amyloid formation process will be discussed [1].

Reference

[1] S. G. Itoh, M. Yagi-Utsumi, K. Kato, and H. Okumura, *J. Phys. Chem. B* **123**, 160–169, 2019.

P-468

***Pseudomonas putida* LapA: A Molecular Dynamics study**

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Pseudomonas putida is a gram-negative bacterium of environmental and industrial interest. Its ability to form biofilms on surfaces depends on the adhesin LapA. This protein is anchored to the outer-membrane, where it can extend to the extracellular milieu. LapA contains a large number of residues --over 8,600-- which makes its structural analysis challenging. Nevertheless, sequencing data reveals a repetitive modular pattern, in which multiple copies of groups of approximately 100 and 219 residues appear. In the present work, these individual groups are analyzed using protein threading and subsequent Molecular Dynamics simulations. Hydrogen bonds, RMSF, secondary structure fluctuations and salt bridges are assessed. To complete a characterization of the protein, other domains present in LapA, such as CalxB and vWF, are also analyzed. The preliminary results point to a dimer of 100 residues --or alternatively a 219 residue group-- as the main functional unit in LapA. The presence of calcium in the medium also seems to regulate LapA structure, and thus the formation of the biofilm.

P-469

Neurotoxic agonists and antagonists docking to M1 GPCR.

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Malaria is a vector-borne disease spread by mosquitoes that affects about 200 million people each year resulting in up to 730 thousand deaths. As the global warming progress, the range of this disease increases. Unfortunately, the most commonly used repellents (i.e. DEET) lose their activity as mosquitoes become resistant. Moreover, they are found to be neurotoxic for humans, especially for children. Therefore, there is a high need for new generation of mosquito repellents.

We use molecular dynamics and docking tools to investigate the conformational changes in muscarinic acetylcholine receptors, which play a crucial role in the activity of both human and insect neuronal systems, in response to ligand binding in orthosteric and allosteric sites. Knowing the molecular basis of these changes is the key to finding compounds that would serve as selective malaria vectors repellents having no side effects in human. Results of docking and dynamical modeling of 5 distinct compounds (DEET, BQCA, IR3535, oxotremorineM, pirenzepine) to human M1 muscarinic acetylcholine receptor model will be presented and discussed.

P-470

Molecular design of the glutathione derivatives for avoidance of decomposition by GGT1.

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Glutathione (GSH) is a tripeptide composed of Glu, Cys, and Gly, whose importance in detoxification is well known. Gamma-glutamyl transpeptidase 1 (GGT1) plays a central role in the metabolism of glutathione and its S-conjugates through the cleavage of gamma-glutamyl linkage. It is said that GSH in blood stream is digested in a few minutes by GGT1 molecules in plasma or on cell surface. Although the investigation of the ways to avoid decomposition of GSH should be an important issue, the 3D structure of GSH-GGT1 complex has not been experimentally analyzed.

In this study, docking simulations were carried out to predict the GSH-GGT1 complex structure using the GGT1 moiety from known structure of glutamate-GGT1 complex (PDB entry: 4ZCG). To avoid decomposition of GSH by GGT1, we tried to design GSH derivatives modified by an amino acid or a monosaccharide on each amino acid. The docking simulations were also employed to verify whether the derivatives are still able to bind to GGT1. As a result, it was suggested that some derivatives have capabilities to avoid being bound and decomposed by GGT1.

P-471

Simulation of Reversible Protein-Protein Binding at an Atomistic Scale

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Virtually all biological processes in nature depend on the interplay between proteins at some point. Knowledge of the structural and energetic interaction mechanisms between proteins at atomic resolution can help to unravel the mechanism of many diseases, for example cancer or hereditary diseases. We have recently demonstrated the simulation of the whole binding process of two small model proteins in a completely reversible manner using Molecular Dynamics (MD) simulations. Calculated binding free energies from these simulations were closely monitored for convergence and in very good agreement with experimentally determined values, with deviations from experiments in the order of thermal noise [1]. To demonstrate the broad applicability of our proposed approach, the binding processes of a diverse set of additional protein-protein complexes were simulated. Some of the complexes were considerably larger than the original model system and well-studied examples like the Barnase-Barstar complex were included in the set. Given the importance of the correct description of the long-range electrostatic interactions during the binding reactions, a correction scheme for the treatment of electrostatic interactions previously described for the calculation of relative binding free energies of small molecules [2] was adapted for the given problem. The applied correction scheme employs continuum electrostatics models to estimate the size of the methodology-dependent error due to the effective electrostatics interaction function and the specific settings of the MD simulations. To our knowledge, this is the first time that such correction schemes have been applied for the calculation of absolute binding free energies of large biomolecular complexes. Our research provides valuable insights in the effort to simulate ever larger biomolecular systems of biological interest.

1. Perthold JW, Oostenbrink C, Simulation of Reversible Protein-Protein Binding and Calculation of Binding Free Energies Using Perturbed Distance Restraints. *J Chem Theory Comput* **2017**, *13* (11), 5697-5708.
2. Reif MM, Oostenbrink C, Net charge changes in the calculation of relative ligand-binding free energies via classical atomistic molecular dynamics simulation. *J Comput Chem* **2014**, *35* (3), 227-243

P-472

Towards Designed Multienzymatic Bioreactions: Changed Assembly Kinetics of Enzyme Complexes by Modifying Flexible Linker Domains

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Multienzyme complexes spatially confine cascade bioreactions, enabling concerted action on metabolites. In case of so-called substrate channeling, diffusion losses, adverse side reactions and inhibition of products are mostly avoided by directed passing of intermediates. The accompanied efficiency of bioconverison indicates the potential for industrial biotechnology. Targeted design of structural dynamic is also essential for therapeutic attempts.

In the human Pyruvate Dehydrogenase Complex (hPDC), an important structural feature for the spatiotemporal coordination of reactions is provided by the flexible linker domains of E2 and E3-Binding Protein (E3BP) [1]. Their role in regulation and self-assembly of hPDC make them suitable for rational reengineering [2]. As biophysical approach, we perform molecular dynamics simulation on this large complex, which assembles to a 60-meric core unit and a surrounding cloud of more than 30 subcomponents [2]. A coarse-grained model of full hPDC [3] is used to run reasonable time scales of simulation on such system of up to 9 MDa in size. As smallest stoichiometric entity of the four enzymes, the actual model system is a trimer of two E2 and one E3BP with either E1 or E3 as binding partner. Truncation on different sites of the flexible linker domains leads to clear changes in binding affinity between the trimer and either E1 or E3. The changes differ for truncation of lipoyl, linker or binding domains along the flexible arm. Affected residues, compactness of structures and structural dynamics of subcomponents play a role in interpreting the data. We compare the results to experimental data from biolayer inferometry (BLI) of corresponding mutants, allowing for verification, model improvement and, furthermore, integration into a multiscale model of enzyme agglomeration [4]. With this combined simulation-experimental approach, hPDC with its flexible domains may be suitable as scaffold for tailored bioprocesses with similarly modified linker domains [5].

[1] Perham, R.N. et al., Annual review of biochemistry 2000
[2] Guo, J. et al., Biochemical Journal 2017
[3] Hezaveh, S. et al., Journal Chemical Information Modelling 2018
[4] Depta, P.N. et al., Journal Chemical Information Modelling 2019
[5] Ruiz, D.M. et al., Scientific Reports 2016

P-473

Gamma-Secretase Substrate Recognition and Prediction

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The intramembrane aspartyl protease γ-secretase (GSEC) cleaves single-span transmembrane proteins like the C-terminal fragment (C99) of the amyloid precursor protein (APP) and the Notch1 receptor within their membrane-spanning helical domain. Aberrant cleavage of these substrates leads to development of Alzheimer's disease and several types of cancer. Processing of the C99 fragment of APP is known to be a multi-step reaction, involving recognition, binding, positioning and stepwise cleavage. Thus, knowledge of the substrate binding sites is essential for understanding the later steps. Recent experimental work has shown that the encounter of C99 and GSEC involves initial binding to an exosite and subsequent translocation of C99 to the active site located in the Presenilin subunit (PS1). However, multiple routes for substrate entry to the active site are discussed, and the involvement of PS1 transmembrane domains (TMDs) in gatekeeping is still elusive. Considering also the broad range of ~100 different substrates processed by GSEC it is so far unknown how the enzyme distinguishes between substrate and non-substrate.

To reveal how substrates and the few known non-substrates interacts with GSEC, we use the DAFT protocol (Docking Assay For Transmembrane components), which applies the Martini coarse-grained force field in combination with varying start geometries and a large number of replicates (>800) each simulated for 1 microsecond. Questions to be addressed include (i) the variability of the contact sites, (ii) the characteristic amino acid determinants for the protein-protein interactions and (iii) how the GSEC recognizes its substrates.

Our in-silico approach shows good agreement with the experimental data from photo-crosslinking experiments and structures of C99 and Notch1 complexed with GSEC as determined by cryo-electronmicroscopy. The hydrophilic loops L1 and L2 of PS1 TMD2 could be identified as main binding sites. The presenilin enhancer 2 (PEN-2) subunit of GSEC provided a secondary binding site. On the part of substrates, the regions flanking the membrane spanning domain were identified as major binding sites. Based on our results we were not only able to distinguish between substrates and non-substrates, but also between two groups of substrates.

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PypKa: A flexible Poisson-Boltzmann-based python API for pKa calculations

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The structure and function of biomolecules are highly dependent on the ionization state of titrable sites. In an average globular protein, ~25% of all residues are ionizable in water [1] and their pK_a values haves a direct influence on its physicochemical properties, such as polarity or lipophilicity. *In silico* cost-effective pK_a calculation tools have numerous applications, ranging from optimization of chemical leads, to building QSAR models or understanding structural/functional properties of biological systems.

Here, we present an open source python API for pK_a calculations with a valuable trade-off between fast and accurate predictions, that can be used to extend existing protocols by adding two extra lines of code and a few minutes of computation time. This module streamlines pK_a calculations by providing validated radii [2], charge distributions [3], and default PB parameters [4], and by pipelining the intrinsic pK_a, site-to-site interactions calculations and Monte Carlo sampling. Although default values are provided, user-defined models are allowed and easily implementable.

PypKa supports CPU parallel computing on anisotropic (membrane) and isotropic (protein) systems. As an example, we show how to easily calculate pK_a values of a membrane interacting peptide using a linear response approximation (LRA) approach.

References

[1] Kim, J., Mao, J., & Gunner, M. R. (2005). Are Acidic and Basic Groups in Buried Proteins Predicted to be Ionized? Journal of Molecular Biology, 348(5), 1283–1298.

[2] Teixeira, V. H., Cunha, C. A., Machuqueiro, M., Oliveira, A. S. F., Victor, B. L., Soares, C. M., & Baptista, A. M. (2005). On the use of different dielectric constants for computing individual and pairwise terms in poisson-boltzmann studies of protein ionization equilibrium. Journal of Physical Chemistry B, 109(30), 14691–14706.

[3] Schmid, N., Eichenberger, A. P., Choutko, A., Riniker, S., Winger, M., Mark, A. E., Van Gunsteren, W. F. (2011). Definition and testing of the GROMOS force-field versions 54A7 and 54B7. European Biophysics Journal, 1−14.

[4] Teixeira V.H., Vila-Viçosa, D., Reis, P.B.P.S. , Machuqueiro, M. (2016). pKa Values of Titrable Amino Acids at the Water/Membrane Interface. Journal of chemical theory and computation 12 (3), 930-934

P-475

Getting the MARTINI Coarse-Grain Model to Make Pores in Membranes

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The formation of membrane pores is a key step in the action of different drugs — namely, those that need to puncture or dissolve a membrane (like the antimicrobial peptides) or those that need to cross it, perhaps carrying along a payload (like the cell-penetrating peptides). Molecular dynamics simulations of poration processes could clarify mechanisms and be a basis for rational drug design and/or improvement. However, the size- and time-scales involved in poration puts this kind of simulations at the limit of the possibilities of conventional molecular dynamics.

The widely-used MARTINI coarse-grain model can be employed to mitigate the computational cost of simulating pores, and therefore open the door to large-scale efforts in membrane-porating drug design. However, a number of reports highlight the inaccuracy of MARTINI regarding the propensity of pore formation in that pores are exceedingly difficult to form in MARTINI membranes.

Using a recently-developed reaction coordinate of pore formation (Hub and Awasthi, JCTC 2017) we set out to characterize the energy profile of poration in MARTINI vs. an all-atom representation (CHARMM36). We find that reaching the transition state of pore formation in the MARTINI model is 90 kJ/mol more expensive, free-energy-wise, than in the atomistic counterpart. Such a high energy difference agrees well with the observations of difficult pore formation in MARTINI. We then separate the entropic and enthalpic contributions to the process, further discriminating the enthalpy into the several bonded and non-bonded components of each force-field. From this we are able to identify which parameters in the MARTINI force-field are causing the overestimation of pore formation energies and should therefore be adjusted.

Our diagnosis approach, albeit computationally expensive due to the need to converge enthalpy values for an atomistic system, proves to be a valuable investment in the improvement of the MARTINI force-field as a tool to tackle the promising field of pore-forming drug design.

P-476

Quantitative structural determination of small saccharides in solution combining Raman and Raman optical activity (ROA) spectroscopies with computer modeling

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Carbohydrates play a pivotal role in numerous functions in all organisms. However, contrary to other biomolecules, such as DNA or proteins, where techniques for their structural determination are abundant, saccharides are usually harder to study, because of their high conformational flexibility in solution. Raman and Raman optical activity spectroscopies are highly sensitive to any structural changes and hydration. Therefore, they are promising tools to study subtle conformational changes of saccharides in solution.

A critical drawback of Raman and ROA techniques is that the interpretation of their spectra by direct analysis of the obtained peaks and bands is impractical. Computer modeling techniques can help to interpret the experimentally recorded Raman and ROA spectra, providing a natural complementarity to these techniques. These modeling techniques usually aim to calculate the same spectra. When the calculated spectra successfully compare to the experimental ones, we can assume that the sugar structures used for the calculations are the relevant ones present in solution. Previous attempts to calculate Raman and ROA spectra for monosaccharides resulted in reduced or, at best, qualitative agreement between the spectra. Furthermore, the computational techniques used are demanding, which restricts the general adoption of the used protocols, for example, to study larger saccharides.

In this work, we have designed a new protocol that calculates Raman and ROA spectra resulting in a nearly quantitative agreement with experiments. Our new protocol also reduces the computational demands drastically, making it suitable for small oligosaccharides. This protocol combines classical molecular mechanics simulations (MM) to obtain an ensemble of saccharide structures with the usage of the QM/MM onion method on each structure to calculate the Raman and ROA spectra. In the QM/MM calculations, the sugar is treated in a quantum mechanics level using density functional theory. Instead, waters in contact with the sugar are treated as MM charges, while the rest are modeled using a continuum approach (cosmos). The result is an efficient protocol which ends in a quantitative agreement between the computed and experimental spectra. The developed method allows us to study the conformations of monosaccharides in solution, their anomeric ratio, or their propensity to aggregate. Our method has the potential to be applied to oligosaccharides too.

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Folding of cyclic peptides stabilized by halogen bonds

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The folding process of proteins and peptides is often stabilized by hydrogen bonds. In fact, most secondary structures observed in proteins are stabilized by these non-covalent, and mainly electrostatic, interactions (namely, alpha-helices and beta-sheets). Recently, it was proposed that, when halogen atoms are present in non-natural amino acids, the stabilization of folded structures can also be associated with halogen bonds (HaB) [1]. These are highly directional non-covalent interactions explained by the existence of a positive region on the electrostatic potential (ESP) of heavier halogens, called sigma-hole, which is available to interact with electron-rich species (i.e. Lewis bases). The improvement of computational methods to model the charge anisotropy of halogenated compounds is therefore mandatory to describe HaB interactions. The simplest approach to describe the ESP anisotropy in halogenated species involves the addition of an off-centre positive extra-point of charge mimicking the sigma-hole.

Here, we studied a family of cyclic peptides with 8 common residues and 2 that can interact via hydrogen bonds, HaB or none: NVXAGPVXQG (where X indicate the variable residues and P is a D-Proline) [1]. When the two X residues are serines, the folded state is stabilized by hydrogen bonding. On the other hand, when replaced with non-natural amino acids, (the -OH group of each serine is replaced by -Me and -OMe, respectively), the folded state became unstable, owing to the impossibility to form hydrogen bonds. With the replacement the -Me group with a chlorine atom, the folded state is recovered via a HaB interaction. These three peptides are studied using different force fields, with and without the addition of an extra-point to describe the sigma-hole. The folding equilibrium and the prevalence of stabilizing non-covalent interactions, are the main focus of this study.

[1] Danelius, E. et al; 2017, Biochemistry 56:3265

P-478

Tackling halogenated species in biomolecular systems with force field methods: MD sampling and PBSA solvation energies

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Halogen atoms have a prominent role in drug design and are also becoming a tool in protein-ligand recognition given the ability to form non-covalent interactions known as halogen bonds (R−X···B with X = halogen, and B = Lewis base), where the halogen acts as an electrophilic species interacting with electron-rich partners. The interaction is ascribed to the existence of a positive site on the electrostatic potential (ESP) of the halogen, termed σ-hole, and has found a multitude of applications [1].

In force field methods, the anisotropy of the ESP at the halogen is often emulated via an extra-point (EP) of charge introduced at a given distance from the halogen.

Herein we show that MM/MD sampling of halogen bonding interactions in protein-ligand systems is more accurate when the EP placed at a distance corresponding to the halogen Lennard-Jones parameter R_{min} [2]. The resulting trajectories could eventually be used in MM-PBSA calculations to estimate protein-ligand binding free energies, however, the effect of adding an EP to the PBSA-calculated solvation free energies is not known. Notice that standard halogen PB radii are smaller than the typical X-EP distances thus placing the EP within the solvent dielectric, corresponding to an unphysical model. We, therefore, show how to obtain a new optimized set of halogen PB radii (Cl, Br, I) which can be used for PBSA calculations when the EP is located at R_{min}. For that, we conducted a radii optimization (minimizing the error against experimental values) for a set of 142 halogenated compounds for which the experimental values are known [3].

[1] Costa, P. J. Phys. Sci. Rev. 2017, 2, 20170136.
[2] Nunes, R.; Vila-Viçosa, D.; Machuqueiro, M.; Costa, P. J. Chem. Theory Comput. 2018, 14, 5383-5392.
[3] Nunes, R.; Vila-Viçosa, D.; Costa, P. J. submitted 2019, preprint available in ChemRxiv (DOI 10.26434/chemrxiv.7660550.v1.)

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pH Replica Exchange simulations of pHLIP peptides

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The pH (low) insertion peptide (pHLIP) is an alpha-helical transmembrane peptide with the ability to insert into membranes in a pH-dependent manner [1]. The insertion process can be divided in two components: thermodynamic and kinetic. The thermodynamic component dictates the populations of each state and is mainly regulated by the protonation equilibrium of the Asp14 residue at the membrane interface [2]. The kinetic component relates to the speed at which state transitions occur, which is dependent on the full protonation of the C-terminus domain acidic residues [3]. The stochastic constant-pH molecular dynamics (CpHMD) method [4] was previously used to study *wt*-pHLIP and it allowed a detailed molecular description of the inserted configuration of this system. However, the method suffers from sampling issues at deep membrane regions where ionized states are less frequent, thus obtaining less precise estimates of pK_a values.

In this work, we present our study of the pHLIP system using a pH replica-exchange (pHRE) method, coupled to the CpHMD methodology to solve the previous sampling limitations. We calculated pK_a profiles for several key residues of pHLIP using different system parameters for both the CpHMD and RE simulations. By varying the number of replicates and pH values, it allowed us to make a fair comparison between simulations' speeds, sampling quality and the predictive ability of the two methodologies, comparing them with the experimental pK of insertion.

References

1. Andreev, O.A., Dupuy, A. D., Segala, M., Sandugu, S., Serra, D. A., Chichester, C. O.,Engelman, D. M., Reshetnyak, Y. K., PNAS, 104 (2007) 7893.
2. Vila-Viçosa, D., Silva, T.F.D., Slaybaugh, G., Reshetnyak, Y.K., Andreev, O. Machuqueiro, M., J. Chem. Theory Comput.,14(6):3289–3297, 2018.
3. Baptista, A. M.; Teixeira, V. H.; Soares, C. M., JCP, 117 (2002), 4184
4. Weerakkody, D.; Moshnikova, A.; Thakur, M. S.; Moshnikova, V.; Daniels, J.; Engelman, D. M.; Andreev, O. A.; Reshetnyak, Y. K. PNAS, 110 (2013), 5834

P-480

Investigation of binding energies of histidine-modulated arginine and tryptophan-based peptides in membrane models: *In silico* and spectroscopic studies

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Antimicrobial peptides (AMPs) are an ancient type of innate defense mechanism against pathogens. Because they show a high selectivity for bacterial membranes over mammalian ones, they are a good alternative for developing future AMP-based drugs. Starting from a previously reported highly efficient tryptophan- and arginine-rich AMP (RRWWRWWRR) several new analogs were designed by substitution of tryptophans or arginines with histidines. We performed non-equilibrium molecular dynamics simulations of one of the proposed peptides (HRWWRWWRR) to assess its membrane-binding behavior. We used potential of mean force (PMF) calculations to characterize AMPs free energy profiles with respect to their distance to the membrane surface. The obtained PMFs suggest higher affinity of the peptide for the bacterial membrane model compared to the mammalian model. Fluorescence experiments were performed by adding increasing concentrations of liposomes over peptides and monitoring the shift of tryptophan residues fluorescence. The fraction of peptides bound to the lipid membrane and the binding energies were determined for all proposed peptides. The experiments validated the in silico predictions of peptide membrane affinity.

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NUCLEOTIDE SPECIFIC AUTOINHIBITION OF FULL LENGTH K-RAS4B STUDIED BY MOLECULAR DYNAMICS WITH EXCITED NORMAL MODES

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RAS proteins have been a hot topic in cancer research for the past decades as RAS is frequently found mutated in cancer cells. Despite the intense efforts of researchers, no efficient RAS inhibitor has been discovered so far. Mutated RAS proteins can be found locked in their active, GTP-bound form and may not respond to GAP stimulation resulting a constantly active signal. Due to the high intracellular GTP concentration and the protein's high affinity to bind it, and also due to the lack of suitable small molecule binding sites on the surface of Kras the protein is thought to be 'undruggable'. It has been pointed out that our view of RAS structure and mechanism through which the protein is regulated is mainly based on static structures. This approach overlooks conformational dynamics and allosteric effects.

The goal of the presented research is to thoroughly map the conformational space of GDP-bound inactive, and GTP-bound activated fullength human KRas proteins, to identify the effects of the Hyper Variable Region (HVR) onto the catalytic domain in its two activation states, and to understand the nucleotide specific autoinhibition mechanism of KRas with the help of the recently developed conformational mapping method: Molecular Dynamics with Excited Normal Modes (MDeNM).

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Characterization and identification of Ampicillin and Amoxicillin binding sites within the multidrug transporter MexB of *P. Aeruginosa*

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Efflux systems of the Resistance Nodulation-cell Division (RND) superfamily are transmembrane transport proteins playing a key role in Multi-Drug Resistance in Gram-negative bacteria. MexAB-OprM, the major RND efflux pump in *Pseudomonas Aeruginosa,* can extrude a wide range of chemically unrelated compounds, including antibiotics[1]. Up to date, no X-ray MexB structures, in complex with antibiotics, have been solved and the dynamics of the transport process remain largely unknown. Henceforth, the understanding of the molecular determinants regulating recognition, binding, transport and extrusion of the efflux substrates is crucial to design more effective antibiotics and/or inhibitors.

In this work, as part of an extensive research activity on RND transporters of Gram-negative bacteria[2], we present a comparative investigation on the interaction between MexB and two penicillins, namely amoxicillin and ampicillin.

Amoxicillin differs from ampicillin by a hydroxyl group on the 2-amino-2-phenylacetamide substituent bound to the penicillin core. Despite this subtle chemical difference, different experimental studies revealed that only ampicillin is a substrate of MexB[3]. In order to rationalize this different behaviour at an atomistic resolution, we applied a combination of different computational techniques. Several molecular docking poses found inside of the so-called distal binding pocket (DP) of MexB have been selected as starting points to run classical molecular dynamics simulations, followed by binding free-energy calculations. For each of the two antibiotics, our study revealed a preference for the different DP sub-regions, along with a favoured orientation and representative key interactions with the DP residues. Our main findings, in agreement with microbiology and mutagenesis studies, could provide useful information for the design of new compounds able to evade or inhibit the efflux process.

References*:*

1. Masuda, N.; Sakagawa, E.; Ohya, S.; Gotoh, N.; Tsujimoto, H. *Antimicrob Agents Chemother,* **2000***, 12, 3322-3327*
2. Atzori, A.; Malviya, V. N.; Malloci, G.; Dreier, J.; Pos, K. M.; Vargiu, A. V.; Ruggerone, P. *Biochimica et Biophysica Acta – Biomembranes,* **2019**, *1861*, 62-74

Cooper, S.J.; Krishanamoorthy, G.; Wolloscheck, D.; Walker, J.K.; Rybenkov, V.V.; Parks, J.M; Zgurskaya, H.I. *ACS Infect Dis*, **2018***, 4, 1223-1234*

P-483

Combining molecular docking and molecular dynamics simulations to propose novel antimicrobial peptides

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The World Health Organization revealed that antimicrobial (AM) resistance is an increasing threat to world public health. One important cause is the bacteria developing resistance strategies against naturally occurring AM peptides, which are host defensive peptides that present high selectivity of interaction with bacterial cells over mammalian cells. First, we applied molecular docking simulations of specifically selected peptides from PepBank peptide database to bacterial and mammalian membranes, in order to reduce the large amount of available peptides. Second, we used molecular dynamics simulations in order to propose a list of candidates for potential further testing (e.g. using potential of mean force calculations). We found that combining these two methods is a very efficient, and relatively fast, *in silico* approach that can be used to propose a reasonable number of potentially effective antimicrobial peptide candidates to be validated by means of experimental techniques.

P-484

Improved modeling of halogen-protein interactions in biomolecular simulations using a GROMOS force field

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Halogen bonds (XBs) are non-covalent interactions, were heavier halogens (Cl, Br, I) interact with electronegative species, that have been predominantly explained by the existence of a positive region on the electrostatic potential of halogenated species, named σ-hole. These interactions have been gaining attention in chemical biology and rational drug design where they can be employed as tools to modulate biomolecular recognition phenomena [1]. For that reason, the development of molecular modeling methodologies that appropriately describe XBs is mandatory [2]. While quantum-mechanical methods are typically only attainable in the context of model systems, force field (FF)-based methods used in biomolecular simulations are most commonly chosen in computer-aided drug design routines. Regrettably, traditional FFs typically fail to describe XBs since the halogen is represented by a point charge not accounting for its charge anisotropy. This limitation can be circumvented through the addition of a positive extra-point (EP) of charge to mimic the σ-hole. This approach reproduces experimental geometries and energetics, but their application to molecular dynamics (MD) simulations has been limited.

In this work, we developed an EP-based strategy compatible with the united-atom GROMOS 54A7 FF, using bacteriophage T4 lysozyme in complex with iodobenzene ligands as a prototype system [3]. In contrast with previous reports, our analysis is based on the capability of different EP parameterization schemes to sample XBs and other relevant intermolecular interactions during MD simulations. The results showed that the implementation of an EP at distance from iodine corresponding to the Lennard-Jones parameter R_{min} provides a good qualitative description of XBs in MD simulations. This methodology can be employed as a valuable tool in the framework of computer-aided drug discovery and is currently being extended to probe the role of XB interactions in other relevant biomolecular systems by MD simulations and free energy calculations.

1. Costa, P. J. Phys. Sci. Rev. 2017, 2, 20170136.
2. Kolar, M. H.; Hobza, P. Chem. Rev. 2016, 116, 5155-5187.
3. Nunes, R.; Vila-Viçosa, D.; Machuqueiro, M.; Costa, P. J. Chem. Theory Comput. 2018, 14, 5383-5392

P-485

Rethinking hydrophobicity: dangling OH groups as molecular fingerprints

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The hydration shell of hydrophobic molecules and moieties has more non-hydrogen bonded hydroxyl groups than bulk water. These “free” hydroxyls, or defects, are thought to be relevant both for hydrophobic hydration, hydrophobic collapse and for the stabilization of transition states in some reactions taking place at hydrophobe-water interfaces (“on-water”), thus acting as catalysts. Measuring and manipulating defect formation is thus a promising strategy to investigate and tune hydrophobic hydration and collapse, and on-water catalysis. Perfluoroalkyl groups – with their larger size and presumed lower dispersion interactions with water than the corresponding alkyl groups – offer a pathway to manipulation. Here we compare the thermodynamics of defect formation around methyl and trifluoromethyl substituents by investigating dilute aqueous solutions of 2,2,2-trifluoroethanol (TFE) and ethanol (EtOH). Using molecular dynamics simulations, first-principles calculations and SC-Raman-MCR spectroscopy, we find that defect formation is dramatically more probable near the more hydrophobic TFE than near EtOH. In both cases, the hydroxyl group involved in such defects is directed towards the methyl/fluoromethyl group in the solute. The enthalpy of defect formation is unfavorable for both solutes, particularly for EtOH (by several kJ/mol), whereas the entropy of defect formation is favorable for both solutes, particularly for EtOH. These results suggest that the hydration shell of EtOH is more ordered than that of TFE, which is confirmed by calculating the entropy of solvation using free energy perturbation simulations. First-principles calculations demonstrate - contrary to prior reports - that weak water-solute electrostatic interactions are the main driving force for enhanced defect formation near TFE relative to EtOH, with dispersion interactions and with changes in volume having a secondary role. These results indicate we should step away from the classical picture of hydrophobe for fluorinated substituents: fully-fluorinated substituents are better thought of as a different class of hydrophobe, containing an intrinsic length and energy scale dictated by the polarity of the individual CF bonds. Defect formation emerges as an experimentally quantifiable fingerprint for the identification of different classes of hydrophobes.

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If you can’t win them join them: Understanding new ways to target STAT3

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Signal transducer and activator of transcription 3 (STAT3) is an important cancer target and a potential novel candidate for therapeutic intervention. However, STAT3 is one of the most challenging cancer-related proteins to target by a small molecule ligands. Although several inhibitors have been published to date just very few of them are still going through clinical trials. Additionally, there is a lack of knowledge of their binding mechanism. SH2 domain has been the main target for drug design but afterwards no crystallographic data was provided that could support most weak computational models based mainly in molecular docking.

It has been described by Mertens et al that a series of mutations in residues that are placed between the linker and DNA-binding domain can decrease (I.e K551A, W546A) or enhance (e.g. D570K) DNA binding with the protein dimer. These residues seem to form key inter-domain interactions responsible for the proteins stability and/or proper DNA binding. Anyhow, the data obtained is not able to explain which possible conformational changes can be occurring on these mutated systems to suffer these activity variations. Understanding what are these mutations triggering could forward to the unveiling and/or targeting of new potential binding sites, therefore completely changing how the system has been targeted the last years.

It has been recently identified that BBI-608, a phase 3 STAT3 ligand, inhibitor patent shows that the ligand binds between the linker and DNA-binding domain. Its overlap with the mutated K570 residue might indicate that the ligand triggers a similar behaviour.

Through a series of computational procedures such as Molecular Dynamics (MD) and Umbrella Sampling (US) simulations we have been able to determine the behaviour of these mutated systems from an atomistic point of view. Furthermore with the same proceedings we could correlate BBI-608’s mode of action.

P-487

Specific interactions between membrane protein glycans and GM3

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Abnormal glycosylation of membrane receptors is associated with various pathological conditions, including neurological diseases and cancer. Intriguingly, recent studies have shown that membrane protein glycans can modulate the visibility and activity of membrane receptors by interacting with the cellular membrane interface (1,2). In particular, it has been demonstrated that a number of crucial cell receptors are modulated by the membrane glycolipid GM3 (3). One of the prevalent hypotheses suggests that GM3 binds to specific termini of protein glycans and maintains functional receptor conformations at the membrane interface. Moreover, experiments have shown that the protein-GM3 binding strongly depends on the sequence of the protein glycans. The binding affinity was reported to be the highest for proteins with GlcNAc termini and much lower for other glycan types. However, due to the limitations of experimental approaches, the details of how the protein glycans interact with GM3 remain unclear. In this work, using atomistic molecular dynamics simulations of membrane systems with typical functional protein glycan termini, we clarified how specific protein glycan structures interact with GM3. Our results show that glycans with sialylated termini behave in a distinctly different manner compared to other termini often found in glycans attached to membrane receptors. The simulation results are in good agreement with experimental data. Overall, the results provide an accurate picture of glycan-GM3 interactions to better understand the mechanisms by which glycans control membrane receptors’ function and activity.

1. A. Polley, A. Orlowski, R. Danne, A. A. Gurtovenko, J. Bernardino de la Serna, C. Eggeling, S. J. Davis, T. Rog, I. Vattulainen. J. Phys. Chem. Lett. 8, 1060 (2017).
2. K. Kaszuba, M. Grzybek. A. Orlowski, R. Danne, T. Rog, K. Simons, Ü. Coskun, I. Vattulainen. Proc. Natl. Acad. Sci. U.S.A. 112, 4334 (2015).
3. A. Ernst, B. Brugger. Biochim. Biophys. Acta 1841(5), 665-670 (2014).

P-488

Improvement of Protein classical force fields by introducing Electronic Continuum Correction in non-polarizable force fields.

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Proteins play a number of critical functions in every organism. However, it should be highlighted that it is the interaction between these proteins and their environment (cytosol, membrane and so on) that often dictates their function. Many essential biological functions depend on the proper binding between proteins or between proteins and cellular membranes, and this binding is often selective to a specific lipid type. Recently, an important biological role has been recognized to Intrinsically Disordered Proteins (IDP), whose function depends on the plasticity of their secondary and tertiary structures. In all of these phenomena, a key role can be played by ions, which can modulate inter- and intra-molecular forces.

Molecular dynamics simulations have been intensively used to achieve knowledge of these molecules and the mechanism behind their biological roles, yet despite efforts, exhaustive description of some properties by MD simulations are still elusive. One of the most important limitations of MD simulation arises due to the use of non-polarizable force fields. These force fields often lead to overestimation of binding among ions, proteins, and membranes. Furthermore, IDPs show a more compact structure compared with some experiments, leading to rigid structure (alpha-helix or Beta-sheet mostly).

Recently, the Electronic Continuum Correction (ECC) was introduced to take into account the polarization effect in standard (non-polarizable) force fields. This leads to a better description of the interaction between ions or between ions and membrane surface. The next logical step is to introduce ECC in commonly used protein force fields, too.

In this work, we show how the introduction of the ECC correction into CHARMM36 force field in single amino acids and in very short peptides improves the force field simulation of these systems. First, using osmotic coefficient calculations, we demonstrate that the ECC correction improves interactions among amino acids. Second, with comparison to NMR data, we show that the ECC correction also leads to a better description of the amino acid structure. Third, we also discuss how the ECC correction improves the description of the interplay between lipids, proteins and ions.

P-489

Evaluation of different computational approaches to measure water permeabilities in Aquaporins

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Aquaporins are a class of membrane water channels whose function is to facilitate the passive transport of water across membranes of cells in response to osmotic gradients (1). These membrane proteins are essential for water homeostasis and cell volume control, and members of this protein family have been implicated in numerous physiological processes such as trans epithelial fluid transport, CNS function, cell migration and proliferation. These membrane proteins have been shown to be mechanosensitive (2), since they are able to sense subtle changes in the membranes properties and be affected in their function if changes in these properties are observed (3).

Within the framework of an on-going project being developed on our research group focused on the identification of membrane PAINS, in this work, we compare and further develop different computational approaches to accurately measure water permeability rates through these protein's channel. The sensitivity of the evaluated methods to different membrane perturbing agents affecting the function of the protein is of utmost importance. Therefore, different membrane perturbing vehicles acting directly on the membrane where the Aquaporin protein is embedded will be tested, and the accuracy of the different selected approaches will be evaluated. All the obtained results will have an experimental counterpart, whose results will be used to validate the computational predictions.

(1) Agre, P. et al. (2003), FBBS Lett. 555:72-78
(2) Soveral, G. et al. (2008), Biochim. Biophys. Acta – Biomembr., 1778:2573-2579
(3) Andersen, O.S et al. (2007), Annu. Rev. Biophys. Biomol. Struct, 36:107-130

P-490

Styrene-Dopamine receptor affinity: a Molecular Dynamics study

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Styrene is an organic molecule which has several industrial applications. It is extensively used in industrial processes, for example as a reactive diluent in epoxy resins, as an intermediate in the preparation of a variety of agricultural and biological chemicals, cosmetics, surface coatings, treatment of textiles and fibers and as a raw material for the production of phenyl stearyl alcohol in perfume industries. Significant scientific evidence has been collected in the last decades demonstrating that exposure to styrene, either alone or in concert with noise exposure, has severe ototoxic effects. Occupational medicine studies, performed among workers exposed to this solvent, show a significant decrease of the DPOAE (distortion product otoacustic emissions) levels in styrene exposed ears [1, 2, 3]. Neurotransmitters such as dopamine have been supposed to be implicated in the mechanism of styrene neurotoxicity. In normal ears, in the outer hair cells membrane proteins able to bind dopamine have been indeed detected. They exist in five variants, known as "dopaminergic receptors" (DR) D1, D2, D3, D4 and D5. Interestingly, D1 and D2 knockout mouse models showed slight, but visible suppression of cochlear responses [4], suggesting a role of these two receptors in the response. In this work we have used molecular docking to identify the poses on the DRD2 receptor where either styrene or dopamine have the highest binding affinity. We find that the relevant poses are located in the Transmembrane 3, Transmembrane 5 and Transmembrane 6 domains. Our results confirm the data of ref. [5] where the contact loci of dopamine on the DRD2 receptor were identified. We are now performing extensive classical molecular dynamic simulations in order to provide a quantitative evaluation of the binding affinity of styrene as well as dopamine for the D2 dopaminergic receptor.

[1] B. W. Lawton, *et al.*, *Int. Arch. Occ. Env. Hea.,* 2 (2006): 93-102.
[2] R. Sisto, *et al.*, *J. Acoust. Soc. Am.,* 134.5 (2013): 3739-3748.
[3] R. Sisto, *et al.*, *Int. J. Audiol.,* 55.9 (2016): 523-531.
[4] S. F. Maison, *et al.*, *J. Neurosci.,* 32.1 (2012): 344-355.
[5] M. Y. S. Kalani, *et al.*, *PNAS,* 101.11 (2004): 3815-3820.

P-491

Molecular dynamics simulation and structure analysis reveal mechanistic insight into protein crystallization

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For decades, crystallization of proteins has been an essential procedure for structure analysis. A new approach uses technical crystallization to purify proteins as an alternative to preparative chromatography. As molecular processes during protein crystallization are barely understood, it requires high empirical screening effort for finding crystallization conditions.

Thermodynamically, the crystallization process is the transition from a solute phase (solvated protein) to a solid crystal phase (symmetrically bound proteins) with enthalpic and entropic contribution to the associated free energy change. We aim to explore and understand these intermolecular mechanisms by investigating altered crystallization behavior of engineered protein variants by means of X-ray structure analysis and molecular dynamics (MD) simulations.

Lactobacillus brevis alcohol dehydrogenase (*Lb*ADH) (PDB-IDs 6h07, 6h1m [Hermann *et al.*, 2018]) was engineered at the crystal contacts and the resulting altered crystallization behavior was examined [Nowotny *et al.*, 2019]. We structurally analyze these crystals in X-ray diffraction experiments and reconstruct the crystal structures *in silico* to identify possible mechanisms of improved crystallizability and provide an input structure for MD simulations. Free energy differences between crystallization of *Lb*ADH variants are calculated with alchemical free energy perturbations and altered intermolecular interactions are analyzed with end point method mm-gbsa (molecular mechanics [mm] with generalized born [gb] surface area [sa]). For example, in *Lb*ADH variant K32A (PDB-ID 6hlf) we revealed a crystal contact enforcement in the measured crystal. MD simulations allowed us to identify a unfavorable intramolecular interaction of Lysine32 in the wildtype enzyme. We calculated an entropy reduction and enthalpy gain at the surface upon mutation which matches the examined crystallization behavior of K32A. With the experimentally measured X-ray crystal and theoretically calculated thermodynamic quantities, we are able to explain the underlying mechanisms on an atomistic level. In the future, this may allow us to deduce general rules and to predict amino acid mutations which facilitate technical protein crystallization.

Hermann J, Nowotny P, Schrader TE, Biggel P, Hekmat D, Weuster-Botz D (2018). *Acta Cryst F*74: 754-764.
Nowotny P, Hermann J, Li J, Krautenbacher AE, Klöpfer K, Hekmat D, Weuster-Botz D (2019). *Cryst. Growth and Des.*

P-492

Molecular dynamics investigation of protein-membrane association for lipid transfer.

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Lipid Transport Proteins (LTPs) provide a rapid non-vesicular pathway for the transport of lipids between membranes. One such superfamily of LTPs are the Tubular lipid binding proteins (TULIPs), characterised by a distinctive tubular fold, and an internal pocket with a hydrophobic lining to enable binding of hydrophobic molecules[1]. Some examples of proteins belonging to the TULIP super-family include the Bactericidal/Permeability-Increasing (BPI) protein, the Cholesteryl Ester Transfer Protein (CETP), and the Extended Synaptotagmins (E-Syts 1-3). However, all available crystal structures of LTPs have been obtained in the absence of a membrane environment, and hence do not provide a complete understanding of how these proteins associate with membranes at the molecular level. Consequently, the mechanisms by which these proteins are able to transfer lipids also remain poorly understood.

Here, we employ all-atom (AA) and coarse-grained (CG) molecular dynamics simulations to understand the mechanism of membrane association of multiple proteins of this superfamily. Our results highlight the role of membrane properties in protein binding, and provide a rationale for the low membrane binding affinity of this protein family observed in biochemical binding assays.

References
1. K.O. Kopec, V. Alva, A.N. Lupas, Bioinformatics of the TULIP domain superfamily, Biochem. Soc. Trans. 39 (2011) 1033–1038

P-493

Computational characterization of membrane PAINS

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Pan-assay interference compounds (PAINS) are promiscuous molecules with apparent bioactivity that can interfere with the result of biological assays. These compounds are often mistakenly flagged as positive hits [1], acting as burden agents in drug screening processes. There are several categories of PAINS, but an especially problematic and underestimated category are the so-called membrane PAINS [2]. These compounds interact directly and nonspecifically with lipid membranes, promoting changes in their biophysical properties and ultimately affecting the function of mechanosensitive membrane proteins. Despite developed efforts, the identification of these compounds in initial compound screening phases of drug discovery is still very imprecise.

We will describe a new computational protocol to identify and characterize membrane PAINS. This protocol is based on an already validated method [2], featuring atomic detail potential of mean force (PMF) calculations using umbrella sampling (US) techniques. These calculations allow the estimation of the perturbing effect of these compounds on membrane permeability and stability. Our validation set comprises molecules with reported minor, mild and major membrane PAINS activity [2,3]. Since one of the main concerns limiting the accuracy of these calculations is related with long equilibration times and insufficient sampling in each US window, we will also show new advances to the initial protocol based on longer simulation times and the use of enhanced sampling methods.

[1] Baell, J.B. et al. (2010) JMC 53(7):2719 doi.org/10.1021/jm901137j
[2] Ingólfsson, H.I. et al. (2014) ACSCB 9(8):1788 doi.org/10.1021/cb500086e
[3] Jesus, A.R. et al. (2017) JMC 60(2), 568-579 doi.org/10.1021/acs.jmedchem.6b01134

P-494

Long-range Electron-Electron Interaction and Charge Transfer in Protein Complexes

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With application to the membrane-anchoring heterohexameric cytochrom *c* nitrite reductase protein complex $NrfH_2A_4$ of *Desulfovibrio vulgaris,* we suggest a strategy to compute the energy landscape of electron transfer in large systems of biochemical interest. For small complexes, the energy of all electronic configurations can be scanned completely by a numerical solution of the Poisson-Boltzmann equation [1, 2], while larger systems have to be treated using a pair approximation. Effective Coulomb interactions between neighboring sites of excess electron localization my become as large as 200meV, and they depend in a nontrivial manner on the intersite distance. We discuss the implications of strong Coulomb interactions for the thermodynamics and kinetics of charging and decharging a 28 cytochrom *c* center containing protein complex. Finally, we turn to the influence of embedding the system into a biomembrane.

[1] Li, L. et al., *BMC Biophysics*, **2012**, *5*, 9, 1-11.
[2] Gnandt, D. et al., *Biophys. Chem.*, **2018**, *241,* 1-7.

P-495

Strategies to improve the description of metal-ligand interactions in biomolecular simulations.

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Metalloproteins play an important role in many biological processes and are thus not only interesting targets for drug design, but are also of increasing importance for biotechnological applications due to their function as biocatalysts, catalyzing a large variety of reactions (Bornscheuer 2018). About one third of all proteins in the Protein Data Bank contain a metal ion, illustrating the importance of these enzymes (Putignano et al. 2017). Therefore, approaches which allow an accurate treatment of the metal-binding properties in such proteins during molecular docking and molecular dynamics simulations are of increasing importance in the field of computational biophysics.

However, it still remains challenging to properly describe the complex nature of metal ions, mainly due to their strong polarization effects, their ability to adopt multiple oxidation states, and the formation of specific coordination geometries. Several strategies have been proposed, ranging from modifications of the Lennard-Jones potential to account for ion-induced dipole interactions to ‘dummy-atom’ models for an improved description of the metal’s coordination geometry. Additionally, hybrid Quantum Mechanics/Molecular Mechanics (QM/MM) methods are regularly applied to model the metal’s environment, however due to the high computational costs of these methods, their applicability is still limited (Li and Merz 2017).

We evaluated several of these methods with respect to their potential to describe metal-ligand interactions properly. We looked into their capability to reproduce and sample the metal’s environment correctly as well as their usefulness for binding free energy calculations. Finally, we used this information to develop a new molecular docking and simulation protocol for metal-containing binding sites, which we applied in molecular docking studies of Metallo-β-Lactamases and Amidohydrolases, both containing bimetallic binding sites.

Bornscheuer UT (2018) The fourth wave of biocatalysis is approaching. Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences 376:20170063

Li P, Merz KM (2017) Metal Ion Modeling Using Classical Mechanics. Chemical Reviews 117:1564-1686

Putignano V, Rosato A, Banci L, Andreini C (2017) MetalPDB in 2018: a database of metal sites in biological macromolecular structures. Nucleic Acids Research 46:D459-D464

P-496
Atomistic investigation of phosphonium dications interacting with simple models of bacterial membranes
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The pharmaceutical potential of room temperature ionic liquids (RTIL) is actively investigated since at least ten years ago (Hough WL., et al., New J. Chem. 2007, 31, 1429). Moreover, it is largely recognised that many bactericidal agents are ionic at physiological conditions, including non-protein polypeptide chains that represent a broad class of antibiotics. The di-cation component of a RTIL compound consisting of a short aliphatic chain terminated by two phosphonium groups has been shown to possess high and broad bactericidal activity, being effective against both gram-positive and gram-negative bacteria (O'Toole, et al., Cornea 31, 810 (2012)). Moreover, its quantitative effect sensitively depends on the length of the neutral aliphatic moiety.
By atomistic MD simulations we investigate the interaction of phosphonium dications with a bilayer made of: (i) POPC, and of (ii) a phospholipid binary mixture representing a closer mimic of a bacterial membrane. Dications of different chain length (from 6 to 12 CH2 groups) are considered and compared. The computational investigation aims at elucidating the structural, thermodynamic and dynamical properties of chain-like multivalent organic ions adsorbed on lipid bilayers. More in general, our study aims at investigating the biomembrane role in the antibacterial activity of multivalent cations. The simplicity of the aliphatic-dication species holds the key to discriminate effects of length and ionic charge from those of chemically specific interactions.

P-497
Structural characterization of Zebrafish Prestin
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Prestin is a membrane protein located in the cochlea outer hair cells (COHC) of mammals. It acts as a voltage-dependent motor protein, and it is responsible for hearing process. In fact, in response to electrical stimuli, it converts the signals into mechanical ones by elongating and contracting itself (electromotility). Its malfunctioning might causes several diseases, including deafness. Mammalian prestin is unique in its kind. It belongs to the solute carrier transmembrane protein 26A family, but it does not carry out any transport-like function. Prestin orthologs of the same family show, instead, an anion exchanger/transporter behaviour. Their transport mechanism is still matter of debate. In this work we focused on the zebrafish ortholog. Zebrafish prestin, in fact, is an ideal candidate to study the transport function of the family and to allow a comparison with the mammalian case, since its amino-acid sequence shares a high similarity with the mammalian counterpart. To shed a light on the zebrafish prestin function we performed molecular dynamics simulations of the protein in several conformations. We refined the initial structures obtained by homology modelling and assessed their quality with respect to the available experimental data.
We identified a putative binding site for the solute and gained an insight of possible residues actively involved in the transport mechanism. These new elements would improve as well the understanding of the mammalian prestin case.

P-498
Density Functional Theory calculations of the main components of the yellow pigment from mycorrhizal roots
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Mycorrhizae refers to the symbiotic relationship between fungi and plant roots. The types of mycorrhizae most studied worldwide are ectomycorrhizae and arbuscular mycorrhizae (AM). Some of the benefits of the AM is their use as biofertilizers, bioprotectors and bioremediators. When the AM fungi colonize the roots of some plants, a yellow coloration occurs in the roots, this coloration is known as yellow pigment which can be an indicator to estimate the degree of mycorrhization. It has been reported that the main components of the yellow pigment from arbuscular mycorrhizal roots are two apocarotenoids: mycorradicin (an acyclic C14 polyene) and blumenol C cellobioside (a C13 cyclohexenone diglucoside). We report on the first Density Functional Theory (DFT) calculations of each reported component. Absorption and photoluminescence spectra were calculated from previously optimized geometries using Time-Dependent Density Functional Theory (TD-DFT). These calculations are in good agreement with our experimental measurements and those previously reported by other authors and will be further discussed.

Tuesday 23rd July
LIVE IMAGING AND OPTICAL MICROSCOPY

P-499 (O-135)
Strong cytoskeleton activity on millisecond timescales upon particle binding revealed by ROCS microscopy
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Cells change their shape on the scale of seconds, cellular protrusions even on subsecond timescales enabling various responses to stimuli of approaching bacteria, viruses or pharmaceutical drugs. Typical response patterns are governed by a complex reorganization of the actin cortex, where single filaments and molecules act on a much smaller and faster timescale. These dynamics have remained mostly invisible due to a superposition of slow and fast motions, but also due to a lack of adequate imaging technology. Whereas fluorescence techniques require too long integration times, novel coherent techniques such as ROCS microscopy can achieve sufficiently high spatiotemporal resolution. ROCS uses rotating back-scattered laser light from cellular structures and generates a consistently high image contrast at 150nm resolution and frame rates of up to 100 Hz - without fluorescence or bleaching. Here, we present an extension of ROCS microscopy that exploits the principles of dynamic light scattering for precise localization, visualization and quantification of the cytoskeleton activity of mouse macrophages. The locally observed structural reorganization processes, encoded by dynamic speckle patterns, occur upon distinct mechanical stimuli, such as soft contacts with optically trapped beads. We find that a substantial amount of the near-membrane cytoskeleton activity takes place on millisecond timescales, which is much faster than reported ever before.

P-500 (O-136)
Multiplexed single-molecule fluorescence imaging by FRET-PAINT
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We combined DNA-PAINT imaging with single-molecule FRET read-out and demonstrate multiplexed detection with sub-diffraction optical resolution [1]. For this purpose, we designed pairs of short oligonucleotides labeled with donor and acceptor fluorophores for different FRET efficiencies, and integrated the design into the concept of DNA-PAINT. We demonstrate this FRET-PAINT approach by designing and imaging DNA origami, on which several target binding sites were spaced ~ 55 nm. We resolved the individual binding sites, and in addition determined the FRET efficiency for each site in single and mixed populations. The combination of FRET read-out and DNA-PAINT allows for multiplexed super-resolution imaging with low background, in conjunction with distance sensitive readout in the 1-10 nanometer range. We apply FRET-PAINT as a multiplexing imaging tool in combination with RNA-FISH.
[1] Deussner-Helfmann et al. (2018). Correlative Single-Molecule FRET and DNA-PAINT Imaging. Nano Letters *18*, 4626-4630.

P-501 (O-137)
In vivo single-molecule imaging of DNA gyrase
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DNA gyrase is a type II topoisomerase that performs a vital function in bacteria of introducing negative supercoils and relaxing positive supercoils generated by DNA replication and transcription. This essential role of DNA gyrase has resulted in the development of several antibiotics that kill the cell by specifically targeting it and interfering with its function. Although there have been extensive biochemical, structural and genetic data, we know little of how it operates in complex cellular environments.
Here, we used genetic techniques to attach specific fluorescent proteins to the subunits of gyrase and to other parts of the cellular molecular machinery, which are involved in the activities of gyrase. We tracked gyraseA (GyrA) and gyraseB (GyrB) subunits in real time using high-speed single-molecule fluorescence microscopy in living *Escherichia coli* cells, allowing us to explore accurately where in the cell these molecules act to super-resolution precision and how many of them are involved in their cellular activities. We correlated the data on GyrA and GyrB to assess if both gyrase subunits are permanently coupled or if they assemble during catalytic engagement with DNA. We also studied how the activities respond to antibacterials, which target gyrase, to address how gyrase poisons can be tolerated by cells and lead to antibacterial resistance.
Our single-molecule approach with DNA gyrase using advanced light microscopy in living cells is allowing us to address fundamental questions concerning the role of ATP hydrolysis in general topoisomerase activity in order to perform its vital role of relaxing torsional stress in DNA.

P-502

Probing PIP_2-induced aggregation of Tau filaments by tip-enhanced Raman spectroscopy

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Tip-enhanced Raman spectroscopy (TERS) is a powerful technique combining the high sensitivity of surface-enhanced Raman spectroscopy (SERS) and the nanoscale lateral spatial resolution of scanning probe microscopies, such as atomic force microscopy (AFM) and scanning tunneling microscopy (STM). AFM-TERS has been already employed to achieve nanoscale chemical characterization of biomolecules and biomaterials [1]. In particular, the amino sequence and the secondary structure of various proteins have been reported.

Here, the morphology and secondary structure of peptide fibers formed by aggregation of tubulin-associated unit (Tau) fragments (K18) are determined with nanoscale (<10 nm) lateral spatial resolution using TERS [2]. The aggregation process is investigated in the presence of two cofactors, namely the inner cytoplasmic membrane phosphatidylinositol component (PIP_2) and heparin sodium (HS). The inclusion of PIP_2 lipids in fibers is demonstrated based on the observation of specific C=O ester vibration modes. Furthermore, analysis of amide I and amide III bands suggests that the parallel beta-sheet secondary structure content is lower but the random coil content is higher for fibers grown from the PIP_2 cofactor instead of HS. These observations highlight the occurrence of some local structural changes between these fibers. This study constitutes the first nanoscale structural characterization of Tau/phospholipid aggregates, which are implicated in deleterious mechanisms affecting neural membranes in Alzheimer's disease.

[1] S. Bonhommeau, S. Lecomte, *ChemPhysChem* **2018**, *19*, 8-18.
[2] D. Talaga, W. Smeralda, L. Lescos, J. Hunel, N. Lepejova-Caudy, C. Cullin, S. Bonhommeau, S. Lecomte, *Angew. Chem. Int. Ed.* **2018**, *57*, 15738-15742.

P-503

***In vitro* nanoparticle tracking in live LA-4 epithelial cells using super-resolution one-photon and two-photon excitation STED microscopy**

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Assessment of nanomaterial effect frequently relies on nanomaterial localization within living cells. While localization of nanomaterials can be attained in fixed cells using high-resolution transmission and scanning electron microscopy, the localization within living cells still poses an enormous challenge. Optically based methods such as fluorescence microscopy are currently best suited for such studies under physiological conditions.

Tracking of nanomaterials using fluorescence microscopy has recently been enabled by development of super-resolution stimulated emission depletion (STED) fluorescence microscopy. We have applied one-photon (1PE) STED microscopy in study of dynamic interactions of the cell surface with the nanomaterial. While 1PE imaging yields good resolution when imaging in xy plane, xz plane (cross-section) imaging resolution in thicker samples is hindered by point spread function and beam distortion due to interchanging refraction indexes on the borders between lipids in the cell membranes and liquid outside the cell membrane. This results in reflection like effects where the nanomaterial sits on the cell membrane, due to cone-shaped nature of 1PE excitation, even when using 3D STED.

We have solved this using two-photon excitation (2PE) instead of 1PE, where excitation is limited to focal volume of the beam. This 2PE STED method also enables recording of cross-sectional dynamics of nanomaterial with increased resolution, such as recordings of nanomaterial passing through the membrane. Due to excitation volume selection 2PE STED increases detection dynamic range which enables us to resolve both large aggregates as well as individual nanoparticles. Some of the challenges and results of nanomaterial tracking and recording of system dynamics using 1PE and 2PE STED in planar and cross-sectional imaging will be presented.

P-504

Super-Resolution Photo-Activated Thermography

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Conventional thermal imaging provides temperature spatial maps based on the intensity of infrared radiation emitted by the sample and detected by a microbolometer-based thermal camera under the assumption of grey body radiance. The typically low numerical aperture of collecting Germanium lenses sets a diffraction-limited spatial resolution of ~100-500 µm. However, the nominal limit is effectively worsened to ~1 mm by the thermal waves diffusion in the sample, so that high sensitivity (~0.1°C) temperature mapping with tens-of-microns resolution across extended (mm-/cm- sized) fields of view is not routinely achieved.

Sub-diffraction thermography is demonstrated here by the development and validation of a super-resolution imaging approach, that combines the photo-thermal effect induced by the sample absorption of modulated focused laser light with the automated a posteriori localization of the resulting laser-induced temperature variations. By the non-linear surface fit of the isolated temperature peaks in the acquired thermal camera frames, light-absorbing and heat-releasing centers get localized and rendered in the final super-resolution image. Best-fit amplitudes color-code for local temperature values, while peak coordinates provide morphological information on the absorbing sample with a resolution assigned by the ~10-µm excitation laser-spot size.

We initially validate the proposed approach on synthetic ink samples. By comparing our results with conventional transmitted-light images of the same structures, we confirm accurate imaging capability at 60-µm resolution. On the adopted setup configuration, we prove therefore a resolution gain of a factor of 6 and 20 with respect to the diffraction-limited prediction and the effective (1200±180)-µm resolution of our thermal camera in conventional operation.

We further perform proof-of-principle experiments on complex biological samples. We image explanted murine skin biopsies treated with Prussian blue 30-nm nanocubes, and provide temperature-based super-resolution maps of the distribution of the absorbing nanostructures across mm-sized tissue sections. Our results suggest potential applications and future impact of photo-activated super-resolved thermal imaging in the characterization of the homogeneity, morphology and functional state of both biological tissues and synthetic materials.

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Diffusion dynamics of the cytosolic peroxisomal import receptor PEX5

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Peroxisomes are cell organelles with a size of 0.3-0.6 µm in mammalian cells, fulfilling many different functions like the oxidation of fatty acids, detoxification of reactive oxygen species as well as the biosynthesis of different lipids. The import of proteins into peroxisomes is unique since peroxisomes import already folded and even oligomerized proteins from the cytosol. Any malfunction on the import process will impair the function of peroxisomes and lead to severe related diseases. Most proteins destined for the peroxisomal matrix are characterized by a PTS1 (Peroxisomal Targeting Sequence 1) that is recognized by the peroxisomal import receptor PEX5, which binds them in the cytosol and directs them to the peroxisomal membrane.

We here present our extended study to characterize the molecular diffusion and interaction dynamics of PEX5 and PTS1 cargo proteins in the cytosol of live human fibroblasts using Fluorescence Correlation Spectroscopy (FCS) in combination with multi-colour (FCCS) and super-resolution (STED-FCS) detection. We observed an unexpectedly slow diffusion of PEX5 with and without cargo (2-times slower than for similarly sized cytosolic proteins). A deep analysis on different variants of PEX5 link this slowed down diffusion to the protein's N-Terminal part, which is known to be involved in the in integration of PEX5 into the peroxisomal membrane. To further characterize the dynamics of PEX5 in the cytosol, we implemented aberration corrections using adaptive optics, which helped us to precisely determine the diffusion mode of cytosolic PEX5.

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Nanoscale multi-colour fluorescence cross-correlation spectroscopy on living cell membranes with plasmonic antennas

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Following the fate of individual molecules in living cells is crucial to understand biological functions. However, detecting this task requires working at diluted labelling concentrations because of the diffraction limit of light. Recently, 2D-plasmonic antennas have emerged as excellent candidates to confine and enhance light at the nanoscale enabling the dynamic investigation of individual lipids in both mimetic and living cell membranes at high labelling conditions [1]. Nevertheless, extending this approach to multi-colour applications has been challenged by the fact that field enhancement occurs at a narrow bandwidth where the antenna is maximally resonant. Here, we applied broadband bowtie nanoaperture antennas (BNA)-on-probes [2] to follow the diffusion of individual lipids and membrane receptors in living cells in a multi-colour fashion. We first demonstrate that BNA-o-probes achieve similar degree of nanoscale light confinement in the blue and in the red regions of the visible spectrum. We combine this approach with fluorescence correlation spectroscopy (FCS) to follow the diffusion of PE lipids on living cell membranes, obtaining confinement volumes 1000-fold smaller than in confocal microscopy. Second, we demonstrate simultaneous dual-colour detection of individual receptors in living cells, and resolve receptor nanoclustering by means of fluorescence cross-correlation spectroscopy. Hence, our combined approach allows investigation of individual molecules and their interactions at the nanoscale, with ultra-high temporal resolution at endogenous expression levels.

[1] P. M. Winkler et al. *The Journal of Physical Chemistry Letters* (2018)
[2] M. Mivelle et al. *NanoLetters* (2012)

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Binding affinity of membrane-inserted epitope to HIV-1 antibody and its dependency on lipids quantified by fluorescence correlation spectroscopy

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Binding affinity of membrane-inserted epitope to HIV-1 antibody and its dependency on lipids quantified by fluorescence correlation spectroscopy

Broadly neutralising HIV antibodies (bnAbs) are defined by their ability to neutralise diverse HIV isolates. Among all described bnAbs, the ones that target the membrane-proximal external region (MPER) show the highest breadth (98% of tested isolates neutralised). MPER is localised in the interface of the viral membrane and thus, anti-MPER bnAbs have evolved to include membrane-interacting regions that permit MPER recognition in a lipid environment. Moreover, these antibody (Ab)-lipid interactions seem to be essential for the neutralizing activity of anti-MPER bnAbs, although their contribution to epitope binding is not fully understood.

In this work, we quantitatively study Ab-MPER interaction in its membrane context by means of fluorescence correlation spectroscopy (FCS). We have quantified the partition coefficient (K_p) of several 10E8 variants bound to MPER-bearing large unilamellar vesicles (LUV) of different compositions through the evaluation of the Ab diffusion regime. Our work builds on previously reported methodology used to assess peptide-membrane partitioning [1, 2], adding to it the complexity of a third component. Reliable K_p values were obtained upon careful quantification of the amount of accessible lipid on the vesicles. The uncertainty of the determined K_p was computed through support-plane analysis. In conclusion, our measurements set a robust method for the quantitative determination of membrane partitioning in intact systems. We foresee that its application will shed light over the functional relevance of interactions between bnAbs and MPER in a lipid membrane environment.

References:

[1] Rusu L, Gambhir A, McLaughlin S, Rädler J. Fluorescence correlation spectroscopy studies of peptide and protein binding phospholipid vesicles. Biophys J. 2004
[2] Melo AM, Prieto M, Coutinho A. The effect of variable liposome brightness on quantifying lipid-protein interactions using fluorescence correlation spectroscopy. Biochim Biophys Acta - Biomembr. 2011

P-508

Molecular quantification of the stress response protein OmpR under osmotic and acidic stress in *E. coli*

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The archetype stress response system OmpR/EnvZ plays a crucial role in bacterial osmoregulation. The transcription factor OmpR regulates the porin expression in the outer membrane of *E. coli*, depending on salt concentration and pH in the surrounding medium. OmpR is phosphorylated by EnvZ, a histidine kinase located at the bacterial membrane, to coordinate the porin expression [1]. It remains open whether the increase in phosphorylated OmpR under osmolar and acidic stress is a consequence of enhanced activity of EnvZ, or of upregulation of OmpR expression level in *E. coli.*

We aim to derive a quantitative, mechanistic description of the bacterial stress response by investigating the temporal and spatial distribution of OmpR using super-resolution microscopy. We developed an *E. coli* K-12 MG1655 strain in which OmpR is chromosomally labeled with photoactivatable mCherry to extract protein copy numbers applying a quantitative single-molecule approach [2]. Furthermore, we combine PALM (photo activated localization microscopy) and PAINT (photoactivation localisation microscopy) to generate multi-colour super-resolution images and investigate the nano-scale spatial organization of OmpR relative to DNA and membrane [3].

[1] Foo, Y. H., Spahn, C., Zhang, H., Heilemann, M. & Kenney, L. J. Single cell super-resolution imaging of *E. coli* OmpR during environmental stress. *Integrative biology: quantitative biosciences from nano to macro* **7,** 1297–1308 (2015).
[2] Endesfelder, U., Finan, K., Holden, S. J., Cook, P. R., Kapanidis, A. K. & Heilemann, M. Multiscale spatial organization of RNA polymerase in Escherichia coli. *Biophysical journal* **105,** 172–181 (2013).
[3] Spahn, C., Glaesmann, M., Grimm, J. B., Ayala, A. X., Lavis, L. D., and Heilemann, M. A toolbox for multiplexed super-resolution imaging of the *E. coli* nucleoid and membrane using novel PAINT labels. *Scientific Reports* **8**, 14768 (2018)

P-509

Study of the modulation of $GABA_A$ receptor by RuBi-GABA uncaging using electrophysiology and non-linear photoactivation in rat cerebellar granule cells *in vitro*

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Ruthenium – bipyridinetriphenylphosphine – GABA (RuBi – GABA) is a caged compound that allows to study the neuronal transmission in a well-defined area of a neuron.

The molecule is composed of the inhibitory neurotransmitter GABA bound to a caged group that inhibits the interaction of the neurotransmitter with its receptor site.

After one (UV-VIS light) and two – photon (700-900 nm) excitation, the covalent bond of the caged molecule is broken, GABA is released with a control in time, space and amplitude of intensity and it interacts with its receptor.

We studied the modulation of $GABA_A$ receptor by photoactivated caged GABA in rat cerebellar neurons, coupling the electrophysiological technique of the patch clamp in the whole cell configuration with the confocal and two – photon microscopy.

We analyzed how the activation of the receptor changes after one and two – photon excitation, considering several parameters such as laser power, time of exposure and distance of the uncaging point from the cell of interest along the X, Y, Z axis. In particular, this approach is useful to detect a selected biological target in a temporally and spatially confined way.

Our purpose is to consider biological events separating them from physical parameters and verify that the results does not depend on changes of them.

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Super resolution microscopy studies provide insights into FGFR dynamics and cell surface densities

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Fibroblast growth factor receptors (FGFRs) belong to the family of receptor tyrosine kinases. Four different FGFRs have been identified (FGFR 1 – 4), with various isotypes [1]. Their assembling and activation are facilitated by fibroblast growth factors (FGFs) and heparin sulfate (HS). FGFRs control crucial cell responses like proliferation, migration, and cell survival which makes precise receptor regulation essential.

Our aim is to understand how FGFRs regulate cellular signal initiation, and how specific chemicals act on FGFR action. As read-out, we measure (i) the diffusion dynamics and (ii) receptor cell surface densities for multiple FGFRs in a single cell. For this purpose, we use single-molecule localization in combination with single-particle tracking [2,3] and points accumulation for imaging in nanoscale topography (PAINT) [4], respectively.

Our studies reveal the diffusion behavior of FGFRs in dependence of different ligands and endocytosis inhibitors in human cancer cells on an endogenous level. By combining FGFR dynamics with receptor distributions and densities, we aim to unveil mechanistic details of FGFR signaling.

1. Eswarakumar, V.P., Lax, I. & Schlessinger, J. Cellular signaling by fibroblast growth factor receptors. *Cytokine & growth factor reviews* **16,** 139–149 (2005).
2. Harwardt, M.-L.I.E. *et al.* Membrane dynamics of resting and internalin B-bound MET receptor tyrosine kinase studied by single-molecule tracking. *FEBS open bio* **7,** 1422–1440 (2017).
3. Winckler, P. *et al.* Identification and super-resolution imaging of ligand-activated receptor dimers in live cells. *Scientific reports* **3,** 2387 (2013).
4. Jungmann, R. *et al.* Multiplexed 3D cellular super-resolution imaging with DNA-PAINT and Exchange-PAINT. *Nature methods* **11,** 313–318 (2014).

P-511

SIMPLE: Structured illumination localization microscopy

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Superresolution (SR) imaging has become an enabling technology to access sub-diffraction information on the nanoscale structure and dynamics of molecular building blocks. Different SR concepts have been introduced such as PALM/STORM to process information in the optical detection path to estimate single particle positions of sparse emitters by centroid fitting to the diffraction-limited photon distribution (with s the size of the PSF). Higher number of photons N results in a higher localization precision given by the standard error of the mean $\Delta x = s/\sqrt{N}$.

MINFLUX was recently introduced as a radically new concept for particle localization with up to 8-fold improvement in localization precision compared to SMLM. In MINFLUX, a single molecule emitter is probed with the minimum of a doughnut-shaped illumination pattern over a restricted area of length L~50nm. Here we present a Structured Illumination Microscopy based Point Localization Estimator (SIMPLE), that allows for the simultaneous localization of isolated emitters via detection of photon count modulations on a camera-based system. We show that phase-shifted sinusoidal excitation patterns can be used as nanometric rulers for deriving the actual positions of multiple single molecule emitters in a micron-sized FOV, thereby overcoming current spatial and temporal sampling restrictions of MINFLUX.

Phase shifting of the illumination pattern can be performed with nanometer precision (<1nm) to probe the actual particle position through photon number variations for different phase shifts. The measured photon counts can be fitted to the known illumination pattern to derive the actual particle localization. We validate SIMPLE in silico and experimentally on a TIRF-SIM setup using DMDs as spatial light modulators revealing 6.5 nm localization precision at 50 photon counts.

Altogether we present a Structured Illumination Microscopy based Point Localization Estimator (SIMPLE) that reveals a 2- to 6-fold increase in single molecule localization precision compared to conventional centroid estimation methods.

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A super-resolved view of the *Trypanosoma brucei* plasma membrane

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African trypanosomes are the causative agents of human sleeping sickness and Nagana in livestock. In the bloodstream of their host, they express a dense coat of variant surface glycoproteins (VSGs). Fluidity of this coat is fundamental for the evasion of the hosts immune system and thus for the survival of the parasite. However, VSG dynamics is also limited by the physical properties of the lipid matrix. We have recently introduced super-resolution imaging of intrinsically fast-moving flagellates based on cyto-compatible hydrogel embedding [Glogger et al. JPD: Appl Phys 17]. Building on this work, we applied single-molecule fluorescence microscopy to study the structure of the inner membrane leaflet using lipid-anchored eYFP as a probe. We found specific domains where the probe accumulates or appears diluted rather than being homogenously distributed [Glogger et al. Exp. Parsitol. 17]. The next step is to check for the presence of inter-leaflet coupling and potential influence of the domains on VSG diffusion. Homodimeric VSGs are anchored to the outer leaflet of the plasma membrane by two GPIs. The maintenance of the VSG coat is accomplished by shuffling the VSGs through the tiny flagellar pocket, which is the sole site for endo- and exocytosis and makes up for only 5% of the cell surface. Nevertheless, the turnover was determined to be surprisingly fast [Engstler et al. J Cell Sci 04]. We want to elucidate the dynamics of single VSGs in relation to the flagellar pocket with a two-color experiment.

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Tracking oligomerization of mutated NPM tagged with fluorescent proteins in live cells

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Nucleolar phosphoprotein nucleophosmin (NPM) shuttles between nucleolus, nucleoplasm and cytoplasm mainly in form of oligomers. The oligomerization, mediated by N-terminus, is critical for NPM function and corresponds to its ability to bind other proteins, including tumor suppressors. NPM is frequently overexpressed in solid tumors, whereas it is often mutated or fused to other proteins in hematological malignancies. Mutations of the *NPM1* gene represent the most frequent genetic aberration in acute myeloid leukemia (AML). Due to modification of C-terminus, the mutated NPM (NPMc+) is dislocated from the nucleolus to the cell cytoplasm, which is likely an important event in leukemogenesis. Inside live cells, the NPM can be tracked by tagging with fluorescent proteins, either on C-terminus or N-terminus. We proved that the NPM oligomerization can be observed in live cells expressing the tagged NPM by fluorescence lifetime imaging microscopy (FLIM) or fluorescence fluctuation spectroscopy (FFS). To have a positive control for the FFS experiments, we prepared a plasmid for expression of a bi-color NPM, labeled with mRFP1 on the N-terminus and eGFP on the C-terminus. In FLIM experiments, we observed shortening of eGFP lifetime in nucleus of cells expressing two color mix of single tagged NPM. The shortening indicates FRET occurring within NPM oligomers, between eGFP tagging one NPM molecule and mRFP1 on the other. We made a similar observation for the NPMc+ localized in cytoplasm. FRET presence in cytoplasm was further proved by eGFP lifetime increase after bleaching the mRFP1 fluorescence. We tracked the NPMc+ oligomerization in cytoplasm also by FFS. For these experiments, we prepared a bi-color NPMc+, i.e. mutated NPM labeled with fluorescent proteins again from both termini. Accordingly to a single color NPMc+ labeled with eGFP on the modified C-terminus, the bi-color construct was located to the cytoplasm. The bi-color constructs were found to be suitable for cross-correlation fluorescence measurements. All our *in vivo* results correspond to those obtained in our *in vitro* immunoprecipitation experiments. Usage of these live-cell-imaging techniques provides us a platform to track the NPMc+ dynamics in response to anticancer drugs treatment for the purpose of understanding the NPMc+ leukemogenic potential..

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Directed manipulation of membrane protein motion by fluorescent magnetic nanoparticles

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The location and movement of proteins in the plasma membrane indicate their specific function in diverse cellular processes. Strikingly, the plasma membrane is organized into compartments which are often partitioned by barriers to the lateral movement of membrane molecules, the so-called diffusion barriers. The exact nanoscopic location of such diffusion barriers is mostly unknown so far. Ensemble methods such as fluorescence recovery after photobleaching (FRAP) and genetic perturbations have been used to detect them. To directly localize diffusion barriers at the nanoscale, we could combine high-accuracy tracking of membrane proteins with active control over their movement. However, few methods exist that allow the direct manipulation of the motion of individual particles on cells. Optical tweezers can be used, but require sophisticated equipment. Here, we present fluorescent magnetic nanoparticles (FMNP) as a straight-forward solution to this problem. We couple FMNPs to membrane proteins on cultured cells and pull them over the cell membrane using a magnetic needle. The motion of the single FMNPs can be monitored with 10 nm spatial and 5 ms temporal resolution. Lipid-anchored as well as transmembrane proteins could be dragged over the plasma membrane for tens of μm until they stopped at the cell edge. The magnetic forces exerted on the particles were calibrated on supported lipid bilayers and found to be in the fN range. We conclude that we have developed a means to apply directional control over protein motion in the plasma membrane of living cells that is compatible with high-speed, high-resolution single-particle tracking. In the future, we hope to use this method to investigate the exact spatial localization of diffusion barriers in the plasma membrane.

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Monitoring the interaction of nucleotides with DODAB cationic vesicles : a discussion about Laurdan as a suitable fluorescent probe

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Considering the possible use of cationic DODAB vesicles as carriers for genetic material, the present work analyses the structural changes caused by different concentrations of a small single-stranded oligonucleotide (ODN) on the structure of DODAB vesicles. Here, we extend a previous work where ODN was used in high concentrations only (Rozenfeld et al., 2015, Phys. Chem. Chem. Phys, 7498), and exam the recently proposed fluorescent probe Laurdan as a probe for DODAB bilayer surface alterations (Masukawa et al., 2019, J. Photochem. Photobiol., 238). Varying the ODN concentration, three different behaviors were observed. For low concentrations of ODN, ([ODN]/[DODAB]) $\leq$ 0.025, the dispersion is stable, clear, but vesicles are slightly larger, an increase in turbidity is observed, and small angle X-ray scattering (SAXS) already shows the presence of a few multilamellar structures. The mixed vesicles display positive surface potential, similar to the potential measured for pure DODAB vesicles. Calorimetry shows the coexistence of regions of pure DODAB bilayer with DODAB-ODN domains, the latter being more stable, presenting a higher gel-fluid transition temperature. The shape and position of the fluorescent band of Laurdan incorporated into the vesicles are not altered by the presence of the oligonucleotide, indicating minor variations in the polarity and surface structure of the mixed membrane monitored by the probe. A second behavior is observed for ([ODN]/[DODAB]) $\approx$ 0.05, where the presence of pure DODAB domains is no longer detected by calorimetry, and the dispersion is unstable, cloudy, displaying vesicle aggregation/coalescence. Finally, a third behavior is detected at high concentrations of ODN, ([ODN]/[DODAB]) $\geq$ 0.075, where a negative surface potential is measured, therefore with predominance of the charge of the oligonucleotide, and the dispersion is stable, exhibiting low turbidity. In this region, calorimetry indicates a great stability of the gel phase, SAXS measurements show the formation of multilamellar structures, but the average diameter of the vesicles is not much altered. In this region, the Laurdan probe monitors variations at the surface of the membrane, possibly indicating the decrease in the amount of water molecules on the surface and/or a stiffening of the bilayer.

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Tracking mechanisms of nanomaterial uptake using live-cell STED microscopy

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Nanomaterials (particles smaller than <100 nm in diameter) dispersed into the air are considered as a major contributing factor to air pollution. Many studies proved that exposure to some nanomaterials can lead to systemic inflammation and increase the risk of heart disease and stroke. Due to define the dangers associated with human exposure to a nanomaterial, the understanding of respiratory toxicity pathways and identification of key events at the bio-nano interface is necessary.

In this study, we try to understand the effect of TiO_2-nanotubes on alveolar lung epithelial cells (LA-4) using high-resolution stimulated emission depletion microscopy (STED) and advanced image analysis method. We focused on an identification of the mechanisms of uptake of nanomaterial. For this purpose, we imaged the dynamic of uptake and colocalization of fluorescently labeled TiO_2-nanotubes with endosomes, lysosomes, and microtubules in live LA-4 cells.

We observed intracellular uptake of TiO_2-nanotubes by the cells. Obtained data suggest that the way and time evolution of nanomaterial uptake depend on the size of TiO_2-nanotubes aggregates. We observed colocalization of TiO_2-nanotubes with endosomes and lysosomes and their movement along microtubules but mostly for small aggregates. We suspect that the mechanism of uptake of larger aggregates of TiO_2-nanotubes might be different.

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Improved spatial resolution using Localization STED microscopy (LocSTED)

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Super resolution microscopy helps to achieve detailed information on structural features that are smaller than the diffraction limit which has been the limitation in optical microscopy for many years. As a method to overcome this limitation, Stefan Hell proposed STimulated Emission Depletion (STED) microscopy in which a second laser called the depletion laser is used to confine the effective point spread function (PSF). This is achieved by engineering the PSF of the depletion beam to form a doughnut (for higher resolution in lateral direction) or bottle beam (for higher resolution in axial direction) with a zero central intensity, overlapped on a regularly focused excitation beam. In general, STED microscopy is considered as a targeted optical imaging method in contrast to STochastic Optical Reconstruction Microscopy (STORM), which is based on localization and position accuracy of single molecules. In this work, we would like to present a combination of both - resolution enhancement by STED microscopy and localization of single molecules by STORM technique i.e. Localization STED microscopy (LocSTED) and show that the photophysical properties of organic fluorophores can be studied efficiently.

A custom built Continuous Wave (CW) STED microscope with excitation of 530 nm and depletion of 660 nm is used to study single molecules of Alexa Fluor 555 (AF-555). Photobleaching has been a limiting factor in STED microscopy because of the rather high intensity of the depletion beam. An enzymatic oxygen–scavenging system was used in order to improve the photostability of AF-555. Regions of subdiffraction extent were imaged to study especially the photoblinking of single molecules of AF-555. The following two samples have been used for this purpose. a) Nanodots fabricated by STED lithography with subsequent functionalization with fluorophores using Laser-assisted Adsorption by Photobleaching, where AF-555 is specifically anchored onto the biofunctionalized nanodots and b) DNA origamis as nanorulers labelled with AF-555. It is thus shown that using an inexpensive and easy to implement CW STED microscope, the blinking events of AF-555 can be effectively studied. Using LocSTED, a resolution of sub-20 nm with a localization precision of ~5 nm was achieved. This can be further applied, for example, to study the stoichiometry of membrane proteins in endothelial cells.

P-518
Study of responsiveness of algae to environmental stress by microscopy methods
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Short-term responses of the endogenous fluorescence of different algae types to various stress agents are evaluated by means of spectrally- and time-resolved microscopy. We have employed advanced spectrally-resolved confocal microscopy and time-resolved fluorescence imaging and observed fast, in terms of minutes, responsiveness of the algae cells to environmental stressors, which included change in pH, active chlorine, or active oxygen. Endogenous red chlorophyll fluorescence underwent bleaching by active chlorine, with contribution of fast shortening of the chlorophyll fluorescence lifetimes. We also recorded rise in the green fluorescence in the presence of the stressors. We specifically focus to the effect of acidification on the algae endogenous fluorescence. Gathered data will help to better understand pathophysiological changes in algae under conditions of stress induced by industrial and environmental pollution. *Supported by APVV-15-0227, VEGA 2/0123/18,* Visegrad No 21720055 and *LASERLAB-EUROPE IV.*

Tuesday 23rd July

ACTIVE MATTER AND BIOLOGICAL SELF-ORGANIZATION

P-519 (O-141)
Design principles for robust self-assembly of multiple biological structures from limited resources
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Controlling the size of sub-cellular structures is very important for proper execution of physiological functions of the cell. While it is well known that the sizes of intracellular structures (filaments, organelles, networks) scale with cell size, the underlying physical mechanisms for size control remains poorly understood. The limiting pool hypothesis- the assembly rate of macromolecular structures scales with the available amount of resources- provides a robust mechanism for size control of a single structure. However it fails to capture size control of multiple structures assembled from the same pool of resources. Examples include, assembling multiple protein structures from a limited pool of ribosomes, assembly of multiple organelles, multiple distinct cytoskeletal structures etc.
Here we present a physical model for active self-assembly and size regulation of 3-dimensional sub-cellular structures, which provides a mechanism for robust size regulation of multiple structures in the presence of stochasticity and competition for resources. Using this model we predict size regulation of multiple organelles, filaments and networks in quantitative agreement with experimental data. We also discuss how competition for resources between multiple networks can lead to cell polarization.

P-520 (O-142)
Active phase separation in mixtures of chemically-interacting particles
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Microorganisms and cells can chemotax in response to gradients of chemicals that they themselves produce or consume. The same behaviour has been recently observed at the nanoscale for individual enzymes, and can be mimicked in synthetic systems using catalytically-active phoretic colloids. Importantly, when many such particles are placed in solution, they interact with each other through their influence on the chemical's concentration field. We show that mixtures of chemically-interacting particles can undergo macroscopic phase separation, displaying a wealth of different configurations that are intimately related to the active, non-equilibrium character of the interactions. The fundamentally new class of phase separation behaviour arises generically from the chemical interactions, making our results directly applicable to self-organisation in heterogeneous populations of microorganisms and cells (e.g. quorum sensing and competition for nutrients in bacterial ecosystems, or cell-cell communication via chemokines); to aggregation of enzymes that participate in common catalytic pathways into a metabolon, which may be harnessed in the design of better synthetic pathways; or to the development of new active materials using catalytic colloids. Our results are also of relevance to fundamental studies of active phase separation phenomena, given that in our system the activity arises from the non-equilibrium nature of the interactions between particles that are otherwise non-motile, rather than from the intrinsic activity of self-propelling particles as commonly studied.

P-521 (O-143)
Self-organization of ciliary beats in bronchial epithelium
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In the lungs, the bronchial epithelium is covered by motile cilia whose coordinated beating drives the transport of mucus along the bronchial tree. This large-scale transport requires a global directional organization of ciliary beats. Yet the self-organization mechanisms leading to a collective dynamics of cilia remain to be explored.
In vitro experiments on reconstituted bronchial epithelium show that the mucus flow exhibits multi-scale swirly patterns during ciliogenesis. These patterns are closely connected to the underneath ciliary-beat organization. The nature of the fluid has a major impact on the spontaneous emergence of these patterns, as shown by the re-organization of the ciliary-beat orientations when replacing the mucus by a model fluid. This emphasizes the prominent role of the active response of cilia to their hydrodynamic interactions with mucus.
A simple physical model of ciliary-beat organization is proposed to investigate the possible emergence of large-scale ciliary patterns due to hydrodynamic interactions. The mucus flow over the modeled epithelium is computed using numerical simulations. Depending on ciliary density and mucus properties, the model solutions can exhibit several ciliary patterns, including a swirly pattern similar to that observed experimentally and a fully aligned pattern that is optimal for mucus transport. This supports that the flow can carry the necessary information for large-scale ciliary-beat organization.

P-522

Fragment molecular orbital (FMO) method for studying actinide/lanthanide interaction with DNA/protein

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Due to its potential health and environmental impacts, actinide binding to biomolecules has been a subject of intensive investigations. A large number of experimental works have been carried out but our understanding remains mostly in a macroscopic scale. Modeling actinide interaction with large biomolecules using *ab initio* quantum chemical calculations may drastically expand our molecular level knowledge but is challenged by a demand for huge computational resources.

Our strategy to overcome this difficulty is to apply fragment molecular orbital (FMO) method. In FMO, the molecular system of interest is partitioned into small fragments. Each fragment and fragment pair is subject to self-consistent field calculations under environmental electrostatic potentials and the electronic structure of the whole system is reconstituted [1]. This procedure drastically reduces computational cost of Hartree Fock calculations from N^3 to N^2 (or less) and is readily parallelizable. FMO has been extended to MP2 and to DFT to include electron correlation and was successfully applied to the systems such as hydrated DNA [2].

Currently we are upgrading the FMO program Abinit-MP [3] to implement 5f elements into the program. We first choose uranyl-bound DNA for a case study. Calculations are performed as follows. UO_2^{2+}-bound $d(CGCGAATTCGCG)_2$ (Dickerson-Drew dodecamer) with 20 Na^+ ions and SPC/E water shell with 10 Å thickness is first thermally equilibrated and subsequently submitted to MD simulation at 300 K for 100 ns interval using AMBER 14 program. Force field parameters for UO_2^{2+} and coordinating water are those developed by Pomogaev et al. [4]. At each 1 ns time step of MD simulation, the structure is extracted and submitted to FMO single point energy calculation at the MP2 level. In FMO, nucleic unit is appropriately divided into sugar, base, and phosphate fragments. Inter-fragment interaction energy analysis is performed to explore the binding affinity of uranyl to DNA and its influence on base pairing.

[1] K.Kitaura et al. (1999) Chem. Phys. Lett. 313, 701–706.
[2] K.Fukuzawa et al. (2015) Comput.Theor.Chem. 1054, 29–37.
[3] S.Tanaka et al. (2014) Phys. Chem. Chem. Phys. 16, 10310–10344.
[4] V.Pomogaev et al. (2013) Phys. Chem. Chem. Phys. 15, 15954–15963.

P-523

Active matter in confined geometries – biophysics of artificial minimal cortices

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The actin cortex is an important component of eukaryotic cells and essential for many cellular processes, like maintaining cell shape, cell division and cell motility. In order to be motile, the cortex – attached to the plasma membrane - needs to be remodeled. Therefore, the actin network consisting of filamentous actin (F-actin) and various actin binding proteins (ABPs) constantly needs to rearrange. Until now little is known about this highly dynamic process and how it influences mechanical properties of cells.

We aim at investigating the impact of actin network architecture, actomyosin contractility and the dynamic attachment of F-actin to the plasma membrane on the viscoelastic behavior of the cortex using different bottom-up and top-down approaches.

Firstly, three bottom-up model systems are analysed. As a basic model system, a 3D actin network in presence and absence of different ABPs is polymerized between two glass slides and for further investigations of the confinement, the networks are analysed inside a droplet-based microfluidic system. These artificial systems are investigated using confocal laser scanning microscopy (CLSM) and video particle tracking (VPT). A light-induced softening of the F-actin network could be seen with fluorescent beads. First dark field imaging measurements do not show any influence on the network mechanics. In presence of myosin II and ATP an active process could be observed. Furthermore, the influence of ABPs on the rheological properties of an actin network attached to a $PtdIns(4,5)P_2$ containing solid supported membrane (SSM) *via* the physiological cross-linker ezrin is studied. The network is crowded on the SSM with methylcellulose. To investigate the frequency dependent viscoelastic properties of the minimal actin cortex (MAC), VPT is used. First results show an increased stiffness of the network with higher $PtdIns(4,5)P_2$ concentration.

Secondly, as a top-down approach, mechanical properties of native apical MDCK II cell membrane fragments on a porous silica substrate in dependency of the ATP concentration are analysed using force cycle experiments. First results show a higher fluidity of the membrane fragments with addition of ATP.

P-524

A biophysical study of the aggregation of a functional amyloid protein

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A biophysical study of the aggregation of a functional amyloid protein

Amyloid proteins are fibrillar appendages that are related with neurodegenerative disease in humans. However, in some cases, particularly in low organisms ranging from bacteria to fungi, these fibers have a structural and a protective role that is beneficial to the organism. These proteins are then termed functional amyloids. TasA is a functional amyloid that is also the major protein component of the extracellular matrix in *Bacillus subtilis* biofilms. It is different than many amyloid proteins since it can be isolated in a structured form. In order to understand the mechanism that underlies its aggregation into fibers, we perform a comprehensive study of the aggregation of TasA in solution using various biophysical methods. In particular, we study formation of TasA fibers from oligomers as well as protein segments and their interaction with lipid membranes that mimic the cell surface. Furthermore, using an Atomic Force Microscope (AFM) we study the basic interactions between TasA segments of high propensity to form fibers, that lead to fiber formation. We have found that en-route to fiber formation, these peptides form islands that upon disruption exhibit a unique F-D receding trace. Using soft matter ideas, we attribute these unique F-D traces to binding and unbinding intermolecular events occurring when the peptide islands are being pulled off. Our results may contribute to better understanding the formation of functional amyloid formation and to the development of anti-biofilm drugs that target these fibers.

References

Abbasi et al., Biochemistry, 57, 5230, 2018
Lester Zer et al., unpublished (in preparation)

P-525

Bacterial cell growth dynamics inside giant lipid vesicles for bottom-up synthetic biology

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In recent, many researchers have interests to design and create artificial cell-like systems to mimic biological cellular organizations. In this study, we constructed a method to bacterial cell growth at the single-cell level inside giant lipid vesicles (GLVs)[1] toward the construction of an artificial cell including an artificial organelle like structure[2]. A single bacterial cell was encapsulated into 10–30-μm GLVs by the droplet-transfer and size-filtration (DSSF) method[3]. The bacterial cell encapsulated GLVs were immobilized on a supported lipid membrane for direct observation of cell growth dynamics. *E. coli* cells were cultured as model bacteria inside GLVs. The single *E. coli* cell could stably and actively grow to a great number of cells at inside GLVs. We successfully observed bacterial cell growth dynamics inside GLVs for 2 days. Our method is a potentially useful tool for culturing other types of bacteria. Moreover, cell encapsulated GLVs can offer hybrid systems of an artificial cell-like system and natural cells for a bioreactor of bottom-up synthetic biology.

1) M. Morita, *et al*., *ChemistryOpen* 2018, 7, 845–849., 2) Y. Elani *et al*., *Scientific Reports*. 2018, 8, 4564., 3) M. Morita, *et al.*, *ChemBioChem* 2015, 16, 2029–2035.

P-526

Study of Collagen self-assembly by molecular dynamics and UV spectroscopy techniques

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Collagen is a fibrous protein representing the main constituent of connective tissue in mammals, with a basic structural unit called tropocollagen that is a triple helix consisting of Gly-Xaa-Yaa repetitions, in which one-thirds of the X and Y residues are either prolines or hydroxyprolines. Collagen triple helices associate in fibrils, where tropocollagens are staggered side-by-side with a shift of 67 nm (234 residues) between two neighbours.

To investigate the assembly mechanisms, collagen aggregation was studied by means MD simulations both at physiological conditions and at low ionic strength.

Two tropocollagen fragments with different hydropathic profiles were chosen and built from *Rattus norvegicus* type I collagen sequence. Other fragments were selected from the same sequence with a shift of 234 residues upstream and downstream of it. Repeated MD simulations suggest that tropocollagens prefer to associate in pairs, with first approach between hydrophobic regions, suggesting that the mechanism is mainly driven by hydrophobic effect and mediated by hydroxyprolines. Association of two, three or four fragments shows that the amino-acidic composition of the triple helices strongly influences their assembly propensity: poorly charged (PC) segments easily associate at 0.1 M salt concentration, contrary to highly charged (HC) ones. As expected, HC fragments are more suited to self-assembly at low ionic strength. Collagen self-assembly was monitored *in vitro* by measuring the turbidity changes of the solution as observed from the increase in absorbance at 310 nm. Rat tail tendon collagen was prepared at low temperature and at different pH. Curves of aggregate fractions vs time display a sigmoid profile, indicating, according with literature, a cooperative process with a lag phase whose length depends on the solution pH.

P-527

Lipid bilayer nanodiscs to study "in-membrane" features of amyloid peptide oligomerization

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Membrane-associated amyloid peptide oligomerization is commonly seen as leading to the onset of neurodegenerative diseases (ND) such as Alzheimer's and Parkinson's diseases. Recently, our group has shown that while membrane mimetics containing sphingomyelin promote amyloid aggregation, the monosialoganglioside GM_1 is able to counteract this effect at near physiological concentrations, suggesting that deregulation of gangliosides within neuronal membranes is potentially a key feature of ND development [1,2]. This is further supported by molecular dynamics simulations showing that the C-terminal of $A\beta_{40}$ has a higher tendency to adopt a β-sheet structure in the presence of sphingomyelin. However, experimental evidence confirming this hypothesis is still missing [1].

Diisobutylene/maleic acid lipid particles (DIBMALPs), due to their small size (reduced scatter) and reduced UV absorption [3], can be ideal lipid systems to study membrane-associated Aβ oligomerization through circular dichroism (CD). Here, we optimize the formation of DIBMALPs of different lipid composition, up to four components (DOPC, cholesterol, sphingomyelin and GM_1). Using dynamic light scattering, transmission electron microscopy, generalized polarization and time-resolved emission spectra measurements, we thoroughly characterize the formation of DIBMALPs and their membrane properties. Moreover, we assess their potential application on the study of "in-membrane" amyloid peptide oligomerization using CD, in the presence of sphingomyelin and/or GM_1.

1. M. Amaro *et al.*, *Angew. Chemie Int. Ed.* **55**, 9411–9415 (2016).
2. M. Cebecauer *et al.*, *Biophys. J.* **113**, 1194–1199 (2017).
3. A. O. Oluwole *et al.*, *Angew. Chemie Int. Ed.* **56**, 1919–1924 (2017).

P-528

3D STED microscopy reveals that cell membranes can deactivate nanomaterial by forming bio-nano agglomerates

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Our daily exposure to nanomaterial in our air, food, water and cosmetics has been linked to the development of various diseases. Despite the increasing variety and amount of nanomaterial produced each year, we are still unfamiliar with the basic principles of interaction between them and our cells. Our group has recently shown that nanoparticles can wrap themselves in cell membranes, leading to cell damage (1). However, many cells survive even when exposed to large doses of nanomaterial, indicating our poor understanding of the relationship between dose, cell damage and cell survival.

While studying the effect of TiO2-nanotubes on lung epithelial cells (LA-4) using fluorescence microscopy, we observed large formations on the outside of the cell plasma membrane, consisting of at least two components: TiO2-nanotubes and cell membranes. More of them formed at higher doses of nanomaterial and with increasing time of exposure.

By applying a specially-designed "Interaction filter" to the images, we show that these lipid-nanomaterial composites are a direct consequence of the affinity between nanomaterial and cellular membranes. Closer inspection using 3D stimulated emission depletion (STED) microscopy reveals the formations are composed of sheets of phospholipids and small, fairly monodispersed nanotubes aggregates, implying that the structures are built over time. They are stiff enough not to be moved by the STED beam, leading us to believe the microvilli and/or cytoskeleton components might be included in the agglomerates as well.

Due to the stiff nature of the agglomerates and the thick coverage of the nanoparticles with phospholipids, the nanomaterial in the agglomerates seems to be hindered from further interacting with cells. The appearance of nano-bio agglomerates poses more questions: is their formation driven purely by biophysical interactions without the involvement of the cell? Or – shockingly – do cells actively passivate the nanomaterial as part of a cell self-defense mechanism? If so, this would completely change our view on nanotoxicology as we know it –cells would not only deal with the reparation of nanomaterial-caused damage, but could also take an active role in defending themselves.

Urbančič, I. et al., Nano Letters 2018 18 (8), 5294-5305. DOI: 10.1021/acs.nanolett.8b02291

P-529

Archaerhodopsin-3: A photoswitching study

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Archaerhodopsin-3 A photoswitching study

A novel bioinspired sensing material has been developed based on the well-established photoinduced pumping of protons by bacterial photoreceptors. Over the past 50 years, silicon devices have been miniaturised, and it is predicted that the size limit of silicon will be reached within the next decade - Moore's Law. It is thus necessary to find alternatives, and using functional biomolecules is currently one of the most promising options for novel bottom-up designed sensing, information storage and imaging devices.

Previous utilization of bacteriorhodopsin (bR) in potential device configuration has suffered from sensitivity issues, asymmetry of the molecular architecture and intimate coupling of the photoreceptor to a conducting material. All these hurdles have been overcome by strategically introducing a M163C mutation into bR, partially delipidating to effect intimate coupling to gold surfaces [1,2,3]

Here, we extend this strategy to other, more efficient photoreceptors (AR3), and spectrally tune the absorption using mutational variances around the retinal binding site. Additionally, hydrogel droplet technology reveals large and wavelength electrical phtotoswitching[4]. The potential for image detection at variable specific wavelengths, as well as colour tuned retinal replacement technology, is discussed.

[1] Berthomieu O., Patil A., Xi W., Aslimovska L., Davis J.J. and Watts A., *Molecular scale conductance photoswitching in engineered bacteriorhodopsin,* Nano Lett. **2012** 12(2):899-903.
[2] Patil A., Premaruban T., Berthomieu O., Watt A. and David J.J., *Enhanced photocurrent in engineered bacteriorhodopsin monolayers,* J Phys Chem B. **2012** 116(1):683-689.
[3] Patil A., Premaruban T., Berthomieu O., Watt A. and David J.J., *Engineered bacteriorhodopsin: a molecular scale potential switch*, Chem. Eur. J. **2012** 18(18): 5632-5636
[4] Restrepo V., Booth M.J., Box S.J., Olof S.N., Mahendran K.R., Bayley H., *Light-patterned current generation in a droplet bilayer array,* Scientific Reports. **2017** 7(46585): 1-9

P-530

Minimal Condition for Metachronal Wave Patterns of Cilia Arrays

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Cilia are ubiquitous in biological systems, and they can be used for liquid transportation in mammalian tubes and self-motion of microorganisms. Independently beating cilia can coordinate with each other, beating in the form of a metachronal wave. In order to understand the physical mechanism underlying the collective beating of the cilia, we construct an analytical theory to deal with the emergent dynamics of an infinite array of cilia. In the model, a cilium is treated as a sphere moving along a circular trajectory in presence of a substrate, and the cilia interact with each other hydrodynamically. By performing analytical analyses, we provide the dispersion relation of the system, and predict the achievable stable wave vector region by linear stability analyses. Numerical simulations are also performed, the results of which matches well with our analytical predictions. Not only providing the minimal condition for achieving metachronal wave patterns of cilia, the model can also serve as a guide in future applications of how to control the wave properties such as the wave frequency and vector.

P-531

Bidirectional FtsZ filament treadmilling promotes membrane constriction via torsional stress

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FtsZ is a key component in bacterial cell division, being the primary protein of the presumably contractile Z ring. Reconstituted in vitro, it shows two distinctive features that could so far however not be mechanistically linked: self-organization into directionally treadmilling vortices on solid supported membranes, and shape deformation of flexible liposomes. In cells, circumferential treadmilling of FtsZ was shown to recruit septum-building enzymes, but an active force production remains elusive. To determine direct contributions of FtsZ to membrane constriction, we designed a novel in vitro assay based on soft lipid tubes pulled from FtsZ decorated giant lipid vesicles (GUVs) by an optical tweezers. FtsZ actively transformed these tubes into spring-like structures, where GTPase activity promoted spring compression. Operating the optical tweezers in lateral vibration mode and assigning spring constants to FtsZ coated tubes, we found that that FtsZ indeed exerts pN forces upon GTP hydrolysis, through torsional stress induced by bidirectional treadmilling.

P-532

Playing lateral charge of G-quadruplex fibers to modulate the properties of guanosine-based hydrogels

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Hydrogels are attractive biomaterials finding a diversity of applications including biomedical and pharmaceutical fields. Self-assembly of small molecules such as guanosine is an efficient way to form stable supramolecular hydrogels. Guanosine form cyclic planar units, called G-quartets, through hydrogen bonds (Hoogsteen). In presence of monovalent cation, G-quartets stack through π-π interactions resulting in columns: the G-quadruplex. Depending on the conditions, G-quadruplex fibers are able to entangle into a 3D-network with high capacity to entrap water (Carducci et al., 2018).

Our study has focused on obtaining stable hydrogels by the self-association of two precursors: Guanosine (G) - neutral and Guanosine 5'-monophosphate (GMP) – negatively charged. The ability of these gels to entrap and release bioactive molecules as Methylene Blue (MB) was also assayed.

Three different proportions G:GMP (1:6; 1:2 and 1:1) were used to address our goals. Small Angle X-Ray Scattering (SAXS) was performed to infer about the hydrogel structure. The results reveal that G:GMP 1:1 gel presents the strongest lateral interaction between the fibers. Morphological aspects of the gels obtained by Atomic Force Microscopy (AFM) are in agreement with SAXS results. Depending on the lateral charge the inner structure of hydrogel is altered. However, for all cases, G-quadruplex fibers layed parallel to substrate surface and the height was compatible with G-quadruplex diameter. In some cases knots can be observed (Nava et al., 2019). The 3D-network influenced the velocity of penetration of the model drug and also its release profile. Due to the lateral charge dependence, the release is also modulated by pH. For higher negative charge, less interlaced is the network and faster is the release. In conclusion, the lateral charge regulates the entanglement degree of G-quadruplex hydrogels, which makes this a pH-responsive biomaterial. Therefore, this sort of hydrogel biomaterial is a potential candidate for drug delivery system, being biocompatible and biodegradable.

References: Carducci F, Yoneda JS, Itri R, Mariani P. Soft Matter. 2018; 14(15):2938-2948.
Nava G, Carducci F, Itri R, Yoneda JS, Bellini T, Mariani P. Soft Matter. 2019 Mar 13;15(11):2315-2318.

P-533

Out-of-equilibrium active membranes: incorporation of bacteriorhodopsin in a floating lipid bilayer

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Cell membranes exhibit thermal fluctuations that can be enhanced by transmembrane protein activity leading to out-of-equilibrium fluctuations. Active fluctuations have been widely described theoretically [1], but to a lesser extent experimentally. We will present our recent results on the investigation of out-of-equilibrium fluctuations of phospholipid membranes induced by active transmembrane protein. In this context, bacteriorhodopsin (BR) was used as a light-driven proton pump, which's activity can be triggered by visible light. Model systems such as solid-supported single and floating phospholipid bilayers are suitable to study phospholipid membranes and their interactions. A detergent-mediated incorporation method [2] was adapted to perform the insertion of BR into the phospholipid bilayer at the interfaces, using a sugar- based detergent such as DDM. The combination of neutron reflectometry, QCM-D, fluorescence microscopy and AFM demonstrated that it is possible to insert BR in model bilayer systems without losing their structural integrity. Recent specular and off-specular X-ray reflectometry experiments demonstrated an activity of the incorporated proteins through its effect on the structure and on the fluctuations of a double bilayer system. These results open the way to investigate, for the first time, the fluctuation spectrum of a planar membrane-protein system at the nanoscale and to access the physical properties of the system such as bending modulus, surface tension and interaction potential between adjacent membranes.

References:

[1] Prost J., Manneville J.-B., and Bruinsma R., EPJ B, 1:465–480 (1998).
[2] Dezi M., Di Cicco A., Bassereau P. and Lévy D., PNAS, 7276, 110727 (2013).

P-534

Characterisation and measurement of HIV-1 capsid protein interactions through artificial assembly using DNA scaffolds

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The mature capsid of the human immunodeficiency virus 1 (HIV-1) self-assembles from hundreds of copies of a single capsid (CA) protein into a peculiar conical shape. In an assembled capsid, CA proteins are predominantly in a hexameric arrangement but also consist of an irregular distribution of pentamers: five on the 'pointed' end of the capsid and seven on the 'round' end. High resolution structures of pentamers and hexamers have been elucidated using X-ray crystallography with the aid of cysteine-mediated crosslinks, and electron microscopy. But how these structures actually form and, in particular, what drives the formation of the pentamer at the crucial encapsulating steps remains unknown.

To approach this, we have designed a system which allows us to visualise the assembly process, and therefore measure interactions contributing to the assembly of the hexamer and pentamer structures. We have achieved this using DNA scaffolds specifically designed to template the assembly of CA proteins into pentamer or hexamer arrangements. The CA proteins are conjugated to DNA strands, which allows for their attachment to specific complementary sites on the scaffold via DNA hybridisation. Moreover, binding affinities can be tuned by altering the DNA sequence. Association and dissociation kinetics are measured between protein and different DNA templates with surface plasmon resonance (SPR). The data can then be fit to mathematical models to extract and compare the kinetics of interactions between protein subunits in hexameric or pentameric arrangements. The information gained will allow us to determine the relative stability of hexamers and pentamers, and whether dynamic transitions are likely to occur in assembled capsids.

Importantly, the use of a template removes the requirement for artificial crosslinks, which prevent observation of dynamic behaviour. Thus, DNA templates will also allow us to study the dynamics and structure of isolated pentamers and hexamers in their relaxed state for the first time with small-angle X-ray scattering (SAXS) and transmission electron microscopy (TEM).

By using this bottom-up approach to visualise the assembly of capsid substructures, we hope to provide insight into the biological features and behaviours of the capsid, and shed light on its role in the infectivity of HIV-1.

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Evaluation of Predicted Protein–Protein Complexes by Binding Free Energy Simulations

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The accurate prediction of protein-protein complex geometries is of major importance to ultimately model the complete interactome of interacting proteins in a cell. A major bottleneck is the realistic free energy evaluation of predicted docked structures. Typically, simple scoring functions applied to single complex structures are employed that neglect conformational entropy and often solvent effects completely. The binding free energy of a predicted protein-protein complex can, however, be calculated using Umbrella Sampling (US) along a predefined dissociation/association coordinate of a complex. We employed atomistic US-Molecular Dynamics simulations including appropriate conformational and axial restraints and an implicit Generalized Born solvent model to calculate binding free energies of a large set of docked decoys for 20 different complexes. Free energies associated with the restraints were calculated separately and yielded an absolute binding free energy. In principle, the approach includes all energetic and entropic contributions to the binding process. The evaluation of docked complexes based on binding free energy calculation was in better agreement with experiment compared to a simple scoring based on energy minimization or MD refinement using exactly the same force field description. Even calculated absolute binding free energies of structures close to the native binding geometry showed a reasonable correlation to experiment. However, still for a number of complexes docked decoys of lower free energy than near-native geometries were found indicating inaccuracies in the force field or the implicit solvent model. Finally, we introduce a novel refinement technique that combines Hamiltonian Replica Exchange (H-REMD) simulations with an explicit water description that is able to overcome some of the inaccuracies encountered previously. Although time consuming the approach may open up a new route for realistic ranking of predicted geometries based on calculated free energies of binding.

P-536

Polymer simulations to understand the structure and dynamics of mitotic barley chromosomes

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From interphase to metaphase the chromosomes go through intense condensation. This process, which is very conserved within eukaryotic organisms, leads to a two- to three-fold packing, enabling an accurate segregation of the chromosomes. Recent analysis from HiC data have brought light into this impressive process and are helping to unravel the more packed structure of the mitotic chromosome. Complementarily, polymer simulations can produce several models to be tested and their fit to experimental data be verified. Together, these two techniques were able to reconcile two apparently conflicting former views: condensation arising either from loop extrusion or from helical packing. Gibcus et al. have suggested that both models may be true. Mitotic chromosome may be formed by nested loops arranged side by side in a dynamical helical scaffold. This model is in good agreement with the contact probability calculated from Hi-C experiments with animal cells. Our own Hi-C data from flow-sorted chromosomes suggest a similar structure for mitotic chromosomes of barley. Despite the differences between these two organisms regarding genome size and the presence of topologically associated domains during interphase, they both seem to share the same packing strategy, relying mainly on the different roles of condensins I and II. With polymer simulations, we can also infer structural aspects specific to barley, such as the loop lengths, the height of a helix turn, or the relative concentrations of condensins. These are all features that may help us understand how the large barley genome is organized during the cell cycle. Furthermore, *de novo* modelling may indicate which forces are acting on the polymer and are driving chromosome condensation. This may shed a light into the regulation of the process and how the involved proteins work and assemble during chromosome condensation.

(1) Gibcus, Johan H., et al. "A pathway for mitotic chromosome formation." *Science* 359.6376 (2018)

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Computational design and experimental validation of novel Self-Assembling Peptides

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Besides their biomolecular relevance, amyloids, generated by the self-assembly of peptides and proteins, are highly organized structures useful for nanotechnology applications. In this work we performed molecular dynamics simulations of Calcitonin protein core-sequence DFNKF, in order to assess the key intermolecular interactions responsible for self-assembly. Subsequently, we computationally designed novel peptides based on the calcitonin core sequence xFxxF, where x is any natural amino acid, leading to 8000 possible new peptides. We first calculate the aggregation propensity of each peptide using coarse-grain MD simulations based on MARTINI force field by modelling a 10x10x10 nm box of peptides at a concentration of 0.1 mM and monitoring the Solvent Accessible Surface Area over the course of 400 ns MD simulations. Since the best aggregating peptides are rich in hydrophobic residues and likely insoluble in water solution, we then calculated the solvation free energy of each peptide using the Bennett Acceptance Ratio method, in order to rule out the insoluble peptides.
The best candidate peptides in terms of both aggregation propensity and solubility are then experimentally tested to verify the ability of the new peptides to form nanostructures.

P-538

DNA droplets: self-assembly and phase transition of DNA nanostructures

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In the past few decades, DNA has been received much attention as programmable bio-materials because of their sequence-specific hybridization. Two- or three-dimensional nanostructures have been constructed using sequence-designed DNAs. In addition, micrometer-sized structures can be constructed by the self-assembly of DNA nanostructures. In living cells, droplet-like structures are formed through weak (or moderate) interaction of biomolecules. We expect that the sequence design of DNAs enables us to control the self-assembly of DNA nanostructures into “DNA droplets” similar to that occurring in living cells because interaction strength between DNA molecules can be changed depends on their base sequence. We herein report creation and control of DNA droplets based on a phase transition phenomenon and sequence design of DNAs. We designed Y-shaped DNA nanostructures (Y-motifs) with three sticky-ends capable of interacting with each other. Experimental results showed that Y-motifs self-assembled into micrometer-sized hydrogels and “droplets” that exhibit fusion with one another at a specific temperature range. We revealed that the phase transition temperatures can be determined by sticky-end sequences. In addition, we successfully created two distinct DNA droplets that exhibit selective fusion by elaborately designing the sticky-end sequences. Furthermore, we demonstrated the fission and Janus-shaped segregation of DNA droplets with the aid of enzymatic reaction. These results provide a novel finding in DNA nanotechnology and biophysics to create micrometer-sized droplets with designated-phase transition temperature and fusion/fission behavior. We believe that our study leads to various future applications such as stimuli-responsive gene expression in DNA droplets via phase transition, creation of artificial organelles, and fabrication of asymmetric gel/droplet particles.

P-539

Stability of Poly (2-Oxazoline) Based Micellar Drug Formulations

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Amphiphilic copolymers based on 2-alkyl-2-oxazolines are next generation polymers widely used for the effective formulation of hydrophobic drugs and their delivery to the target site.[1, 2] The library of six gradient copolymers differing in the alkyl chain length and copolymer composition was prepared. The one-pot living copolymerization of 2-ethyl-2-oxazoline with 2-(4-alkyloxyphenyl)-2-oxazolines was leading to the formation of gradient copolymers what was demonstrated by kinetic study of copolymerization. Self-assembly of prepared copolymers in presence or absence of hydrophobic drugs like, curcumin and hypericin provided polymeric micelles of different size, morphology, and loading capacity. Increased thermodynamic stability of drug-loaded polymeric micelle compared to the unloaded polymeric micelle was possibly due to the incorporation of highly lipophilic drugs and thereby increasing the hydrophobic fraction in the core of the micelles. The stability of polymeric micelles in the presence of biological medium is an important factor which decides its stability in circulation.[3] By encapsulating curcumin and hypericin into a single polymeric micelle which can act also as Forster resonance energy transfer pairs and following their release by fluorescence, the thermodynamic and kinetic stability of these polymeric micelles were monitored in the presence of different concentration of bovine serum albumin, fetal bovine serum and γ-globulin. The result demonstrated excellent dependence of polymeric micelle stability on the alkyl chain length of gradient copolymers.

1. Lorson, T.; Lubtow, M. M.; Wegener, E.; Haider, M. S.; Borova, S.; Nahm, D.; Jordan, R.; Sokolski-Papkov, M.; Kabanov, A. V.; Luxenhofer, R., Poly(2-oxazoline)s based biomaterials: A comprehensive and critical update. *Biomaterials* **2018,** *178*, 204-280.

2. He, Z. J.; Schulz, A.; Wan, X. M.; Seitz, J.; Bludau, H.; Alakhova, D. Y.; Darr, D. B.; Perou, C. M.; Jordan, R.; Ojima, I.; Kabanov, A. V.; Luxenhofer, R., Poly(2-oxazoline) based micelles with high capacity for 3rd generation taxoids: Preparation, in vitro and in vivo evaluation. *J Control Release* **2015,** *208*, 67-75.

3. Owen, S. C.; Chan, D. P. Y.; Shoichet, M. S., Polymeric micelle stability. *Nano Today* **2012,** *7* (1), 53-65

P-540

A versatile framework for Brownian dynamics simulations of biopolymer networks

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To complement our experiments on actomyosin networks, we develop a versatile Brownian dynamics simulation framework to study the active dynamics of 3D biopolymer networks. Filaments are simulated as a highly coarse-grained linear bead-spring model where all beads experience harmonic potentials for stretching and bending modes while all other bead/bead interactions are reduced to hard-sphere potentials.

Crosslinking proteins and myosin motors are not simulated as explicit entities - they rather are special "states" of beads, allowing these beads to form bonds with nearby beads of other filaments. While the whole spatial dynamics of the network is simulated via Langevin equations of motion, the kinetics of crosslinking and the myosin motor motions are handled via Gillespie algorithms modeling the temporal state changes in form of a reaction system.

In this way the filaments can nucleate, assemble and disassemble, crosslinks can be formed and can be broken, myosin motors can translate along the filaments and thus extort and distribute forces in the network. The extreme coarse-grained structure of our model will allow us to study the active filament dynamics and the network relaxation as a response to external distortions covering many orders of magnitude in time scales.

P-541

Spectroscopic Studies of Dual Fluorescence in *2-(4-fluorophenylamino)-5-(2,4-dihydroxybenzeno)-1,3,4-thiadiazole*: Effect of Molecular Aggregation in a Micellar System

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This work reports on spectroscopic investigation into a selected 1,3,4-thadiazole derivative from the series of *2-(4-fluorophenylamino)-5-(2,4-dihydroxybenzeno)-1,3,4-thiadiazole* (FABT). The experiments consisted on preparation of a micellar system with use of a non-ionic detergent Triton X-100 and the subsequent spectroscopic measurements of the micelles formed. The steady state fluorescence revealed a dual fluorescence phenomenon emerging from the particular molecular organisation of the compound, which in turn depends both on the concentration of the detergent and the concentration of the compound itself. Dual fluorescence emission in FABT micelles was observed in methanol-water mixture, wherein the dual fluorescence of this compound had never been reported before. It has been noted that the occurrence of this effect was related to the process of molecular aggregation taking place between FABT molecules in the micellar system in question. This hypothesis was evidenced based on a series of spectroscopic experiments, namely the UV-Vis electronic absorption, resonance light scattering (RLS), emission and excitation fluorescence spectra, as well as measurements of dynamic light scattering (DLS) and Principal Component Analysis (PCA). Moreover, the measurements carried out at various temperatures demonstrated a reversibility the effects observed. Results obtained from steady state fluorescence spectra together with those from time-correlated single photon counting (TCSPC) measurements suggest that dual fluorescence occurs at detergent concentrations necessary to form micelles, facilitating the aggregation of FABT molecules. The comparison of fluorescence effects occurring in methanol-water mixture to those obtained from previous measurements performed on various FABT analogues suggests the possibility of charge transfer (CT) within the range of detergent concentrations at which the aforementioned fluorescence effects are observed.

P-542

On the concentration-dependent clustering of trehalose in aqueous media: a spectroscopic study

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Trehalose is a disaccharide of no reducing power, widely distributed in nature (bacteria, fungi, insects, invertebrates and plants), protecting cells and organisms against various stresses such as dryness, freezing and osmopressure [1]. Therefore, it presents diverse functional properties with applications namely in biomedicine, as a nontoxic excipient for freeze-drying in processes for cryopreservation and/or dehydration of cells. In those applications, the concentration trehalose solutions can be as high as 0.5-1 M (below the solubility limit of 1.82 M), presenting problems for its accumulation inside cells to act as a cryoprotectant.

The aqueous solutions of trehalose show a concentration-dependent clustering tendency, due to its ability to self-association in water forming clusters of various sizes. MD simulations showed that concentration 1.5-2 M, allow trehalose molecular clusters to percolate and form continuous aggregates [2]. In this study, we show that for water solutions from up to 1.5 M, there is an increase in absorbance at 220 nm, which is in accordance with what is expected for absorption spectra of aqueous suspensions containing supramolecular aggregates. To further characterize the clustering process, we used the well-known fluorescence method based on the excimer formation of 1-methylpyrene [3]. We found that no excimer formation is observed, as the trehalose clustering in water is not ruled by the hydrophobic effect that leads to amphipilic aggregates such as micelles. Instead, since there is no increase in the concentration of 1-methylpyrene that would allow the excimer formation, one may conclude that this experimental finding supports the formation of a continuous array of percolating aggregates. Further characterization of trehalose aqueous solutions through light scattering experiments is ongoing. We also discuss the effects of continuous percolation clustering in water in the accumulation process of trehalose inside cells within the framework of biomedical applications.

[1] A. D. Elbein *et al.* (2004) *Glicobiology* **13**:17R-27R.
[2] L. Sapir, D. Harris (2011) *J. Phys. Chem. B* **115**:624-634.
[3] F. Mouffouk *et al.* (2011) *Biosens. Bioelectron.* **26**:3517-3523.

P-543

Spectroscopic and Theoretical Studies of Dual Fluorescence in 2-Hydroxy-N-(2-phenylethyl)benzamide Induced by ESIPT Process. Solvents Effects

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In this work we discusses the theoretical ([TD]DFT) and molecular spectroscopic investigation of *2-Hydroxy-N-(2-phenylethyl)benzamide* (SAL3). We also discuss its physiochemical behaviour in the selected organic solvents (polar and non-polar). The compound demonstrated a very interesting dual fluorescence effect in alcoholic solutions (e.g.: methanol, ethanol or isopropanol), while in acetonitrile (ACN), dimethylsulfoxide (DMSO) and non-polar solvents (like: n-Hexane, n-Heptane, chloroform) only a single emission maximum was observed. The noticeable shortening of average fluorescence lifetime (TCSPC), the employed detailed analysis of solvatochromic shifts of absorption spectra in the function of polarizability related to a change in the induction polarization of the environment and, above all, the performed quantum-mechanical calculations [TD]DFT with a detailed analysis of excited states, clearly indicate a connection between the observed fluorescence effects and processes related to changes in the system's structure in the excited state. We also noticed that the observed fluorescence effects (dual fluorescence) are quenched by aggregation effects of this compounds.

Based on these experimental and theoretical studies, it has been proposed that the environment (solvent) polarity-induced dual fluorescence effect in SAL3 is related to the Excited-State Intramolecular Proton Transfer (ESIPT) process. Also, the quantum-mechanic studies [TD] DFT point to the specific conformation of SAL3 molecule characteristic of dual fluorescence emission. The results of spectroscopic studies on a selected compounds from SAL3 in a micellar and liposomal systems will be presented.

P-544

Small-angle neutron scattering with contrast variation reveals RNA localization in reassembled Hepatitis B capsids.

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The genetic code of viruses, RNA or DNA, are typically protected in an icosahedral capsid, which is primarily assembled from over a hundred subunits of the same protein in a spontaneous self-assembly process. Similar highly efficient assembly processes are ubiquitous in biological systems, and viral capsids in particular present a unique platform to exploit for therapeutic advances in the targeted cellular delivery of cargo packaged within the capsid. Our research aims to provide a more detailed understanding of how this precise viral capsid protein assembly process occurs from a pool of single building blocks, and specifically how the RNA or DNA is incorporated into the capsid. Here, we present results from small-angle neutron scattering experiments using contrast variation to reveal the final assembled structural organization of both the protein and nucleic acid components from recombinant Hepatitis B virus (HBV) capsid protein and a synthetically prepared RNA containing the capsid protein binding domain. These data revealed that RNA was localized along the inner capsid surface. Time-resolved small-angle x-ray scattering (SAXS) experiments were also used to determine the structure during HBV capsid assembly in the presence and absence of RNA. We employed Bayesian statistics-based computational methods to extract kinetic parameters of assembly and the overall size and shape of the dominant structural intermediates from the SAXS data. Additional single-particle cryoEM reconstructions are provided to assess the effect of RNA (none, 40, or 90 nts) on the resulting assembled capsid structure. The combination of time-resolved scattering data, Bayesian statistics, and cryoEM structural analysis, provides a framework which not only describes the viral self-assembly process, but can be extended to other hierarchical assemblies in biology.

P-545

Microrheological experiments with actomyosin networks under the influence of external white noise

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The linear response of a system to external forces is accessible through the measurement of equilibrium fluctuations. In the case of active matter, like living cells or ATP driven, reconstituted biopolymer networks, the system is driven out of equilibrium by energy conversion. We imbed colloidal particles in active and passive actin networks and study the particle response to induced and thermal fluctuations in order to explore the applicability of the fluctuation dissipation theorem in active matter far from equilibrium. Additionally, we want to compare in vivo measurements with artificial systems to evaluate to which extent cellular mechanics are a phenomenon emerging from the interaction of hundreds of different biomolecules. Simplified model systems are a useful and popular way to reduce the immense complexity and to access crucial information about living systems, but they also bear the risk of oversimplifying properties that arise from a variety of interactions. Another challenge that we try to overcome is the wide range of elasticities and frequency dependencies of microscopic, biological network structures, which exceed the accessible range for many microrheological techniques. One of our approaches is the usage of external white noise for elevated kinetic temperatures. This enables, for example, active measurements in strongly cross-linked actin networks with optical tweezers without high laser intensities, which would heat the sample locally and drive the system further out of equilibrium. So far we studied the influence of network structures modeled by cross-linker proteins like α-actinin and fascin on the mechanical properties of active actomyosin networks in cell sized confined structures and implemented a variety of measuring techniques to verify our results. The comparison between one-point and two-point microrheology with optical tweezers and video particle tracking, for example, gave us information about inhomogeneitys in the local particle environment. Comparing active and passive measurements on the other hand gave us access to information about active processes.

Further experiments will hopefully reveal more insight into the nonequilibrium mechanics of cell sized artificial biopolymer networks.

P-546

Phase separation in mixtures of active and passive particles: Dynamic control over the patterning formation and growth

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Theoretical models of active matter can be used to study the behavior of a wide range of out-of-equilibrium system, ranging from flocks of birds to cells. In this talk we are going to present simulation results of the emergent behaviors of active particles within dense passive environments.

First, we explore the behavior of Active Brownian Particles (ABPs) within dense monolayers of passive Brownian particles [1]. In this model, ABPs translate at constant speed while changing their direction of motion according to a rotational diffusion coefficient (D_r). We show that the direction of motion of self-propelled particles strongly depends on the structure and properties of the passive media [2]. Moreover, we observe three well differentiated collective behaviors as a function of D_r. In this talk I will focus on the case of high D_r, where we observe a phase separation into a solid-like phase composed only by passive particles and a fluid-like phase with a mixture of active and passive particles. We observe different dynamical regimes for this phase separation as a function of the propulsive force of the active particles that produce different structures. One that resembles to a coalescence process and another one that resembles a coarsening process. We exploit these dynamical regimes to propose dynamic actuation protocols that allow us to control the formation of patterns.

We also study the behavior of active particles that undergo an oscillatory translational motion in one dimension for which an experimental realization is much more feasible. Using numerical simulations, we demonstrate how it is possible to control the shape of the passive phase by dynamically changing frequency, amplitude and direction of the oscillators. This active synthetic system could be further developed into a powerful tool to process materials at microscopic scale.

[1] J. Stenhammar, R. Wittkowski, D. Marenduzzo and M.E. Cates, *Physical Review Letters*, **114**, 018301 (2015).
[2] J.L. Aragones, S. Yadzi and A. Alexander-Katz. *Physical Review Fluids*, **3**, 083301 (2018).

P-547

Magnetically-actuated particles find their way through collagen networks

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Cells are very sophisticated motile machines engineered by Nature to move through complex environments; this motion enables vital biological processes such as tissue remodeling and immune response. Mimicking the motion of cells is thus of outmost importance in the design of motile synthetic systems intended to be use in imaging applications or to serve as carriers to deliver payloads to targeted regions of the body. Moreover, understanding the physical mechanisms that enable cell-like motion in synthetic systems may shed light on additional mechanisms, besides chemotaxis, that cells may be exploiting to move. Therefore, we study the potential of a synthetic system consisting of commercial superparamagnetic microparticles to move through a collagen network under actuation with a rotating magnetic field. We explore the movement through collagen of microparticles that do not interact specifically with collagen. As a function of microparticle size, we observe rotation in place for large particles, caging for intermediate particle sizes and movement through the pores of the collagen network for small particles. Complementarily, we study the role of the structure and mechanical properties of the collagen networks on the motion of these microparticles. This synthetic system will enable to explore the possible role of friction, understood as the formation and breakage of either chemical or physical bonds, on microparticle motion and motion direction,[i] through the incorporation of ligands on the microparticles that specifically bind collagen receptors.

P-548

Membrane induced oligomerisation of proteins: the role of membrane size

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A number of important processes involve binding of monomeric proteins to plasma membrane surfaces, their oligomerisation and subsequent conformational transition to a transmembrane pore. Examples are bacterial toxins, cytolysins of complement system or pores formed by proteins of the cytosol in the context of programmed cell death.
The relation between the concentration of monomeric proteins in solution and lysis of the cell depends on a number of parameters: monomer binding affinity, oligomerisation equilibrium, rate of conformational transition to the pore, size of the pore and finally cellular responses to the increased permeability of the plasma membrane. In order to address the different steps of protein/membrane interaction, usually different types of model membranes are employed, and the results are discussed in context of available cell-based data. However, the very different size of the membrane systems involved can lead to very different relative contributions of binding, oligomer formation and conformational transition to the observed rate of pore formation. This might be relevant when comparing the dose/response curve of differently sized membrane systems, e.g. for the role of protein receptors for pore formation. Simulations based on the data available for the interaction of α-toxin from *S.aureus* are employed to give an overview of the different scenarios possible.

P-549

Microscopic machines within anisotropic environments

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Cells execute vital biological processes such as tissue remodeling, wound healing, and immune responses. In most of these different processes cells are required to move through complex environments and respond to external stimuli. We are designing microscopic machines able to mimic how cells move through complex environments and spontaneously respond to chemical and/or physical stimuli. These microscopic machines are superparamagnetic particles rotating under the actuation of an external rotating magnetic field that in the presence of a solid substrate translate through friction against the substrate. By tuning the interactions between the active particles and the solid substrate we are able to control the migration speed and direction of our microscopic machines. Moreover, by creating anisotropies in the interactions driving their movement, our micromachines behaves as *ratchets* [1]. The thermally fluctuating nature of their propulsion force may induce transient decreases of entropy [2]. Our experimental system opens up a new avenue of research in the mobility of microscopic machines in complex environments.

[1] J.P. Steimel, J.L. Aragones and A. Alexander-Katz. *Artificial tribotactic microscopic walkers:*
walking based on friction gradients. Phys. Rev. Lett. **113**, 178101 (2014)
[2] G.M. Wang, E. M. Sevick. *Experimental Demonstration of Violations of the Second Law of*
Thermodynamics for Small Systems and Short Time Scales. Phys. Rev. Lett. **89**, 050601 (2002)

P-550

Hydrodynamics of quartz microbalance of liposome-DNA complexes

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Quartz microbalance (QMB) is a wide spread technique in the experimental biophysics. It samples the elastic and viscous response of different analytes (from proteins to cells) when adsorbed to the surface of the quartz crystal, a piezo-electric resonator which oscillates at dozens of megahertzs. Despite several decades several of usage, the fundamentals of QMB are still not well described or fully understood. In this work we investigate the complex hydrodynamics of QMB when the analyte consists on liposomes tethered to DNA strands. We use the immersed boundary method to couple the fluid motion with the vibration of the bio-molecules, described using flexible coarse-grained models. We find excellent agreement with the experiments carried out within the same EU project CATCH-U-DNA, whose objective is to use QMB to detect minute bulk concentrations (femto to atto-molar) of mutant DNA in the serum of patients. Extrapolating to higher frequencies (to be developed within CATCH-U-DNA), we forecast that an hydrodynamic resonance should drastically increase the QMB signal which is essential to the main objective.

Tuesday 23rd July

IONIC LIQUIDS AND BIOMOLECULES

P-551 (O-147)

Probing the effect of a room temperature ionic liquid on self-assembled structure of phospholipid membrane

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A cellular membrane, which is mainly composed of phospholipids and proteins, is the outer layer of a cell that protects the inner components from the adverse effects of any foreign molecule. It also plays important roles in many physiological activities of cell including communication and endocytosis-exocytosis process. Any deviation from its structure, dynamics, and stability can influence the biochemical and physiochemical activities of the membrane. The interaction of inorganic salts is well known to generate such deviation and thereby affect the cell functionality.

Recently, there has been significant research interest in understanding the interaction of room temperature ionic liquids (ILs) with the cell membrane. An IL is a salt in the liquid state below 100°C. These molecules are non-explosive, non-flammable, and are having good electrical and thermal conductivities. They do not pollute air as they have low vapour pressure. Because of these properties, they have a large number of industrial applications. But some recent studies have revealed the lysing effect of ILs on environment friendly microorganisms living in soil and water.

The molecular mechanism of toxic activities of ILs is yet to understand. In this work, multilamellar vesicles (MLV) of phospholipids have been used to shed light on the effect of an IL on the structure of cellular membrane. The MLVs formed by zwitterionic lipid, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) are found to shrink as a consequence of interaction with an imidazolium-based IL, 1-decyl-3-methylimidazolium tetrafluoroborate ([DMIM][BF4]). The absorbed IL significantly modify the surface charge of the MLVs. While these observations indicate a strong membrane-IL interaction, synchrotron-based small angle x-ray diffraction (SAXD) measurements have provided a structural description of the interaction. SAXD and Fourier transform infrared spectroscopy studies have clearly revealed disordering effect of the IL on the conformational organization of lipid chains. The presence of negativily charged lipid 1,2-dipalmitoyl-*sn*-glycero-3-phospho-L-serine sodium salt (DPPS) in DPPC MLVs plays an important role in disordering the chains in membrane and inter-bilayer interaction.

P-552 (O-148)

The effect of imidazolium-based ionic liquids on human insulin aggregation/amyloid fibrillization

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Proteins are one of the most important molecules in living organisms possessing immense amount of functions in their native state. Under certain conditions, native proteins could aggregate. These misfolded proteins may accumulate in a form of protein aggregates called amyloid fibrils. Amyloid fibrils are elongated unbranched fibers consisting of β-structures of separate monomers positioned perpendicular to the fibril axis and stacked strictly above each other. Amyloid aggregates are often correlated with diseases known as amyloidoses, including Alzheimer's, Parkinson's or insulin-derived amyloidosis. Moreover, amyloid fibrils have also been recently tested as novel biomaterials due to their specific properties such as high stability, strength, elasticity or resistance against degradation [1]. Solvent conditions play an important role in controlling the amyloid aggregation *in vitro*. Ionic liquids (ILs) are a new class of media with interesting properties and low melting-point (< 100°C), that may substitute the volatile organic solvents. They can be used neat or diluted in water, eventually in other solvents. ILs consists of large organic cations combined with various anions and can be designed to match desired properties for various applications. In this work, we have studied the effect of ILs with 1-ethyl-3-methyl imidazolium (EMIM) cation and various anions (NO_3^-, BF_4^-, Cl^-, $CH_3CO_2^-$, HSO_4^-) on fibrillization kinetics and morphology of insulin fibrils using ThT fluorescence assay, calorimetry, CD and FTIR spectroscopy and AFM. We have found that the effect of ILs strongly depends on their concentration and the extent is not correlated to the position in Hofmeister series. At lower concentration of ILs, the acceleration of kinetics is observed, and polymorphic fibrils are formed. At higher concentration, amorphous aggregates are prevalent for all studied ILs. It is important to recognize and define the relations between physico-chemical properties of ILs and the kinetics of amyloid fibrillization or morphology of fibrils in order to expand our understanding of the amyloid fibrillization process.

This work was supported by the research grant from the Slovak Grant Agency VEGA No. 2/0030/18, 2/0145/17, bilateral project SAS-MOST JRP 2015/5 and MVTS COST 083/14 action BM1405.

[1] Assenza, S., et al., *Phys Rev Lett.* 113 (2014) 268103

P-553 (O-149)

Effect of Ionic Liquids on the Mechanoelasticity of Biomembranes: from Model Phospholipid Bilayers to Living Cells

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The interaction between ionic liquids (ILs) and biomolecules is an emerging area of research [1]. Among biological structures, model biomembranes such as phospholipid bilayers have been the first to be investigated in relation with ILs. Recently, by neutron reflectivity and computer simulations, it has been shown that ILs dispersed at low concentrations at water-biomembrane interfaces diffuse into biomembranes without disrupting their overall integrity [2,3]. Computer simulations [3] also suggested that the mechanoelasticity of biomembranes is affected by the presence of ILs. To study this experimentally, we recover to atomic force spectroscopy (AFS) by which we measure the mechanoelasticity of supported lipid bilayers interacting with water solutions of ILs. Different lipids and different ILs have been employed in these investigations. As a result, we found that different ILs change the mechanoelasticity of phospholipid bilayers in different ways. More precisely, the same ILs can make phospholipid bilayers either softer or stiffer depending on the lipid composition of the bilayer.

We have also indications that this effect is IL dependent. In my contribution, I will present these experimental results, partially published recently [4], and show how the understanding of the microscopic mechanism behind this behaviour can be used to study the effect of ILs on living cells. This research holds the promise for new applications in bio-nanotechnology [5].

References:

[1] A. Benedetto, P. Ballone (2016) Room temperature ionic liquids meet bio-molecules: a microscopic view of structure and dynamics. ACS Sustainable Chem Eng 4:392
[2] A. Benedetto, et al (2014) Structure and stability of phospholipid bilayers hydrated by a room-temperature ionic liquid/water solution: a neutron reflectometry study. J Phys Chem B 118:12192
[3] A. Benedetto, R.J. Bingham, P. Ballone (2015) Structure and dynamics of POPC bilayers in water solutions of room temperature ionic liquids. J Chem Phys B 142:124706
[4] C. Rotella, P. Kumari, B.J. Rodriguez, S.P. Jarvis, A. Benedetto (2018) Controlling the mechanoelasticity of model biomembranes with room-temperature ionic liquids. Biophys Rev 10:751
[5] A. Benedetto, P. Ballone (2018) Room-Temperature Ionic Liquids and Biomembranes: Setting the Stage for Applications in Pharmacology, Biomedicine, and Bionanotechnology. Langmuir 34(33):9579

P-554

Thermodynamics of Ion Pair Formation Between Charged Poly(Amino Acid)s and Linear Chain Surfactants

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Due to unique composition and structure proteins show a variety of interactions with other macromolecules and ligands. It is often difficult to dissect energy contributions of constituent parts in real protein-ligand systems, thus we study the interaction mechanisms and energies in structurally simpler model systems.

Here we present results of ion pair formation between homo-polymers and linear-chain surfactants – a system that partially mimics interactions in real protein-ligand systems. We used isothermal titration calorimetry to measure thermodynamic parameters between anionic poly(amino acid)s–polyglutamic and polyaspartic acid–and charged cationic linear-chain surfactants–nonylamine, decylamine, undecylamine, dodecylamine, and tridecylamine. The data showed that hydrophobic and electrostatic interactions drive the binding of surfactants to homopolymers. The increased length of surfactant's chain increases the enthalpy contribution to the interaction. All studied interactions had negative constant-pressure heat capacities. We also applied a binding model to describe the cooperative interactions between polymer-bound surfactants.

P-555
Room-Temperature Ionic Liquids in Protein Amyloidogenesis: a Combined Neutron Scattering, Atomic Force Microscopy and Optical Tweezer Study
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Iomic liquids (ILs) are a vast class of organic non-aqueous electrolytes whose interaction with proteins is receiving great attention. Recently, it has been shown that ILs can affect protein amyloidogenesis in different ways: whereas some ILs favour the formation of amyloids, others inhibit it [1]. To better understand the microscopic picture behind these two very much different effects, we are combining neutron scattering with atomic force microscopy and optical tweezers. In this contribution, we are presenting a set of data on the interaction between the model protein lysozyme with two different ILs, i.e. ethyl ammonium nitrate (EAN) and tetramethylguanidinum acetate (TMGA). These ILs have been chosen because the former has shown to favour the protein amyloidogenesis, whereas the latter to inhibit it. The way in which these ILs affect the mechanical stability of lysozyme has been investigated by optical tweezers and atomic force microscopy, and linked with the dynamical behaviour at the protein-solvent interface accessed by neutron scattering.
[1] V.V.S. Pillai, A. Benedetto (2018) Ionic liquids in protein amyloidogenesis: A brief screenshot of the state-of-the-art. Biophys Rev 10:847

Wednesday 24th July
LIPID AND LIPIDOME BIOPHYSICS

P-556 (O-153)
Formation of membrane domains: insight from simulations on different scales
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The formation of domains in lipid membranes is a key requisite for signal-processing in cells. In this contribution a profound understanding will be provided from a simulation perspective. Results will be presented for lipid mixtures with and without transmembrane domains for a variety of different lipid types.
First, extensive Molecular Dynamics (MD) simulations of lipid membrane mixtures are presented on the atomistic as well as the coarse-grained level (MARTINI) [1]. A close comparison of both levels of description involve the spatial properties of the domain formation (in case of mixtures of saturated and unsaturated lipids and cholesterol) and the time evolution of characteristic observables such as the chain order parameter, indicating the emergence of domain formation [2,3]. Among others, we check to which degree the coarse-grained representation matches the atomistic one from a thermodynamic perspective and get quantitative insight into the enthalpic and entropic change of the gel-to-liquid transition of a specific saturated lipid in good agreement with experiments. In this context, the individual enthalpic and entropic driving forces of the lipid mixtures are quantified [4].
Second, we can map the systems on a lattice model which is exclusively characterized by properties, inherited from the MD simulations (see [4,5] for first results). In this way, it is possible to reach length- and timescales which extend the typical MD scales by orders of magnitudes. Furthermore, due to the direct accessibility of enthalpic and entropic contributions a close thermodynamic understanding of the domain formation becomes accessible.
[1] H. J. Risselada and S. J. Marrink, Proc. Natl. Acad. Sci. U. S. A 105, 17367 (2008).
[2] D. Hakobyan and A. Heuer, J. Phys. Chem. B 117, 3841 (2013).
[3] D. Hakobyan and A. Heuer, PLoS One 9, e87369 (2014).
[4] D. Hakobyan and A. Heuer, J. Chem. Phys. **146**, 064305 (2017).
[5] R. Friedman et al., J. Membr. Biol. **251**, 609 (2018).

P-557 (O-154)
Monte Carlo and molecular dynamics simulations to explain biomembrane meso-patterning by a composition-curvature coupling mechanism
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Plasma membrane forms a selective barrier for the cell, yet its role goes far beyond a simple frontier. Indeed, it plays a crucial role in biological functions such as endo and exocytosis, cell communication or adhesion. It is now widely agreed that membrane lipid and protein spatial repartition is not homogeneous but that these components are organized into nanodomains. These domains have proven to be key players in the above-mentioned biological functions. Combining statistical physics analytical tools and numerical simulations, we propose a physical mechanism for this membrane organization in a simple model vesicle. At the mesoscale, we describe the membrane with a composition-curvature coupling mechanism. We use an elastic description of the membrane surface via a Helfrich Hamiltonian and study the species mixture with an Ising model, relevant in describing phase transition phenomena. We perform Monte Carlo simulations for different membrane parameters (temperature, composition, spontaneous curvature, surface tension) and study its equilibrium states. Depending on the range of parameters, we get systems either undergoing a macrophase separation, either mixing homogeneously, or featuring domains. We characterize the range of parameters leading to these phase modulations by drawing phase diagrams from the simulation results. We compare this phase diagram with the one previously obtained by analytical field-theoretic techniques. In the case of meso-patterning, different observables are computed such as correlation functions and domain size distributions to extract information about the emerging membrane domains, such as their typical shape, size or spacing. In order to propose a valid rationale for membrane structuring at a lower scale, we also perform coarse-grained molecular dynamics simulations (MARTINI) of lipid bilayers including curvature-generating components. To assess the validity of our results, we compare them to available analytical predictions and experimental data.

P-558 (O-155)

Biophysical properties of 1-deoxyceramides in ordered and disordered bilayers

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Membrane sphingolipids have attracted a considerable attention in the last decades due to the discovery of the sphingolipid signaling pathway and its importance in regulating important processes such as cell growth and apoptosis. Our views on the complexity and variety of this lipid class have been enriched with the recent discovery of a novel sphingolipid subclass, the (1-deoxy) sphingolipids that lack the 1-hydroxy group. These lipids differ in their properties from the canonical (or 1-hydroxy) sphingolipids and they are toxic when accumulated in cells, inducing neurodegeneration and other dysfunctions. (1-Deoxy)ceramides, (1-deoxy)dihydroceramides, and (1-deoxymethyl)dihydroceramides, the latter two containing a saturated sphingoid chain, have been studied in this work using differential scanning calorimetry and atomic force microscopy to evaluate their behavior in bilayers composed of mixtures of three or four lipids. When compared to canonical ceramides, C16:0 (1-deoxy)ceramides show a lower miscibility in mixtures of the kind pSM/Chol/XCer (54:23:23 mol ratio), where XCer is any 1-deoxyceramide, giving rise to the coexistence of a liquid-ordered phase and a gel phase. The latter resembles, in terms of thermotropic behavior and nanomechanical resistance, the gel phase of the pSM/Chol/pCer mixture [Busto et al., Biophys. J. 2014, 106, 621-630]. Differences are seen between the different C16:0 (1-deoxy)ceramides under study in terms of nanomechanical resistance, bilayer thickness and bilayer topography. When examined in a more fluid environment (an ER-mimicking model membrane composed of ePC:ePE:ePI, 1:1:1 mol ratio), segregated gel phases are still present. Interestingly, and probably related to segregated gel phases, (1-deoxy)ceramides preserve the capacity for membrane permeation, but their effects are significantly lower than those of canonical ceramides. Moreover, C24:1 (1-deoxy)ceramides show significantly lower membrane permeation capacity than their C16:0 counterparts. The above data may be relevant in the pathogenesis of certain sphingolipid-related diseases, including certain neuropathies, diabetes, and glycogen storage diseases.

P-559

Toxic and essential metals influence membrane packing and size of biomimetic biological membranes

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Toxic and essential metals influence membrane packing and size of biomimetic biological membranes

The use of metals for daily anthropogenic activities has become widespread since the industrial revolution and has expanded to a multitude of applications in modern times. At the biomolecular level, divalent metals can interact with proteins, DNA, and lipids. Toxic heavy metals, like mercury (Hg) and cadmium (Cd), serve no physiological function in living organisms, and are known to be carcinogenic and neurotoxic. In contrast, the essential trace metal manganese (Mn), serves important physiological functions, as it is a cofactor for metabolic enzymes necessary for energy production of living cells. It is abundant in bones, where its required for proper function. However, chronic overexposure to Mn may result in a neurologic disorder known as *Manganism*. Previous studies have recognized lipids as toxicological targets of these divalent metals, which can alter lipid-lipid interactions.

The focus of this study is investigating the interactions of mercury, cadmium, and manganese with lipid membranes, by using fluorescence spectroscopy and dynamic light scattering. Metal speciation was considered, and the calculations showed that cadmium and manganese formed cationic species under physiological conditions, while mercury formed anionic chloro-complexes. For fluorescence spectroscopy, the dye laurdan was used, which display shifts in its emission spectrum as a function of the phase properties of the labelled lipid membrane. These shifts are quantified by calculating the Generalized Polarization (GP) and can be plotted as a function of temperature. The resulting GP curve allows assessing metal-induced changes to membrane fluidity and/or the shift in phase transition temperature. Additionally, dynamic light scattering was used to test for metal-induced changes to the liposome size. Phospholipids containing a net negative charge on their head group showed strong interactions with Cd and Mn and were identified as preferred targets. Whereas, plasmalogen lipids were identified as prime targets for Hg, which catalyzed an irreversible cleavage of these lipids resulting in a lysolipid and hydrophobic aldehyde products. This work provides good insight into the interaction of divalent metals with lipid model system and a better understanding of their potential effects on biological membranes.

P-555

Room-Temperature Ionic Liquids in Protein Amyloidogenesis: a Combined Neutron Scattering, Atomic Force Microscopy and Optical Tweezer Study

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Iomic liquids (ILs) are a vast class of organic non-aqueous electrolytes whose interaction with proteins is receiving great attention. Recently, it has been shown that ILs can affect protein amyloidogenesis in different ways: whereas some ILs favour the formation of amyloids, others inhibit it [1]. To better understand the microscopic picture behind these two very much different effects, we are combining neutron scattering with atomic force microscopy and optical tweezers. In this contribution, we are presenting a set of data on the interaction between the model protein lysozyme with two different ILs, i.e. ethyl ammonium nitrate (EAN) and tetramethylguanidinum acetate (TMGA). These ILs have been chosen because the former has shown to favour the protein amyloidogenesis, whereas the latter to inhibit it. The way in which these ILs affect the mechanical stability of lysozyme has been investigated by optical tweezers and atomic force microscopy, and linked with the dynamical behaviour at the protein-solvent interface accessed by neutron scattering.

[1] V.V.S. Pillai, A. Benedetto (2018) Ionic liquids in protein amyloidogenesis: A brief screenshot of the state-of-the-art. Biophys Rev 10:847

P-560

Simple vs. complex lipid bilayers: Balancing compositional simplicity and behavioral complexity of mammalian and bacterial membrane models

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Overcoming antimicrobial drug resistance will provide an important contribution to ensure global health security. In addition to their high therapeutic potential, natural antimicrobial peptides also present a high selectivity of interaction with bacterial cells over mammalian cells. Because the lipid diversity in membranes is essential for each cell of a living organism, we modeled membranes as bilayers with different compositions. The models need to be as simple as possible to understand membrane behavior, yet complex enough to reproduce real cellular membranes properties. We employed atomistic and coarse-grained molecular dynamics simulations to identify both simple and complex models for both mammalian and bacterial membranes. The lipid bilayer models were characterized by various parameters (thickness, number density, order parameter, lipid tilt angle). Our proposed models allow for improved methods to identify antimicrobial peptides with high activity against bacterial membrane models and low cytotoxicity and binding affinity to mammalian membrane models.

P-561

Gramicidin A and lipid environment: interplay between membrane deformations and peptide functioning

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Gramicidin A (gA) is an ion channel that forms through transbilayer dimerization of gA monomers. In a number of experimental works it was shown that parameters of gA channel, such as a lifetime of the conductive state and its formation probability, depend on properties of surrounding lipids, namely bilayer thickness, spontaneous curvature and etc.

Because there is a mismatch between the length of hydrophobic part of gA monomer and equilibrium thickness of the lipid monolayer, there are deformations of lipid membrane near the gA monomer. If two gA monomers are close enough, deformations produced by them start to overlap and monomers interact through these deformations.

In the framework of theory of liquid crystals adapted to lipid membranes, we calculated the energy of membrane deformations arising near gA in case of one and two monomers and in case of conducting dimer. Obtained results are in a good agreement with available experimental data. Calculated monomer interactions could explain gA clusterization in some experiments.

P-562

Influence of cholesterol-phospholipid interactions on lateral segregation

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The formation of lateral membrane domains is believed to play a role in a number of cellular processes. Still the mechanisms driving this lateral segregation are not known in detail. One important modulator of the structure and function of mammalian cell membranes is cholesterol. The influence of cholesterol is mediated through the interactions between cholesterol and the phospholipids present in the membrane. According to recent results, the relative affinity of cholesterol for the various phospholipids in lipid bilayers may be an important determinant of the degree of lateral lipid segregation. By expanding our previous study (Biophys J. 111(3):546-556) to include a broad variety sphingomyelin molecules together with different unsaturated phospholipids and cholesterol we have gained new insights into how the presence of cholesterol, in combination with other factors drive the lateral structuring of membranes. Using methods including time-resolved fluorescence, FRET, deuterium NMR, and scanning calorimetry, we gathered information about the formation of lateral membrane domains and the properties of these. The results from these experiments offer insights into the mechanisms driving the formation of lateral domain formation that will help us understand the processes that occurs in cellular membranes.

P-563

Fungal membrane lipid interactions study by means of Molecular Dynamics simulations

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In agriculture, the intensive use of chemical pesticides is environmentally unfriendly and potentially harmful to our health. Therefore, the development of new approaches with less environmental and health impacts is quite a critical challenge. Within this context, this project is focused on a family of natural amphiphilic glycolipids called rhamnolipids. Because they are produced by certain microorganisms and present antibacterial and / or antifungal properties, while remaining harmless to humans and stimulating plant defenses, they can be considered as a reasonable alternative to agrochemicals. However, even though it is most likely based on direct interactions with the lipids from the target cell membrane, the mode of action of these molecules is barely known in details.

To study this mode of action, solid-state NMR experiments were carried out in our laboratory and showed a fluidification of liposomes when the rhamnolipids were added to these model membranes containing ergosterol, a fungal-specific membrane sterol. This was not observed for plant models containg sitosterol or stigmasterol. Here, Molecular Dynamics (MD) simulations are used with the objective of studying these rhamnolipids/ plasma membrane lipids interactions from an atomistic point of view. Thus, the all-atom Slipids ("Stockholm lipids") force field (FF) seems to be the most suitable for our purposes. However, because these sterols topologies were not described on this FF, the cholesterol was used as a reference molecule since it is very similar to these sterols and is already described. This approach was validated by comparing order parameters from simulations of membranes studied in the literature composed by 70% phospholipid (DMPC, DOPC or DPPC) and 30% sterol (cholesterol, ergosterol, sitosterol or stigmasterol) and NMR experiments. A comparison with other FFs is currently being achieved.

Then, in order to study the effect of sterol nature on lipid dynamics more complex models with 53% POPC, 23% POPG and 25% sterol have been simulated without and with rhamnolipids. We are currently carrying out other simulations with different sterol concentrations in addition to systems without POPG for analysing the consequence of a charged membrane.

P-564

Intrinsic curvature and large head group of co-lipids influence segregation of ceramides in bilayer membranes

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Ceramide is a sphingomyelin precursor that is normally present only in minute amounts in cell membranes. Increases in ceramide content has been linked to apoptosis and cell stress responses. Due to its lack of a large head group, ceramide does not form non-stacked bilayers on its own and thus, when present in bilayer membranes, needs co-lipids to shield it from unfavourable interactions with interfacial water. Ceramide also has a quite low monomeric miscibility in phospholipid membranes, causing it to segregate into ceramide-rich domains. The lack of a large head group also imparts negative curvature on membranes, and general destabilization. When ceramide is included in membranes that also contains other lipids that have negative intrinsic curvature, such as phosphatidylethanolamines and cardiolipin-Ca^{2+}, segregation into ceramide-rich domains is facilitated. On the other hand, lysophospholipids with positive intrinsic curvature also facilitate the formation of ceramide-enriched ordered phases. Although ceramide segregation can be facilitated by lipids which impose either negative or positive curvature stress to bilayers, the mechanisms of action are different, and will be discussed.

P-565

Lipid Interactions and Membrane Localization of Common Lipid-Conjugated Fluorescence Probes

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Lipids may laterally segregate in membranes, especially in the presence of cholesterol. This lateral segregation in membranes has been extensively studied using a comprehensive set of experimental techniques. Most methods require a probe to report on the biophysical properties of a specific molecule in the lipid bilayer. Because such probes can adversely affect the results of the measurements, a detailed understanding of the influence of the probe on the membrane properties is important. Using ^{2}H and ^{31}P NMR spectroscopy, we have studied how different fluorescence probes affect the membrane properties of 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine (POPC) bilayers. Probes chosen were *trans*-parinaric acid (tPA), diphenylhexatriene (DPH) and 1-oleoyl-2-propionyl[DPH-*sn*-glycero-3-phosphocholine (O-DPH-PC). In addition, using 2D ^{1}H magic-angle spinning (MAS) nuclear Overhauser enhancement spectroscopy (NOESY) NMR, we have determined the distribution of the probe moiety in the POPC membrane relative to the z-direction. Results showed that the different probes exhibit different membrane localizations and distributions, *e.g*. DPH seemed to predominantly exist in two orientations while tPA is arranged perpendicular to the membrane surface and distributed in the lower half of each membrane leaflet. Further, tPA was conjugated to sphinogomyelin (tPA-SM) as a substitute for the acyl chain in the SM. The interaction of the tPA-SM with cholesterol in comparison to POPC-cholesterol interaction was studied with ^{1}H NOESY NMR in membranes composed of ternary lipid mixtures. tPA-SM showed a strong favorable and very temperature dependent interaction with cholesterol, especially compared to POPC. In conclusion, NMR techniques can explain probe behavior and measure affinities between different lipid segments in complex bilayers, relevant to understanding nanodomain formation in biological membranes.

P-566

Towards reconstituting a biosynthetic pathway within compartmentalized GUVs

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Eukaryotic cells are complex systems defined largely by the presence of different sub-compartments. Compartmentalization of cells is considered as a key step in the biological evolution, providing a physical boundary which enables cells to perform distinct metabolic activities. Our aim is to construct a complex model cell system using giant unilamellar vesicles (GUVs) to reconstitute a biosynthetic pathway between multiple membrane compartments. Several techniques including microfluidics have been recently developed to construct artificial compartments from bottom-up principles. However, widespread application of these techniques is lacking due to the time-consuming and complex instrumental setup. The inverted emulsion-based method can be used to produce GUVs containing internal components such as proteins, enzymes, cells and even microspheres. In this study, we first optimize this method for (i) yield, (ii) size and (iii) to produce GUVs containing internal vesicles, referred to compartmentalized GUVs [Moga et al, 2019, submitted].

To illustrate the compartmentalization, we chose a multi-step enzymatic reaction: the biosynthesis of the molybdenum co-factor (Moco). Moco biosynthesis is a highly conserved and ubiquitous pathway divided into three major steps, which start in the mitochondrial matrix and terminates in the cytosol. Not only because the entirety of the pathway spans across two different compartments, but also for its biological significance in disorders induced through its malfunctioning - this pathway is ideal for our study. To comprehend the role of biological sub-compartments, encapsulation of enzymes and other cofactors involved in the pathway is required. Our preliminary results have shown promise with reproducibility of stable GUVs encapsulating the initial reactant compounds and proteins. We optimized the inverted emulsion method not only for encapsulating macromolecules and other small GUVs, but also for the required physiological buffers. Finally, we show a novel fluorescence detection system for tracking of Moco production within the GUVs via confocal microscopy in real-time.

P-567

Illuminating the spatio-temporal dynamics of lipopolysaccharide in the Gram-negative bacterial outer membrane

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The Gram-negative bacterial outer membrane (OM) is an asymmetric bilayer with an inner leaflet composed of phospholipid and an outer leaflet of lipopolysaccharide (LPS). The LPS leaflet creates an impermeable barrier to environmental challenges including many commonly used antibiotics. A bacterial population must continually turnover its OM in response to changes in its environment, e.g. to enable colonisation during infection of the host.

It has previously been shown that OM proteins (OMPs) are turned over via binary partitioning; new OMPs are inserted at the mid-cell and are pushed to the poles by cell growth[1]. OMPs do not diffuse within the OM, in contrast to inner membrane proteins[1,2]. It is not known whether LPS composition is altered via binary partitioning or if it is inserted uniformly across the cell surface. Previous studies of LPS lateral diffusion are contradictory with both mobile and immobile behaviour observed[3,4].

We have visualised LPS on the surface of bacteria using a metabolic labeling technique to incorporate a sugar analogue into LPS. This analogue can be fluorescently labeled with a specific bio-orthogonal click-it reaction[5]. Using both fluorescence recovery after photobleaching and single-molecule tracking experiments we have demonstrated that LPS is unable to diffuse laterally in the OM. This immobility is independent of LPS polysaccharide structural complexity and dependent on metal ion mediated LPS-LPS interactions. By pulse labeling bacteria we have shown that new LPS is inserted at discrete locations. These observations are consistent with binary partitioning of LPS during cell growth.

1. Rassam, et al., *Nature* 523(7560), 333-336 (2015)
2. Ursell, et al., *PLoS Comput. Biol.*, *8*(9), e1002680 (2012)
3. Mühlradt, et al., *Eur. J. Biochem.* 43.3 (1974): 533-539.
4. Ghosh, et al., *J. Bacteriol.* 187.6 (2005): 1913-1922.
5. Dumont, et al., *Angew. Chem. Int. Ed.*, *51*(13), 3143-3146 (2012)

P-568

LATERAL HETEROGENEITY IN BILAYERS OF TERNARY LIPID COMPOSITIONS CONTAINING CERAMIDE AND CHOLESTEROL

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Sphingomyelin (SM) in the cell plasma membrane is known to be degraded to ceramide (Cer) by acid sphingomyelinases under stress conditions. Since cholesterol (Chol) is abundant in the plasma membrane, the study of ternary mixtures SM:Chol:Cer is interesting from the point of view of membrane biophysics, and it might be physiologically relevant. In previous studies we have described the homogeneous gel phase formed by phospholipid:Chol:Cer at 54:23:23 mol ratio, where phospholipid was either SM or dipalmitoyl phosphatidylcholine (DPPC). We now provide new data, based on *trans*-parinaric acid and diphenylhexatriene fluorescence, supporting that the gel phase includes all three components in a single bilayer. The main question addressed in this paper is the stability of the ternary gel phase when bilayer composition is changed, specifically when the SM proportion is varied. To this aim we have prepared bilayers of a composition phospholipid:Chol:Cer at X:Y:Y ratios in which phospholipid increased between 54 and 70 mol%. The N-palmitoyl derivatives of SM (pSM) and Cer (pCer) have been used. We observe that for X = 54 or 60 mol%, a gel phase is clearly predominant. However when the proportion of phospholipid increases beyond 60 mol%, i.e. in 66:22:22 or 70:15:15 mixtures, a lateral phase separation occurs at the μm scale. These data can be interpreted in terms of a pCer:Chol interaction, that would predominate at the lower phospholipid concentrations. The putative pCer:Chol complexes (nanodomains?) would mix well with the phospholipid. At the higher SM or DPPC concentrations pSM:pCer and pSM:Chol interactions would become more important, giving rise to coexisting gel and liquid-ordered phases respectively. Heterogeneity, or lateral phase separation, occurs more easily with pSM than with DPPC, indicating a higher affinity of SM over DPPC for Chol or Cer.

P-569

Partitioning of DNA nanochannel between Lo/Ld phases in a lipid membrane: Relevance of lipid anchors.

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DNA origami is emerged as a powerful and versatile method to fabricate highly controllable 3-dimensional structures at the nanometer-scale. One example of those powerful nanostructures is a DNA ion channel, which attracts attention of many researchers. DNA channel exists often in a cylinder-like structure of six-helix bundle attached with a lipid anchor in order to lower the energy barrier of DNA insertion into lipid bilayer. The membrane-spanning DNA channel exhibited the capability of DNA molecule in mimicking the biological channels for biomedical applications(Burns et al. 2016). However, the relevance of the lipid anchor in controlling the preferential interaction of DNA with different lipid compositions has not been studied. *In vivo*, for instance, palmitoylated proteins that contain saturated C16 fatty acid are enriched in rafts, whereas prenylated proteins that have a bulky branched structure segregate into non-raft lipids (Melkonian et al. 1999). Here we address a question of whether the spatial configuration of lipid anchor could play a role in targeting the DNA channel to a distinct lipid domain phase.

We fabricated DNA nanopores of six DNA double helices arranged in parallel with a 9-13 nm-length and decorated with hydrophobic tags derived from fatty acid tail of lipids. These fatty acid chains were chosen based on their potency in partitioning the membrane-bound proteins *in vivo* into lipid rafts (liquid-ordered phase, l_o) and non-raft phases (liquid-disordered, l_d).

The DNA channels with various lipid anchors were incubated with giant unilamellar vesicles (GUVs) with Lo + Ld phase coexistence and the lipid phase preference of DNA channels was visualized under confocal microscope. Moreover, the transport activity of these artificial channels was assessed by a release technique of a fluorescent dye encapsulated into SUV liposomes.

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A lipid based nanoparticle design and activity for targeted delivery of a fibrinolytic agent

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According to ever-growing evidence, a denser fibrin clot structure highly correlates with clot resistance to degradation (fibrinolysis) and cardiovascular diseases (CVDs) and (athero)thrombotic disorders persistence and severity. CVDs account for nearly one-third of deaths worldwide and there is an urgent need to overcome this scenario. During fibrin polymerization small residue sequences in fibrin molecules are exposed, knobs A and B, and interact with their respective binding pockets on the C-terminal region of the Aα- and γ-chains of another fibrin molecule, leading to the formation and growth of protofibrils, which culminates in fibrin fibers. Liposome nanoparticles have drawn a lot of interest as pharmaceutical nanocarriers due to their stability and content release in a controlled manner. The aim of the work is to develop an encapsulated fibrinolytic agent into lipid nanoparticle, to be incorporated in the clot structure, leading to lower bleeding risk. A lipid nanoparticle, composed of POPC:Chol:DSPE-PEG (65:30:5) was tested, with a COOH or Maleimide groups as pegylated lipid terminal. We first evaluated fundamental safety of this lipid formulation studying the impact of the empty liposome nanoparticle on clot formation and lysis, and demonstrating no effects on haemostasis by recording clot polymerization and lysis kinetics. Turbidimetry studies showed that the presence of the nanoparticles reflected a non-significant increase in fibrin fiber radius, protofibril packing and fibrin content with increasing lipid nanoparticle concentrations. No structural changes, in a fully formed clot, was observed by atomic force microscopy after addition of empty liposome nanoparticles. Dynamic light scattering and zeta potential assays assured that the lipid nanoparticles are stable, without any measurable aggregation or change in its surface charge, for 28 days. The fibrinolytic agent tissue plasminogen activator (tPA) was added as liposome cargo, using two different methods of encapsulation, with one of them achieving around 70% efficiency. Ultracentrifugation was used to separate non-encapsulated material without triggering liposome nanoparticle aggregation. Preliminary results demonstrated a controlled release of tPA in a solid emulation of a clot, without activity loss. The work is now focused on optimizing the liposome nanocarrier by surface decoration with a targeting element toward fibrin clots.

P-571

A molecular view of crowded and complex membranes

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Cellular membranes are asymmetric mixtures of lipids and membrane proteins that separate the cell interior from the outside environment.[1] Plasma membranes have hundreds of lipid species whose actual composition is regulated by the cell and varies between organelles and organisms.[2] The question then arises as to the structural heterogeneity and lateral organization of lipids around the membrane proteins. This is a challenging problem as malfunctioning at the level of lipid-protein interaction and bilayer properties in the crowded cellular membranes are implicated in numerous diseases.[3] Thus, in-silico modeling of prototypical crowded membranes provides insight into the detailed microscopic understanding of the large-scale cell membrane behavior. We have used coarse-grained molecular dynamics simulations to study the lipid environment of 10 different proteins in a prototypical plasma membrane of 60 lipid species in a membrane patch of 150 nm x 150 nm. To provide a view of overall organization of lipid environments in cells, our analyses focus on characterizing the specific and non-specific lipid interactions with different proteins, impact of lipid composition on protein-protein interactions, protein cluster formation as mediated by lipids, and diffusion of various species of the system under the crowded condition.

References:
1) Ingólfsson H.I. et al., J. Am. Chem. Soc. 2014, 136, 14554–14559
2) van Meer G., EMBO J. 2005, 24, 3159– 3165
3) Holthuis J.C.M.; Menon A.K., Nature 2014, 510, 48– 57

P-572

POPC/Cholesterol lipid bilayers: A matter of polarity

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Water is an important component of lipid membranes and contributes for the stabilization of the bilayer structure. It determines the polarity gradient in lipid bilayers, since the amount of water at the lipid/water interface is higher than in the acyl chains region. Therefore, these different bilayer environments are characterized by different dielectric constants. The estimated dielectric constant (ε) values for the interface section is ranged 10-45 and for the hydrophobic core it must be around 2-4 [1].

The study of lipid bilayers polarity requires the use of suitable fluorescent probes such as Pyrene (Py), since this polycyclic aromatic hydrocarbon displays a variation on the ratio of emission band intensities (I_1/I_3) which is related with the polarity of its environment [2]. The polarity is estimated through the ratio of the intensities of the third (I_3) and the first (I_1) fluorescence bands. I_3 is insensitive to the solvent polarity, and the ratio I_1/I_3 increases as the solvent polarity augment, since the polarity of solvents is directly related with I_1 [2].

The structural and physical-chemical properties of Py settle its location in the ordered section of the methylenic palisade of lipid bilayers, stating values of ε, averaged transversally in space (the longest axis of Py, 9.2 Å) and laterally in time (due to lateral diffusion of Py during its excited state lifetime, ~150 ns in aerated media) [3]. From the correct measurements of the ratio I_1/I_3 in lipid bilayers, one can estimate their ε, provided that a calibration plot is constructed with standard alcoholic solvents [4]. The bilayer polarity of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and its binary mixtures with cholesterol (Chol) on multilamellar vesicles (MLV) were monitored using the Py Ham Effect (I_1/I_3). Pure POPC MLV exhibit higher ε than the mixtures at high Chol proportions, pointing to features observed in the available thermal phase diagrams.

References:
[1] E. Pérochon, *et al*. (**1992**) *Biochemistry* **31:**7672-7682.
[2] D. S. Karpovich; *et al*. (**1995**) *J. Phys. Chem.* **99:**3951-3958.
[3] L.M.S. Loura *et al*. (**2013**) *Biochim. Biophys. Acta* **1828:**1094-1101
[4] D. Arrais; J. Martins (**2007**) *Biochim. Biophys. Acta* **1768:**2914-2922.

P-573

How two Sterols Affect the Biophysical Properties of Membrane Mixtures: Insights from Molecular Dynamics Simulations

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Cholesterol and ergosterol are two dominant sterols in the membrane of eukaryotic cells and yeast cells, respectively, and their specific chemical structure influences the structural and dynamical properties of membranes. In this work we have explored the effect of different percentages of these sterol molecules on 1,2-Dipalnitoyl-sn-glycerol-3-phosphocholine (DPPC) lipid bilayer system, employing molecular dynamics simulations. The simulations reveal that at high temperatures, bilayers containing cholesterol have higher order than the membranes including ergosterol. This impact likely arises from the higher rigidity of ergosterol with respect to cholesterol. The ordered and stretched lipid tails in the membranes containing cholesterol give rise to a slightly higher thickness of the membrane compared to the systems with ergosterol. Concerning the energetics of the system, the simulations indicate that irrespective of the type of sterol and temperature the DPPC-DPPC interactions become more favorable with increasing order of the lipid. The DPPC-sterol interaction is, however, different in the two systems. Indeed, at high temperatures the DPPC-ergosterol interaction is more favorable at lower order parameters, resulting in a lower tendency of ergosterol to increase the order of the system as compared to cholesterol. A likely explanation for this behavior is the existence of extra double bonds in the molecular structure of ergosterol and their inclination to interact with lipid tails which in turn deceases the order of lipids. These results may have implications in the field of cell membranes.

P-574

Underlying mechanisms of cholesterol in bilayers – Highlighting its fundamental properties

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Cholesterol is essential for, e.g. , the domain formation of lipid membrane mixtures and is thus at the heart of many basic properties of lipid membranes. Using molecular dynamics simulations we found cholesterol to be effective inside only in the chain region of lipid bilayers, surprisingly not changing the mean distance of phospholipid heads along the bilayer both in DPPC and DLiPC cholesterol mixtures. This corroborates cholesterols condensing effect. Furthermore we found the phospholipid interaction to be impaired in vicinity to cholesterol. This effect intensifies with increasing number of cholesterol molecules present. Using the derived data, we propose different approaches to model phospholipid/cholesterol mixtures. Their choice is guided by the required input of a recently introduced lattice model of lipid membranes [1]. On this basis the underlying properties to describe phase and segregation behavior of those mixtures can be extracted.

[1] D. Hakobyan, A. Heuer, *J. Chem. Phys.* **146,** 064305 (2017)

P-575

Mistic senses lateral pressure distribution in lipid bilayers

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Mistic is a unique protein among membrane-interacting proteins: it displays a high number of negatively charged residues on its surface, but surprisingly avidly associates with lipid-bilayer membranes. *In vivo*, it promotes biofilm formation in *Bacillus subtilis* but its role remains unclear. From previous studies in detergent micelles, it is known that Mistic's conformational stability is promoted by both polar interactions with detergent headgroups and hydrophobic contacts with moieties of the micellar core. However, little is known about Mistic's structural stabilization in lipid-bilayer membranes. In the present work, single-molecule FRET in combination with ensemble spectroscopy was used to monitor Mistic's conformational behavior in lipid-bilayer membranes. By reconstituting the protein into phospholipid vesicles of varying composition, we show that Mistic assumes two conformational states when interacting with lipid bilayers, while the secondary structure seems hardly affected. Strikingly, by systematically varying acyl chain length, the degree of saturation, and membrane composition using binary lipid mixtures, the results suggest that the lateral pressure profile of the bilayer determines Mistic's conformational behavior and its switch between the two states. In the presence of bilayers with a higher lateral pressure on the acyl chains, Mistic is more likely to be found in an open, extended conformation, whereas in bilayers with a high lateral pressure on the headgroups, Mistic adopts a more compact state. Here, a model is proposed in which expanded Mistic resides within the interfacial region of the membrane, while the more compact conformation inserts deeper into the hydrophobic core of the bilayer.

P-576

Combining NMR Experiments and MD Simulations with Open Collaboration to Model Complex Biomembranes

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Chemical details of lipid headgroups and their interactions with ions are of paramount importance in biological signaling processes. NMRlipids (nmrlipids.blogspot.fi) is an Open Collaboration project aiming to describe lipid headgroup structures and ion binding to lipid bilayers with atomistic detail by combining NMR data and molecular dynamics simulations. Unfortunately, the massive amount of MD simulation and NMR data collected using the Open Collaboration method revealed that none of the available MD simulation models correctly reproduces the lipid headgroup structures or cation binding details, the main artefact being the overestimated cation binding affinity to lipid bilayers [1-3]. Recently we were able to improve the cation binding details to PC lipid bilayers by implicitly including the electronic polarizability into the MD simulation models using the electronic continuum correction [3]. Automation and extension of the process to other membranes containing biological lipids, like cholesterol, PE, PS and PG, is ongoing within the NMRlipids project [3,5]. Our longer term aim is to accurately describe the intermolecular interactions between different lipid species, which can be used for accurate modeling of complex biomembranes. We have also collected the largest publicly available database of MD simulations which can be accessed via www.nmrlipids.fi. The database can be used not only in force field comparison and development, but also in novel machine learning applications. It also brings the high-quality MD simulation data available for wide range of audience beyond MD simulation experts.

References

[1] Botan et al., J. Phys. Chem. B **119**, 15075–15088 (2015).
[2] Catte et al., Phys. Chem. Chem. Phys., **18**, 32560-32569 (2016).
[3]https://github.com/NMRLipids/NMRlipidsIVotherHGs/blob/master/Manuscript/manuscriptPS.pdf
[4] J. Melcr, H. Martinez-Seara, R. Nencini, J. Kolafa, P. Jungwirth, and O.H.S. Ollila J. Phys. Chem. B, DOI: 10.1021/acs.jpcb.7b12510 (2018)
[5]https://github.com/NMRLipids/NmrLipidsCholXray/blob/master/MANUSCRIPT/manuscript.pdf

P-577

Effects of Whole Body Hypothermia on pulmonary surfactant performance: from *ex vivo* to *in vitro* evidences

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Whole Body Hypothermia (WBH) is an effective treatment for neonates with encephalopathy due to perinatal asphyxia. During WBH, respiratory mechanics improves and lung tissue inflammation is reduced. Upon breathing, the presence of pulmonary surfactant (PS), a lipid-protein complex, is essential to stabilize the delicate structure of mammalian alveoli by reducing the surface tension at the respiratory air-liquid interface. In mammals, PS activity is adapted to be functional at a physiological temperature of 37°C. Interestingly, as a result of cooling, surfactant seems to modify its organization and composition both in heterothermic animals (able to live at different body temperatures) and neonates with and without lung injury. In detail, PS from newborns under WBH undergoes a time-dependent improvement of its tensioactive properties with a simultaneous increase of saturated dipalmitoylphosphatidylcholine (DPPC) and a reduction of certain unsaturated phospholipid (PL) species.

To explain these results, we further investigated on the structural and molecular mechanisms governing the improvement of biophysical activity in cooled PS. In a Langmuir-Blodgett trough, we tested surfactant purified from porcine lungs, subjected or not to meconium inactivation with and without compression-expansion cycles, at either 37°C or 33.5°C (WBH condition). In parallel, we studied the temperature-dependent changes of the lateral structure of the interfacial films, by testing PS films doped with a fluorescence probe under an epifluorescence microscope. Finally, to define how 1) the proportion of DPPC and 2) the number of double bonds in PL acyl chains could influence the surface-active properties of the material, we designed an appropriate *in vitro* model. We prepared protein-lipid mixtures containing DPPC (35% or 65% w/w), POPG (35% w/w) and surfactant protein SP-B (1% w/w) in the absence or presence of POPC (30% w/w) or DOPC (30% w/w). We tested their temperature-dependent biophysical activity and thermotropic profile by Captive Bubble Surfactometer and Differential Scanning Calorimetry, respectively.

Overall, the decrease in temperature seems to improve *ex vivo* and *in vitro* PS activity by means of two concurrent mechanisms: the facilitated and selective loss of uncompressible species from the air-liquid interface and the simultaneous reduction of DPPC loss from the interfacial film.

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Stability of membranes containing different types of anionic lipids

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Membrane integrity is fundamental to sustaining life. When subjected to strong mechanical, osmotic or electric stimuli, pores open in the membrane. Usually, these pores reseal after that and membrane integrity is restored. However, giant unilamellar vesicles (GUVs) containing significant fractions of charged lipids display a different response to strong DC electric pulses: Some micron-sized pores (macropores) open indefinitely leading to vesicle burst within less than a second after the pulse while GUVs that apparently restore their integrity after macropore closure can exhibit a long-lasting high permeability revealing the persistence of sub-microscopic pores minutes after the end of the pulse. These phenomena correlate with the fraction of the anionic lipid used, PG (phospatidyl glycerol). Here, the response of GUVs composed of the neutral lipid PC (phosphatidyl choline) and increasing fractions of other physiologically relevant anionic lipids, such as cardiolipin (CL) and phosphatidyl inositol (PI), is investigated with phase contrast optical microscopy. First, the occurrence frequency of bursting and long-lasting permeability events is quantified in a large population of GUVs. Then, the dynamics of vesicle contrast loss due to long-lasting permeability is assessed on individual GUVs. Finally, the pore edge tension, which gives the energy penalty per unit length to arrange lipids in the pore rims, is measured from the dynamics of macropore closure. The results obtained are compared with previous data with the PC:PG system. Overall, membranes containing higher fractions of charged lipids are more unstable and prone to vesicle bursting and long-lasting permeability, as a result of a reduced pore edge tension. For the PG and CL systems, a significant membrane destabilization occurs already at 20 mol% PG or CL, whereas for PI-containing membranes vesicle bursting was rarely observed and significant long-lasting membrane permeability was induced only at 50 mol% PI. The edge tension values of all equimolar mixtures were substantially reduced when compared to pure PC membranes. The results obtained are important to understanding the response of cells to electroporation, a widely used protocol to render biomembranes transiently permeable for several purposes, such as gene transfer.

P-579

Towards TATA-Box Melting: A Computational Study of DNA Unwinding

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DNA unwinding is of high relevance for many biological functions. It can trigger a sequence specific response of DNA, such as the localization of damages, and hence plays an important role in recognition by proteins. Furthermore, strong unwinding can also result in local strand separation of the DNA that is essential for several biological processes (e.g. transcription). However, how torsional stress propagates through DNA and how it leads to a mechanically induced phase transition has still remained elusive. In order to investigate sequence specific behavior of DNA upon unwinding, we have performed all-atom MD simulations for 50-bp long double stranded DNA sequences. Employing an external torque during Umbrella Sampling (US) simulations allowed us to systematically unwind the DNA molecules. From the simulations, we have then calculated the absorbed elastic energy along the sequence by means of a harmonic model. Intriguingly, most elastic energy is absorbed in C/G rich segments, while a T/A rich segment remains rather relaxed. As stereo-chemical explanation therefore we could identify that thymine's methyl group clashes with the 5' neighboring sugar, which locks the local geometry into a low twist state. C/G rich segments, in contrast, show a high degree of twist-bimodality and hence can more easily absorb higher proportions of elastic energy. However, further increase of torsional stress to a specific level induces a phase transition and hence abruptly changes the character of energy absorption: For all sequences, we observe local melting of the T/A rich segment, which then absorbs almost the whole global deformation energy, whereas other parts of the DNA relax towards an equilibrated B-DNA structure. In addition to the simulations, we calculated changes in the free energy upon unwinding by means of a nearest-neighbor Ising model that is based on experimentally determined parameters. The free energy profiles based on the Ising model are in good agreement with those obtained from MD simulations.

P-580

Teardrop and blinking: is surfactant activity the key?

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Keeping the ocular surface wet during inter-blink periods is necessary for both optical integrity and normal function of the eye. The tear fluid (TF) needs to move and spread quickly from the narrow excretory ducts of principal and accessory lacrimal glands to the large ocular surface. In this context, the stability of the ultrathin TF film at the air-liquid interface as well as its capability to reduce surface tension may play a crucial role.

During breathing, lung alveoli are subjected to repeated opening and closing cycles with a blinking-like speed of around 20 breaths per minute. In lungs, surfactant protein SP-C, with the contribution of several lipid species, is essential to stabilize pulmonary surfactant (PS) structures at the air-liquid interface, ensuring the correct spreading of material. Interestingly, the presence of SP-C and several PS lipids was described in the canalicular system and the tear drop. However, the biophysical activity of TF film has not been investigated yet.

We performed a detailed study on TF surface-active properties upon blinking-like compression-expansion cycles in a Captive Bubble Surfactometer. Investigating on the potential presence of SP-C by MALDI TOF-TOF mass spectrometry, we detected two peaks with masses corresponding to non-palmitoylated and palmitoylated SP-C and a third peak that could be associated to an intermediate in the proteolytic processing of pro-SP-C to mature SP-C. We assayed samples from healthy people and patients with dry-eye syndrome, using 2 different collyrium as references. Within healthy subjects, two biophysical behaviors (confirmed at different timing of sample collection) were observed: 1) a group with more active TFs containing a major rate of palmitoylated SP-C and 2) another group of samples with a lower biophysical activity. In this second group, 70% of TFs did not show peaks at the mass spectrograms that could be consistent with the presence of mature SP-C, but with a higher molecular mass. Moreover, TFs from patients did not show a proper biophysical activity and contained a major rate of non-palmitoylated SP-C.

These preliminary results illustrate the importance to further investigate on the connection between dry-eye syndrome and the impaired biophysical properties of TF films at ocular air-liquid interfaces.

P-581

THE INFLUENCE OF PROTEOLIPOSOME PHASE STATE ON THE ALKALINE PHOSPHATASE ACTIVITY AND MINERAL PROPAGATION

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Osteoblasts are responsible for the bone biomineralization and the process is mediated by release of matrix vesicles (MVs). The MV membrane has high levels of Tissue Nonspecific Alkaline Phosphatase (TNAP), Cholesterol (Chol), and Sphingomyelin (SM) when compared to the plasma membrane. Lipids in different states of organization can influence the functionality as well as the properties of the proteins present in the membrane. TNAP is a phosphomonohydrolase capable of generating inorganic phosphate (Pi) through the ATP hydrolysis, thus initiating biomineralization. In this study, we evaluated the incorporation of TNAP into liposomes constituted by lipid mixtures that form "rafts", in order to study the kinetic properties of TNAP and the ability of these proteoliposomes to induce the biomineralization in vitro. TNAP was reconstituted into liposomes of DPPC, DPPC:Chol (9:1), DPPC:SM (9:1), DPPC:Chol:SM (8:1:1), DMPC, DMPC:Chol (9:1), DMPC:SM (9:1) and DMPC:Chol:SM (8:1:1) (molar ratios), as previously described by Favarin et al. (2017). The presence of SM or Chol:SM in the DPPC proteoliposomes did not alter the values of catalytic efficiency for ATP hydrolysis. However, these proteoliposomes increased the dissemination of the mineral by about 4.5 and 8-fold, respectively, compared to neat DPPC. For DMPC vesicles, proteoliposomes containing DMPC:SM and DMPC:Chol:SM presented an increase in catalytic efficiency compared to neat DMPC. The mineralization in vitro assays using DMPC-containing mixtures showed an increase of 7.2- and 4-fold compared to DPPC proteoliposomes. Thus, with respect to the mineralization in vitro, DMPC proteoliposomes were more effective when compared to proteoliposomes containing neat DPPC. Taking together the kinetics parameters and mineralization in vitro results, it is possible to observe that the ternary raft-like proteoliposomes (DPPC:Chol:SM and DMPC:Chol:SM) had higher values of catalytic efficiency, and are more efficient in the mineral propagation in comparison with DPPC and DMPC proteliposomes.

P-582

Size regulation of membrane domains

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Ordered domains (rafts) in plasma membranes are smaller than 200 nm in size. In contrast, ordered domains in artificial lipid bilayers may be orders of magnitude larger. The size difference is often attributed to the lack of membrane proteins in giant unilamellar vesicles or planar bilayers. Yet, how membrane proteins regulate domain size is not known. Here we show that the coalescence of smaller domains that lead to the formation of micrometer sized domains is primarily determined by the spontaneous curvatures of the coexisting liquid ordered and liquid disordered phases. For this purpose we formed freestanding planar lipid bilayers that contained the photoswitchable lipid PhoDAG-1. The presence of an azobenzene switch in one of the acyl chains enabled light controlled conformational changes between the cis and trans states of the lipid. In the cis state lipid packing density is low and, consequently, PhoDAG-1 mainly distributes into the disordered phase. In contrast, trans PhoDAG-1 partitions into the ordered state. Thus, illumination by light offers control over the elastic characteristics of lipid domains. In one conformational state PhoDAG-1 allows rapid domain fusion so that almost every collision of two smaller domains results in their merger and thus the formation of a larger domain. In its other conformation, PhoDAG-1 inhibits domain coalescence. As a result, well preserved domains remain visible. These non-fusing domains may form chained aggregates. We used the elasticity theory of lipid membranes to describe the interactions of lipid domains. Our model shows that the interaction energy of domains nontrivially depends on the distance between their boundaries. In turn, the latter is governed by the spontaneous curvature of the different lipid phases. Photoswitching PhoDAG-1 drastically alters the curvature, thereby regulating domain fusion. A similar mechanism may operate in plasma membranes, i.e. the spontaneous curvature of rafts may act to prevent domain fusion in living cells.

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Incorporation of lipoprotein particles into lipid bilayers depends on local physical properties of the target membrane

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Here, we show via direct imaging techniques that High Density Lipoprotein (HDL) particles becomes integrated into the lipid bilayer upon membrane contact and amphiphilic cargo is immediately transferred to the bio-membrane. However, cholesterol content and the basic structure of lipids of the target membrane decide whether lipoprotein particles are integrated into the membrane and if cargo is released. In particular, we investigated how alterations of the cholesterol content, fatty acid chains and glycerol backbone to the head group of lipids influence the interaction. Particle incorporation and cargo transfer are abolished at increased membrane cholesterol concentrations and interleaflet flexibility as a consequence of the reduced elasticity of the lipid bilayer. Once in proximity, elastic membrane properties regulate the HDL particles' fusion with the bilayer and subsequent cargo transfer. We conclude that the biomembrane itself regulates the cholesterol transfer: high cholesterol levels act repulsive, low cholesterol levels fusogenic. Moreover, a more flexible head group at identical fatty acid composition has a positive effect on membrane interactions. Our observations reveal a new mechanism for regulation of lipid uptake based on sensing of the physical membrane properties and how mechanical cell properties are contributing to the nature of cargo transfer.

P-584

The role of lipid microenvironment in the pore-forming activity of cyclic lipopeptides

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The mechanisms of action of cyclic lipopeptides, syringomycin E from Pseudomonas syringae, fengycin from Bacillus subtilis, polymyxin B from Bacillus polymyxa, and daptomycin from Streptomyces roseosporus, on model lipid membranes were investigated. The electrophysiological study of planar lipid bilayers modified by lipopeptides was combined to fluorimetry of calcein leakage from lipid vesicles and a differential scanning microcalorimetry. The influence of the lipid microenvironment on the lipopeptide-membrane interaction was assessed using low molecular weight amphiphiles, flavonoids, alkaloids, styryl and xanthene dyes, thyroid hormones, and local anesthetics, as the factors initiating changes in the physical properties of model lipid membranes.

(1) Three ways to regulate the pore-forming activity of syringomycin E were found: by changing the transmembrane distribution in the electric potential, by modifying the lateral pressure profile, and by potential-dependent blockage of single pores.

(2) The ability of fengycin to form cation-selective channels in model lipid membranes mimicking target cell membranes was demonstrated. The key role of negatively charged lipids was shown. Lipid packing density affected macroscopic fengycin-induced membrane conductance. Cooperativity of the interaction of fengycin with membranes was revealed.

(3) The possibility to regulate the channel-forming activity of polymyxin B in negatively charged lipid bilayers by modifying the membrane dipole potential was found.

(5) The decrease in the membrane dipole potential was accompanied by an increase in the macroscopic current induced by complexes of daptomycin with calcium in lipid bilayers composed of phospholipids with branched saturated hydrocarbon chains. Plant polyphenol phloretin was found to be a synergist of the pore-forming activity of daptomycin.

The theory of reversible electrical breakdown was applied to the description of the dependence of the macroscopic current induced by lipopeptides on the transmembrane voltage. A classification of lipopeptides according to the mechanisms of interaction with cellular membranes was proposed.

The work was supported by the Russian Science Foundation (#19-14-00110).

P-585

Effects of general anesthetics on lipid membranes

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General anesthetics have been with us for more than 150 years. Despite their widespread use little is known about their molecular mechanism of action. Protein-bound theories alone are inadequate considering the enormous chemical variety of known anesthetics. These theories also fail to explain the experimentally observed phenomenon of pressure reversal; the fact that anesthetized animals regain consciousness under increased external pressure. What is known is that general anesthetics partition preferentially into the membrane; they follow the so-called Meyer-Overton rule. Therefore, there is a potential accumulation of anesthetics in the membrane interior that may modify its physicochemical properties. This observation led to the development of the lipid hypotheses. Such hypotheses can easily explain the aforementioned large variety of anesthetic molecules However, the precise way in which the presence of anesthetics modify the behavior of the membrane and in turn alter the conformation of the membrane-bound proteins is still unknown and is hypothesis dependent. In this work, we conducted a series of molecular dynamics simulations of various general anesthetics in protein-free lipid bilayers, in order to investigate the predictions of the lipid hypotheses. The primary strategy of the investigation is to search for membrane properties that are modified similarly by the addition of any general anesthetic. Moreover, the increase of the external pressure should revert the change of these properties towards the anesthetic-free values, following the pressure reversal phenomenon. Among the quantities considered are the density profiles of the various molecular moieties, the volume of the membrane, the free-volume inside the membrane, and the lateral pressure profile. For the latter, the computation requires the localization of an inherently non-local quantity, the pressure profile, and is of utmost interest to Cantor's hypothesis of general anesthesia. Finally, for a better understanding of the anesthetic-induced changes in the membrane properties, these properties were studies not only for different anesthetics but also for various membrane compositions. Our results suggest that if anesthesia arises indeed as a result of the conformational changes of certain membrane-bound proteins induced by changes in the lateral pressure profile, the relevant changes are expected to occur in the membrane region where the ester groups are located.

P-586

Probing antimicrobial mechanisms for effective strategies to overcome resistance

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Biophysical measurements are vital in the characterisation of peptides and proteins especially when they form sophisticated self-assembled nanostructures. Complexity of these structures poses demanding criteria for detailed measurements. In addition, obtaining mechanistic insights of peptide-membrane interactions is another challenging task. Functional performance of peptide structures along with real time measurements of its interaction with membranes cannot only be achieved by high resolution techniques. Therefore, detailed informations are plausible if a continuum of complementary techniques are employed. The case studies highlighted in this poster contribute to the notion how these low and high resolution techniques can be utilised to obtain the highest demand for applications in industry. As a result, bio-physical analysis of peptide-membrane may undergo a renaissance in providing more meaningful descriptions of biological mechanisms in approaching molecular therapy and diagnosis more rationally.

Wednesday 24th July

BIG DATA IN BIOPHYSICS

P-587 (O-159)

Real-time dynamic changes in serum metabolome during the anticancer treatment by means of NMR-based metabolomics

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Head and neck squamous cell carcinomas (HNSCC) are mainly located in larynx, pharynx and oral cavity, which play crucial roles in respiratory, nutritional, social and communicative functions. The standard organ preservation treatment method for HNSCC is sequential and/or concurrent radiotherapy (RT) and chemotherapy (CHT). However, it is associated with significant temporary or permanent toxic side effects in normal tissue and/or involved regions (Acute Radiation Sequelae, ARS). We aimed to investigate the real-time (during-treatment) changes in the serum metabolome and to correlate these changes with available patients' clinical data (ARS, laboratory blood tests, weight/BMI).

230 HNSCC patients were enrolled into the study. Patients were treated radically with RT and CHT-RT. CHT was realized as induction and/or concurrent treatment. Blood samples were collected weekly, starting from the day before the treatment and stopping within the week after the treatment completion, resulting in a total number of approximately 1900 samples. Patients were clinically monitored until the resolution of all ARS symptoms. Serum samples were analyzed using 1H-NMR spectroscopy followed by multivariate projection techniques (MPT) and batch analysis (BA).

Significant metabolic alterations correlated with ARS escalation were successfully identified. Patients with significant treatment induced weight loss showed increased serum ketone bodies (KB) concentrations. The increase of KB preceded signaling from the clinical nutritional parameters (albumin, BMI) and may be used as a prognostic marker. Furthermore, significant changes in concentrations of inflammatory and energy involved metabolites were observed during the RT/CHRT treatment.

The application of MPT and BA methods allows to track the trajectories of temporal (during-treatment) changes in the serum metabolome of HNSCC patients. Which, in turn, gives the opportunity to predict metabolic alterations and treatment response in new patients. Multivariate models trained and validated on big datasets may be useful in introducing personalized medicine to anticancer treatment.

P-588 (O-160)

Decrypting interaction fingerprints in protein molecular surfaces

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Interactions between proteins and other biomolecules are the basis of protein function in all biological processes. Many proteins bear functional signatures that can be effectively inferred from sequence and structure, but require the existence of sequence or structural homologues functionally annotated. A higher-level description of a protein structure is presented by its molecular surface, which models a protein as a continuous molecule where geometric and chemical features are presented, to some extent, independently of the underlying protein sequence. We hypothesized that molecular surfaces carry fingerprints that can be identified and enlighten their interaction with other biomolecules. Thus, we develop a computational algorithm to identify molecular surface interaction fingerprints (MaSIF). MaSIF exploits geometric deep learning tools to extract geometric and chemical features from protein surfaces. We used MaSIF to study three fundamental aspects related to biomolecular interactions: I) determining the binding specificity of enzymes, where we achieve a 74% accuracy on highly similar cofactors; II) predicting protein-protein interaction sites, where we outperform state-of-the-art site predictors with a ROC AUC of 0.85; III) large scale protein docking, a new paradigm based on surface fingerprints that outperforms other methods by a factor of 1000 in computational time, enabling multitarget docking campaigns. In summary, we present a novel approach - MaSIF – that leverages deep learning techniques to identify patterns in protein surfaces and from those infer important function-related properties. We anticipate that these emerging modeling techniques will be the next generation tools to improve our understanding of protein function and design.

P-589 (O-161)

Bayesian inference and machine learning approaches to determine protein copy number from localization microscopy

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Single-molecule localization microscopy has become an important tool for nanoscale imaging and a considerable effort has been devoted to quantifying protein copy number in super-resolution images. In localization microscopy, the imaging procedure results in the mapping of a labeled protein into a stochastic number of molecular localizations, randomly placed around the actual protein position. Therefore, the determination of the exact protein copy number is impaired by the stochasticity of the labeling and the complex photophysics of the fluorescent probes. High protein density and tight arrangement in oligomeric structures at the nanoscale further complicate this task. Consequently, even when using *ad hoc* calibration standards, the inverse problem of determining protein copy number and positions from the collected localizations is technically and computationally challenging.

We present two methods to tackle this problem at different levels. The first consist in a brute force approach, based on a convolutional neural network architecture to create the most-likely protein spatial arrangement compatible with an experimental localization map. The neural network is trained on a calibration image dataset, based either on experiments or numerical simulations, thus offering the possibility of transfer learning. We discuss performance and limitations of the method at varying imaging parameters.

While the former approach is directly applied to raw localization lists, other methods previously described involve the application of a segmentation algorithm to cluster nearby localizations and then the fit of the distribution of localization per cluster to infer the relative abundance of different oligomerization species. In these cases, the latter step is complicated by the lack of knowledge about the largest oligomeric structure and the increasing number of free parameters. To improve the fitting step, we describe a method for Bayesian model comparison and parameter inference via a nested sampling algorithm that robustly estimate the weight of each oligomeric population without overfitting. The method has a wide applicability and - in the presence of a proper calibration - can be also applied to STED, confocal and TIRF imaging.

We gratefully acknowledge the support of NVIDIA Corporation with the donation of the Titan Xp GPU used for this research.

P-590

ADAPTABLE: a web platform for antimicrobial peptides

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Antimicrobial peptides (AMPs) are small molecules produced by all living systems, which can be considered part of the innate immune response to pathogens. Due to their extraordinary variety of biological and chemical activities (antibacterial, antibiofilm, antiviral, antifungal, antiparasitic, anticancer, anti-inflammatory, immunomodulatory...), they have received significant attention, especially as candidate drugs to face the threat of super-bacteria. The study of their multiple modes of action requires a deep understanding of their properties, which are often hidden in their sequence.

We present a web platform, ADAPTABLE (http://gec.u-picardie.fr/adaptable), able to cluster sequence-related peptides after collection, uniformization and data-merging of most of the main databases available on the web. Our methodology is the only one standardizing modified amino acids, often a key feature for the activity of AMPs. ADAPTABLE is a flexible tool that allows the researcher to tailor the analysis by choosing among a variety of optional parameters (covering multiple chemical and biological features and including target organisms and standardized experimental activity concentration against them). The performed clustering can highlight the potential properties of already existing and/or user-supplied peptides.

In the era of antimicrobial resistance, ADAPTABLE can assist the research community in the complex field of AMPs. With thousands of existing sequences and hundreds discovered every year, a unifying and standardizing platform is urgently needed to combine a large amount of information, currently scattered among different web-resources (more than 25 AMPs, chemical and microbiological databases). ADAPTABLE provides tools that can be used to: i) design new peptides using motifs responsible for the specificity towards a specific organism; ii) predict several properties of a generic peptide sequence; iii) discover new potential activities of pre-existing sequences not yet tested experimentally; iv) generate an optimal scaffold for drug design, thanks to the mathematical analysis of the clustered families.

P-591

Investigating Native State Fluorescence Emission of Immunoglobulin G (IgG) using polarized Excitation Emission Matrix (pEEM) spectroscopy and PARAFAC

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Intrinsic protein fluorescence is a potentially useful, non-destructive, rapid, method for measuring protein structure changes and variance. However, the presence of multiple fluorophores results in complex spectral overlap, making it hard to separate the emission from different fluorophores, thus preventing the extraction of potentially useful information. The use of multidimensional fluorescence (MDF) measurements with polarization can be used to exploit the intrinsic anisotropy of protein emission. When polarized measurements are combined with chemometric methods (e.g. Parallel Factor (PARAFAC) analysis), we have a methodology entitled Anisotropy Resolved Multidimensional Emission Spectroscopy (ARMES). This combination of MDF spectroscopy, anisotropy and chemometric analysis enabled accurate recovery of individual fluorophore emission in relatively simple proteins like human serum albumin or insulin. However, Immunoglobulin G (IgG), which is the main antibody used for therapeutic purposes, is much more complex with 70+ fluorophores. Here we explore, for the first time, the efficacy of ARMES for the spectral analysis of IgG in its native and stressed states. The first issue to overcome was the critical issue of data pre-processing for correcting non-trilinearity caused by Rayleigh/Raman scatter, and inner filter effects. Once the correct data pre-processing was implemented, PARAFAC analysis could only resolve one main component (PFC1=native state, ≥99%) and a minimum contribution of a second component (PFC2<1%), and this was due to the extensive FRET between tryptophan and tyrosine. Although there was insufficient fluorescence fluctuation in the native state for PARAFAC analysis to resolve different fluorophores populations, this study served as a baseline for further investigations into physically and chemically stressed IgG using ARMES. PARAFAC analysis of thermally (20−75°C), freeze-thawed (1−3 cycles), and chemically (guanidine hydrochloride) stressed IgG showed significant differences in the recovered components scores and thus provides a different insight into fluorescence emission changes induced by stressing conditions. Freeze-thaw was found to be the mildest stress, followed by thermal and chemical stresses, with PFC1 scores decreasing by 2%, 15%, 25%, and PFC2 increasing by 12%, 40%, and 75%, respectively. This indicates that PARAFAC/ARMES might provide a different approach to the analysis of protein stresses via intrinsic fluorescence emission measurements.

P-592

Using Anisotropy Resolved Multidimensional Emission Spectroscopy (ARMES) and chemometric modelling to study Förster Resonance Energy Transfer (FRET) processes.

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Polarized Excitation Emission Matrix (pEEM) measurements potentially offers more information rich methods for analysis of intrinsic protein emission and therefore analysis of structural change or variance. pEEM and fluorescence anisotropy yields information about size, mobility, and photophysical processes.[1] Combining pEEM measurements with chemometric methods like parallel factor analysis (PARAFAC) or Tucker3 is referred to as anisotropy resolved multidimensional emission spectroscopy (ARMES).[2, 3] Here we investigate the efficacy of ARMES to quantify energy transfer between the intrinsic fluorescence of HSA to 1,8-anilinonaphathalene sulfonate (ANS). The objective was to determine if ARMES can provide more information about FRET by resolving the constituent fluorophores. The pEEM data ($EEM_{\parallel}$, $EEM_{\perp}$, and *aniso*-EEM maps) from 240-440 nm (excitation) and 260-570 nm (emission) was recorded over the full HSA-ANS emission space. pEEM data for the donor region was modelled using PARAFAC whereas the global EEM, including acceptor region, required Tucker3. For the donor region, simple PARAFAC extracted the FRET donor signal (mainly Tryptophan) from non-interacting fluorophores (mainly Tyrosine) of the intrinsic HSA emission during 1,8-ANS (acceptor) addition. Decomposition of HSA donor fluorescence into three-dimensional FRET interacting and non-interacting signals facilitated the calculation of FRET efficiency in a new way which uses the full emission space. FRET efficiency values calculated using this approach showed marginally higher values than those calculated conventionally using the raw EEM data. The underestimation of the FRET efficiency using the conventional technique can be attributed to spectral overlap with the emission from the non-interacting tyrosine. Modelling of the global EEM was complicated by FRET induced non-linearity of the acceptor region, thus required a more flexible Tucker3 model. *A priori* system knowledge was used to restrict the Tucker3 model- and the FRET signal was successful extracted using a 3-component model of the global EEM. The ability to extract the different emission components is of major significance for interpretation of complex emission from multi-fluorophore mixtures.

1. D. M. Jameson and J. A. Ross, Chem. Rev. **110**, 5, 2685 (2010).
2. Y. Casamayou-Boucau and A. G. Ryder, Methods Appl Fluoresc **5**, 3, 037001 (2017).
3. R. C. Groza, B. Li, and A. G. Ryder, Anal. Chim. Acta **886**, 133 (2015).

P-593

Structural characterization of *Aspergillus fumigatus* cell wall architecture by solid-state NMR

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The filamentous fungus *Aspergillus fumigatus* is one of the major fungal pathogen of the respiratory system. Aspergillosis displaying both high incidence and mortality rates, is becoming a massive public health issue. The spores of *Aspergillus fumigatus* are surrounded by a cell wall, essential for their growth and allowing them to resist against host defense mechanisms. The cell wall is composed of a set of polysaccharides, covered by melanin and a layer of proteins called hydrophobins [1,2], characterized by their amphipathic properties and their ability to self assemble at hydrophilic/hydrophobic interfaces. Seven hydrophobins were identified in the genome of *Aspergillus fumigatus*. Among them, RodA is the most studied hydrophobin and it forms a layer of amyloid fibers (with a rodlet morphology) at the spores' surface to prevent the cells from the desiccation.

In this study[3], we aim at investigating the structural architecture of *Aspergillus fumigatus* cell wall at atomic resolution using magic-angle spinning solid-state NMR spectroscopy. We use multidimensional solid-state NMR to identify the cell wall components, decipher their rigidity / mobility and understand their interaction with bulk water molecules. By comparing wild type conidia, swollen conidia and germinating conidia we investigate the composition of the cell wall during the germination process. Moreover, using *Δags* conidia (α-glucan deficient), we analyze the compensatory mechanism[4] occurring when α-glucan is deleted.

[1] Beauvais, A., Fontaine, T., Aimanianda, V., Latgé, J. P. Apergillus cell wall and biofilm. Mycopathologia 178, 371-377 (2014).
[2] Wosten, H. A., Asgeirsdottir, S. A., Krook, J. H., Drenth, J. H. & Wessels, J. G. The fungal hydrophobin Sc3p self-assembles at the surface of aerial hyphae as a protein membrane constituting the hydrophobic rodlet layer. Eur J Cell Biol 63, 122-129 (1994).
[3] Supported by the ANR project HYDROFUN to I. G.
[4] Henry, C., Latgé, J. P., Beauvais, A. α-1,3 Glucans Are Dispensable in Aspergillus fumigatus. Eukaryot Cell 11, 26-29 (2012).

P-594

DEVELOPMENT OF AUTOMATED ANALYSIS OF BIOMEDICAL SIGNALS OBTAINED FROM CALCIUM IMAGING

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In a series of previous studies we demonstrated the effect of purified immunoglobulins G from ALS patients (ALS IgGs) on Ca homeostasis in rat astrocytes in culture. In order to develop automated diagnostic screening of these IgGs as compared to healthy IgGs, the raw data for the analysis came from the primary rat cortical astrocytes recorded for calcium imaging. The first step in the automated analysis was to determine the boundaries for each cell Region of Interest (ROI). We implemented the methods called Adaptive Histogram Equalization and Active Contour Model in order to segment the cells in the video. For this analysis, we used images created by two projection methods instead of using the whole video. These projection methods use each frame in the video to create a single new image thus making the boundaries of every cell more pronounced while also decreasing the computational cost since one image is being processed instead of all frames. Furthermore, we compared the results obtained using combinations of different methods and then extracted traces from each ROI. Next, in order to assess the performance of Supervised Machine Learning algorithms in discriminating between calcium traces, we extracted traces for (1) baseline (pre-treatment, with perfusion on), (2) IgG treatment, (3) IgG wash (with perfusion on) and (4) ATP application. Each trace for the given group covered the same amount of time. In addition, we utilized Unsupervised Machine Learning algorithms in order to: (1) characterize traces and extract significant features, (2) cluster the traces and (3) if any groups could be found, train a classifier to reliably predict a class for any given test trace. Clustering was performed with Decision Trees by utilizing Block Bootstrapping for time series to model the traces, as well as k-Nearest Neighbors. All methods and performance measurements were implemented in MATLAB. In the future, we plan to implement and compare other methods for segmentation and Machine Learning algorithms like Watershed algorithm and Majority-Voting Classification.

P-595

ANISOTROPY RESOLVED MULTIDIMENSIONAL EMISSION SPECTROSCOPY (ARMES) AS A TOOL FOR CHARACTERIZATION OF MIXED PROTEIN SOLUTIONS

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Here, we investigate the use of Anisotropy Resolved Multidimensional Emission Spectroscopy (ARMES) [1] for the characterization (quantitative and qualitative) of mixed protein solutions. ARMES seeks to resolve the excitation and emission spectra of the constituent fluorophores using multivariate data analysis methods like PARAllel FACtor analysis (PARAFAC). Intrinsic emission from mixtures of human (HSA) and bovine (BSA) serum albumin in PBS buffer were measured using polarized excitation-emission matrix (pEEM). This generated five sorts of multidimensional data: parallel ($EEM_{\parallel}$), perpendicular ($EEM_{\perp}$), and total emission maps (EEM_T); anisotropy (*aniso*-EEM), and a separate light scatter map.

Spectral analysis revealed tryptophan (Trp) environment changes and quenching of tyrosine (Tyr) contribution indicating an increased energy transfer process with concentration not due to inner filter effects. The light scattering data confirmed some form of protein-protein interaction with a non-linear increase in scattering at λ>330nm where albumins did not absorb. These were accompanied by changes in the *aniso*-EEM maps which were caused by two competing factors: increased anisotropy due to larger protein size, but also depolarization via energy transfer. Thus, the shape change in the *aniso*-EEM maps is a better indicator of protein-protein interaction in this case than simple anisotropy.

To validate this, PARAFAC analysis of pEEM data [2] was undertaken and this resolved three, different Trp emission contributions, with very different *aniso*-EEM maps, indicating different origins. The first component (~90%) originated from Tyr to Trp-213/214 intramolecular FRET, which increased (as did its overall anisotropy) as the HSA:BSA ratio increased. The second Trp component (~5.5%) represented Trp-134 (BSA) emission generated via FRET whereas the third component (4.5%) was ascribed to directly excited Trp (most probably Trp-134) because of its high average anisotropy. Changes in the scores and anisotropy of this component show evidence of protein-protein interactions which are under further investigation. This multivariate ARMES methodology potentially offers a more comprehensive analytical method for identifying subtle changes in intrinsic protein emission caused by protein-protein interactions in solution.

References:

[1] Groza, RC, Li B, and Ryder AG. *Anal. Chim. Acta* 2015; 886: 133-142.

[2] Steiner-Browne, M, Elcoroaristizabal,S, Casamayou-Boucau, Y, Ryder, AG. *Chemom. Intell. Lab. Syst* 2019; 185:1-11.

P-596

Multi-dimensional Excitation Emission Spectroscopic analysis of viscosity effects on tryptophan emission in solution.

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Tryptophan (Trp) is the most important fluorophore involved with intrinsic protein emission and changes in its emission can be used to monitor both structural and environmental changes of proteins. A huge number of studies are devoted to Trp fluorescence in proteins, however, there is still no definitive, agreed explanation for its photophysical behaviour because of its complex emission. In the literature, there are many controversies and contradictions concerning electronic transitions (^1L_a and ^1L_b), rotamers, and the complex lifetime decays of Trp. Using multi-dimensional fluorescence measurement techniques such as Anisotropy Resolved Multi-dimensional Emission Spectroscopy (ARMES)[1, 2] and Excitation Emission Fluorescence Lifetime Matrix (EEFLM)[3], we undertook a more comprehensive analysis of the fluorescence intensity, anisotropy, and lifetime behavior of Trp in solutions of varying viscosity. Measurements were made over the full excitation and emission spectral range ($\lambda_{ex}/\lambda_{em}$ = 240-320/290-450 nm) using a tunable, frequency doubled, pulsed supercontinuum laser. This is the region involved in intrinsic Trp emission from proteins and the goal is to identify and resolve emission contributions from electronic and rotamer sources and their effect on anisotropy in particular. For example, increasing solution viscosity led to significant increases in the contribution to the total Trp emission from one rotamer. Its lifetime increased from 0.68 to 4 ns and the amplitude from 11 to 35%. Multivariate analysis (PARAFAC) of the steady-state data further enables the resolution of the characteristic excitation and emission spectra of the various sources for Trp emission in solution. This combination of steady-state, time-resolved, and anisotropy data with multivariate data analysis offers a unique methodology for resolving some of the controversies about Trp emission which will better facilitate the use of intrinsic protein emission analysis.

References:

1. Y. Casamayou-Boucau and A. G. Ryder, Anal. Chim. Acta **1000**, 132 (2018).

2. M. Steiner-Browne, S. Elcoroaristizabal, Y. Casamayou-Boucau, and A. G. Ryder, Chemometrics Intellig. Lab. Syst. **185**, 1 (2019).

3. D. Melnikau, S. Elcoroaristizabal, and A. G. Ryder, Methods Appl Fluores **6**, 4 (2018).

Wednesday 24th July

LIQUID-LIQUID PHASE SEPARATION IN BIOLOGICAL SYSTEMS

P-597 (O-165)

General sol to gel transition of liquid-liqud phase separated protein under shear

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It has been discovered that protein undergo reversible liquid-liquid phase separation (LLPS) forming membraneless compartments with condensed protein in living cells. This process is essential for information transportation and spatial organization. However, irreversible phase transition caused by protein aggregation or fibrillation results in neurological diseases, such as Amyotrophic lateral sclerosis (ALS) and Frontotemporal lobar degeneration (FTLD). Nonetheless, the aspects affecting this irreversible phase transition are not well understood. We have discovered, for several biological relevant proteins, such as FUS, Annexin A11 and Ded1, that shear stress triggers the sol-gel transition and can further transform condensed liquid protein droplets into solid fibres. The process is remarkably similar to the silk formation. In past studies, a few attempts have been made to generate fibres mimicking silk spinning but limited only to silk proteins. Our results have shown that proteins containing intrinsic disordered regions undergo LLPS, gelation and form solid fibres upon the response to the shear stress. To well study this phenomenon, microfluidic techniques have been applied to observe protein phase transition under controlled laminar flow. We characterized the change of structural conformation and mechanical properties of these materials of the different phases. All these discoveries are suggesting the possible mechanism of irreversible phase transition of liquid-liquid phase separated droplets in biological systems and opened a door to new sources of shear sensitive biomaterials.

P-598 (O-166)

Recapitulation of nucleocytoplasmic transport with phase-separation of engineered protein repeats

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Nuclear pore complexes (NPCs) in eukaryotes are equipped with a selective permeability barrier, which conducts transport between nucleus and cytoplasm. The barrier is composed of ~10 subtypes of FG domains, which are low-sequence-complexity, intrinsically disordered proteins (IDPs) containing numerous phenylalanine-glycine (FG) dipeptide motifs. The barrier allows passive diffusion of small molecules, but the diffusion of larger macromolecules (≥ 5nm) is restricted. Transport of the latter is mediated by binding to the shuttling Nuclear Transport Receptors (NTRs), like Importinβ. Such kind of "facilitated translocation" is achieved by the interactions between NTRs and FG domains.

We previously demonstrated that many FG domains, including the functionally important Nup98 FG domains, are "cohesive": they phase-separate spontaneously from aqueous solutions to form a hydrogel-like protein rich phase with remarkable NPC-like selectivity: it favours the fast partition of NTRs but excludes inert large proteins. We proposed that FG motifs within FG domain molecules interact with each other by polyvalent π-π/ hydrophobic interactions and these interactions lead to phase-separation. The phase can be locally and transiently disrupted by the binding of NTRs but not by inert molecules, thus allowing selective passage of NTRs and the associated cargos.

One challenge for systematic analyses of FG domain sequences is that they have very degenerated repeats. Here we attempted to engineer the simplest possible sequence of FG domain which still confers functions. Along this line, we discovered that both phase-separation propensity and selectivity remain unchanged if all xxFG motifs are converted to GLFG motifs; however, they are correlated with the density of FG motifs. We also compared different natural existing xxFG motifs systematically: GLFG and SLFG motifs are of similar cohesiveness. FSFG motifs, however, make the phase hyper-cohesive and NTRs fail to enter.

Finally, we designed an ultimately simplified FG domain comprising a perfectly repeated 12mer peptide. Remarkably, it also phase-separates to form a selective barrier. This dataset establishes that, although NPCs contain different FG domains with diverse repeat units, sequence heterogeneity is no fundamental requirement. Instead, the overall cohesive property of FG domains is essential. The simplified variants set the stage for structural/ atomic-level analyses.

P-599 (O-167)

Protein phase transition: from biology towards new dynamic protein materials

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It is now recognized that cells can form dynamic membraneless compartments by liquid-liquid phase separation (LLPS) of proteins and nucleic acids. Most of the proteins associated with this process contain low complexity domains (LCDs) or low complexity sequences (LCSs), which are intrinsically disordered domains enriched in specific amino acids. These sequences play a crucial role in tuning weak, attractive intermolecular interactions that compete with the entropic cost associated with de-mixing.

Here, we demonstrate the possibility to generate a new class of bio-inspired dynamic protein materials by conjugating low complexity domains to soluble globular regions. Specifically, we derived low complexity domains from a series of DEAD-box proteins strongly associated with the formation of processing bodies (P-bodies) in yeast. We show that these biologically derived molecular velcros enable the self-assembly of globular proteins into supramolecular architectures via a multistep process. This multistep pathway involves an initial liquid−liquid phase transition, which creates protein-rich droplets that mature into protein aggregates over time. These protein aggregates consist of permeable structures that maintain activity and release active soluble proteins. We further demonstrate that this feature, together with the dynamic state of the initial dense liquid phase, allows one to directly assemble different globular domains within the same architecture, thereby enabling the generation of both static multifunctional biomaterials and dynamic microscale bioreactors.

References

Faltova L., Küffner A. et al, "Multifunctional Protein Materials and Microreactors using Low Complexity Domains as Molecular Adhesives", ACS Nano, 2018, 12, 9991-9999

P-600

A Dielectrophoretic Microfluidics Integrated with Aqueous Two-phase System for Circulating Tumor Cells Capture and Recovery

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To improve patient recovery from cancer therapy, molecularly targeted therapy that blocks the growth and spread of cancer has been used to treat cancer in recent years. However, the critical problem in cancer therapy is to prevent a patient's cancer from relapsing. Therefore, the detection and monitoring of circulating tumor cells (CTCs) in patients who accepted cancer therapy have become a topic of study in clinical cancer therapy. In this study, we developed a dielectrophoretic microfluidics (DEP-microfluidics) device integrated with an aqueous two-phase system (ATPS) and magnetic beads to capture CTCs from whole blood. First, to avoid red blood cells clogging microchannels, the whole-blood sample is separated using the ATPS. Polymorphprep was selected as the solvent for the isolation of human polymorphonuclear leucocytes and CTCs from whole blood in the ATPS, and the parameters influencing the ATPS were also investigated and optimized. Second, the DEP-microfluidics device fabricated using photolithography captures polymorphonuclear leucocytes and CTCs. Magnetic beads with anti-EpCAM (Epithelial cell adhesion molecule) are injected to bind the EpCAM expressed on the surface of the CTCs captured in the DEP-microfluidics device. Finally, the CTCs bound by magnetic beads are recovered for detection through an external magnetic field. The device developed in this study can be used in clinical cancer therapy to help monitor the dynamic range of CTCs using a fluidics platform.

P-601

Mechanical characterization of phase separated Nucleoporin droplets using Atomic Force Microscopy techniques

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While keeping nuclear and cytoplasmic contents separated, protein complexes—like the nuclear pore complex (NPC) of eukaryotic cells—conduct massive transport mediated by shuttling nuclear transport receptors (NTR) across the nuclear envelope.

Aqueous Nup98 FG domain solutions rapidly phase-separate into characteristic assemblies, called FG particles, on the micrometer scale; likely to be driven by multiple weak interactions involving interplay among electrostatic, dipolar, and short-range directional interactions. We study this soft matter as a hydrogel submerged in water using atomic force microscopy techniques (AFM) allowing us to reveal its intrinsic mechanical characteristics such as stiffness or viscoelastic behavior and stress relaxation under load while simultaneously obtaining topographical information. We use force-clamp force mapping to measure the mechanical response, using sub-micrometer spatial resolution by combining force-distance curves with an added force clamp phase during tip-sample contact in which quantitative viscoelastic or poroelastic sample properties are extracted. Besides adhesion upon retraction we analyze viscoelasticity in the analysis of conventional force distance curves by fitting the entire force cycle.

Here we directly validate a poroelastic model approach to explain rheology at short timescales of nucleocatoplasmic transport and deriving physical parameters of the meshwork using microindentation tests in conjunction with protein engineering. Our results show influences of different engineered GFP variant to the water redistribution through the solid phase of the hydrogel and its fundamental role in nuclear transport at short timescales.

P-602

The effect of alkaloids on the phase transition and membrane permeability of lipid bilayers

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Using differential scanning calorimetry we studied the phase behavior DPPC in the presence of alkaloids. The lipid:alkaloid ration was equal to 10:1. It was shown that the addition of the capsaicin to the DPPC liposomes leads to the decrease in the main lipid phase transition (T_m) on 5.7 degrees, while the width at half-width of main peak in the endotherm ($T_{1/2}$) increased on 3.7 degrees. The introduction of the melatonine, quinine, hordenine, and berberine slightly decreases T_m on the 0.7, 0.7, 1.4 and 1.9 degrees, respectively, and does not practically change $T_{1/2}$. Caffeine and pentoxifylline do not influence the phase behavior of DPPC. The ability of alkaloids to promote calcein leakage from large unilamellar POPC-vesicles was decreased in the following order: capsaicin >> berberine ≈ hordenine ≈ quinine > melatonine ≈ caffeine ≈ pentoxifylline. Both the differential scanning calorimetry and calcein leakage indicate that interact of capsaicin, berberine, and hordenine with a lipid head-groups increases the area per lipid molecule and consequently enhances the chain mobility. The effects of alkaloids and related compounds on the membrane activity of gramicidin A, nisin and syringomycin E are tested. The study was supported by RSF (#19-14-00110) and scholarship SP-484.2018.4.

P-603

Role of seipin in lipid droplet biogenesis

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Lipid droplets (LDs) serve as reservoirs of neutral lipids for metabolic energy and membrane components[1]. However, excess storage of neutral lipids underlies metabolic diseases, such as obesity[2]. Synthesis of LDs is initiated in the endoplasmic reticulum (ER) from where it buds into the cytoplasm[3]. This is driven by seipin which is an oligomeric integral membrane protein with two transmembrane domains connected by a large ER luminal loop[4]. Seipin plays an essential role especially in LD formation and maturation, although the underlying mechanism is largely unknown[5]. However, the structure of the oligomeric form of seipin was recently elucidated[4,6].

Based on structural homology of the β-sandwich fold in the ER luminal domain with other lipid-binding proteins, it has been suggested that seipin also binds lipid molecules. Indeed, it has been shown that seipin interacts with phosphatidic acid and phosphatidylinositol-3-phosphate lipids albeit with different affinities[4]. We have investigated the interaction of seipin with lipid molecules of the ER membrane using multi-scale molecular dynamics simulations. The findings from this study reveal a detailed view into seipin selectivity towards specific lipid species, which is essential for LD formation and maturation.

References

1. Welte, M.A, *Curr. Biol.* **25**, R470-481 (2015).
2. Krahmer, N., Farese, Jr, R.V., Walther, T.C., *EMBO Mol. Med.* **5**, 905-915 (2013).
3. Walther, T., Chung, J., Farese, Jr, R.V., *Annu. Rev. Cell Dev. Biol.* **33**, 491-510 (2017).
4. Yan, R. *et al*., *Dev. Cell* **47**, 248-256 (2018).
5. Salo, V.T. *et al*., *EMBO J* **24**, 2699-2716 (2016).
6. Sui, X. *et al*.; *J. Cell Biol.* **217**; 4080-4091 (2018).

P-604

The Influence of Cholesterol in a myelin-like Monolayer on Myelin Basic Protein

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Myelin basic protein (MBP), an intrinsically disordered protein (IDP), may play a decisive role in the research on the elucidation of multiple sclerosis, in particular on the progression of this highly debilitating, demyelinating autoimmune disease. However, the exact order of events on the conformation, orientation and self-assembly of MBP on a single membrane surface is not known. Former studies have shown that the composition of the monolayer and the amount of protein within are important for the outcome and may be a good indication of the role in lipid bilayers such as myelin.[1,2] Therefore the interactions between a lipid monolayer (with different amount of cholesterol) and MBP are investigated in this study.

The main emphasis was laid on the changing behavior of a monolayer during compression on a Langmuir trough observed by fluorescence microscopy. The method of measuring the surface pressure with a Wilhelmy balance in a Langmuir trough provides information about the interaction between protein and the lipid monolayer at the air/water interface.[3] The applied lipid mixture was a combination of different natural lipids similar to the cytoplasmic leaflet of myelin with varying cholesterol content. Interestingly, the native amount of 44 % cholesterol in the membrane seems to be important for the interaction between lipid membrane and protein and shows unique features, which are presented.

[1] C.M. Rosetti, B. Maggio, Biophys. Journal 2007, 93, 4254-4267.
[2] C.M. Rosetti, B. Maggio, N. Wilke, Biochim. Biophys. Acta 2010, 1798, 498-505.
[3] H. Brockmann, Curr. Opin. Struct. Biol. 1999, 9, 438-443.

P-605

Liquid-liquid and liquid-solid phase separation competition in plant protein-polysaccharide systems

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Liquid-liquid phase separation is a universal phenomenon occurring spontaneously in nature. For example, mussels (*Mytilus edulis*) and sandcastle worms (*Phragmatopoma California*), two sessile marine organisms, are able to secrete glue displaying strong underwater adhesives properties. The key step in this adhesion mechanism is a liquid-liquid phase separation between several oppositely charged proteins. Moreover, the hypothesis that liquid-liquid phase separation underlies cellular compartmentalization re-emerged lately. Within cytoplasm and nucleus, transient non-membrane-bound compartments have been highlighted over the past few years. These non-membrane bound compartments, displaying a liquid-like behaviour can be seen as micro-reactors, concentrating reactive components and allowing a reaction to occur. High mobility, diffusion and exchange were also reported within these liquid compartments, highlighting similarities with a protein-protein bulk mixture subjected to liquid-liquid phase separation, where continuous diffusion and exchange between the two phases was evidenced. Liquid-liquid phase separation is also important from a technological point of view: it is commonly used as a process for protein separation or for microencapsulation of oil or active ingredients with potential applications in cosmetics, pharmaceutics or food. In the present study, we investigated the assembly of a plant protein, rapeseed napin (NAP), mixed with a plant polysaccharide, highly methylated pectin (PEC). Screening of interactions was probed using microplates, droplets-based millifluidic, optical microscopy and phase composition at thermodynamic equilibrium. The optimum pH for NAP/PEC interactions was found at pH 4.0 for which the charge difference between the two biopolymers is the highest. Two types of phase transition were observed depending on pH and mixing ratios: liquid-solid and liquid-liquid phase separation. We showed that liquid-solid transition was favored by strong electrostatic attraction whereas liquid-liquid phase separation was promoted by weaker attraction. In addition, we highlighted a solid-to-liquid phase transition overtime for ratios with excess of proteins. We showed that polysaccharide charge neutralisation was a requisite for the transition as no rearrangement was observed when residual charges remained. The underlying mechanism leading to this transition remains to be explored. To the best of our knowledge, such solid-to-liquid transition has never been reported for protein-polysaccharide mixtures.

P-606

Self-Emerging Cooperative Localization of DNA and Actin in Cell-Sized Aqueous/Aqueous Droplet

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Living cells maintain their lives through the generation of micro-compartments entrapping significant amounts of various bio-macromolecules. Here, we report that cell-sized water droplets entrapping bio-macromolecules such as DNA and actin are generated in a self-organized manner through simple procedure of sample mixing with solvable polymers such as polyethylene glycol (PEG) and dextran (DEX). [1, 2] It will be shown that biomolecules such as long DNAs and actin proteins are spontaneously entrapped inside the micro-compartments. For the preparation of cell-sized water/water droplets, we adapted the solution of PEG and DEX, under the compositions near the binodal line of the phase separation. It was found that the bio-macromolecules, actin and DNA, exhibit specific localization in Cell-sized Aqueous/aqueous Micro Droplets (CAMD), when we adopt the polymer compositions near criticality of phase-separation. Under the coexistence of CAMD, long double-stranded λ-DNA (49kbp) localized in the DEX-rich microdroplet. In contrast, the short single-stranded oligomer DNA (11nt) distributed homogeneously inside and outside microdroplet. Interestingly, the distribution of actin inside the droplets changes markedly depending on the state of actin; G-actin, or dispersed F-actin, or bundled F-actin. Here, we controlled the state of actin by changing the concentrations of potassium or magnesium ions. Actin monomer, G-actin, was distributed evenly inside and outside the droplets. When G-actin monomers were polymerized, F-actin was localized inside the microdroplet and distributed homogeneously. Furthermore, the bundled state F-actin located at the interface of microdroplet. We also show the specific spatial corporation of long DNA and F-actin in the microdroplet. We confirmed that λ-DNA distributes homogeneously inside the microdroplet. Similar experimental trend was encountered also with F-actin. Whereas, under the coexistence of DNA and F-actin, F-actin fibers tend to align in a parallel manner and then deplete λ-DNA molecules for the both poles in a micro droplet.

(References)

[1] K. Tsumoto, *et al.*, *Life* **5**, 459 (2015).
[2] N. Nakatani, H. Sakuta, *et al.*, *ChemBioChem* **19**, 1370 (2018).

P-607

Translocation of cell penetrating peptides through droplet interface bilayers

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Cell Penetrating Peptides (CPPs) are small polycationic peptides able to cross the cell membrane even if they are attached to a cargo. They are interesting candidates as efficient vectors to deliver drugs directly into the cytoplasm. It has been shown that this crossing can occur through endocytosis pathway or direct translocation, however the mechanism of the latter remains unknown [1].

In our group, we focused on the molecular description of translocation mechanisms and the determination of the peptide's partners for this translocation. In this work, we focused on the role of lipids in the interaction and internalization of a well-known CPP: Penetratin. For this purpose, we used a model lipid bilayer formed at the interface of two adhering aqueous droplets in oil, each covered with a lipid monolayer [2]. By introducing the fluorescently labelled CPP into one of the droplet, we can monitor its translocation through the bilayer. Moreover, this system allows us to form asymmetric bilayers, as in cell membranes, and thus provides a versatile model for studying the effects of lipid composition on CPP translocation and obtaining insights on the mechanism involved.

Thus, we have showed that anionic lipids are required on both leaflets and that the nature of the head group on the proximal leaflet (or "outer leaflet") has a crucial importance to induce translocation of the studied CPP. To pursue this study, we are currently developing a microfluidic device to form and trap the two-droplets population. This system, under development, will allow us to screen a lot of parameters and to evaluate the role of lipids in CPP internalization mechanisms. We also aim to introduce electrodes into droplets in order to detect possible modifications of the bilayer in presence of the CPP and study the effect of the membrane potential.

These tools, under development, will provide more insights into the mechanisms of translocation of cell penetrating peptides.

[1] C. Bechara et al. *FEBS Letters*, **2013**, *587*, 1693-1702
[2] H. Bayley et al. *Molecular BioSystems*, **2008**, *4*, 1191-1208

P-608

Assessment of a potential novel interaction between alpha-synuclein and p62

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Alpha synuclein (AS) is the major protein component of Lewy Bodies (LBs), the characteristic intracellular neuronal inclusions associated with Parkinson's disease (PD) and other neurodegenerative disorders. Indeed, the process of AS oligomerization and aggregation has been associated with the pathogenesis of LBs diseases and is believed to play a central role in the aetiology of PD. However, the mechanisms by which AS starts to aggregate into toxic oligomers and fibers in neuronal cells remain largely unknown.

AS suffers extensive post-translational modifications, including ubiquitination, which is highly frequent in the protein found in the LBs, although it is not clear if any of these modifications are relevant for the initiation of the aggregation process.

p62 is a key protein involved in the degradation of ubiquitinated proteins either by their presentation to the proteasome or their sequestration into large condensates generated by phase separation and directed to autophagy. Interestingly, p62 is highly abundant in LBs and mutations in the p62 gene have been associated with a number of neurodegenerative diseases due to the accumulation of misfolded proteins.

In this work we present a preliminary assessment of a potential novel interaction between AS and p62 by single-molecule experiments which could play a role in AS aggregation and LBs formation in vivo.

P-609

Understanding Membrane Protein Interactions Using Phase Diagrams

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A major bottleneck in determining the structure of membrane proteins is the formation of crystals that diffract at high resolution. Screening many different conditions including detergent and/or lipid concentration/type, pH, ionic strength and PEG concentration is generally the standard approach to crystallizing membrane proteins. While often successful, it is time consuming and expensive and even when crystallization is observed, it is generally not clear why one condition resulted in crystallization and many others did not. Protein phase diagrams can be used to understand what conditions lead to membrane protein crystallization (and why), and therefore increase the probability of future success. Here, we present work for outer membrane proteins of Gram-negative bacteria (Porins) to assess how crystallization conditions, determined from phase diagrams relate to membrane protein interactions and use this as an approach for rationalising membrane protein crystallization.

P-610

The interaction of α-Tocopherol with model membranes

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We report on studies of the interactions of α-Tocopherol, a form of Vitamin E, with synthetic phosphatidylcholines. α-Tocopherol is recognised as a major antioxidant in biological systems and is known to slow down the rate of lipid oxidation of food products such as omega-3. Although its activity is well known, the effect the molecule has on biological assemblies still remains unclear. Here we have focussed on characterising the structural effect of Vitamin E on model membrane systems (phosphatidylcholines) using calorimetry, X-ray diffraction and solid-state NMR.

We have found that α-Tocopherol tends to induce interfacial curvature in systems comprised of unsaturated phospholipids. Small angle X-ray scattering (SAXS) patterns of DOPC and α-Tocopherol mixtures indicate the presence of inverse hexagonal and Im3m cubic phases. ^{31}P-NMR has shown that α-Tocopherol perturbs the gel phase of DPPC, inducing disordering of the phospholipid headgroups. Wide angle X-ray scattering (WAXS) profiles acquired from various amounts of α-Tocopherol mixed with DPPC suggests the formation of a liquid-ordered (L_o) phase. This phase is unique as it possesses attributes of both gel (ordered chain conformations) and fluid (disordered lateral packing with rapid lateral diffusion) lamellar phases. Until now the L_o phase has only ever been observed in mixtures of phospholipids with sterols such as cholesterol. Further evidence of the formation of this unique phase comes from our observation of phase-separated domains in giant unilamellar vesicles comprised of hydrated mixtures of α-Tocopherol, DPPC and DOPC.

P-611

Characterization of liquid-liquid phase separation of FUS with EPR spectroscopy

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Electron paramagnetic resonance (EPR) in combination with site-directed spin labelling has gained recognition as a tool that provides information on side chain dynamics and distance distributions between two paramagnetic centers.[1] Here, we present an approach to characterize the low-complexity domain of the RNA/DNA-binding protein Fused in Sarcoma (FUS) in its dispersed state and during liquid-liquid phase separation with EPR spectroscopy.

FUS localizes to stress granules, which are membraneless organelles formed upon cellular stress.[2] In vitro, monomeric FUS can liquid-liquid phase separate to liquid droplets, which can mature to hydrogels and fibrils.[3] Mutations associated with the neurodegenerative diseases ALS and FTLD lead to irreversible stress granule formation and exacerbate the liquid-to-solid transition in vitro.

Our methodology for studying these dynamic protein assemblies in vitro with EPR spectroscopy includes continuous wave (CW) measurements at ambient temperature, and pulse dipolar spectroscopy measurements at cryogenic temperatures. The mobility of the spin label, which can be estimated from the lineshape of the CW spectrum, changes upon phase separation. The double electron-electron resonance (DEER) measurement provides information on the mean distance between two paramagnetic centers in the nanometer range as well as on the width of the conformational ensemble.

We will discuss the design of spin labeling sites for FUS, as well as the first ambient- and low-temperature EPR results, demonstrating the applicability of EPR spectroscopy to study such disordered systems at the molecular level.

[1] G. Jeschke, Annu. Rev. Phys. Chem., 2012, 63, 419-446.
[2] C. Lagier-Tourenne, M. Polymenidou, D. Cleveland, Hum. Mol. Genet., 2010, 19, R46-R64.
[3] T. Murakami et al., Neuron., 2015, 88, 678-690.

P-612

Optogenetic platform for photoactivated control of proteins clusterization

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According to modern concepts, the organization of many intracellular processes is associated with the membraneless organelles, which are highly dynamic structures that assemble/disassemble as a result of a reversible highly controlled in biological systems liquid-liquid phase separation. Thus, addressing the membraneless organelles is a way to achieve a finely regulated spatiotemporal control of intracellular processes. It is known that light exposure of plant cells initiates the formation of photobodies containing phytochromes photoreceptors absorbing in the red/far-red spectral regions. The formation of phy-bodies in plant cells is triggered by the interconversion of phytochromes between the ground Pr form (absorption maximum at 660 nm) and the biological active Pfr form (absorption maximum at 730 nm) due to photoisomerization of the phycocianobilin cofactor under red light illumination. We developed optogenetic platforms based on modified plant phytochromes for light-dependent control of intracellular processes in animal cells. Plant phytochromes have been chosen for designing the optogenetic tools as they absorb light in the so-called near-infrared "transparency window" of biological tissues (650 – 900 nm), beneficial for non-invasive monitoring and control of various cellular processes in tissues and organs. Using of sequences, that are inherent for the core proteins of known membraneless organelles combined with phyB allowed us induce light-activated phase transition of developed constructs. It is also planned to demonstrate the possibility of controlling the intracellular signaling pathways using the designed optogenetic tools. The work was supported by grant form Russian Science Foundation 18-75-10115

P-613

Liquid-glass transtion of water confined under simplified intracellular conditions

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Much attention has been paid to the fact that cytoplasm shows glass-like properties [Fabry et al., Phys. Rev. Lett. (2001) ; Zhoua et al., Proc. Natl. Acad. Sci. USA (2009) ; Parry et al., Cell (2014) ; Nishizawa et al. Sci. Rep. (2017)]. This is believed to be attributed to molecular crowding in cells. This raises a question of how intracellular water contributes to the glassy behavior, because water is a major constituent and essential for biological reactions. Intracellular water is thought to be confined owing to molecular crowding, and so its state should be different from that in dilute aqueous solutions. Moreover, the state of water surrounding biopolymers is crucial for their biological functions. However, the glassy behaviors of cytoplasm have hardly been studied at the molecular level, owing to the complexity of intracellular contents. A liquid–glass transition is a change from a liquid to a solid state, while maintaining the structure of the liquid at the transition point, that is, random structures. We use reverse micelles to realize simplified intracellular conditions. A reverse micelle is a nanoscopic water droplet covered by a membrane formed by the self-assembly of surfactant molecules whose hydrophilic group faces the water, and its size can be controlled experimentally [Murakami et al., J. Phys. Chem. B (2011); Murakami et al., Chem. Phys. Lett. (2012)]. In addition, a water-soluble molecule, such as dye and protein molecules, can be dissolved in the water droplet. By optical spectroscopy of a probe molecule within a reverse micelle with a water-pool size of ~2 nm, we have recently shown that water surrounding the probe molecule is in a glassy state at room temperature [Murakami et al., Phys. Rev. E (2013); Murakami et al., J. Chem. Phys. (2018)]. In the present study, we have conducted size-dependent measurements of the reverse micelle. As a result, it has been found that water changes from a liquid to glass state at a water-pool size of ~4 nm, and that the glassy state is ascribed to the confinement effect. This result suggests that water can vitrify locally on the nanoscale in cells.

P-614

Role of protein conformation and weak interactions on γ-gliadin liquid-liquid phase separation.

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Wheat storage proteins, gliadins, were found to form in vitro spherical droplets (condensates or coacervates) in 55% ethanol/water mixture by decreasing temperature. The possible role of this liquid-liquid phase separation (LLPS) process on the in vivo gliadins storage is elusive and remains to be explored. γ-gliadin as a model of wheat proteins to probe gliadins behavior in aqueous media is used in the present study. Bio-informatics analyzes suggest that γ-gliadin could be a hybrid protein with N-terminal domain predicted to be disordered and C-terminal domain predicted to be ordered. We developed an in vitro approach consisting to first solubilize γ-gliadin in 55% ethanol (v/v) and to progressively decrease ethanol ratio in favor of increased aqueous solution. Our results show the ability of γ-gliadin to self-assemble into dynamic condensates through LLPS, with saturation concentrations ranging from 66 µM (35% ethanol) to 3.1 µM (2.5% ethanol). We demonstrate the importance of the predicted ordered C-terminal domain of γ-gliadin in the LLPS by showing the protein condensates transition from a liquid to a solid state under reducing conditions. We also demonstrate the contribution of hydrogen bonds and electrostatic interactions in the LLPS process. Finally, we discuss the importance of gliadins condensates in their accumulation and storage in the wheat seed.

P-615

Microfluidic reconstruction of compartmentalized cytoplasm mimics within a lipid membrane

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Microdroplets and giant unilamellar vesicles (GUVs) generated by microfluidic technology are useful platforms for the characterization of biological systems, allowing the controlled reconstruction of different combinations of elements aimed at identifying their structural and functional organization. Here we show the implementation of this technology to model the intracellular compartmentation by direct encapsulation of a binary phases system into microdroplets delimited by a lipid membrane matching that in *Escherichia coli*, and describe the behavior of diverse elements of the bacterial division machinery. The advantage of these *in vitro* systems is that they enable the encapsulation of molecules in hundreds of microdroplets, identical in size and composition, stabilized by a boundary mimicking the lipid composition of the cellular membrane. Their interior was designed to reproduce the cytoplasm crowded nature, physiological osmolarity and compartmentation, the latter recently emerging as nonspecific modulator of the reactivity of biological macromolecules. We also show the conversion of microdroplets into permeable vesicles, which allows triggering reactions in their interior by external addition of ligands. The structures of proteins or nucleic acids reconstructed in these systems (fibers, condensates, etc.) were analyzed using confocal fluorescence microscopy.

P-616

Exploring and reconstructing protein phase separation in bacterial systems

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Biomolecular condensation linked to phase separation is emerging as a new mechanism involved in the regulation of many biological processes with important consequences in physiology and pathology. While this phenomenon has been widely demonstrated in the last years in eukaryotes, it is still poorly understood in prokaryotes. Using biochemical reconstruction approaches and fluorescence methods we have found that the nucleoprotein complexes of two key bacterial division proteins in *E. coli*, the tubulin homolog FtsZ and the nucleoid occlusion factor SlmA, form crowding-driven condensates. The factors determining the formation of these condensates and their interconversion with nucleotide elicited fibers were studied. The implications of these dynamic structures in the modulation of bacterial division ring assembly and positioning by the nucleoid occlusion factor were analyzed. Our results suggest that phase separation leading to biomolecular condensation may play a role in the overall regulation of bacterial processes such as cytokinesis.

P-617

Interacting active droplets

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Liquid-liquid phase separation plays an important role in organizing material inside biological cells. Examples are membrane-less organelles, which can be described as liquid droplets. To regulate intracellular processes, precise control over the droplets' properties is necessary. Droplets can be affected by diffusive fluxes created from driven chemical reactions. By varying the reaction rates, the size and growth of such active droplets can be controlled.

We want to understand how active droplets behave in heterogeneous environments, e.g. inside biological cells. We study this by numerically solving a modified Cahn-Hilliard equation and comparing the results with analytical predictions. We start by considering a single active droplet in an external chemical gradient. The results can be used to examine how two or more droplets interact. This will help explain how cells can use chemical reactions to control their membrane-less organelles.

P-618

The physical chemistry of lipid droplet biogenesis

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Lipid droplets (LDs) are intracellular organelles that serve as the main cellular site of metabolic energy storage. Besides their metabolic functions, LDs also play a central role in numerous pathological processes, including lipotoxicity, cancer development, endoplasmic reticulum (ER) stress and viral attack[1]. Despite the multiple functions of LDs in the cell, a basic understanding of their molecular properties is still missing, mostly because of their unique structure: a core of neutral lipids, such as triacylglycerols and steryl esters, surrounded by a single monolayer of phospholipids, that makes them, in essence, intracellular oil emulsions[2]. Furthermore, little is known about the formation process of this organelles, that occurs in the ER. Here we present our results on the molecular mechanism of LD biogenesis using both existing and newly-developed methodologies based on molecular dynamics (MD) simulations and *in vivo* experiments. Using these approaches, we could identify the relevant parameters driving the spontaneous phase separation between triglycerides and phospholipids, leading to the formation of oil lenses in the ER bilayer.

1. Walther, T. C. & Farese, R. V. Lipid Droplets and Cellular Lipid Metabolism. (2012). doi:10.1146/annurev-biochem-061009-102430
2. M'barek, K. Ben, Ajjaji, D., Thiam, A. R. & Al., E. ER membrane phospholipids and surface tension control cellular lipid droplet formation. *Dev. Cell* **41,** 591–604.e7 (2017).

P-619

Reorganization of liquid ordered lipid domains dampens changes in membrane tension

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The cell's lipid membrane is known to play a crucial role in determining cell fate and behaviour. As a biophysical transducer, the mechanical properties of this structure are known to be altered in a number of diseases including cancer, malaria and atherosclerosis (AS). As the leading cause of death worldwide, cardiovascular diseases are a major research target and there is an increasing interest in understanding the mechanism of the atherosclerosis process.

From a mechanical point of view, variations in the arterial geometry during AS lead to alterations in blood flow, changing the blood-caused shear stress experienced by the endothelial cell's membrane. Consequently, membrane tension and viscosity will be changed too. However, this response is not yet fully understood, with discrepancies existing between current studies.

In order to address this issue, we test viscosity sensitive fluorescent probes (termed molecular rotors or MRs) to map changes in lipid packing under external stress. In this work we investigate two thiophene-based dyes[1] which, uniquely, equally partition between liquid disordered (L_d) and liquid ordered (L_o) membrane domains[2] that have different levels of lateral order. Fluorescence Lifetime Imaging (FLIM) results suggest both molecule types allow us to distinguish lipid organization, although only the triple-bonded (olefinic) molecule appears to be viscosity sensitive while the other responds to polarity.

Using the viscosity sensitive MR, we have explored how membrane viscosity and domain organization are altered when the membrane is subjected to tensile and compressive forces, with particular interest on the effect of membrane cholesterol content (known to increase during AS). Results using lipid bilayers of different composition suggest viscosity changes are minimized for those membrane compositions that exhibit L_d - L_o phase coexistence. It appears membrane tension buffering is mediated by L_o domain coalescence and, ultimately, lipid diffusion into the L_d matrix resulting in a single phase at sufficiently high tension.

1. López-Duarte, I. *et al.* Thiophene-based dyes for probing membranes. *Org. Biomol. Chem.* **13**, 3792–3802 (2015).
2. Dent, M. R. *et al.* Imaging plasma membrane phase behaviour in live cells using a thiophene-based molecular rotor. *Chem. Commun.* **52**, 13269–13272 (2016).

P-620

Registration of nanometer-sized lipid domains

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Micrometer-sized liquid ordered and disordered domains from the two membrane leaflets adopt matching positions in a lipid bilayer. This kind of registration is thought to occur also in the plasma membrane, albeit these domains are orders of magnitude smaller. The reasoning is that lipid domains would otherwise be unable to act as signaling platforms. However, experimental estimates of the driving forces for the registration of micrometer-sized domains would not suggest that 10 or 20 nm large bilayer spanning domains may exist. To clarify the conundrum, we performed fluorescence correlation spectroscopy to measure lipid mobility within domains. We combined these experiments with wide field fluorescence microscopy of planar lipid bilayers to trace single domain diffusion. Here we show that the addition of photo-switchable lipids (ceramide PhoDAG-1) to the membrane forming lipid mixture allowed solubilizing and re-assembling domains by illuminating free-standing planar lipid bilayers at 365 nm and 475 nm, respectively. Differently colored fluorescent labels adopted matching distributions in the two monolayers indicating that domains from the two leaflets were always in register. Domain mobility revealed perfect agreement with the Saffman-Delbrück equation. The registration of domains as small as 10 nm in diameter is in line with the predicted role of line tension as a driving force for alignment (1). It reduces (i) the hydrophobic mismatch at domain boundary and (ii) the strain that arises in a flat membrane at the boundary between phases that display different spontaneous curvatures. The gain in energy due to the reduction of line tension scales with the radius of the domain. In contrast, registration of micrometer sized domains is driven by membrane undulations (3). In this case the energy gain scales with domain area.

1. Galimzyanov, et al. *Phys. Rev. Lett.* **2015**, 115:088101.
2. Galimzyanov et al. *Phys. Rev. Lett.* **2016,** *116* (7), 079802.
3. Galimzyanov, et al. Biophys. J. **2017**, 112:339.

Wednesday 24th July
INSTRUCT-ERIC: INTEGRATING ACCESS TO BIOPHYSICS AND STRUCTURAL BIOLOGY IN EUROPE

P-621 (O-172)
Crystal structures of bacteriophage receptor binding proteins
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Bacteriophages have specialised receptor binding proteins (RBPs) for initial, reversible, host cell wall recognition. Once a suitable host is found, the phage commits to infection by irreversible attachment via a secondary receptor interaction. The crystal structures of several of these receptor-binding proteins have been solved and have been shown to be mainly beta-structured, but structurally highly diverse and containing several new protein folds. Here we present structures of the receptor-binding proteins of the Escherichia coli phages T4, T5 and T7, and of Staphylococcus phages S24-1 and K. Bacteriophage receptor-recognising proteins may be used for bacterial detection, while modification by natural or experimental mutation of bacteriophage receptor-binding domains may allow retargeting of phages to alternative host bacteria. Their shape and stability may also allow their use in nano-technological applications.

P-622 (O-173)
The Cryo-EM Structure of a Non-toxic Greek Key Oligomer of Alpha Synuclein
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Aggregation of the intrinsically disordered protein alpha-Synuclein (α-Syn) into insoluble fibrils with a cross-β sheet amyloid structure plays a key role in the neuronal pathology of Parkinson's disease (PD). The fibrillation pathway of α-Syn encompasses a multitude of transient oligomeric forms differing in size, secondary structure, hydrophobic exposure, and their ability to inflict toxicity. According to a recent ssNMR study, an amyloid fibril of α-Syn contains the core residues of the protein arranged into in-register parallel β sheets with a unique C-terminal Greek key topology. We report that the physiologically available small molecule heme (hemin chloride) when added at sub-stoichiometric ratios to either monomeric or aggregated α-Syn, inhibits fibril formation by stabilizing a population of 'mace'-shaped oligomers. Using cryo-EM, we observed that these mace-oligomers consist of approximately four monomers, which complements previous reports of a physiologically stable tetramer. Incidentally, these heme-stabilized oligomers contain the Greek key topology and are essentially the smallest fundamental nuclei/ units that make up the ssNMR Greek key fibril model. This 'Greek key oligomer' fits well as a segment of the previously-described annular oligomers and appears to be its structural predecessor in the hierarchical pathway of fibril formation. This heme-stabilized oligomer also shares resemblances with the recently-determined cryo-EM structure of 'protofilament kernels.' However, these oligomers differ from the conventional Greek key model due to a 'distortion' in the Greek key architecture at the C termini of the protein molecules. We propose that this distortion prevents further appending of the twisted units into annular oligomers as well as protofilaments. Furthermore, heme inhibits fibrillation by binding to a crucial histidine (His50) residue located in the inter-protofilament preNAC interface, thus interfering with a salt bridge formation with a Glu57 residue located in the opposite protofilament, thereby weakening the inter-protofilament steric zipper integrity. When compared with the untreated fibril-forming on-pathway oligomers, the heme-treated 'distorted Greek key oligomers' showed lesser liposome permeation and toxicity to neuroblastoma cells.

Wednesday 24th July
MOLECULAR MOTORS

P-623 (O-177)
Kinesin-2 stepping reflects its heteromeric nature
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The functional significance of heteromerization in kinesin-2 motors remains largely unknown. Having coevolved with cilia, this motor is a prime example for environmental adaptation. Performing dual-color superresolution microscopy (dcFIONA), we followed the two different heads of the KLP11/20 motor from *C. elegans* walking on microtubules. Observing the stepping of both heads for the first time in a kinesin, we show that the heads have distinct stepping behaviors. Looking for the cause of this irregularity, our data unexpectedly points towards the C-terminal tails of the motor. We observe a potential allosteric interaction, that holds the promise to deliver key insights into the specialization of kinesin-2 for IFT.

P-624 (O-178)
Reverse stroke of cardiac myosin revealed by single molecule microscopy is essential for heart function
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In order to elucidate the molecular mechanism of how dynamics of cardiac myosins contribute to heart function, we measured forces of synthetic β-cardiac myosin filaments using optical tweezers and revealed stepwise displacements of actin filaments driven by myosins under a wide range of loads. The stepping ratio, which is the ratio of the numbers of forward steps relative to backward steps, under unloaded conditions decreased with increasing ATP concentrations. Compared with skeletal myosin, the stepping ratio of cardiac myosin is much lower than that of skeletal myosin, indicating cardiac myosin shows frequent backward steps. Meanwhile, the peak forces generated by cardiac myofilaments with ~15 interacting molecules were 1.5-2 times higher than those observed in skeletal myofilaments with nearly the same number of interacting molecules. Based on these findings, we developed a simulation model to understand which molecular properties critically affect on stepping behaviors and force outputs in cardiac myofilaments. The simulation suggested that reverse stroke in ADP states is a key feature to cause frequent backward steps at higher ATP concentrations, resulting lower stepping ratio. Moreover, switching between two ADP states associated with the alternate execution of power and reverse strokes keeps many myosins populated in force-generating states, enhancing the duty ratio and force outputs. Therefore, we further investigated whether single cardiac myosin can execute the power and reverse strokes in ADP state under a variety of loading conditions. When single cardiac myosins interacting with single actin filaments were stretched by optical tweezers, beads' positions were occasionally switched between two discrete levels for high loads, implying the load-dependent execution of power and reverse strokes. To know physiological meaning of reverse stroke, we simulated dynamics of myosins in sarcomere and found that the reverse stroke plays a crucial role in reducing the rate of ATP consumption during isometric contraction. Also, we implemented such molecular properties into a whole heart simulator and found that the reverse stroke is a unique future of cardiac myosin and essential for maintaining high systolic blood pressure and a rapid relaxation of diastolic blood pressure.

P-625 (O-179)

Artificial Assembly of the Bacterial Flagella Motor on DNA Scaffolds

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The bacterial flagella motor (BFM) is an 11 MDa protein complex, composed of ~13 different component proteins, which powers the rotation of the bacterial flagella filament. Generation of torque and rotational switching of the BFM are mediated in the rotor by the proteins FliG, FliM and FliN. These proteins assemble into ring-shaped oligomers to form a multi-layered cytosolic ring called C-ring. The assembly of these proteins is controlled by inter- and intra-molecular interactions. In solution, FliG as well as the complex of FliM/N exist predominantly as monomers. However, after binding to a structural template these proteins can self-assemble into the ring-shaped polymers observed in the BFM. This study aims to control C-ring assembly using DNA nanostructures as an artificial template to functionally and structurally characterize interactions in the C-ring *ex vivo.*

Rationally-designed DNA templates were used to immobilise an arbitrary number of protein molecules in a spatial configuration similar to that in a functional motor *in vivo*. This was achieved via the hybridisation of a single stranded DNA that was covalently attached to the protein. The use of DNA templates allowed us to measure the kinetics of intra- and intersubunit interactions in a C-ring assembly for the first time with surface plasmon resonance (SPR).

Furthermore we characterized rationally designed point mutations and truncations of FliG using small-angle X-ray scattering (SAXS) and showed that these new protein constructs exhibit different conformations in solution compared to the wild-type protein. Additionally, SPR measurments with these constructs on DNA templates revealed the influence of these structural changes on the kinetics of the protein assembly, which allowed us to develop a model for self-assembly of the C-ring.

This study demonstrates the use of synthetic DNA templates to probe molecular mechanisms underlying the self-assembly of the BFM and other complex protein machinery from their component parts.

P-626

Motor recruitment and activation for intraflagellar transport

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Cilia are one of the most functionally diverse organelles in the human body, and defects in their functions are implicated in many diseases collectively known as ciliopathies. This functional diversification is attributed to the highly conserved intraflagellar transport (IFT) building machinery. In *Caenorhabditis elegans* sensory cilia, the continuous movement of IFT trains towards the ciliary tip is driven by kinesin-2 motors, and by dynein-2 motors back to the ciliary base. We used the bottom-up approach to reconstitute the first in vitro functional multi-component IFT complex. We identified the key IFT subunit that recruits the homodimeric kinesin-2 into its relevant IFT complex and activates it for functional transport. Our results pave the way towards understanding the molecular mechanism of IFT regulation that has been eluded for several decades.

P-627

Insights in the mechanism by which ATP induces movements of the nucleotide binding domains of ABCB1 and energizes transport

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P-glycoprotein (ABCB1) is expressed in barrier tissues and causes resistance against chemotherapy if expressed in cancer cells. The pseudo-symmetric ABCB1 consists of two transmembrane domains (TMD) that bind and transport the substrate, and of two nucleotide binding domains (NBDs) that energize the transport by ATP binding and hydrolysis. Our molecular dynamics simulations showed that the presence of Mg and ATP is enough to trigger dimerization, forming a stable NBD dimer. Potential of mean force profiles indicate that a deep energy minimum (~ -42 kJ/mol) is reached in the presence of MgATP as the result of orchestrated electrostatic, hydrophobic and water mediated interactions between the nucleotides and the NBDs. In the post-hydrolytic state, interactions between the NBDs, the hydrolysis products MgADP and Pi and solvent result a high energy state in the closed NBD dimer and therefore is unstable, pushing ABCB1 to reopen and to proceed through the transport cycle. Thus, ATP binding and its subsequent hydrolysis provide the energy both for dimerization and dissociation, energizing transport by ABCB1 by converting the chemical energy stored in ATP into mechanical forces and transporter motions.

This work was supported by the Austrian Science Fund (FWF), Special Research Program SFB 3524 and the stand alone grant P32017.

P-628

A landscape-based view on the stepping movement of myosin VI

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Myosin VI walks towards the minus end of the actin filament with a large and variable step size of about 25-36 nm. Because it has only two calmodulin-binding motifs within each neck domain, the stepping movement with a large step size cannot be fully accounted for with the lever-arm-like conformational changes within the two heads. So far, two competing models have been put forward to explain this large step size. Spudich's model (Spink et al., Nat. Struct. Mol. Biol. 15, 591 (2008)) assumes that two myosin VI monomers associate at distal tail near the cargo-binding domain, forming a myosin dimer, which makes two full-length Single Alpha Helix (SAH) domains serve as long legs in the dimer. In contrast, the Houdusse-Sweeney model (Mukherjea et al., Mol. Cell 35, 305 (2009)) assumes that the association occurs in the middle (between residues 913 and 940) of the SAH domain and that the three-helix bundle unfolds to ensure the large step size. Each of these models has experimental grounds, but their consistency with the large and variable step size has not been examined quantitatively. Stepping movement is the result of Brownian motion of the leading head of myosin VI which is connected with the rear head bound to the actin filament. Using a same computational method as we have used for myosin II (Nie et al., PLoS Comput. Biol. 10, e1003552 (2014)), we have theoretically characterized the free energy landscape experienced by the leading head to compare the two proposed models of myosin VI. Our results showed that the leading head is pulled toward the minus end of the actin filament according to the energy bias in the actin-myosin interactions, which gives rise to the variable step size of movement in both two models. However, the large stepping size is realized only in the Spudich model, because in the Houdusse-Sweeney model, unfolding of the three-helix bundle gives rise to the entropic force as in rubber elasticity to shorten the distance between two heads. The stiffness of the SAH domain is a key factor for giving strong free energy bias toward longer stepping distance.

P-629

Synthetic cells, a reductionist's approach: Reconstitution of ATP synthases in giant unilamellar vesicles

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Understanding the evolution of multicellularity is one of the most important and challenging targets in modern research. Within this project we try to investigate communication and translocation in multicellular organisms built from unicellular compartments by means of synthetic biology. To study such complex processes in living systems, the formation of an interconnected system of minimal cell compartments (MCCs) in a bottom-up fashion is a promising strategy. This should be realized by implementing essential biological components in giant unilamellar vesicles (GUVs) via droplet-based microfluidics. Hereby we seek to build up artificial cells and reach a collective behavior to produce a synthetic tissue. The creation of these so called living foams has some challenges like establishing a reliable way to produce GUVs with a defined size and the physiological reconstitution of different proteins allowing for adhesion, connectivity and communication as well as stabilization by cytoskeletal filaments and energy production. To tackle these challenges modular engineering approaches, relying on a microfluidic system to generate monodisperse GUVs in copolymer stabilized water-in-oil droplets, are appropriate. These copolymer-stabilized GUVs can be equipped by microfluidic setups with different proteins in a gentle manner and released afterwards in a physiological environment [1]. Here we present a way to reconstitute a F_OF_1 ATP synthase from *thermophilic Bacillus* in GUVs by using the above mentioned microfluidic methods. The insertion of ATP synthases within the GUVs is one of the most important requirements towards obtaining artificial tissues since they can act as a renewable energy source ensuring the production of chemical energy in the form of ATP by a conversion of electrochemical gradients. To monitor and study the activity of the ATP synthases in vesicles, pH sensitive assays with fluorescent dyes or the highly sensitive Luciferin-Luciferase assay are used. The successful integration of the ATP synthase in GUVs will be the first step towards a living foam.

P-630

Mimicking Cell-to-Cell Communication: Reconstitution of Connexin-43 in Minimal Cell Compartments (MCCs)

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Gap-junctions support multicellular life providing a unique direct cell-to-cell communication system that allows the transport of molecules to neighboring cells. Assemblies of hexameric connexins, also referred as connexons or hemichannels, dock with connexons of adjacent cells to form a direct pathway to connect the cytosol of both cells. Connexin-43 (Cx43) is the most ubiquitous isoform and by far the best characterized connexin. In multicellular systems, cell-cell interactions are highly complex with numerous proteins playing vital roles in cell signaling or cell contacts. Therefore, we are employing a bottom up approach to mimic cell-to-cell communication in synthetic cell models. The aim of this study is to reconstitute Cx43 into Giant Unilamellar Vesicles (GUVs) as Minimal Cell Compartments (MCCs) to build artificial cells capable of forming multicellular aggregates and mimic intercellular communication by the formation of gap-junctions. For this purpose, Cx43 has been expressed using *E. coli* and cell-free expressions systems, and reconstituted or directly inserted into liposomes. The functional characterization of connexin-43 will be performed with Bilayer Lipid Membranes (BLM) by the fusion of proteoliposomes with pure lipid bilayers to gather insight on the connexon's properties at the single-channel level. Finally, the formation of gap junction channels will be investigated by coupling Cx43-doped GUVs to Cx43-doped pore spanning membranes in a dye transfer assay for functional characterization of cell-cell communication in artificial cell models.

P-631

Global domain flexibility in Human Argonaute studied by advanced sampling simulations

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Argonaute (Ago) proteins are the key players of the RNA-induced Silencing Complex (RISC). During RISC formation, Ago binds and unwinds small RNA duplexes. It discards one of the strands and binds tightly to the other one, called guide strand. Agos are guided by the 5'-phosphorylated guide RNA strands to their complementary RNA targets. There have been reports suggesting that the N domain is the initiator of the unwinding process by wedging through the duplex. Nevertheless, there is little known on the dynamical aspects of this process. In the present study, we investigate the flexibility of Human Ago domains using an all-atom Hamiltonian replica exchange simulation (H-REMD) scheme. The H-REMD methodology employs specific biasing potentials to accelerate the sampling of relative domain motions along replicas. It allows us to extract a model of the N-domain induced unwinding process. In addition, we scrutinize the relation between N domain flexibility and Ago mutations that are unwinding deficient and we will discuss the molecular pathways that lead to unwinding of the RNA duplex.

Wednesday 24th July

TISSUE BIOPHYSICS AND MORPHOGENESIS

P-632 (O-183)

Biomimetic emulsions probe the mechanics of tissues

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We study tissue-mimetic systems to understand the physical basis of collective remodeling in biological tissues. In particular, we seek to understand how the interplay between adhesion and forces controls the emergence of tissue architecture during morphogenesis. Indeed, during morphogenesis the homogeneous cell aggregate is subjected to large movements that give rise to the highly organized 3D structures found in the embryo. Using a bottom-up approach would thus allow to identify the minimal ingredients necessary to reproduce such collective processes by isolating the passive mechanical pathways of self-assembly in adhesive synthetic tissues.

In particular, we use biomimetic emulsions that were shown to reproduce the minimal mechanical and adhesive properties of cells in biological tissues. These emulsions are stabilized with a monolayer of phospholipids that reproduce the fluidity of the cell membrane, and can be functionalized with adhesive proteins to mimic cell-cell adhesion in tissues. We then study the mechanical behavior of these emulsions under mechanical perturbations. In particular, we impose a global compression of the emulsions by flowing them in microfluidic constrictions with controlled geometries. Image analysis allows us to distinguish between two types of behavior in the emulsion: (1) the droplets can keep their respective positions in the packing and only be elastically deformed by the perturbation; (2) the droplets can adapt to the perturbation by rearranging positions with their neighbors, thus exhibiting an irreversible plastic response.

In order to correlate these observations with *in vivo* measurements we also use emulsions as biocompatible force probes inside developing embryos. Since the droplets exhibit mechanical properties that are close to those of tissues they are visibly deformed by cellular forces. This technique is useful to map out forces in the tissue and shed light on mechanical processes at stake during development.

Altogether, those findings will reveal the underlying regulations of adhesion and mechanical properties that take place in cells during the remodeling of tissues, and will more generally shed light on the physical processes at stake during embryonic development.

P-633 (O-184)

Mechano-transduction and coordination of epithelial cells during Drosophila morphogenesis

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Epithelial cells are capable of sensing and reacting to the forces and movements of their neighbors. These forces are multicellular forces at the tissue level and are transmitted by multi-protein complexes at the cell-cell adhesion sites. We hypothesize that mechano-sensitive ion channels could behave as molecular switches and respond to change in force at the cell-cell junction. The resulting biochemical signaling could be crucial for the maintenance of morphology and coordination in epithelial cells.

We investigate the coordination of cells by fully quantifying the dynamics of the Amnioserosa tissue in Drosophila using a novel high-throughput image analysis pipeline based on deep neural networks. This method allows near-complete segmentation, yielding feasible analysis of a large ensemble of embryos.

Inspired by graph theory, we decompose cell-cell interactions into three distinct coupling types. With this approach, the epithelium can be represented by a planar graph of cell couplings whereby cells are interpreted as vertices and junctions between cells as edges. We compare wild type embryos and mutants with impaired ion-channel functionality (TMC^{Gal4}) and weakened adhesion complexes (xit), revealing significant differences in e.g. composition of coupling types and spatial distributions. In contrast to the wild type, we find tension in those mutants to be anisotropically distributed, indicating that local cell-cell coordination through mechano-sensing is essential for the function of an epithelium as force-generating tissue.

P-634 (O-185)

A hydraulic instability underlies oocyte size selection in *C.elegans*

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The process of making an oocyte starting from a germline tissue is a fundamental cellular process that also demonstrates remarkable mechanical as well as hydrodynamic phenomena across organisms. In various cases it involves heterogenous growth of germcells within a collective structure leading to elimination of a significant proportion of germcells, this eludes to fundamental questions of dynamic size regulation and mechanical symmetry breaking. The roundworm *C. elegans* has a tubular syncytial (tissue architecture with connected cytoplasm) germline, which achieves germ-cell growth by hydrodynamic flows that range across 400 microns. We unravel the physical basis of oogenesis and cell elimination by combining cellular mechanics and hydrodynamics. We develop a novel theoretical framework that couples mechanics of a syncytial tissue with active fluid dynamics. By quantitative analysis and theoretical modeling, we discover that germcells actively generate long-range hydrodynamic flows along the germline, while also locally maintaining their homogenous size. The coupling of cell mechanics and hydrodynamic fields lead to active pressure-tuning, which yields a hydraulic instability setting a critical size for the germ-cells in the absence of active sources. This mechanism ensures selection and growth of germcells beyond a critical size at the expense of smaller cells and is independent of the apoptotic machinery.

P-635

Biomimetic emulsions to probe the role of adhesion in tissue remodeling processes

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We use biomimetic systems to understand the physical basis of tissue remodeling in a simplified framework. In particular, we study how cell-cell adhesion controls the behavior of tissues under mechanical constraint. To this end, we use biomimetic emulsions that were shown to reproduce the minimal mechanical and adhesive properties of cells in biological tissues. The adhesive forces between the droplets are introduced in two different ways: non-specific depletion attraction forces between the droplet surfaces can be introduced by tuning the concentration of SDS micelles in the continuous phase; or we can induce specific adhesion between the droplets by grafting their surface with binding molecules. We then study the effect of adhesion on the mechanical behavior of such emulsions as a response to an applied mechanical perturbation. To this end, we flow the emulsions through microfluidic constrictions with controlled geometries. Image analysis allows us to characterize the elastic response of the emulsions by measuring the level of deformation of each droplet in the channel. We find that, for similar surface tensions, higher depletion attraction forces induce larger deformations of the droplets. This shows that the elastic response of adhesive assemblies is favored when the plastic response is impaired by adhesion, even for seemingly low attraction forces like depletion. Preliminary results on specific adhesion also indicate that adhesion modifies the flow of compact biomimetic tissues through mechanical constrictions. In the future, this work should thus allow us to predict tissue behavior as a function of cell-cell adhesion.

P-636

The Influence of Mechanical Cues on Pancreatic Organoid Branching

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Branching is a phenomenon exemplified in numerous animal organs where the developing epithelium directionally extends into the neighboring mesenchyme, forming tree-like topologies. Branching has been studied mainly from a molecular perspective, identifying important genes and molecules enabling the process (using the lung, kidney, mammary gland, and pancreas as primary models). Although numerous roles of biophysical and mechanical stimuli on cellular processes have been reported, little has been characterized on the tissue/organ level. To overcome the inaccessible and invasive challenges of biophysical experiment on *in vivo* organs, we use *in vitro* pancreatic organoids embedded in 3D Matrigel which mimics *in vivo* branching and ductal-network morphogenesis.

The growing organs/tissues (e.g. stomach and spleen) surrounding the pancreas may impose a physical stress on the pancreas, therefore we initially performed a compression assay. Through osmotic pressure, by substituting dextran (2MDa) into the organogenesis medium, the Matrigel and the pancreatic organoid within were compressed. We find that, pancreatic organoids grown in compressed conditions display no branching. Moreover, we observed that these pancreatic organoids undergo cellular reorganization and their surface cells at the periphery display higher migration capabilities. Additionally, the microlumens within the pancreatic organoids, which later morph into ductal networks *in vivo*, increase in size under compressed conditions.

These preliminary observations indicate that the compression may hinder branching *in vivo*. However, whether or not this is achieved by changing intercellular processes (i.e. migration and reorganization) and microlumen morphology has yet to be confirmed. Further biophysical and biological assays will be carried out, to dissect the role of the mentioned observations in the mechanics of pancreatic branching morphogenesis.

P-637

Erythrocyte membrane fluidity in normal and obese subjects

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The physical properties of erythrocyte membranes from 146 normal and obese patients from Hospital de Cruces (Bilbao, Spain) were examined using a variety of fluorescence techniques, as well as atomic force microscopy (AFM). All subjects signed informed consent documents and the study was authorized by the Hospital de Cruces Ethics Committee. The subjects included 82 adults and 64 children. In children, the force required to penetrate the plasma membrane, measured with AFM in the force spectroscopy mode, was 6.0 ± 1.20 nN, while in adults it was 5.9 ± 1.35 nN. 2-photon confocal microscopy was used to measure the generalized polarization of the probe Laurdan, that reports on membrane molecular order. The average values for children were 0.058 ± 0.021, and in adults they were 0.056 ± 0.026. Aliquots from the same erythrocytes were studied by Laurdan fluorescence spectroscopy, in cuvettes, with similar results to those found by fluorescence microscopy. The fluidity of erythrocyte membranes was further studied measuring the polarization of the fluorescence emission of either DPH or TMA-DPH. The latter probe reports on the lipid-water interface, while DPH is distributed in the whole of the lipid matrix. Average DPH polarization was 0.298 ± 0.0033 (children) and 0.298 ± 0.0042 (adults). Average TMA-DPH polarization in children was 0.334 ± 0.0037. In all the above studies, no statistically significant differences were found between normal and obese subjects at room temperature or at 37ºC. However, when the fluorescent probe TMA-DPH was studied at 37ºC, the probe fluorescence anisotropy was 0.3104 ± 0.0048 for normal-weight subjects, and 0.3047 ± 0.0052 for obese subjects, i.e. the membrane lipids are more disordered/fluid at the lipid-water interface in obese than in normal subjects. Here the difference was extremely significant ($p<0.001$) between both groups. The physiological meaning of these observations remains to be explored. Moreover, the newly designed fluorescent probe PA (Sci. Rep. 6:18870) is a promising tool in the study of erythrocyte membrane fluidity.

P-638

The role of adhesion on the microfluidic flow of biomimetic tissues

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The aim of this study is to design a biomimetic cohesive tissue with a tunable degree of internal adhesion and determine its flow behavior in controlled microfluidic settings. The final goal of the project is to elucidate, by means of a biomimetic system, the role of cellular adhesion on the flow of epithelial tissues. The artificial tissue is obtained by the controlled assembly of giant unilamellar vesicles, which constitute a suitable model system for cells. Intercellular adhesion is mediated by the inclusion of ligand-receptor complexes, which allows us to control the occurrence (or not) of cell-cell assembly, the strength of the adhesion, as well as the typical size of the formed aggregates. Aspiration experiments in microfluidic constrictions are performed in order to characterize the flow behavior of the designed tissues. Our velocimetry results show that, depending on the aspect ratio between the aggregate and the constriction size, the tissue adopts different strategies in order to advance through the constriction.

P-639

Investigation of side effects in polyneuropathy on skeletal muscle by DSC caused by cyclophosphamide treatment

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Polyneuropathy is defined as a simultaneous malfunction of several peripheral nerves, which could be a side effect of cancer therapy as well. Well-known, that polyneuropathy is caused by chemotherapeutic drugs in patients with malignant tumor. Many kinds of drugs supposedly cyclophosphamide also can induce a disease classified as toxic polyneuropathy. Cyclophosphamide is a widely used cytotoxic drug, it can causes polyneuropathy as one of its wide side effect spectrum. Cyclophosphamide importance is shown by the appearance on the most important drugs list published by WHO. In present study we analyze a study on skeletal muscle (*m. gastrocnemius*) by DSC (differential scanning calorimetry), as an established thermoanalitical method, to follow the possible consequence of drug treatment. We used cyclophosphamide treated in vitro animal model (Guinea pig) with a comparable dosage and time handling of human protocol to show evidences of this drug-induced effects. According to our results, we could show a dose-dependent difference between thermal parameters of untreated and treated samples in their contractile proteins (actin and myosin), which can be detected by DSC. It proved that we can create new possibilities in the detection and prognosis of expected and unwanted side effects of cyclophoshamide such as polyneuropathy.

P-640

Development of artificial tissue systems from multiple vesicles using microfluidic manipulation

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The necessity to mimic cells, as the smallest unit of living organisms, steadily increases in the scientific world. While efforts exist in mimicking single-cells, few works have focused on mimicking collections of cells as tissues. Giant unilamellar vesicles (GUVs) are considered the 'gold standard' for artificial cells providing multiple cell-like characteristics and methods should be developed to enable precise handling of these delicate soft objects and to allow precise positioning of defined tissue-like assemblies. A sophisticated method to achieve long-term observation of GUVs with various experimental options is provided by microfluidic platforms (Robinson, *et al.*, 2013). Recently, our group demonstrated a high-throughput microfluidic method where over 23,000 GUVs can be examined per chip by trapping them with unique polydimethylsiloxane (PDMS) micro-post layouts (Yandrapalli & Robinson, 2019). While different GUV populations can be trapped simultaneously, they tend to randomize in the trapping area of the microfluidic device. The herein presented method adds the possibility to bring in an additional barrier to obtain more control over trapping of different GUV species and achieving defined vesicle assemblies. The novel method is based on applying pressure to separate PDMS layers with cavities that will bend and, therefore, actuate features in the underlying microfluidic channel. Creating tissue-like collections of vesicles with this bottom-up approach is a sophisticated way to gain insights into biological processes of multi-cellular systems, such as cell-cell adhesion or communication.

References

Robinson, T., Kuhn, P., Eyer, K., & Dittrich, P. S. (2013). Microfluidic trapping of giant unilamellar vesicles to study transport through a membrane pore. *Biomicrofluidics*, *7*(4), 044105. https://doi.org/10.1063/1.4816712

Yandrapalli, N., & Robinson, T. (2019). Ultra-high capacity microfluidic trapping of giant vesicles for high-throughput membrane studies. *Lab on a Chip*, *19*(4), 626–633. https://doi.org/10.1039/C8LC01275J

P-641

3D polymer scaffolds within a blood-vessel on a chip for transport studies at the nanoscale

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Tissue engineering is a rapidly growing field during the last decade. Cells within an artificial tissue need structural support and guidance for growth. For this purpose, we fabricate polymeric bio-compatible scaffolds by multi-photon lithography (MPL). In MPL, a femtosecond-pulsed laser focused into a photosensitive resin solution initializes polymerization solely within the focal volume of the laser beam. Hence, sub-micrometer resolution can be achieved in three dimensions. The challenge herein is the development of a photoresist that is biocompatible, mechanically stable and is structurable at high writing speed. We use multi-component polymers composed of methacrylates, which improve biocompatibility, lower the auto-fluorescence and speed up the polymerization process. So far, we achieved biocompatible, three-dimensional polymer scaffolds with writing speeds in the mm/second range. For biocompatibility testing, the scaffolds are seeded with endothelial cells and apoptosis-associated caspase presence is measured. This 3D structured cell scaffold within a home-built microfluidic device is seeded with human endothelial cells modelling a blood vessel wall. The apical and basolateral polarity of the cells within the chip as well as the transport of High Density and Low Density Lipoprotein particles will be analyzed at the nanoscale using localization microscopy.

P-642

Study of advanced glycation end products levels (AGEs) using photoluminescence spectroscopy from several feeding types in infants under one year in El Salvador

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Advanced glycation end products (AGEs) are a group of compounds that originate from the non-enzymatic glycation and oxidation of proteins, lipids and nucleic acids. AGEs are formed endogenously, levels increase naturally on aging, and this can also be favored from exogenous sources such as certain food ingestion among others. High AGEs levels also have been reported to be associated with different diseases. Four feeding types can be identified: breast milk, infant formula, mixed lactation (breast milk and infant formula) and ablactated feeding. Using photoluminescence spectroscopy in newborn infants in El Salvador, light emission associated with AGEs levels were measured. From the results, an apparent increase in AGEs levels production in the ablactated feeding is observed, contrary with the breast milk feeding, where the apparent increase is smaller. These results will be further discussed.

Wednesday 24th July

EMERGING BREAKTHROUGH MOLECULAR-SCALE BIOPHYSICS METHODOLOGIES

P-643 (O-189)

Correlative µ-Brillouin and µ-Raman spectroscopy: emerging tool for simultaneous mechanical and chemical analysis of cells and tissues

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In biological tissues and cells, chemical and mechanical properties are strictly correlated: their balance ensures the correct biological functionality. Here we present an innovative label-free microscopy able to jointly characterize the viscoelastic properties and the biochemical composition of biological materials with sub-micrometric resolution [1,2]. Employing Raman and Brillouin spectroscopies, this breakthrough methodology is able to analyse an exceptionally wide spectral range accessing from molecular to collective vibrational dynamics. We report relevant biophysical cases starting from the single living cells analysis to the investigation of ex-vivo tissues to demonstrate the potentiality of this emerging method. In some proof-of-principle experiments, the ability to characterize subcellular compartments and to distinguish cell status has been successfully tested [1] as well as, on ex-vivo tissues, the link between morphological structures and biomechanics has been evidenced. The results demonstrate the wide applicability of the technique.

1. S. Mattana, et. al. Light: Science & Applications **7**, 17139 (2018).
2. F. Scarponi et al. Physical Review X **7**, 17139; (2017).

P-644 (O-190)

Revisiting the structure-function relationship with mass photometry

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The cellular processes underpinning life are orchestrated by proteins and the interactions they make with themselves and other biomolecules. A range of techniques has been developed to characterise these associations, but structural and dynamic heterogeneity remain a fundamental challenge. I will show that mass photometry based on interferometric scattering microscopy can mass-image single biomolecules in solution with nanometre precision and mass accuracy comparable to native mass spectrometry in the gas phase. Thereby, we can resolve oligomeric distributions at high dynamic range, detect small-molecule binding, and mass-measure polypeptides, glyco- and lipoproteins. These capabilities enable us to quantify the molecular mechanisms of processes as diverse as homo- and hetero-oligomeric protein assembly, amyloidogenic protein aggregation and actin polymerisation [1]. Our results illustrate how single molecule mass imaging provides access to protein dynamics and interactions and introduces a third, light-based approach to measuring mass in addition to the historical mechanical and spectrometric methodologies. This ability to investigate biomolecules in their native state with high mass accuracy and resolution provides critical, complementary information to static structural techniques in the context of protein function and regulation.

1. Young G, Hundt N, Cole D, Fineberg A, Andrecka J, Tyler A, Olerinyova A, Ansari A, Marklund EG, Collier MP, Chandler SA, Tkachenko O, Allen J, Crispin M, Billington N, Takagi Y, Sellers JR, Eichmann C, Selenko P, Frey L, Riek R, Galpin MR, Struwe WB, Benesch JLP, Kukura P. Quantitative mass imaging of single biological macromolecules. *Science* **2018** 360: 423-327

P-645 (O-191)

Hierarchical micro- and nanostructured surface architectures for label-free spectroscopic and microscopic protein interrogation

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The ambition to probe proteins in their most native state creates a high demand for label-free protein interrogation techniques. These techniques are currently limited by their lack of specificity and therefore time-consuming protein purification is needed. We here aimed to develop surface architectures that enable specific interrogation of protein-protein interactions and conformational organization by label-free surface-enhanced spectroscopy and microscopy without the need for protein purification. To ensure structural integrity and full functionality of immobilized proteins, we tailored surface biofunctionalizations for site-specific protein capturing into micro- and nanostructured sensor surfaces in *vitro*, from crude cell lysates and in live cells. Two label-free sensing approaches were explored: hierarchical Silicon micropillar (SiMP) arrays with nanoscale roughness combined with orthogonal surface chemistry were used for high density *in situ* capturing of soluble and membrane proteins from cells cultured on-chip. SiMP arrays serve as IR-resonators allowing for resonant sensing of captured proteins through a strong IR field enhancement. An augmented specificity can be achieved by tuning their resonance frequencys to protein absorption bands. Protein interrogation was carried out with a FTIR spectrometer which enables optimal probing of IR-active secondary and tertiary protein structures by sensing shifts of SiMP IR resonances upon protein deposition. As a second approach gold nanoparticles (AuNP) immobilized onto a glass surface were employed for localized surface plasmon resonance (LSPR) detection. Surface functionalization of immobilized AuNP with tris-(nitrilotiacetic acid) or HaloTag-Ligand yielded site-specific reversible and irreversible capturing of His or HaloTag fusion proteins that allowed real-time monitoring of protein-protein interactions by LSPR reflectance spectroscopy. By micropatterning and integrating of AuNP into SiMP arrays we aim to achieve a strong and spatially confined electromagnetic field enhancement which allows for highly sensitive interrogation of ligand receptor interactions at the plasma membrane of living cells.

P-646

DNA-tagged lipid bilayers: novel nanoscale membrane-mimetic systems

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DNA-tagged lipid bilayers: novel nanoscale membrane-mimetic systems

Nanoscale membrane mimetics play a crucial role in biophysical studies of membrane protein function, its dependence on lipid composition and phase transitions. They are also attractive potential units for the crystallization of membrane proteins in their native lipid environment. Controlling the size and shape or introducing functional elements in a programmable way can tremendously augment the applicability of such systems which are currently based on polymers, peptides or membrane scaffolding proteins (MSPs). In this work we present biochemical approaches to produce lipid bilayers with DNA-encoded programmability to provide a link to DNA nanotechnology. This can be accomplished by thiolreactive modification of conventional nanodiscs (NDs) obtained with MSPs carrying site-specific cysteine mutations. Such systems enable the DNA-directed assembly of oligomeric structures of NDs by hybridization of DNA tags. Ultimately, these systems allow the spatial arrangement of NDs with nanometer precision using programmed DNA-origami. In an alternative approach, MSPs may be replaced entirely if bilayer stabilization can be accomplished by lipid DNA interactions as shown with DNA-encircled bilayers (DEBs), a recently proposed novel membrane mimetic.[1]

1.Iric et al., 2018 ***Nanoscale***, **10**, 18463-18467.

P-647

Fibrinogen-erythrocyte binding as a new cerebrovascular risk factor on stroke patients

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Stroke is the most frequent and mortal cerebro/cardiovascular disease worldwide. It is caused by bleeding from a blood vessel or a blood clot in the brain (ischemic stroke). Blood clots are associated with high fibrinogen levels in plasma. It is important to identify molecular biomarkers to early detect stroke. These biomarkers can have a prognostic value in stroke, helping patients to prevent secondary events. Our main goal was to evaluate the influence of fibrinogen on erythrocyte-erythrocyte adhesion using blood from ischemic stroke patients and compare them with the erythrocytes from healthy donors. Blood samples were analysed by Atomic Force Microscopy (AFM) and through haemorheological parameters. AFM was used to measure fibrinogen-erythrocyte adhesion and erythrocyte-erythrocyte interactions, as well as erythrocyte stiffness. Erythrocytes from stroke patients are less deformable and have higher propensity to aggregate. The blood from stroke patients is also more viscous. AFM-based force spectroscopy measurements showed that stroke patients have higher fibrinogen-erythrocyte binding forces, despite its lower binding frequency. With increasing fibrinogen concentrations, the work and the maximum force necessary to detach two erythrocytes from stroke patients are increased. Higher concentrations of the y' fibrinogen variant in plasma were also found on these patients. These changes could be associated with a higher risk of thromboembolic events and a worst clinical prognostic. Understanding the role of fibrinogen on erythrocyte aggregation may be relevant for potential future drug interventions to reduce aggregation and enhance microcirculatory flow conditions. These findings may contribute to considering the fibrinogen-erythrocyte binding as a new cerebrovascular risk factor for stroke disease.

P-648

Label-free protein detection with an optofluidic lab-on-chip sensor

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Whispering gallery mode (WGM) resonators are promising optical structures for microfluidic label-free biosensors mainly due to their high sensitivity. Their real laboratory diagnostic application however lacks a robust fabrication method that offers a complete device of practical value. Here we report on a monolithic lab on a chip sensor fabricated by a hybrid femtosecond laser micromachining approach, for label-free biosensing of a selected protein. It consists of a polymer WGM microresonator sensor made by two-photon polymerization directly inside a glass microfludic chip prepared by laser-assisted etching. The device, after a thorough geometry optimization, presents a refractive index change sensitivity of 61 nm/RIU. We demonstrate its bio-sensing capability exploiting the biotin-streptavidin binding affinity, obtaining a detectable minimum protein surface density increase of 67 x 10^3 molecules/μm^2.

P-649

A molecular sensor reveals differences in macromolecular crowding between the cytoplasm and nucleoplasm

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We present a molecular sensor that reports, using fluorescence, on the degree of macromolecular crowding in different cellular compartments. The oligonucleotide-based sensor is sensitive to changes in the volume fraction of macromolecules over a wide range in vitro and, when introduced in cells, distributes in various compartments and shows a striking contrast between the cytosol and the nucleus. This contrast can be modulated by osmotic stress, or using a number of drugs that alter chromatin organization within the nucleus. These findings suggest that the sensor can be used as a tool to probe chromosome organization. Further, our finding that the cell maintains different degrees of macromolecular crowding in the cytoplasm and nucleoplasm has implications on molecular mechanisms since crowding can alter protein conformations, binding rates, reaction kinetics, and therefore protein function.

P-650

Rapid detection of AIB1 in breast cancer cells based on fluorescein-labeled aptamers functionalized nanomotors

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We demonstrated ultrasound (US)-propelled graphene-oxide (GO) coated gold nanowires (AuNW), functionalized with fluorescein-labeled DNA aptamers (FAM-AIB1-apt) as nanomotors for qualitative real-time intracellular detection of overexpressed AIB1 oncoproteins in MCF-7 breast cancer cells. The sensing strategy is based on an attractive OFF-ON fluorescent switching. The fluorescence signal is quenched while FAM-AIB1-apt is adsorbed on GO/AuNW (signal OFF). Then, in the presence of the specific target AIB1 the FAM-AIB1-apt is released from the GO/AuNW surface leading to a fluorescence signal recovery. The movement of nanomotors under the ultrasound field facilitated intracellular uptake and resulted in a faster aptamer binding with the target protein and thus faster fluorescence recovery. The propulsion behavior of the aptamer functionalized nanomotors greatly enhanced the fluorescence intensity compared to static conditions (1.09 *vs.* 0.25 a.u.). Negligible fluorescence intensity was observed in negative control HFF-1 cells as well as in positive control MCF-7 treated with a nonspecific fluorescein-labeled aptamer. This new aptamer functionalized nanomotor-based strategy offers considerable potential for further development of sensing methodologies towards early diagnosis of breast cancer and adequate treatment.

Supported by the European Commission within the project FORMILK under grant agreement number 690898 /H2020-MSCA-RISE-2015.

P-651

Molecular scale biophysical methodologies for levo-thyroxine interaction with DNA

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Thyroid hormones are crucial in the regulation of many metabolic processes and their absence requires treatment, therefor administration of levo-thyroxine (LT4) plays an important role. It is presumed, that these hormones exert their effects by activating gene transcription of messenger ribonucleic acid and promote protein synthesis. LT4 enters the cell nucleus and binds to deoxyribonucleic acid-bound (DNA) thyroid receptors, which regulate gene transcription. In this work, we monitor the binding mechanism of LT4 directly to DNA molecules, free into solution and confined to a gold chip, and we analyse its potential damage using several methods.

UV-Vis spectrophotometry is used here as a classical tool, allowing the spectra measurement of each compound separately and its interaction with LT4, based on the absorption of light over a certain range of wavelengths. Surface plasmon resonance (SPR) based on refractive index changes is sensitive to the adsorption of molecules on a gold coated chip enabling the study of affinity and kinetics constants of biomolecular interactions between DNA and LT4. Electrochemistry was used to monitor interfacial changes, at gold coated chip, which occur at confined DNA and study the LT4 binding to DNA by electrochemical impedance spectroscopy (EIS) through variation of electrical parameters.

P-652

A new in vitro screening system for evaluating of anticancer drugs effect

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Reactive oxygen species (ROS) is associated with induction of apoptosis. The study of intracellular ROS levels may represent one possibility to research the effects of drugs in inflammatory cells. ROS are released from cells during apoptosis, play a crucial role in the development of cancer and neurodegenerative diseases. Nowadays, there is the problem of developing methods for treating cancer tumors, and quickly evaluation of the anticancer drugs efficiency is the priority. The ROS determination using nanosensors in single cells has gained increasing attention [1]. However, traditional fluorescent dyes have a number of disadvantages. These dyes are known to be intrinsically cytotoxic and thus can significantly alter cellular metabolism. Here, we have developed an electrochemical method for determining the ROS inside the cells. Using this method, it is possible to evaluate the effect of the developed drugs on the cells [2]. We evaluated the effect of PSMA (Prostate-Specific Membrane Antigen) - specific carrier equipped by Doxorubicin, monomethyl auristatin E or Abiraterone on cell lines with (22RV1) and without (PC-3) PSMA receptors. Our data obtained by using carbon-filled quartz nanopipettes with platinum tips showed a ROS increase using conjugates compared with native drugs. We also studied the effect of drugs based on copper complexes on the cell line MCF-7 and demonstrated that complexes with copper in various degrees of oxidation, depending on the ligands, can have different effects on tumor cells. Complexes with copper in various degrees of oxidation, depending on the ligands, can have different effects on tumor cells. In the future, this method may allow evaluating the effect of drugs in vitro and to help validate drug candidates for preclinical evaluation.

References:

[1] Actis, Paolo, et al. "Electrochemical nanoprobes for single-cell analysis." Acs Nano 8.1 (2014): 875-884.
[2] Erofeev, A., et al. "Novel method for rapid toxicity screening of magnetic nanoparticles." Scientific reports 8.1 (2018): 7462.

P-653

Time-resolved infrared spectroscopy to analyze dynamic interactions of photoreceptors with biomimetic membranes

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While it is generally accepted that the membrane composition has an important influence on membrane protein function, the origin is less understood. We used Bacteriorhodopsin (BR) and Proteorhodopsin (PR) as important membrane proteins to analyze these effects, because their light-driven transmembrane proton pumping mechanisms are well characterized by time-resolved infrared (IR) spectroscopy. The photoreceptors were reconstituted into liposomes thereby providing uniform biomimetic membranes, and the physical properties of the lipids were systematically varied. By applying step-scan FTIR spectroscopy, we could dissect the effect of membrane composition on the individual proton transfer steps and monitor protein conformational changes simultaneously. We observed an intriguing dependence of correlated proton transfer and protein conformational dynamics on the membrane fluidity. Our results emphasize the challenges to mimic key characteristics in artificial membrane systems. The focus has often been set on matching charge and membrane thickness, but our study indicates that membrane fluidity is the most important variable. To further analyze the membrane dynamics, we will apply our home-built IR spectrometer based on quantum cascade lasers and single wavelength detection. The improved signal-to-noise ratio allows to resolve minor changes of lipid vibrational modes.

P-654

Label-free determination of binding affinity using topography and recognition imaging

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Single-molecule and single-cell force spectroscopy are appropriate tools for retrieving accurate dynamic and statistical information about the nanomechanical behavior of molecular bonds involved in adhesion to biotic and abiotic surfaces. In biochemistry, the determination of the equilibrium dissociation constant is key for quantifying the interaction between biological molecules. Despite the wide range of approaches in increasing the measurement sensitivity for minute sample amounts, critical limitations with respect to labelling, fluorescence tags, and low detection signals combined with high noise are difficult to overcome. This intimately leads to requirements of new measurement tools that combine high sensitivity with nano-scale spatial resolution. In recent years, the topography and recognition (TREC) imaging technique, based on force spectroscopy in resonance, has been utilized for mapping bio-molecular recognition events to localize bio-molecules at the nano-scale. In the present work, we fabricated DNA arrays on glass or silicon substrates as platforms capable for sensing single molecular interactions. We employed TREC to characterize the DNA array and quantified the equilibrium dissociation constant K_d of DNA duplexes from recognition images, yielding $K_d = 2.4 \times 10^{-10}$ M. Using TREC we developed an affinity sensing assay, which can be directly assessed without any labelling or secondary binding for detection.

P-655

Deciphering biophysics of biomolecules, functional layers and membranes by Multi-Parametric Surface Plasmon Resonance (MP-SPR)

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Multi-Parametric Surface Plasmon Resonance (MP-SPR) is a surface analysis technique which application goes far beyond the traditional SPR, typically used for biomolecular binding studies. Indeed, this label-free and real-time technique give insight into biophysics of functional layers and surface related interactions. MP-SPR optical set-up combines wide angular scanning and detection at multiple wavelengths thus providing a unique possibility to assess layers from Ångströms up to tens of microns. It enables to characterize a large variety of materials ranging from thin films (polymers, nanocellulose), biomolecular assemblies, lipid bilayers and up to nanoparticles and live cells. Measurements provide information on kinetic parameters from biointerface interactions as well as calculation of layer refractive index (RI) and its thickness (d) without prior knowledge of either parameter. The performances of MP-SPR technique are demonstrated in such studies as: conformation changes in lipid layers or functional polymers [1-3], cell binding kinetics in biomimetic environments [4], antifouling efficacy in serum [5] or protein corona formation on liposomes [6]. With the ability to characterize both kinetics and nanoscale layer properties, MP-SPR is an effective tool for nanomaterial, biomaterial and biochemical interactions research.

[1] Granqvist N., Yliperttula M. et al. Control of the Morphology of Lipid Layers by Substrate Surface Chemistry. *Langmuir* **2014**
[2] Parkkila P., Elderfi M., Bunker A., Viitala T. Biophysical Characterization of Supported Lipid Bilayers Using Parallel Dual-Wavelength SPR and QCM Measurements. *Langmuir,* **2018**.
[3] Ferrand-Drake del Castillo G., Emilsson G., Dahlin A. Quantitative Analysis of Thickness and pH Actuation of Weak Polyelectrolyte Brushes. The Journal of Physical Chemistry C, **2018**.
[4] Soler M., Li X. et al. Two-dimensional label-free affinity analysis of tumor-specific CD8 T cells with a biomimetic plasmonic sensor. *ACS Sensors*, **2018**..
[5] Emilsson G., Schoch R.L., et al., Strongly Stretched Protein Resistant Poly(ethylene glycol) Brushes Prepared by Grafting-To. *ACS Applied Materials & Interfaces,* **2015**..
[6] Kari O.K. et al., Multi-parametric surface plasmon resonance platform for studying liposome-serum interactions and protein corona formation. *Drug Delivery and Translational Research,***2017**.

P-656

Understanding the absorption and the dual fluorescence of Prodan in solution

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Prodan is a fluorescent probe used to monitor biological systems such as lipid membrane, proteins and DNA. Remarkable interest is associated to the interpretation of its fluorescent spectrum. In this paper the sequential hybrid Quantum Mechanics/Molecular Mechanics (S-QM/MM) method was used to establish that the fluorescent emission occurs from two different excited states, resulting in an emission spectrum with two bands. The absorption spectra in several solvents populate two bright excited states (S_1 and S_3) in the low energy band. These excited states were analyzed theoretically using multi-configurational calculations (CASSCF) and second-order multi-configurational perturbation theory (CASPT2). It was found that these excited electronic states are very close in energy and the corresponding equilibrium geometries are all planar. The S_1 state is a π-π* which after the vertical excitation decay to S_2 by internal conversion, while S_3 relaxes to its equilibrium geometry. In gas phase and in solution the fluorescent states are S_2 and S_3 (n-π* and π-π* character respectively). A consistent explanation of the experimental data is obtained with the conclusive interpretation that the two bands observed in the emission spectrum of Prodan in several solvents are due to the emission from the S_2 and S_3 states.

P-657

Potential role of oxygen molecules and cytochrome c oxidase proteins in optical communication in the brain

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Great discoveries have been made in the field of neuroscience; however, the origin of consciousness and many mental abilities of the brain are still unanswered. Quantum physics may help provide some answers. In particular, there is some experimental evidence of spins and photons playing a role in biology. We explore the possibility of the existence of an optical communication network connecting spins in addition to the well-known electro-chemical network in the brain. Photon emission by neurons has been observed experimentally. Also, it has been shown that photons can be guided by myelinated axons. To further support the hypothesis of an optical communication network in the brain, we investigate the possible role of oxygen molecule and cytochrome c oxidase as biophoton sources and receivers, respectively. The oxygen molecule not only has non-zero spin, but also emits light which matches spectrally with the observed biophotons. Cytochrome c oxidase in mitochondria is a light absorbing metalloprotein. This protein has a peak in its absorption spectrum around the frequency of dimol emission of oxygen. The role of this protein in generating singlet oxygen (an excited state of the oxygen molecule) after photon absorption and the fact that this protein has electron and nuclear spin make the possibility of a spin-photon interface stronger, resembling quantum repeaters (key components of quantum communication networks) in some respects. We propose experimental tests for many important aspects of these ideas, including spin-light coupling, absorption cross sections and spin properties of oxygen and cytochrome c oxidase.

P-658

Combination of ruthenium dendrimers and ultrasound propelled-gold nanowires for active drug delivery towards breast cancer cells

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Dendrimers are highly branched nanoparticles with unique molecular properties, which make them promising carriers for delivery of various therapeutically active compounds into the disease affected cells. Recently, a new class of carbosilane metallodendrimers based on ruthenium possessing anticancer activity has been synthetized [1].

At the same time, another group of engineered particles – gold nanowires (AuNWs) are intensively studied as active transporters (motors) in the field of ultrasound propelled drug/gene delivery [2].

In this work, we combined fluorescently labelled ruthenium dendrimers (CRD_{13}FITC) with graphene oxide modified nanowires (GO-AuNWs). Prepared complexes were tested as active transporters being propelled by ultrasound (US) towards breast cancer cells. Energy dispersive X-ray spectroscopy analysis confirmed successful modification of nanowires by dendrimers as shown by the presence of ruthenium corresponding to the ruthenium groups of CRD_{13}FITC. Binding of dendrimers to the surface of GO-AuNWs was accompanied by quenching their fluorescence signal. Upon 5min application of an ultrasound field (2V, 2.66 MHz), the complexes were propelled towards MCF7 breast cancer cells, decomplexed and thus the fluorescence recovery detected. Fluorescence signal from US-treated samples was ~1.5 fold higher compared to passive controls. This suggests that US-powered propulsion leads to a fast internalization, hence to an accelerated delivery of CRD_{13}FITC – dendrimers inside MCF7 cells.

This system gains a potential for higher payload and rapid delivery of therapeutical material.

[1] Maroto-Diaz, M. et al: Synthesis and anticancer activity of carbosilane metallodendrimers based on arene ruthenium (II) complexes, Dalton Trans, 2016, 45(16), 7049-7066.
[2] Esteban-Fernández de Ávila, B. Et al: Acoustically-propelled nanomotors for intracellular siRNA delivery, ACS Nano 2016, 10(5), 4997-5005.

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Novel Biophysics Methodologies for Single Cell Analysis with Electrochemical Nanoprobes

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Nanopipettes have been used in different applications with integration into Scanning Ion Conductance Microscopy (SICM): high resolution topographical imaging of living cells, quantitative delivery of molecules to the surface of living cells. Additionally, nanopipette probes still hold great promises as intracellular biosensors.

Here we describe the fabrication, characterization, and tailoring of carbon nanoelectrodes based on nanopipette for intracellular electrochemical recordings. We demonstrate the fabrication of disk-shaped nanoelectrodes whose radius can be precisely tuned within the range 5-200 nm. The functionalization of the nanoelectrode with platinum allowed the monitoring of oxygen consumption outside and inside of melanoma cell [1].

We applied the nanoelectrode to perform intracellular measurement in cultured melanoma cells, HEK293 and LNCap cancer cell. Upon penetration of the cells the anodic current quickly increases followed by equilibration to a level above the one measured in the cell media. A cell can withstand multiple penetrations and we measured a substantial difference between the electrochemical signal measured inside and outside the cell. We believe these results show the potential of functional nanoelectrode to probe endogenous species into cells and with further improvements they may allow the study of oxidative stress under influence of different drugs and nanoparticles [2].

References:

1. P., Actis, et. al., Electrochemical nanoprobes for single-cell analysis. ACS Nano, (2014) 87

2. A., Erofeev, et. Al., Novel method for rapid toxicity screening of magnetic nanoparticles. Scientific Reports, (2018) 8:7462

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Multi-timescale conformational dynamics of the autophagy related protein GABARAP revealed by fluorescence spectroscopy combined with NMR and simulations

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Mechanistic understanding of the function of proteins requires capturing of time-resolved structures. Different timescales require various experimental techniques: fast dynamics can be studied with MD and NMR, while fluorescence covers nanosecond to second processes but requires additional probes. We demonstrate how our integrated approach captures multi-timescale dynamics of GABARAP, protein involved in multiple cellular processes like membrane trafficking and fusion events. With combination of fluorescence spectroscopy, NMR, and MD we mapped internal flexibility, exposing possible sites of large-scale motions, and hydrodynamic features of GABARAP. FRET spectroscopy was used to study large-scale conformational dynamics occurring on microscecond timescale, revealing functionally-relevant flexibility of N-terminal domain. Our integrated approach allowed us to characterize time-resolved dynamics of secondary and tertiary structure of protein, suggesting mechanisms of GABARAP self-association and ligand binding.

Satellite meeting, Bilbao, 25th-27th July:

PROTEIN LIPID NANOSTRUCTURES: FROM DOMAINS TO DEVICES

Mechanism of lipidic pore formation by helical amphipathic peptides

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Amphipathic alpha-helical peptides are promising antimicrobial agents. Positively charged peptides can selectively recognize and bind outer monolayer of bacterial membranes that bears negative charge. Interaction of peptides with the bacterial membrane results in formation of through pore. When bound to the membrane, the peptide helix exposes hydrophobic residues on its side-surface, and polar or charged residues on the opposite side-surface. The tendency to hide hydrophobic residues from contact with water leads to partial incorporation of the peptide into the lipid monolayer. The incorporation induces elastic deformations around the peptide. When two peptides are far separated, these deformations are independent. Upon approaching, the deformations overlap leading to effective lateral interaction of the peptides. In the framework of theory of elasticity of lipid membranes, we obtained that the total elastic energy of the membrane is minimal when two peptides are in register, parallel to each other, and the distance between their longitudinal axes is about 5 nm. At such separation the electrostatic repulsion of the peptides is negligible. The most stressed region is situated in the middle between amphipathic alpha-helical peptides. Formation of through pore in this region seems to be the most probable. As the equilibrium distance between peptides is about 5 nm, the initial pore should be purely lipidic. We obtained that in the presence of peptides the energy barrier of pore formation decreases by ten-tens of k_BT. After pore formation, the peptides tend to arrange to its equatorial plane: the total elastic energy has local minimum in such configuration. The depth of the minimum (and the life-time of the pore) is influenced by electrostatic repulsion of charged peptides; stronger repulsion results in shallower minima and shorter life-times. Long-living pore can be further stabilized by recruiting additional peptides from the membrane surface. Highly charged peptides rapidly escape from the edge of short-living pore either to the initial or to the opposite side of the membrane. This provides the mechanism of membrane penetration by charged substances without formation of long-living pores.

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Molecular mechanisms behind remorin nanodomain formation by solid-state NMR

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Protein and lipid components in biological membranes act as a dynamic network of subtle molecular interactions segregating the membrane into particular regions called nanodomains. Nanodomains act as functional platforms enriched in specific lipids (such as sterols and phosphoinositides) and proteins to perform their diverse activities. Remorins (REMs) are plant proteins and well-established nanodomain markers and, as such, they can be considered as a paradigm to provide a mechanistic description of membrane organisation into functional nanodomains. Using solid-state nuclear magnetic resonance (ssNMR) and building upon our initial knowledge of *St*REM1.3 and its C-terminal membrane anchor, we reveal the delicate balance between hydrophobic and electrostatic effects leading up to the protein's characteristic affinity for negatively charged phospholipids. In a divide-and-conquer approach, we describe the impact of *St*REM1.3's C-terminal anchor, its oligomerisation domain and its intrinsically disordered region on membrane structure and dynamics. Furthermore, we tackle the structural features of *St*REM1.3 when associated to nanodomain-mimicking membranes. We reveal that *St*REM1.3 drives nanodomain organisation by concerted lipid-protein and protein-protein interactions.

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LC3/GABARAP protein family in mitophagy

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Mitophagy is a selective mitochondrial autophagy in which the phospholipid cardiolipin (CL) has been proposed to play a role. CL externalization to the outer mitochondrial membrane would act as a signal for the autophagic machinery to degrade damaged mitochondria. LC3/GABARAP protein family could mediate both mitochondrial recognition and autophagosome formation, ultimately leading to removal of damaged mitochondria. Previous studies have shown that LC3B binds preferentially to CL enriched membranes. The objective of this project is to understand this interaction in different members of the LC3/GABARAP protein family. Even though LC3/GABARAP family members have similar structures, they show different binding affinities to CL liposomes in our experiments. With the aim of validating these results in eukaryotic cells we have performed binding assays in isolated mitochondria and co-localization assay in SH-SY5Y cells by confocal microscopy. Differences have been observed between the different family members. Our results support the hypothesis that the interaction between LC3/GABARAP protein family and CL plays a role in the recognition of mitophagy cargo.

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Lipid and proteo-lipid nanotubes with adjustable physicochemical and geometrical parameters

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Functionalization of nanopores with lipid bilayers (lipid bilayer coating) has already attracted growing interest going far beyond merely imparting biomimetic properties to them. Lipid bilayer coated nanopores appear to be a novel platform for applications of regulated (controlled) transport of ions and nanoparticles/macromolecules. To improve these emerging applications, we study for the first time the possibility to use 2D fluidity of the lipid bilayer for creating a nanopore with adjustable physicochemical properties and luminal radius. We pulled lipid nanotubes (NT) from planar reservoir membranes to obtain biomimetic elastic nanopores, the lumen radius of which could vary from 10 to 2 nm depending on the proteo-lipid composition of the NT membrane. We showed that the net surface charge inside the NT could be adjusted through accumulation or depletion of charged lipids in the inner monolayer of electrically biased NT. Electric field acts as regulator of ionic selectivity of the NT. Moreover, by conjugating electromigration with curvature-driven distribution of conical lipids, we demonstrated the possibility of making elastic nanopores with voltage-controlled diameters – an effect similar to piezoelectricity. Considering that geometrical parameters of NTs allow detecting single macromolecule transport, simultaneous regulation of both the surface charge and the luminal radius of such nanostructures significantly improves their dynamic functionality and suggests proteo-lipid nanotubular structures as promising tools for single molecule research/detection.

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HIV-1 gag protein sensitivity to membrane curvature and lipid environment

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Among other enveloped viruses, human immunodeficiency viruses (HIV) attracts the most efforts to elucidate molecular mechanisms of its infectivity, mutagenesis and reproduction activity. Lack of vaccine candidates against HIV drive attention to the self-assembly of the virion in order to disturb it. Group-specific antigen (Gag) polyprotein, interacting both viral RNA and plasma (or, later, viral) membrane, is believed to play a pivotal role in the process of HIV assembly. Moreover, this protein alone can produce virus-like particles from infected cells. Despite a loan of studies of Gag interaction with lipid membranes, there are still open questions about the main partners of such interactions among lipids and cell and viral proteins, as well as about the mechanism of viral protein envelope self-organization during budding of progeny virions from the infected cell. In the present study, we focused on the effect of membrane curvature, lipid composition and pH of the environment on self-assembly of Gag protein on the lipid bilayer. Using atomic force microscopy (AFM), we characterized the Gag adsorption in physiological conditions on lipid membranes of various composition, as well as its sensitivity to membrane curvature. We presented a theoretical model describing the protein rearrangements between flat and curved membrane regions. Moreover, we demonstrated the effect of low pH environment on Gag self-assembly to clarify the possible outcomes of the HIV infectivity through endocytic pathway.

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Comparative analysis of membrane constriction by dynamin isoforms

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The proteins of dynamin superfamily are large GTPases widely implicated in fission and fusion of endomembranes. Their activity is necessary for internalizing essential nutrients, organelle transformations and maintenance, dynamins are intimately involved in signalling and membrane trafficking networks in the cell, in life and pathology. The superfamily founding members, dynamins 1 and 2 (Dyn1 and 2), remain the most characterized dynamins primarily involved in orchestrating membrane fission in the clathrin dependent endocytosis. As an endocytic vesicle buds, dynamin molecules are recruited to its neck, where it self-assembles a helical coat generating a dynamin-lipid tube. The helix constriction driven by GTP hydrolysis promotes fission of the neck and release of the vesicle. While the above patterns of dynamin activities common for Dyn1 and 2 are well understood, *in vitro* analyses revealed important functional differences between neuron-specific Dyn1 and ubiquitous Dyn2 isoforms. Here we performed systematic mechanistic comparison of membrane remodelling activities of Dyn1 and 2 reconstituted using lipid membrane nanotubes. By combining fluorescence microscopy and optical tweezers approaches, we quantified the nanotube constriction by dynamin isoform in apo state and in the presence of different nucleotide. Our analyses revealed significant differences between membrane constriction and curvature stabilization activities of Dyn1 and 2. We discuss possible relevance of these differences to physiological functions of the proteins.

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Single-cell resolution of metabolic control over HIV-1 entry and a role for membrane lipid order and tension

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Recent studies have highlighted cellular metabolic activity as a critical factor driving HIV-1 infection in T cells. However, deciphering how the metabolic state of single cells affects virus entry remains to be fully characterised. We developed an assay utilising FRET-based biosensors of various metabolites to evaluate the influence of global metabolic processes on the success rate of virus entry in single cells. Lifetime fluorescence images of single cells were recorded immediately before and after addition of HIV-1 pseudovirions (i.e. HIV-$1_{JR\text{-}FL}$) or non-enveloped HIV-1 with incorporated BLaM-Vpr. Lifetime measurements of cells expressing biosensors for ATP:ADP ratio or lactate were utilised to determine relative metabolite concentrations before and during entry. The same cells were subsequently screened for fusion and productive infection to determine whether baseline intracellular metabolite concentrations were correlated with these processes. Interestingly, cells with a lower ATP:ADP ratio prior to virus addition were less permissive to virus fusion and infection. These results indicated a relationship between host metabolic state and the likelihood for virus-cell fusion to occur. To confirm this, we show that cells treated acutely with 2-deoxy-d-glucose (2-DG), an inhibitor of glycolysis, permitted substantially fewer fusion events. Single particle tracking (SPT) revealed that virions were arrested at hemifusion in 2-DG-treated cells. Interestingly, cells treated with 2-DG also possessed less surface membrane cholesterol, while the addition of cholesterol to the plasma membrane rescued the block to fusion. Further investigation with additional reporters revealed a link between host glycolytic activity and membrane tension and order, with cells treated with 2-DG exhibiting lower plasma membrane lipid order and higher tension values. These data suggest that low glycolytic activity results in a deficiency of membrane cholesterol. Finally, SPT illustrated that virions were less likely to enter cells at areas of high membrane tension. We are currently performing similar experiments in T cells. We have identified a connection between host glycolytic activity and membrane tension which may influence HIV-1 fusion at the single-cell level. Our results indicate that HIV-1 fuses with glycolytically-active cells and that this activity is linked to cell surface membrane cholesterol and membrane tension.

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Membrane tethering by Dynamin-2

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The dynamin superfamily comprises large multi-domain GTPases deeply implicated in intracellular membrane remodeling, fusion and fission. Though fusion and fission have been associated with distinct dynamin species, their functional specialization seems not to be strict. Classical dynamins, particularly Dynamin-2 (Dyn2), mediating membrane fission during endocytosis, have been also linked to membrane tethering and fusion in such processes as viral infection, cell-cell fusion and kiss-and-run exocytosis. In order to understand how the same protein species can meaningfully participate in such different membrane processes as fusion, fission, and tethering, we reconstituted trans-membrane interaction(s), tethering, and fusion, by Dyn2 in a minimal *in vitro* membrane system. We found that Dyn2 promotes trans-membrane interaction and mediates stable membrane tethering between membranes of different curvature and topology. We analyzed the membrane contact sites produced by Dyn2 by fluorescence and cryo-EM and further quantified the stoichiometry of dynamin complexes using single-molecule fluorescence microscopy. We found that tethering strength and efficiency depends on the presence of nucleotide and membrane elasticity. We propose a model linking the different modes of action of Dyn2 to membrane topology and mechanics.

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Lipid domain boundary as universal attractor for various membrane inclusions

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Cell membranes are nonuniform multicomponent systems comprising various types of lipids and membrane proteins interacting with each other. Lipid diversity results in phase separation and formation of liquid-ordered domains also called lipid rafts. They are assumed to be important actors in diverse cellular processes, mainly signal transduction and membrane trafficking. Rafts are thicker than the disordered part of the membrane, thus compensating the hydrophobic mismatch between transmembrane proteins and the disordered lipid environment. That leads to the main cause of the boundary energy, a strained structure of the boundary. Membranes may also contain a wide variety of inclusions, inducing elastic stress: peripheral proteins, nonlamellar lipids, transmembrane proteins, etc. Deformations, induced by these inclusions, can interfere with raft boundary deformations, leading to the specific arrangement of these impurities around the domain edge, relaxing the membrane strain. Using elasticity theory approach, we revealed that the domain boundary serves as an attractor for almost all types of membrane inclusions developed for lipid membranes. We have shown that various types of impurities tend to distribute to the narrow intermediate region at the liquid-ordered domain boundary, falling to the elastic energy well. The magnitude of the potential well equals to 0.4–0.5 k_BT per 1 nm of the boundary for membrane proteins, amphipathic peptides and hydrophobic molecules. For nonlamellar lipids, possessing spontaneous curvature of ±0.25 nm^{-1}, the depth of the energy well equals to 0.15–0.2 k_BT/nm. Moreover, the redistribution of these components dramatically varies the morphology and size of liquid-ordered domains, which is achieved by changing the domain boundary energy.

Thus, we have shown that the boundary of the liquid-ordered domains can act as the universal attractor for a wide variety of membrane minor components, such as various peptides and non-bilayer lipids. Such attractive activity of the domain boundary can explain the mechanisms and suggest the new pathways of the strong influence of the low concentration of membrane impurities on various physiological processes involving rafts and may have an impact on cell signal transduction pathways, viral-induced membrane fusion, aggregation of amphipathic peptides.

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Lipid-gold clusters (Aurora™): a membrane-friendly form of gold nanoparticles

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Lipid model membranes in lamellar phases (bilayers) of different phospholipid compositions have been prepared, in the form of vesicles, or of supported lipid bilayers, and doped with Aurora™ at 0.1 mol%. Aurora™ consists of an Au_{55} gold nanoparticle (about 1.4 nm in diameter) capped with triphenylphosphine ligands and a single diglyceride (distearoyl glycerol) ligand. Gold nanoparticles have been incorporated in the past inside liposomes, or grafted onto their surfaces, with diagnostic or therapeutic aims. Including the gold nanoparticles in a stable form within the lipid bilayers has serious technical difficulties. We have tested the hypothesis that, because of the diglyceride ligand, Aurora™ would allow the easy incorporation of gold nanoclusters into cell membranes or lipid bilayers without significant effects in their biophysical properties. Our results show that Aurora™ readily incorporates into lipid bilayers, particularly when they are in the fluid phase, i.e. the state in which cell membranes exist. Calorimetric, fluorescence polarization or fluorescence confocal microscopy concur in showing that bilayer-embedded Aurora™ hardly changes the physical properties of the bilayers, nor does it perturb the phase equilibrium in lipid mixtures giving rise to lateral phase separation in the plane of the membrane. Atomic force microscopy shows, in fluid bilayers, well-resolved particles, 1.2 – 2.9 nm in height, that are interpreted as single Aurora™ conjugates. Cryo-transmission electron microscopy allows the clear observation of lipid bilayers with an enhanced contrast due to the Aurora™ gold nanoparticles; the single particles can be resolved at high magnification. Our studies support the applicability of Aurora™ as a membrane-friendly form of nano-gold particles for biological research or clinical applications.

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Binding affinity of membrane-inserted epitope to HIV-1 antibody and its dependency on lipids quantified by fluorescence correlation spectroscopy

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Broadly neutralising HIV antibodies (bnAbs) are defined by their ability to neutralise diverse HIV isolates. Among all described bnAbs, the ones that target the membrane-proximal external region (MPER) show the highest breadth (98% of tested isolates neutralised). MPER is localised in the interface of the viral membrane and thus, anti-MPER bnAbs have evolved to include membrane-interacting regions that permit MPER recognition in a lipid environment. Moreover, these antibody (Ab)-lipid interactions seem to be essential for the neutralizing activity of anti-MPER bnAbs, although their contribution to epitope binding is not fully understood. In this work, we quantitatively study Ab-MPER interaction in its membrane context by means of fluorescence correlation spectroscopy (FCS). We have quantified the partition coefficient (K_p) of several 10E8 variants bound to MPER-bearing large unilamellar vesicles (LUV) of different compositions through the evaluation of the Ab diffusion regime. Our work builds on previously reported methodology used to assess peptide-membrane partitioning [1, 2], adding to it the complexity of a third component. Reliable K_p values were obtained upon careful quantification of the amount of accessible lipid on the vesicles. The uncertainty of the determined K_p was computed through support-plane analysis. In conclusion, our measurements set a robust method for the quantitative determination of membrane partitioning in intact systems. We foresee that its application will shed light over the functional relevance of interactions between bnAbs and MPER in a lipid membrane environment.

References:

[1] Rusu L, Gambhir A, McLaughlin S, Rädler J. Fluorescence correlation spectroscopy studies of peptide and protein binding phospholipid vesicles. Biophys J. 2004

[2] Melo AM, Prieto M, Coutinho A. The effect of variable liposome brightness on quantifying lipid-protein interactions using fluorescence correlation spectroscopy. Biochim Biophys Acta - Biomembr. 2011

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Interactions through the membrane-accommodation surface improve the biological function of broadly neutralizing HIV-1 antibody 10E8

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The 10E8 antibody targets a helical epitope in the membrane-proximal external region (MPER) and transmembrane domain (TMD) of the envelope glycoprotein (Env) subunit gp41, and is among the broadest known neutralizing antibodies against HIV-1. Accordingly, this antibody and its mechanism of action valuably inform the design of effective vaccines and immunotherapies. 10E8 exhibits unusual adaptations to attain specific, high-affinity binding to the MPER at the viral membrane interface. Here, we demonstrate that by increasing the net positive charge of the polar surface-patch in contact with the viral membrane, the neutralization potency of the antibody may be significantly enhanced. We found that the optimized 10E8 could interact spontaneously with synthetic-fluid membranes, but did not gain any observable polyreactivity. Binding analyses, including single virion STED microscopy, revealed that the increase in neutralization potency correlated with higher affinity for Env spikes inserted into the rigid viral membrane. Overall, our data provide a proof-of-principle for rational optimization of 10E8 via manipulation of its interaction with the membrane element of its epitope. Moreover, our results emphasize the crucial role played by the viral membrane in the antigenicity of the MPER-TMD of HIV-1.

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Functional nanostructure of NhaA protein in tethered lipid bilayer membranes

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The study of active membrane proteins requires an environment which is as close as possible to their natural environment to retain protein function, while at the same time keeping the system as simple as possible to allow for an experimental characterization and to be able to identify factors which influence the system. Tethered lipid bilayers (tBLMs) represent an experimentally accessible and stable model for biological membranes that offers a high level of control over the structure and can form a more natural environment for membrane protein incorporation than the widely used solid supported bilayers [1]. We report the use of a tBLM system to investigate how the structural factors of the surrounding membrane influence the incorporation and subsequently the activity of the NhaA protein, which is the main sodium proton antiporter of *Escherichia coli*. NhaA serves as the means for *E. coli* to maintain sodium homeostasis and for pH control [2]. Here we present a study on the incorporation of NhaA into PEG-tBLM on gold surfaces. We show the nanostructural characterization of highly covering tBLMs of different lipid composition with large fractions of incorporated NhaA by neutron reflectometry (NR) and how electrochemical impedance spectroscopy (EIS) can be used to investigate its activity.

NR allowed us to determine the structure of the membrane/protein system to monitor bilayer dimensions, completeness and to precisely determine the amount of incorporated NhaA protein. EIS provided functional characteristics like electrophysiological properties related mainly to ion permeability and indicated NhaA activity, as this is associated to an ionic current across the bilayer.

The combination of these two methods enables us to correlate structural and functional information of the NhaA-membrane system in order to understand the mechanisms behind these dependencies.

This study provides the information to optimize the membrane/protein system regarding protein activity to enable further optimization of our biomimetic fuel cells, which take advantage of NhaAs electrogenic properties.

[1] M. Maccarini et al., *Langmuir*, **2017**, *33*, 9988-9996

[2] Etana Padan, *BBA-Bioenergetics*, **2014**, 1837. Jg., Nr. 7, S. 1047-1062

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Molecular mechanisms behind remorin nanodomain formation by solid-state NMR

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Protein and lipid components in biological membranes act as a dynamic network of subtle molecular interactions segregating the membrane into particular regions called nanodomains. Nanodomains act as functional platforms enriched in specific lipids (such as sterols and phosphoinositides) and proteins to perform their diverse activities. Remorins (REMs) are plant proteins and well-established nanodomain markers and, as such, they can be considered as a paradigm to provide a mechanistic description of membrane organisation into functional nanodomains. Using solid-state nuclear magnetic resonance (ssNMR) and building upon our initial knowledge of *St*REM1.3 and its C-terminal membrane anchor, we reveal the delicate balance between hydrophobic and electrostatic effects leading up to the protein's characteristic affinity for negatively charged phospholipids. In a divide-and-conquer approach, we describe the impact of *St*REM1.3's C-terminal anchor, its oligomerisation domain and its intrinsically disordered region on membrane structure and dynamics. Furthermore, we tackle the structural features of *St*REM1.3 when associated to nanodomain-mimicking membranes. We reveal that *St*REM1.3 drives nanodomain organisation by concerted lipid-protein and protein-protein interactions.

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Induction of a local phase transition in lipid membranes by monolayer domains of an ordered phase and protein inclusions

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Cell membranes are structurally inhomogeneous systems with separate domains of the liquid-ordered phase, called rafts. Cell membranes and its organelles are asymmetric in the composition of the outer and inner monolayers. Experimental data on asymmetric bilayers are scarce; the effect of asymmetry on the structure and physical properties of the membrane remains insufficiently studied. It is known that the formation of bilayer domains in asymmetric membranes in some cases may be thermodynamically disadvantageous. At the same time, experimental data indicate the mutual influence of opposite monolayers, leading to averaging degrees of the order of monolayers. Using the available data on the structure of the raft boundary, we have investigated the mechanism of the formation of ordered domains under thermodynamically unfavourable conditions induced by the monolayer domain of the ordered phase or protein inclusion. We determine the finite critical size inclusion or monolayer domain that can induce the transition from monolayer to bilayer domain configuration. We find the dependence of this size on the geometry of protein inclusion. We have also investigated the effect of inter-layer coupling and spontaneous curvature of monolayers on this process. We show that under certain conditions a monolayer domain will tend to grow limitlessly without the formation of a bilayer structure. For calculations, we use the theory of elasticity of a continuous liquid crystal medium, adapted to lipid membranes. When calculating the elastic deformations of the membrane, we utilized the fact that the thicknesses of the raft and the surrounding membrane are different. The energy of elastic deformations caused by smoothing the thickness jump at the boundary with the liquid-disordered phase is shown to be the main driving force for the formation of ordered domains under thermodynamically unfavourable conditions.

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Influence of bacteriorhodopsin activity on the structure and fluctuations of a floating lipid bilayer

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Cell membranes exhibit thermal fluctuations that can be enhanced by transmembrane protein activity leading to out-of-equilibrium fluctuations. Active fluctuations have been widely described theoretically [1], but to a lesser extent experimentally.

We will present our recent results on the investigation of out-of-equilibrium fluctuations of phospholipid membranes induced by active transmembrane protein. In this context, bacteriorhodopsin (BR) was used as a light-driven proton pump, whose activity can be triggered by visible light. Model systems such as solid-supported single and floating phospholipid bilayers are suitable to study phospholipid membranes and their interactions. A detergent-mediated incorporation method [2] was adapted to perform the insertion of BR into the phospholipid bilayer at the interfaces, using a sugar- based detergent such as DDM.

The combination of neutron reflectometry, QCM-D, fluorescence microscopy and AFM demonstrated that it is possible to insert BR in model bilayer systems without losing their structural integrity.

Recent specular and off-specular x-ray reflectometry experiments showed an activity of the incorporated proteins through its effect on the structure and on the fluctuations of a double bilayer system. These results open the way to investigate, for the first time, the fluctuation spectrum of a planar membrane-protein system at the nanoscale and to access the physical properties of the system such as bending modulus, surface tension and interaction potential between adjacent membranes.

References:

[1] Prost J., Manneville J.-B., and Bruinsma R., EPJ B, 1:465–480 (1998).
[2] Dezi M., Di Cicco A., Bassereau P. and Lévy D., PNAS, 7276, 110727 (2013).

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Proteins interacting with the phosphopholipid monolayer of lipid droplets regulate lipid hydrolysis

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Intracellular lipid droplets (LD) are ubiquitous organelles with the characteristic features that the hydrophobic core composed of neutral lipids (triacylglycerols and sterol esters) is shielded from the outer, aqueous phase with a phospholipid monolayer. Often, different proteins regulating access to the interior are bound to this phospholipid monolayer. The size of LDs size varies greatly, ranging from the nanometer to micrometer scale. LDs play important roles in energy homeostasis and lipid metabolism, e.g. they act as storage depots for neutral lipids that can be mobilized upon demand. In mammals, energy mobilization from LDs is a highly conserved process. It is mediated by the action of lipases, which degrade the triacylglycerols step-by-step to molecules of glycerol and three fatty acids. Adipose triglyceride lipase (ATGL) localizes to the LD and hydrolyzes triacylglycerol into diacylglycerol and fatty acid. On a protein level, the activity of ATGL is regulated by interaction of ATGL with the stimulatory proteins “alpha/beta hydrolase domain containing 5” (ABHD5) and the inhibitory proteins termed “G0/G1 switch gene 2” (G0S2) and “hypoxia inducible lipid droplet associated (HILPDA)”. Our goal is to understand the activating and inhibitory mechanism of these protein-protein complexes at the LD-water interphase at molecular detail. Therefore, we apply biophysical, structural and biochemical approaches with native LDs or different nano-structures as LD mimic. We will present ongoing structural characterization of the inhibitory protein G0S2 in dodecylphosphocholine (DPC) micelles and circularized nano-discs as LD mimics. Furthermore, we present the solution-state NMR structure of a peptide derived from the co-activator ABHD5 bound to (DPC) micelles. This peptide anchors ABHD5 to the LD, which is strictly required for co-activation of ATGL.

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Lipid dynamics in nanodiscs probed by solid-state NMR

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Nanodiscs provide a novel tool to embed membrane proteins into native-like lipid bilayers environment allowing high-resolution biophysical studies while maintaining their structure and function. Nanodiscs are composed of a small patch of a lipid bilayer surrounded by a membrane scaffold protein (MSP) creating a belt like shape. Different scaffold proteins can be used resulting in nanodiscs of various diameters and properties. It still remains unclear how lipids dynamically organize inside a nanodisc scaffold compared to their behavior in cellular membranes.

Recently, we compared lipid dynamics in nanodiscs and in liposome [1]. In the present work, we use deuterium solid-state NMR spectroscopy to investigate the organization and dynamic of lipids in various nanodisc constructs with an average size from 8 to 13 nm, assembled in different lipids composition (DMPC, DPPC). The thermotropic behavior, ordering and thickness of lipid membrane in nanodiscs were assessed. The results point out highly variable gel-to-fluid phase transitions and lipid ordering as a function of the nanodisc diameter, suggesting that the scaffold design have a profound impact of the dynamic organization of the lipid bilayer.

1. Martinez, D. *et al., ChemPhysChem* 18, 2651-2657 (2017)

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Lipid bilayer stabilization for the correct exposure of MPER epitope in peptide-liposome vaccines targeting the gp41 subunit of HIV-1 Env

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Antibodies targeting the C-terminal subdomain of the membrane-proximal external region (C-MPER), which is connected to the transmembrane domain (TMD) of the envelope glycoprotein (Env), achieve near-pan neutralization of HIV-1. Thus, recreating the structure that generates C-MPER-targeting antibodies is a major goal of the rational development of HIV vaccines. Here, to assess the TMD section as a potential membrane integral scaffold for the MPER epitope, we first attempted to reconstitute two sequences into lipid bilayers, namely, MPER-TMD1 (gp41 residues 671-700) and MPER-TMD2 (gp41 residues 671-709). Infrared spectroscopy determinations as a function of the cholesterol content, and immunochemical assays revealed that, following our method, MPER-TMD1, but not MPER-TMD2, could be effectively reconstituted adopting a main helical conformation, and exposing the MPER epitope on the surface of phospholipid bilayers. The structural features including the angle of insertion and the effects on membrane order and architecture were further correlated with antigenicity and immunogenicity of liposome formulations containing MPER-TMD1 transmembrane helices. Overall, our results suggest new approaches to the design of effective immunogens directed against MPER.

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Solid-state NMR studies of dynamic mitochondrial protein-lipid nanocomplexes

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Organelle-specific lipid species play critical roles in cellular signalling events as well as the trafficking and targeting of numerous proteins. Frequently, these processes are mediated by lipid-binding or lipid-recognition domains that are soluble and non-membrane bound in absence of their lipid targets. The "peripheral" interactions between lipid bilayers and such conditional membrane-associated proteins remain difficult to probe on the molecular level, even as huge progress is made in tackling integral membrane proteins. One primary challenge stems from the dynamics and heterogeneity inherent in many of these nano-scopic protein-lipid complexes. In recent published and unpublished work, we employ advanced solid-state NMR (ssNMR) methods to probe the structure and dynamics of two different cardiolipin-protein complexes involved in mitochondrial fission and apoptosis. Using multinuclear ssNMR we observe the lipid-specific membrane binding of the dynamic and disordered "variable domain" of dynamin-related protein Drp1. Membrane curvature and non-bilayer phases are instrumental for the function of this mitochondrial protein, which mediates key molecular events in mitochondrial membrane fission and mitochondrial dynamics. We use ssNMR's ability to detect and characterise the structure and dynamics of the lipid bilayer membrane, and especially non-bilayer phases, to experimentally probe for curvature changes in the protein-bound membrane.

In parallel, we have been using ssNMR to probe how mitochondrial cytochrome c binds cardiolipin (CL) and facilitates its peroxidation in presence of mitochondrial reactive oxygen species (ROS). This process of great interest due to the fact that exposure and peroxidation of cardiolipin are pivotal signals in mitochondrial apoptosis, with implications for cancer treatments and neurodegenerative diseases. With the aid of ssNMR and complementary biophysical studies, we gain new insights into the way that the peripherally bound protein engages nanoclusters of CL lipids, which in turn act as dynamic regulators that control the CL-specific lipid oxidation activity of this pro-apoptotic protein-lipid complex. In both cases, a toolkit of advanced ssNMR methods is deployed to access unique structural and dynamical information for both the fluid membrane and the surface-bound protein, despite the dynamics and disorder that is inherent in these mitochondrial protein-lipid nanocomplexes.

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CD300a receptor promotes HIV-cell fusion through the interaction with phosphatidylserine and phosphatidylethanolamine

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The CD300a surface receptor promotes the infection of host cells by several enveloped viruses, such as Dengue, West Nile, Yellow Fever and Chikungunya viruses. The ligands of CD300a, phosphatidylserine (PS) and phosphatidylethanolamine (PE), are exposed in the outer leaflet of the plasma membrane of HIV-infected cells and they are also present in the HIV envelope. It has been described that mutations in the IgV domain of the receptor prevent the binding to PS and PE and abrogate the increase of CD300a-mediated Dengue virus infection. We have previously observed that CD4+ T lymphocytes from healthy donors expressing CD300a were significantly more susceptible to HIV *in vitro* infection. The objective of this work was to investigate the specific role of CD300a molecule and its ligands PS and PE in HIV infection, mainly in the fusion stage of viral entry. Accordingly, we first infected CD300a-transfected TZM-bl cells with the $HIV_{JR\text{-}FL}$ pseudovirus expressing Gag-GFP for 48 hours, and subsequently measured the infection by fluorescence microscopy. In this assay, we used three compounds: A01, an inhibitor of phospholipid scrambling, and MFG-E8 and cinnamycin, which sequester PS and PE respectively from receptor binding. We have observed a decrease in the number of HIV-infected cells after the addition of these three compounds, in particular in cinnamycin-treated cells. Furthermore, we also transfected TZM-bl cells with wild type and mutant CD300a (D95A and F39A). Then, we infected these cells with $HIV_{JR\text{-}FL}$ during 90 min and we performed a β-lactamase assay in order to study the level of HIV-cell fusion. Our results showed a higher level of HIV-cell fusion in cells expressing wild type CD300a than in non-transfected cells or cells expressing mutated CD300a. We conclude that CD300a promotes HIV-cell fusion, which depends on the interaction with its ligands PS and PE. Therefore, CD300a could be a potential therapeutic target in HIV infection.

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Niklas Beata; Poland
Nilsson Daniel; Sweden
Nishio Takashi; Japan
Noe Frank; Germany
Nogales Eva; United States
Nowak Wieslaw; Poland
Nunes Rafael; Portugal
Nuñez Viadero Eider; Spain
Nyholm Thomas Kaj Mikael; Finland
Oh Han Bin; Korea (South, Republic Of)
Oh Yoojin; Austria
Okabe Kohki; Japan
Okazaki Keiichi; Japan
Okumura Hisashi; Japan
Oliveira Ana Sofia Fernandes De; United Kingdom
Ollila Samuli; Finland
Orozco Modesto; Spain
Ortega Cruz Maria; Spain
Ortega Quintanilla Gabriel; United States
Ostroumova Olga; Russian Federation
Oz-Arslan Devrim; Turkey
P. Pelaez Raul; Spain
Pabst Georg; Austria
Páez Pérez Miguel; United Kingdom
Paiva Paiva Daisy; Germany
Palacios Juan; Spain
Panaitov Gregory; Germany
Pancaldi Vera; France
Parisse Pietro; Italy
Park Su-Chan; Korea (South, Republic Of)
Park Jeong-Man; Korea (South, Republic Of)
Parrondo Parrondo Juan Manuel; Spain
Pedrera Puentes Lohans; Germany
Perach Michal; Israel
Pereira Manuela; Portugal
Pereira-Leite Catarina; Portugal
Perez Gil Jesús; Spain
Perez Perez Ruben; Spain
Perez Katia Regina; Brazil
Pérez Del Valle Blanca; Spain
Pérez Illana Marta; Spain
Pérez Lara Francisco Ángel; Germany
Pérez Mar; Spain
Perthold Jan Walther; Austria
Perzanowska Olga; Poland
Pesanelli Jennifer; United States
Petrauskas Vytautas; Lithuania
Petrenčáková Martina; Slovakia
Piechocka Izabela; Poland
Pimenta Gabriela; Germany
Pinigin Konstantin; Russian Federation
Pinto Giulia; Italy
Pizzuto Malvina; Belgium
Plaza Ismael; Spain
Plochberger Birgit; Austria
Pogodaev Aleksandr; Netherlands
Pohl Peter; Austria
Pohl Elena; Austria
Pollack Lois; United States
Pontani Lea-Laetitia; France
Portillo Castellano Yeimar; Germany
Pourjafar Dehkordi Danial; Germany
Prah Alja; Slovenia
Pricolo Maria; Spain
Prieto Manuel; Portugal
Pupkis Vilmantas; Lithuania
Quinn Steven; United Kingdom
R. Pulido Carlos; Spain
Rabdano Sevastyan; Russian Federation
Radotic Hadzi Manic Ksenija; Serbia
Ramaswamy Sriram; India
Ramírez Aportela Erney; Spain
Ramos Martin Francisco; France
Rao Madan; India
Ray Owens; United Kingdom
Redondo-Morata Lorena; France
Reglinski Katharina; United Kingdom
Reidelbach Marco; Finland
Reifs Carmona Antonio; Spain
Reina Francesco; Germany
Reis Ana; Portugal
Reis Pedro; Portugal
Rico Felix; France
Ries Jonas; Germany
Riske A. Karin; Brazil
Ritort Felix; Spain
Rittinger Katrin; United Kingdom
RIVAS CABALLERO GERMÁN; Spain
Rivera Esperanza; Spain
Robalo Joao; Germany
Robinson Tom; Germany
Robles Michel; Spain
Roca-Cusachs Roca-Cusachs Pere; Spain
Rodriguez Moraga Nely; France
Rodríguez Arriaga Laura; Spain
Roig Merino Sara Raquel; Netherlands
Rossi Giulia; Italy
Rossi Michele; Italy
Rousso Rousso Itay; Israel
Rudden Lucas; United Kingdom
Rueda David; United Kingdom
Ruiz Ortiz Irene; Spain
Ruprecht Verena; Spain
Ruysschaert Jean-Marie; Belgium
Ryder Alan; Ireland
S. Baptista Mauricio; Brazil
S. Vignoli Muniz Gabriel; Brazil
Saad Ahmad; France
Sabanes Zariquiey Francesc; United Kingdom
Sachiko Aida; Japan
Saeedimasine Marzieh; Sweden
Safran Safran Samuel; Israel
Sagues Francesc; Spain
Sahli Line; France
Sakai Yuji; Japan
Sakamoto Yoneda Juliana; Brazil
Sakuta Hiroki; Japan
Salassi Sebastian; Italy
Salbreux Guillaume; United Kingdom
Salgado Salgado Gilmar; France
Salgado Jesús; Spain
Salo Janne;
Salvat Natalia; Spain
Samatanga Brighton; Germany
Sanchez-Barrena Maria Jose; Spain
Santiago José Antonio; Mexico
Santos Nuno; Portugal
Santos Seiça Ana Filipa; France
Sanz Maria; Spain
Saric Andela; United Kingdom
Sarkar Niladri; Netherlands
Sarmento Maria J.; Czech Republic
Sasai Masaki; Japan
Sassi Paola; Italy
Sastre Judit; Spain
Satkauskas Saulius; Lithuania
Sato Ryuma; Japan
Sato Yusuke; Japan
Savino Silvana; Italy
Sawczyc Henry; United Kingdom
Sayed Ahmed; Germany
Scarcelli Giuliano; United States
Schahl Adrien; France
Scheerer David; Germany
Schenkel Mathias; Germany
Schiessel Helmut; Netherlands
Schiopu Irina; Romania
Schlotter Til; Switzerland
Schmidt Christoph; United States

Schneider Falk; United Kingdom
Schromm Andra; Germany
Schwarz Ulrich; Germany
Schwebs Marie; Germany
Sébastien Bonhommeau; France
Sebastijanovic Aleksandar; Slovenia
Seddon John; United Kingdom
Sedlak Erik; Slovakia
Seevaratnam Dushanth; United Kingdom
Seifert Udo; Germany
Semeraro Enrico Federico; Austria
Sengupta Kheya; France
Seoane Mateo; Spain
Serpersu Engin; United States
Serrano Israel; Spain
Sewell Sewell Bryan; South Africa
Sewell Bryan; South Africa
Sezgin Erdinc; United Kingdom
Shadfar Zahra; New Zealand
Shalaby Raed; Germany
Shen Yi; United Kingdom
Shimaya Takuro; Japan
Siebenmorgen Till; Germany
Silva Itala; Portugal
Simon Menig; Germany
Simunovic Mijo; United States
Singaraju S Gayathri; India
Singh Anubhuti; Germany
Siu Ho Wah; Germany
Smiatek Jens; Germany
Smith Paul; United Kingdom
Šnipas Mindaugas; Lithuania
Snoj Tina; Slovenia
Soares Claudio; Portugal
Sokolov Valery; Russian Federation
Sophie Sacquin-Mora; France
Sophie Lecomte; France
Sophie Cribier; France
Sot Sanz Jesus; Spain
Sotodosos Laura; Spain
Sotolongo Bellón Junel; Germany
Soudherpally Thirupathi Reddy; Brazil
Sousa F. Carla; Portugal
Souto Martins Ana; Portugal
Souza Câmara Amanda; Germany
Srinivasan Sriraksha; Switzerland
Srivastava Amit; United Arab Emirates
Stadler Andreas; Germany
Stavans Stavans Joel; Israel
Steinem Claudia; Germany
Steiner-Browne Faisca Marina; Ireland
Steplewska Agata; Ireland
Stepp Willi; Germany
Stiel Andre C.; Germany
Stockner Thomas; Austria
Strancar Janez; Slovenia
Strofaldi Alessandro; Ireland
Strutt Robert; United Kingdom
Stulz Anja; Germany
Suarez Gonzalez Lucía; Spain
Suay-Corredera Carmen; Spain
Subjakova Veronika; Slovakia
Subramanian Madhumalar; Germany
Sudarikova Anastasia; Russian Federation
Sugihara Kaori; Switzerland
Suginta Wipa; Thailand
Sule Kevin; Canada
Sulkowska Ida Joanna; Poland
Sung Bong June; Korea (South, Republic Of)
Szatmari David Zoltan; Hungary
Szavits Nossan Juraj; United Kingdom
Szöllösi Dániel; Austria
Tabata Kazuhito; Japan
Tajkhorshid Tajkhorshid Emad; United States
Takarada Masaharu; Japan
Takeuchi Yohei; Japan
Talafová Veronika; Slovakia
Tamás Fekete; Hungary
Tankovskaia Svetlana; Russian Federation
Tapia Rojo Rafael; United States
Tardin Catherine; France
Tempra Carmelo; Czech Republic
Teng-Broug Valerie; Netherlands
Testa Ilaria; Sweden
Thacker Dev; Sweden
Thierry Bizebard; France
Tinao Nieto Berta; Spain
Tognato Riccardo; United Kingdom
Tomaskova Natasa; Slovakia
Tombolesi Niki; Italy
Tonkin Louise; United Kingdom
Torosyan Anahit; Switzerland
Torra Joaquim; Spain
Torreño Juan; Spain
Tosatto Laura; Italy
Toth Katalin; Germany
Träger Jennica; Germany
Trowitzsch Simon; Germany
Trylska Joanna; Poland
Tsushima Satoru; Germany
Turcu Ioan; Romania
Tych Katarzyna; Germany
Tymchenko Ekaterina; Russian Federation
Ukleja Marta; Spain
Ulloa Severino Luisa; Canada
Uriati Eleonora; Italy
Ushakov Dmitry; United Kingdom
V S Pillai Visakh; Ireland
Valbuena Alex; Spain
Valencia Alfonso; Spain
Valenzuela Gómez Fernando; Spain
Valpuesta José María; Spain
Van Der Veen Rozemarijn; Netherlands
Van Der Wel Patrick; Netherlands
Van Der Wijst Jenny; Netherlands
Van Raaij Mark J.; Spain
Van Zanten Camila; Ireland
Vaneev Aleksandr; Russian Federation
Vanik Vladimir; Slovakia
Varlamova Ekaterina; Russian Federation
Vattulainen Ilpo; Finland
Velez Marisela; Spain
Venturini Juárez Víctor; Spain
Venturini Valeria; Spain
Vequi-Suplicy Cintia; United Kingdom
Vera Gómez Andrés Manuel; Germany
Vera Lillo Javier; Spain
Vetri Valeria; Italy
Victor Bruno; Portugal
Viela Felipe; Belgium
Vignolini Tiziano; Italy
Vila-Viçosa Diogo; Portugal
Villalba Riquelme Eva María; Spain
Villegas Hernández Sandra; Spain
Villegas Lupe; Spain
Vinals Camallonga Javier; United Kingdom
Vitkova Vitkova Victoria; Bulgaria
Vivas Palomares Cristofher Victor; Brazil
Vleugel Mathijs; Germany
Volovik Marta; Russian Federation
Von Der Heydt Alice; United Kingdom
Voth Gregory; United States
Vrancken Jeroen; Belgium
Vrel Jean-Patrick; France
Vuletić Tomislav; Croatia
Walczewska Szewc Katarzyna; Poland
Wang Wei; China
Wang Jun; China
Wang Weiqiang; Spain
Wang Xiaobo; United Kingdom
Watts Anthony; United Kingdom
Weber Andreas; Austria
Weber Florian; Austria
Weigt Weigt Martin; France
Weikum Julia; Denmark
Wilkinson Tony; United Kingdom
Willis Simon; United Kingdom
Wingbermühle Sebastian; Germany
Winkler Pamina; Spain
Winter Paul Gregory; United Kingdom
Woodhouse Vanessa; United Kingdom
Xin Yang; Germany
Xu Stephanie; Australia
Yamauchi Masataka; Japan
Yang Yu; Germany
Yang Xiaorong; China
Young Gavin; United Kingdom
Yu Zhiwu; China
Zaburdaev Vasily; Germany
Zacharopoulou Maria; United Kingdom
Zarkeshian Parisa; Canada
Zdorevskyi Oleksii; Ukraine
Zeraik Ana Eliza; Brazil
Zhang Zhuqing; China
Zhang Tongfei; United Kingdom
Zhu Zhu Cheng; United States
Zimova Lucie; Czech Republic
Zoldak Gabriel; Slovakia
Zoni Valeria; Switzerland
Zorrilla López Silvia; Spain
Zubriene Asta; Lithuania