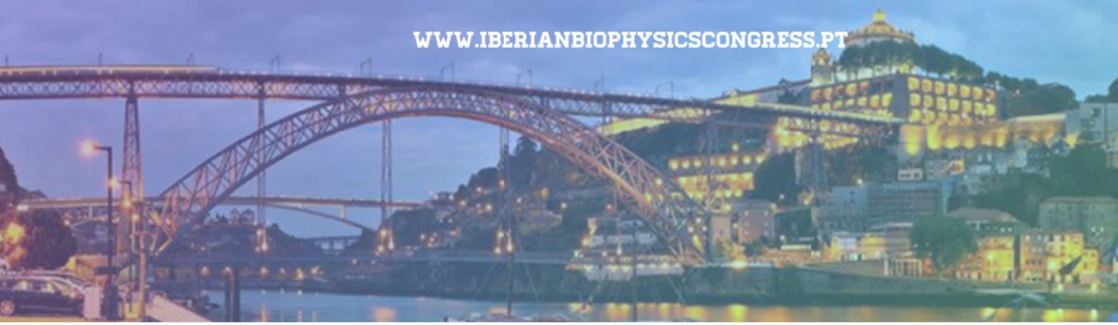
5TH INTERNATIONAL IBERIAN BIOPHYSICS CONGRESS

15-17TH JUNE 2016

PORTO, PORTUGAL

WWW.IBERIANBIOPHYSICSCONGRESS.PT



SYMPOSIA

- Protein Structure, Folding and Dynamics
- Membranes and Protein-Lipid Interactions
- Receptors, Channels and Transporters
- Cell and Tissue Biophysics
- Nanobiophysics and Molecular Recognition
- Computational Biophysics
- Bioenergetics
- Supramolecular Complexes and Biospectroscopies

CONTACT E-MAIL: GERAL@IBERIANBIOPHYSICSCONGRESS.PT

Promotor

LISBONPH
EMPRESA JÚNIOR DA FFUL

SPBf
SOCIEDADE PORTUGUESA DE BIOFÍSICA

SBE
Sociedad de Biofísica de España


SPBf
SOCIEDADE
PORTUGUESA
DE BIOFÍSICA


SBE
Sociedad de Biofisica de España


EBSA
Biophysics in Europe


Sociedade Portuguesa de Bioquímica
SPB


FCT
Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA EDUCAÇÃO E CIÊNCIA


SARSTEDT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High quality. High impact.


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ORGANIZING COMMITTEE

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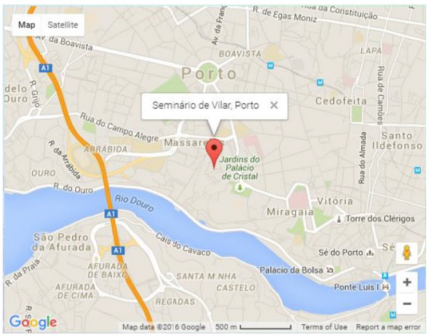
VENUE
SEMINÁRIO DE VILAR
PORTO

Near by:

- **Palácio de Cristal**
- **Torre dos Clérigos**
- **Casa da Música**
- **Faculdade de Letras – Universidade do Porto**
- **Faculdade de Arquitectura – Universidade do Porto**
- **Faculdade de Ciências – Universidade do Porto**
- **Faculdade de Farmácia – Universidade do Porto**
- **ICBAS – Universidade do Porto**



How to get there?

- **Subway – Casa da Música Station**
- **BUS – Palácio stop**
  - **STCP**
    - **200,201,207**
    - **208,302,303**
    - **501,507,601**
    - **12M,13M,ZM**
  - **Resende**
    - **104 and 119**

CONFERENCE DINNER: RESTAURANT "BJEKAS" PORTO

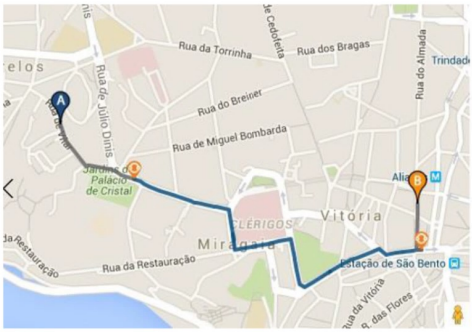
Map:

Rua de Almada, 160, Porto

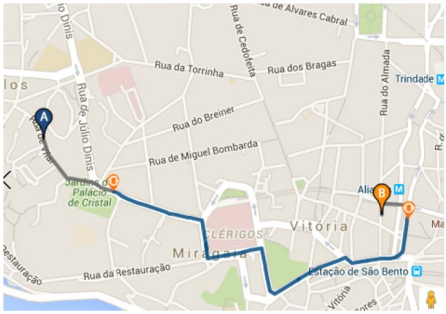
Bus option:

Buses with numbers 200, 201, 207, at stop "Palácio", in front of Palácio de Cristal, 5 min walk distance from the Conference:

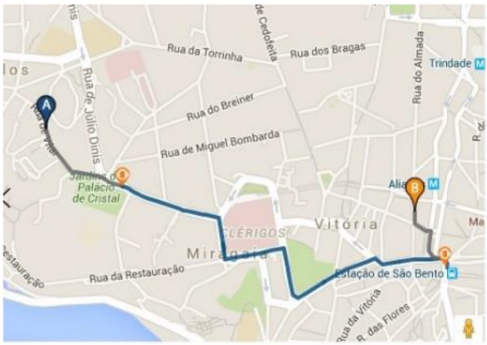
200 (direction: "Bolhão"), leave at stop: "Pr. da Liberdade", walk 7min



201(direction: "Aliados"), leave at stop: "Av. Aliados", walk 3min



207 (direction: "Campanhã"), leave at stop:" "Est. S. Bento", walk 4min



On foot:

PROGRAMME

15th June 2016

10:30 — Satellite Symposium: New and Notable

11:30 — Coffee Break

11:50 — New and Notable (cont.)

12:50

14:30 — Opening

14:50 — Plenary Lecture 1
Wolfgang Baumeister

15:50 — Symposia 1 & 2
Membranes and Protein-Lipid Interactions
Computational Biophysics
2 Invited Lectures

16:40 — Coffee Break

17:10 — Symposia 1 & 2 (cont.)
Membranes and Protein-Lipid Interactions
Computational Biophysics
2 Invited Lectures
+ 4 Short Talks

19:00 — Reception

16th June 2016

09:00 — Symposia 3 & 4
Bioenergetics
Cell and Tissue Biophysics
2 Invited Lectures
+ 4 Short Talks

10:50 — Coffee Break

11:20 — Symposia 3 & 4 (cont.)
Bioenergetics
Cell and Tissue Biophysics
2 Invited Lectures

12:10 — Plenary Lecture 2
Annalisa Pastore

13:10 — Lunch Break

14:30 — Symposia 5 & 6
Receptors, Channels and Transporters
Supramolecular Complexes and Biospectroscopies
2 Invited Lectures
+ 4 Short Talks

16:20 — Coffee Break

16:50 — Symposia 5 & 6 (cont.)
Receptors, Channels and Transporters
Supramolecular Complexes and Biospectroscopies
2 Invited Lectures

17:40 — Poster Party 1

19:30 — Gen. Assemb. SPBf/SBE

17th June 2016

09:00 — Symposia 7 & 8
Protein Structure, Folding and Dynamics
Nanobiophysics and Molecular Recognition
2 Invited Lectures
+ 4 Short Talks

10:50 — Coffee Break

11:20 — Symposia 7 & 8 (cont.)
Protein Structure, Folding and Dynamics
Nanobiophysics and Molecular Recognition
2 Invited Lectures

12:10 — Plenary Lecture 3
Lino Ferreira

13:10 — Lunch Break

14:30 — Poster Party 2

16:00 — Prizes Session

17:30 — Plenary Lecture 4
Luis Liz-Marzan

18:15

18:30 — Closing Ceremony

20:00 — Conference Dinner

Invited Lectures- 20 min + questions
Short Talks- 10 min + questions

WEDNESDAY, 15TH JUNE

10.00 **Satellite Symposium: New and Notable – Room 105**
Chairs: **Cláudio Soares** (Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Portugal) and **María García Parajo** (ICFO, Barcelona, Spain)

10.00 **Catarina Coelho** (FCT-UNL, Portugal) – "The Crystal Structure of human Aldehyde Oxidase and Implications towards Drug Metabolism"

10.25 **Pedro de Pablo** (UAM, Madrid, Spain) – "Visualizing genome release during the mechanical unpacking of single viruses"

10.50 **Filomena Carvalho** (IMM, U Lisboa, Portugal) – "Atomic force microscopy as a tool to evaluate the risk for cardiovascular diseases in patients"

11.15 **Coffee break**

11.35 **Satellite Symposium: New and Notable (Cont.) – Room 105**
Chairs: **Cláudio Soares** (Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Portugal) and **María García Parajo** (ICFO, Barcelona, Spain)

11.35 **Pere Roca-Cusachs** (IBC, IBEC, Barcelona, Spain) – "Mechanical regulation of a molecular clutch defines cell sensing of matrix rigidity"

12.00 **Inês Cardoso Pereira** (ITQB, UNL, Portugal) – "A protein trisulfide is the product of dissimilatory sulfite reduction"

12.25 **Noa Martín-Cófreces** (Hospital la Princesa & CNIC, Madrid, Spain) – "Aurora A drives early signalling and vesicle dynamics during T-cell activation"

14.30 **Opening**

14.50 **Plenary Lecture 1 - Room 105**
Chair: **Nuno C. Santos** (Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Portugal)

Wolfgang Baumeister (Max Planck Institute of Biochemistry, Martinsried, Germany) – "The Challenge of Doing Structural Biology *in situ*"

15.50 **Symposium 1: Membranes and Protein-Lipid Interactions – Room 105**
Chairs: **Juan C. Gómez-Fernández** (Universidad de Murcia, Spain) and **Paula Gameiro** (Requimte, Faculdade de Ciências, Universidade do Porto, Portugal)

15.50 **Banafshe Larijani** (UBF, Bilbao, Spain) – "Effects of Phosphoinositides and their Derivatives on Membrane Morphology and Function"

16.15 **Maria João Moreno** (U Coimbra, Portugal) – "Interaction of bile salts with model membranes mimicking the gastrointestinal epithelium: a study by isothermal titration calorimetry"

15.50 Symposium 2: Computational Biophysics – Room 022
Chairs: **Armindo Salvador** (Centro de Neurociências e Biologia Celular, Universidade de Coimbra, Portugal) and **Miquel Pons** (Universidad de Barcelona, Spain)

15.50 **Federico Gago** (U Alcala Henares, Spain) – "Mammalian tubulin as a paradigm for the binding of natural products to a relevant anticancer target"

16.15 **Isabel Rocha** (U Minho, Braga, Portugal) – "Metabolic Systems Biology: tools for model-based analysis and design of microbial systems"

16.40 Coffee Break

17.10 Symposium 1 (cont.): Membranes and Protein-Lipid Interactions – Room 105
Chairs: **Juan C. Gómez-Fernández** (Universidad de Murcia, Spain) and **Paula Gameiro** (Requimte, Faculdade de Ciências, Universidade do Porto, Portugal)

17.10 **Maria Rosário Domingues** (U Aveiro, Portugal) – "Understanding lipid protein adducts using mass spectrometry based approaches"

17.35 **Ismael Mingarro** (U Valencia, Spain) – "Towards membrane protein de novo design"

18.00 **Cláudia Nunes** (UCIBIO, REQUIMTE, Faculdade de Farmácia, Universidade do Porto, Portugal) – "X-ray diffraction studies to study drug-membrane interactions"

18.15 **Antonio L. Egea-Jimenez** (Institut Paoli-Calmettes, Marseille, France; Department of Human Genetics, Leuven, Belgium) – "High-affinity coincident detection of Frizzled 7 and PIP2 by the PDZ2 domain of syntenin supports Frizzled 7-syntenin trafficking and signaling."

18.30 **Sílvia C. Lopes** (UCIBIO, REQUIMTE, Faculdade de Ciências da Universidade do Porto, Portugal) – "Cardiolipin's importance in mitochondrial membrane model construction. mitochondria membrane, cardiolipin, dynamic light scattering, fluorescence anisotropy"

18.45 **Esperanza Rivera-de-Torre** (Facultades de Química y Biología, Universidad Complutense, Madrid, Spain) – "Synergistic action of actinoporin isoforms from the same sea anemone species assembled into functionally active heteropores"

17.10 Symposium 2 (cont.): Computational Biophysics – Room 022

17.10 **Symposium 2 (cont.): Computational Biophysics – Room 022**
Chairs: **Armindo Salvador** (Centro de Neurociências e Biologia Celular, Universidade de Coimbra, Portugal) and **Miquel Pons** (Universidad de Barcelona, Spain)

17.10 **José Villalaín** (UMH, Elche, Spain) – "Molecular dynamics of biological membranes. What can we get?."

17.35 **Pedro Fernandes** (U Porto, Portugal) – "Computational Proteomics Cocktail"

18.00 **Marcelo Tozo de Araujo** (Institut für Physik, Universität Augsburg, Augsburg, Germany) – "Coupled Particles in a Confined Geometry"

18.15 **Diana Lousa** (Universidade Nova de Lisboa, Oeiras, Portugal) – "Structure and activity of the influenza fusion peptide: A combined simulation and experimental approach"

18.30 **Manuel N. Melo** (University of Groningen, The Netherlands) – "Simulation of a partly atomistc, partly coarse-grain membrane channel"

18.45 **Miguel Machuqueiro** (Universidade de Lisboa, Lisboa, Portugal) – "Computational methods to calculate pKa values at the water/membrane interface"

19.00 **Reception**

THURSDAY, 16TH JUNE

9.00 **Symposium 3: Bioenergetics – Room 022**

Chairs: **Francisco Barros** (Universidad de Oviedo, Spain) and **Manuela Pereira** (Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Portugal)

9.00 **Milagros Medina** (U Zaragoza, Spain) – "The human Apoptosis Inducing Factor: key molecular determinants in cellular respiration, programed cell death and neurodegenerative disorders"

9.25 **Jose Manuel Cuezva** (U Autonoma, Madrid, Spain) – "ATPase Inhibitory Factor 1 (IF1): A main driver of metabolic reprogramming and nuclear signaling"

9.50 **Irene Díaz-Moreno** (cicCartuja, Universidad de Sevilla-CSIC, Spain) – "How Molecules Form Transient Complexes in Photosynthesis and Respiration"

10.05 **A Sofia F Oliveira** (Instituto de Tecnologia Química e Biológica - Universidade Nova de Lisboa, Portugal) – "Coupling between protonation and conformation in cytochrome c oxidase: Insights from Constant-pH MD simulations"

10.20 **Livier de la Rosa** (Institute of Biochemistry and Genetics of the Cell, Université Bordeaux, France) – "Control of mitochondrial biogenesis by free heme in the yeast S. Cerevisiae"

10.35 **Filipe M Sousa** (Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Portugal) – "The role of the strictly conserved E172 in the reaction mechanism of type-II NADH:quinone oxidoreductase of S. aureus"

9.00 **Symposium 4: Cell and Tissue Biophysics – Room 105**

Chairs: **Jesús Salgado** (Universidad de Valencia, Spain) and **Nuno C. Santos** (Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Portugal)

9.00 **Cláudio Franco** (IMM, U Lisboa, Portugal) – "Endothelial cell dynamics in vascular morphogenesis"

9.25 **Jerome Solon** (CRG, Spain) – "Contracting, spreading, folding: How forces shape biological tissues during development"

9.50 **Ana Ester Ventura** (iMed.ULisboa, Universidade de Lisboa, Lisboa, Portugal) – "Ceramide-gel domains in living cells: understanding the role of membrane biophysical properties in cell (patho)physiology"

10.05 **Carlo Manzo** (The Barcelona Institute of Science and Technology, Barcelona, Spain) – "The role of spatiotemporal heterogeneity in the regulation of cellular function"

10.20 **Pedro Gouveia** (CNC, University of Coimbra; Coimbra, Portugal) – "Flexible nanofilms coated with aligned piezoelectric microfibers preserves engineered cardiac tissue's function"

10.35 **Patricia R Pitrez** (Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal) – "Micropatterned substrates to accelerate pathological smooth muscle cells aging"

10.50 Coffee Break

11.20 Symposium 3 (cont.): Bioenergetics – Room 022

Chairs: **Francisco Barros** (Universidad de Oviedo, Spain) and **Manuela Pereira** (Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Portugal)

11.20 **João Laranjinha** (CNC, U Coimbra, Portugal) – "Neurometabolic coupling in hippocampus mediated by neuronal-derived nitric oxide"

11.45 **Ricardo Louro** (ITQB, UNL, Portugal) – "Exploration of the electrical contact between electroactive bacteria and conducting solids relevant for the optimization of microbial electrochemical technologies"

11.20 Symposium 4 (cont.): Cell and Tissue Biophysics – Room 105

Chairs: **Jesús Salgado** (Universidad de Valencia, Spain) and **Nuno C. Santos** (Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Portugal)

11.20 **Ralf Richer** (CIC biomaGUNE, Spain) – "Hyaluronan – from a simple polysaccharide to biological materials/interactions with remarkable properties"

11.45 **Rui Travasso** (U Coimbra, Portugal) – "Mechanics of Sprouting Angiogenesis in Tumors"

12.10 Plenary Lecture 2 – Room 105

Chair: **María García Parajo** (ICFO, Barcelona, Spain)

Annalisa Pastore (King's College London, UK)

13.10 **Lunch Break**

14.30 **Symposium 5: Receptors, Channels and Transporters – Room 105**

Chairs: **Graça Soveral** (iMed.ULisboa, Faculdade de Farmácia, Universidade de Lisboa, Portugal) and **Jose Antonio Lamas** (Universidade de Vigo, Spain)

14.30 **Emília Quinta-Ferreira** (CNC, U Coimbra, Portugal) – "Fluorescence and computational studies of zinc changes in hippocampal mossy fiber synapses"

14.55 **Manuel Palacin** (U Barcelona, Spain) – "The structure of Heteromeric Amino acid Transporters: getting there"

15.10 **Andreia Mósca** (iMed.ULisboa, Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal) – "Exploring the pH gating mechanism of human aquaporin-7"

15.25 **Antonio Reboreda** (Laboratory of Neuroscience, CINBIO, University of Vigo) – "Bradykinin modulation of trek-2 currents in mouse superior cervical ganglion neurons"

15.40 **Nádia Rei** (Institute of Pharmacology and Neurosciences, Faculty of Medicine, Instituto de Medicina Molecular, University of Lisbon, Portugal) – "Molecular characterization of Adenosine and VEGF receptors in SOD1(G93A) mouse model of Amyotrophic Lateral Sclerosis (ALS)"

15.55 **Sara R Roig** (Molecular Physiology Laboratory, Institut de Biomedicina (IBUB), Universitat de Barcelona, Barcelona, Spain) – "Deciphering the KCNE4 function within the Kv1.3 channelosome"

14.30 **Symposium 6: Supramolecular Complexes and Biospectroscopies – Room 022**

Chairs: **Antonio Rey Gayo** (Universidad Complutense, Madrid, Spain) and **Fábio Fernandes** (Centro de Química-Física Molecular and IN-Instituto de Nanociência e Nanotecnologia, IST, ULisboa, Lisbon, Portugal)

14.30 **José María Valpuesta** (CNB-CSIC, Madrid, Spain) – "Structural characterisation of the chaperone Hsp70 folding network"

14.55 **Smilja Todorovic** (ITQB, UNL, Portugal) – "Surface enhanced vibrational spectroscopic view of DNA repair"

15.10 **Joan-Ramon Daban** (Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Barcelona, Spain) – "Nanomechanical data validates a multilayer supramolecular model that explains the shape, dimensions, and cytogenetic properties of metaphase chromosomes"

15.25 **Sofia R. Pauleta** (UCIBIO, REQUIMTE, Dept. Química, FCT-UNL, Portugal) – "Characterization of Proteins Involved the ORP Complex of Desulfovibrio vulgaris Hildenborough"

15.40 **Didier Barradas Bautista** (Barcelona Supercomputing Center) – "A strategy to mix different biophysical scoring functions fusing the ranking power of FFT-based Protein-protein Docking protocols"

15.55 **Cláudia S. Nóbrega** (UCIBIO, REQUIMTE, Dept. Química, FCT-UNL, Portugal) – "Neisseria gonorrhoeae Cytochrome c Peroxidase and the electron donor, LAz, a Lipid-modified Azurin"

16.20 Coffee Break

16.50 Symposium 5 (cont.): Receptors, Channels and Transporters – Room 105

Chairs: **Graça Soveral** (iMed.ULisboa, Faculdade de Farmácia, Universidade de Lisboa, Portugal) and **Jose Antonio Lamas** (Universidade de Vigo, Spain)

16.50 **Vicente Aguilella** (U Castellon, Spain) – "Entropy–enthalpy compensation at the single channel level"

17.15 **Jorge Azevedo** (IBMC, U Porto, Portugal) – "Translocation of proteins across the peroxisomal membrane"

16.50 Symposium 6 (cont.): Supramolecular Complexes and Biospectroscopies – Room 022

Chairs: **Antonio Rey Gayo** (Universidad Complutense, Madrid, Spain) and **Fábio Fernandes** (Centro de Química-Física Molecular and IN-Instituto de Nanociência e Nanotecnologia, IST, ULisboa, Lisbon, Portugal)

16.50 **Carlos Salgueiro** (FCT-UNL, Portugal) – "Engineering of electron transfer components for optimal e-/H+ energy transduction processes: a foundation to improve microbial fuel cells performance"

17.15 **Fábio Fernandes** (Centro de Química-Física Molecular and IN-Instituto de Nanociência e Nanotecnologia, IST, ULisboa, Lisbon, Portugal) – "Analysis of nanoscale

compartmentalization of PI(4,5)P2 in living cells and model membranes through fluorescence spectroscopy and FRET imaging"

17.40 **Company Talk - Hamamatsu (Room 105)**

17.40 **Poster Party 1** - Presentation of posters with even number

19.30 **General Assembies of SPBf (Room 022) and SBE (Room 105)**

FRIDAY, 17TH JUNE

9.00 **Symposium 7: Protein Structure, Folding and Dynamics – Room 105**

Chairs: **João Morais Cabral** (Instituto de Biologia Molecular e Celular, Universidade do Porto, Portugal) and **Marta Bruix** (Instituto Quimica Fisica Rocasolano, Madrid, Spain)

9.00 **Francesc Xavier Aviles** (UAB, Spain) – "Enveiling Interactions and their Specificities between Proteolytic Enzymes, Inhibitors and Protein Ligands"

9.25 **Ivo Martins** (IMM, U Lisboa, Portugal) – "Understanding dengue virus capsid protein for drug design: combining wet-lab and computational biology"

9.50 **Sara E. Cannella** (Institut Pasteur, Chemistry and Structural Biology Department, Paris, France) – "Structure, function and stability of a monomeric adenylate cyclase (CyaA) toxin from Bordetella pertussis"

10.05 **Jorge Alegre** (Centro Nacional de Investigaciones Cardiovasculares Carlos III, Madrid, Spain) – "Nanomechanical phenotypes in familial hypertrophic cardiomyopathy"

10.20 **Fernando Moreno-Herrero** (Department of Macromolecular Structures, CSIC, Madrid, Spain) – "RepC nicking activity is passive and force and twist dependent"

10.35 **Antonio Rey** (Universidad Complutense de Madrid - Dept. Quimica Fisica, Madrid, Spain) – "Computer modeling of protein folding from flexible NMR structures"

10.50 **Coffee break**

9.00 **Symposium 8: Nanobiophysics and Molecular Recognition – Room 022**

Chairs: **Eurico Cabrita** (Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Portugal) and **José María Valpuesta** (CNB-CSIC, Madrid, Spain)

9.00 **Cecília Roque** (FCT-UNL, Portugal) – "Tailored synthetic affinity reagents"

9.25 **Cristina Flors** (IMDEA Nanociencia, Spain) – "Novel correlative microscopy tools to study Biology at the nanoscale"

9.50 **Catarina Praça** (Center for Neuroscience and Cell Biology, University of Coimbra, Portugal) – "Targeting the brain: Enhanced brain accumulation of nanoparticles by fine-tuning surface chemistry."

10.05 **Julene Madariaga-Marcos** (Centro Nacional de Biotecnología, Consejo Superior de Investigaciones Científicas, Madrid, Spain) – "Development and characterization of a hybrid apparatus combining Magnetic Tweezers and TIRF microscopy to study single DNA:protein interactions"

10.20 **Ana S. Martins** (Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal) – "West-Nile Virus capsid protein binds to host lipid systems requiring potassium ions"

10.35 **Sara H. Mejías** (Instituto Madrileño de Estudios Avanzados en Nanociencia, Madrid, Spain) – "Repeat proteins as templates to organize photoactive molecules"

10.50 Coffee Break

11.20 Symposium 7 (cont.): Protein Structure, Folding and Dynamics – Room 105

Chairs: **João Morais Cabral** (Instituto de Biologia Molecular e Celular, Universidade do Porto, Portugal) and **Marta Bruix** (Instituto Quimica Fisica Rocasolano, Madrid, Spain)

11.20 **Sandra Macedo-Ribeiro** (IBMC, U Porto, Portugal) – "Ser or Leu? Structural impact of genetic code ambiguity in Candida albicans"

11.45 **David Rodríguez-Larrea** (U. Oxford, UK) – "Single-molecule analysis of proteins with nanopore technology"

11.20 Symposium 8 (cont.): Nanobiophysics and Molecular Recognition – Room 022

Chairs: **Eurico Cabrita** (Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Portugal) and **José María Valpuesta** (CNB-CSIC, Madrid, Spain)

11.20 **Peter Eaton** (U Porto, Portugal) – "Antimicrobial Effects of Peptides Isolated from Amphibian Secretions"

Marisa Martín-Fernández (STFC, Oxford, UK) – "Determining the geometry of EGF receptor oligomers on cells with 6 nm resolution"

12.10 Plenary Lecture 3 – Room 105

Chair: **Conceição Rangel** (Requimte, UCIBIO, ICBAS, Universidade do Porto)

Chair: **Conceição Rangel** (Requimte, UCIBIO, ICBAS, Universidade do Porto)

Lino Ferreira (CNC, Universidade de Coimbra, Portugal) – "Light-activatable biomaterials for biomedical applications"

13.10 **Lunch Break**

14.30 **Poster Party 2** - Presentation of posters with odd number

16.00 **Prizes Session – Room 105**

Chairs: **Antonio Ferrer Montiel** (Universidad Miguel Hernández, Elche, Spain) and **Cláudio Soares** (Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Portugal)

16.00 **António José Pereira** (IBMC, U Porto, Portugal)
"Young Biophysicist 2016" Prize, awarded by the Portuguese Biophysical Society

16.25 **Xavier Gomis Rüth**
"Manuel Rico-Bruker" Prize

16.50 **Raúl Pérez Jiménez**
"Enrique Pérez-Paya" Prize, sponsored by Prima-Derm and BCN Peptides

17.15 **Lorena Redondo**
"SBE-33" Prize, sponsored by AntalGenics

17.45 **Plenary Lecture 4 - Room 105**

Chair: **Antonio Ferrer Montiel** (Universidad Miguel Hernández, Elche, Spain)

Luis Liz-Marzan (CIC biomaGUNE, Donostia-San Sebastian, Spain) – "Monitoring Bacterial Growth through Plasmonics"

18.30-19.00 **Closing Ceremony**

20.00 **Conference Dinner** – Restaurant "Bjekas"

POSTERS

Poster Party 1 - Presentation of posters with even number
Poster Party 2 - Presentation of posters with odd number

P01 – "Effect of sphingosine on membrane permeability and biophysical properties of lysosomal-mimicking vesicles"
Ana C. Carreira, Rodrigo F. M. de Almeida and Liana C. Silva

P02 – "Fibrinogen-erythrocyte binding as a potential prognosis biomarker in heart failure patients"
Ana Filipa Guedes, Filomena A. Carvalho, Nuno Lousada, Luís Sargento, Nuno C. Santos

P03 – "Effort in neuropsychiatric disorders"
Ana Catarina dos Santos Farinha

P04 – "Probing the interaction between serotonin receptors and model lipid bilayers containing isomeric forms of cholesterol using computer simulation"
Carmen Domene and Victoria Oakes

P05 – "Vehiculizing drugs and liposomes with pulmonary surfactant along the respiratory surface"
Alberto Hidalgo, Francesca Salis, Guillermo Orellana, Jesús Perez-Gil and Antonio Cruz

P06 – "Daunorubicin-membrane interactions with model membranes: relevance for the drug's biological activity"
Ana Catarina Alves, Daniela Ribeiro, Miguel Horta, José L.F.C. Lima, Cláudia Nunes and Salette Reis

P07 – "Influence of unsaturated sphingomyelin and ceramide in the formation of segregated lateral domains in lipid bilayers"
Emilio González-Ramírez, Aritz Garcia-Arribas, Itziar Areso, Jesús Sot, Alicia Alonso and Félix M. Goñi.

P08 – "Erythrocyte-erythrocyte adhesion promoted by fibrinogen in Essential Arterial Hypertension patients"
Catarina Sousa Lopes, Ana Filipa Guedes, J. Braz Nogueira, Carlos Moreira, Filomena A. Carvalho, Nuno C. Santos

P09 – "Structure of a dimeric fragment of the plakin domain of plectin by hybrid methods"
Arturo Carabias, José A Manso, Ana M Carballido, Inés García Rubio, José M de Pereda

P10 – "Structural organization of the guanine nucleotide exchange factor C3G"
María Gómez-Hernández, Arturo Carabias, Beatriz Escudero Paniagua, Patricia González, Carmen Guerrero, José M de Pereda.

P11 – "Unveiling the role of the mutation F508del in cystic fibrosis"
Bárbara Abreu, Emanuel Lopes, A. Sofia F. Oliveira, Cláudio M. Soares

P12 – "The anti-epileptic drug levetiracetam inactivates N-type, but not L-type, calcium channels in culture human neuroblastoma SH-SY5Y cells and rat cerebellar granule neurons."
Sofia Fortalezas, María Berrocal, Isaac Corbacho, Ana M. Mata and Carlos Gutierrez-Merino

P13 – "The pH effect on Marinobacter hydrocarbonoclasticus denitrification genes and nitrous oxide reductase"
Cíntia Carreira, Rute F. Nunes, Olga Mestre, Isabel Moura, Sofia R. Pauleta

P15 – "Cisplatin-induced changes on membrane biophysical properties of colon cancer cells: characterization of the initial steps of cisplatin mechanism of action"
Tânia CB Santos, Ana E. Ventura, Manuel Prieto, Liana C Silva

P16 – "Exploring the conformational and structural properties of the influenza fusion peptide in membrane bilayers: a computational study"
Davide R. Cruz, Diana Lousa, Cláudio M. Soares

P17 – "pH-dependent insertion of pHLIP peptide into lipid bilayers: pKa values of key residues"
Diogo Vila-Viçosa, Vitor H. Teixeira, Miguel Machuqueiro

P18 – "Unraveling the anticarcinogenic effects of resveratrol through a membrane biophysical approach"
Ana Rute Neves, Francisca Mendes Almeida, Cláudia Nunes and Salette Reis

P19 – "The impact of resveratrol in lipid bilayers: a neutron reflectometry study"
Chen Shen, Alexis de Ghellinck, Giovanna Fragneto, Beate Klösgen

P20 – "Stability of nanocomposites over ionic force"
MM Collado-González, MG Montalbán, R Trigo, Jorge Peña-García, Horacio Pérez-Sánchez, G Víllora and F Guillermo Díaz Baños

P21 – "A NMR-based method to quickly probe disordered protein regions via N-H solvent accessibility"
André F. Faustino, Glauce M. Barbosa, Miguel A. R. B. Castanho, Andrea T. Da Poian, Nuno C. Santos, Fabio C. L. Almeida, Ivo C. Martins

P22 – "Characterization of thermo-stable Ktra mutants"
Celso Moura Teixeira-Duarte, Ricardo S.Vieira-Pires and João H. Morais-Cabral

P23 – "Aquaporin involvement in oxidative stress response"
Claudia Rodrigues, Ana P. Martins, Catarina Prista, Ana Čipak, Graça Soveral

P24 – "Use of voltage-clamp fluorometry to determine the relative positioning of ion channel cytoplasmic domains-located fluorescent tags toward the plasma membrane"
Francisco Barros, Pedro Domínguez and Pilar de la Peña

P25 – "Lathnanide DO3A-type complexes conjugated to Pittsburgh compound B as optical and MRI probes for targeting Aβ amyloid aggregates involved in Alzheimer's disease"
Alexandre C. Oliveira, Jean-François Morfin, Éva Tóth, Telma Costa, Licínia L.G. Justino, Hugh D. Burrows, Carlos F.G.C.Geraldes

P27 – "Unraveling the acemetacin effects on phosphatidylcholine bilayers"
Catarina Pereira-Leite, Cláudia Nunes, Débora Grahl, Filipe S. Lima, Shirley Schreier, José C. Bozelli Junior, Iolanda M. Cuccovia and Salette Reis

P28 – "An additional phase transition of DPPC monolayers at high surface pressure confirmed by GIXD studies"
Chen Shen, Jorge B. de la Serna, Bernd Struth, Beate Klösgen

P29 – "Characterization of MEK1 pathological mutations by enhanced molecular dynamics"
Chiara Pallara, Ludovico Sutto, Fabian Glaser, Francesco Luigi Gervasio, Juan Fernández-Recio

P30 – "NMR Solution Structure of the Excalibur domain of CbpL from S. pneumoniae"
D. Pantoja-Uceda, M.A. Treviño and M. Bruix

P31 – "Gastric transport studies and breast cancer apoptosis mediated cell-death by Malvidin-3-O-glucoside and their derivatives."
Hélder Oliveira, Iva Fernandes, Victor de Freitas, Jingren He and Nuno Mateus

P32 – "Aquaglyceroporins expression in endothelial cells and implications in cardiovascular disease"
Inês Vieira da Silva, Madalena Barroso, Rita Castro, Graça Soveral

P33 – "pKa Values of Titrable Amino Acids at the Water/Membrane Interface"
Pedro B. P. S. Reis, Vitor H. Teixeira, Diogo Vila-Viçosa, and Miguel Machuqueiro

P34 – "Proof of pore formation through a 2D amoxicillin-membrane interaction study"
Daniela Lopes, Cláudia Nunes, Philippe Fontaine, Bruno Sarmento and Salette Reis

P35 – "Equilibrum and dynamic interfacial properties of pulmonary surfactant studied by new micropipette techniques"
Elisa Parra, Koji Kinoshita, David Needham

P36 – "Amyloid-formation in food allergens endorse epitope multivalency for optimal IgE binding"
Rosa Sánchez, Felix Arribas, Javier Martínez, María Pedrosa, Santiago Quirce, Rosa Rodriguez-Perez and María Gasset

P37 – "In bacterial FAD Synthetases little structural variations produce big catalytic differences"
María Sebastián Valverde, Ana Serrano, Marta Martínez-Júlvez, Milagros Medina

P38 – "Immunotargeting of E.coli TolC outer membrane channel"

João Laranjeira, Rui Cruz, Rita Vilaça, Marta V. Mendes, Rita Rocha and Ricardo S. Vieira-Pires

P39 – "Ubiquitin-mediated endocytosis of the voltage-gated potassium channel Kv1.3"
Katarzyna Styrczewska, Ramón Martínez-Mármol, Albert Vallejo-Gracia, Mireia Pérez-Verdaguer, Jesusa Capera, Irene Estadella, Alexander Sorkin and Antonio Felipe

P40 – "Analysis of nanoscale compartmentalization of PI(4,5)P2 in living cells and model membranes through fluorescence spectroscopy and FRET imaging"
M.J. Sarmento, A. Coutinho, M. Prieto, F. Fernandes

P41 – "Conformational transitions and membrane binding properties of human and salmon calcitonins: a time-resolved fluorescence study"
J.C. Ricardo, P. Caldas, A. Fedorov, F. Fernandes, M. Prieto, A. Coutinho

P42 – Study of interaction of the Rifampicin with lipid bilayers
Jaime Amadeu Samelo, Maria Julia Mora, Maria João Moreno

P43 – "Phase coexistence in DMPC/Chol bilayers and lateral partitioning of DPH"
Joana Cristo and Jorge Martins

P44 – "CellectAb - Engineering the Next Generation of Antibody Discovery"
Joana Ministro, Soraia Oliveira, Sofia Côrte-Real, João Gonçalves

P45 – "Pyrene probes in quantifying dielectric constants and excimer formation in unsaturated lipid bilayer: the importance of their molecular properties"
Jorge Martins, Miguel Manuel and Dalila Arrais

P46 – "Pulmonary surfactant: A heterogeneous mixture of membranes with different structural and functional properties."
Castillo-Sanchez, J.C., Cerrada, A., Conde, M., Cruz, A., Perez-Gil, J.

P47 – "Studying the interaction of the inactive form of Topotecan with cell membranes using 3D and 2D biomimetic models"
José Lopes-de-Araújo, Cláudia Nunes and Salette Reis

P48 – "Characterization of the calcium binding mode of rabphilin 3a"
Dolores Pérez-Sánchez, Teresa Coronado-Parra, Jesús Baltanás Copado, David López-Martínez, Juan C. Gómez-Fernández and Senena Corbalan-Garcia

P49 – "Anchoring of a plant protein, Remorin 1.3 to lipid domains: role of phosphoinositides"
Magali Deleu, Mehmet Nail Nasir, Jean-Marc Crowet, Julien Gronnier, Sébastien Mongrand, Laurence Lins

P50 – "Glucosylceramide-induced biophysical changes in artificial and cell membranes"
Ana R.P. Varela, Ana E. Ventura, André Sá Couto, Aleksander Fedorov, Anthony H. Futerman, Manuel Prieto, Liana C. Silva

P51 – "Combining antimalarial drugs with the cell-penetrating peptide TP10: betting on intracellular delivery"
Luísa Aguiar, Nuno Vale and Paula Gomes

P52 – "Structural and functional characterization of the cationic antimicrobial peptide EcAMP1R2"
Marcin Makowski, Mário R. Felício, Octávio L. Franco, Nuno C. Santos, Sónia Gonçalves

P53 – "Ternary complexes of fluoroquinolones as new metalloantibiotics: a biophysical and biological approach"
Mariana Ferreira, Carla F. Sousa, Cláudia Matias, José Roberto S. A. Leite, Paula Gameiro

P54 – "Study of the molecular interactions of antimicrobial peptides with bacteria"
Mário R. Felício, Octávio L. Franco, Nuno C. Santos and Sónia Gonçalves

P55 – "Biophysical analysis of lipid-conjugated peptide fusion inhibitors that target paramyxoviruses"
Marta C. Marques, Matteo Porotto, Anne Moscona, Nuno C. Santos

P56 – "Cholesterol-conjugated peptide inhibitors of influenza A virus: biophysical characterization"
Patrícia M. Silva, Marta C. Marques, Marcelo T. Augusto, Matteo Porotto, Anne Moscona, Nuno C. Santos

P57 – "Human SP-A1 enhances interfacial properties of lung surfactant and restores a proper behavior in the presence of inhibitory agents"

Raquel Arroyo, Mercedes Echaide, Elena Lopez-Rodriguez, Alicia Pascual, Joanna Floros, Jesús Pérez-Gil

P58 – "Toxin-induced pore formation is hindered by intermolecular hydrogen bonding in sphingomyelin bilayers"
Sara García-Linares, Juan Palacios-Ortega, Tomokazu Yasuda, Mia Åstrand, José G. Gavilanes, Álvaro Martínez-del-Pozo, J. Peter Slotte

P59 – "Exploring the structural features of antibacterial rhodamine labelled 3,4-HPO chelators that favour permeation through liposomes and macrophages"
T. Moniz, A. Leite, T. Silva, P. Gameiro, M. S. Gomes B. de Castro and M. Rangel

P60 – "Quantifying biomolecular-lipid membrane interactions using surface plasmon resonance equilibrium and kinetic data"
T.N. Figueira, J.M. Freire, A.S. Veiga, M.A.R.B. Castanho

P61 – "Deoxycholic acid modulates cell death signaling through changes in mitochondrial membrane properties"
Tânia Sousa, Rui E. Castro, Sandra N. Pinto, Ana Coutinho, Susana D. Lucas, Rui Moreira, Cecília M.P. Rodrigues, Manuel Prieto, Fábio Fernandes

P62 – "Type-II NADH:quinone oxidoreductase from Staphylococcus aureus has two distinct binding sites and is rate limited by quinone reduction"
Filipa V. Sena, Ana P. Batista, Teresa Catarino, José A. Brito, Margarida Archer, Martin Viertler, Tobias Madl, Eurico J. Cabrita and Manuela M. Pereira

P63 – "Protein-based biomaterials mimicking muscle tissue"
Carla Huerta-López, Diana Velázquez-Carreras and Jorge Alegre-Cebollada

PLENARY LECTURES

PLENARY LECTURE 1: THE CHALLENGE OF DOING STRUCTURAL BIOLOGY *IN SITU*

Wolfgang Baumeister

Traditionally, structural biologists have approached cellular complexity in a reductionist manner by characterizing isolated and purified molecular components. This 'divide and conquer' approach has been highly successful, as evidenced by the impressive number of entries in the PDB. In spite of this, awareness has grown in recent years that only rarely can a complex biological function be attributed to an individual macromolecule. Rather, most cellular functions arise from their acting in concert. Hence there is a need for methods developments enabling studies performed *in situ*, i.e. in unperturbed cellular environments. Sensu stricto the term 'structural biology *in situ*' should apply only to a scenario in which the cellular environment is preserved in its entirety; sensu largo it may refer to situations where the local environment is either preserved or reconstituted in such a manner that some functionality is maintained.

Electron cryotomography has unique potential to study the supramolecular architecture or 'molecular sociology' of cells. It combines the power of three-dimensional imaging – eventually at near-atomic resolution – with the best structural preservation that is physically possible. Key methods developments, such as correlative LM/EM, Focussed Ion Beam Milling (cryo FIB) or phase plate imaging, will be discussed as well as applications highlighting the potential of this post-reductionist approach to structural biology.

References:
Asano, S., Fukuda, Y., Beck, F., Aufderheide, A., Förster, F., Danev, R., Baumeister, W.: A molecular census of 26S proteasomes in intact neurons, Science 347:439-442 (2015)

Mahamid, J., Pfeffer, S., Schaffer, M., Villa, E., Danev, R., Kuhn-Cuellar, L., Förster, F., Hyman, A. A., Plitzko, J.M., Baumeister, W.: Visualizing the molecular sociology at the HeLa cell nuclear periphery, Science 351:969-972 (2016)

PLENARY LECTURE 2: FRATAXIN: NOT ANY LONGER A FUNCTION ORPHAN PROTEIN

Annalisa Pastore[1]
[1]The Wohl Institute, King's College London, 5 Cutcombe Rd London (UK)

Iron sulphur cluster formation is an essential and yet poorly understood metabolic pathway common to all living organisms. Frataxin, an essential and highly conserved mitochondrial protein whose reduced expression causes Friedreich's ataxia (FRDA) in humans, is an active part of this assembly: using a bacterial model and different biochemical and molecular biology techniques, we have proven that frataxin acts as an iron concentration dependent inhibitor of cluster formation. This suggests that frataxin is an iron sensor which acts as the gate keeper for Fe-S cluster formation and fine tunes the quantity of Fe-S clusters to the concentration and/or possibly the distribution of the available acceptors. I will review our work and the more recent advancements of our research. Our observations provide a new perspective for understanding FRDA and a mechanistic model which rationalizes the available knowledge.

PLENARY LECTURE 3: LIGHT-ACTIVATABLE BIOMATERIALS FOR BIOMEDICAL APPLICATIONS

Lino Ferreira[1], Emanuel Quartin[1], Miguel Lino[1], Susana Simões[1], Adrian Jimenez[1], Josephine Blersch[1], Sonia Pinho[1], Carlos Boto[1], Sandra Pinto[1], Ricardo Neves[1]
[1]CNC- Centro de Neurociencias e Biologia Celular, Universidade de Coimbra

The advent of molecular reprogramming and the associated opportunities for personalised and therapeutic medicine requires the development of novel systems for on-demand delivery of reprogramming factors into cells in order to modulate their activity/identity. Such triggerable systems should allow precise control of the timing, duration, magnitude and spatial release of the modulator factors. Furthermore, the system should allow this control even in vivo, using non-invasive means. Nanoparticles (NPs) are very promising for the intracellular delivery of proteins, non-coding RNAs, siRNAs and small molecules. They offer several advantages over the viral vectors including high biomolecule carrying capacity, low risk of immunogenicity, low cost and ease of production. In addition, the possibility of making NPs that disassemble by light make them excellent candidates as triggerable systems. During my talk, I will give several examples of technologies that under development to address this challenge in the context of therapeutic and regenerative medicine.

Keywords: biomaterials, light, nanomedicine, stem cell modulation

PLENARY LECTURE 4: MONITORING BACTERIAL GROWTH THROUGH PLASMONICS

Luis M. Liz-Marzán[1,2,3]

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[2]Ikerbasque, Basque Foundation for Science, 48013 Bilbao, Spain
[3]Biomedical Research Networking Center in Bioengineering, Biomaterials, and Nanomedicine (CIBER-BBN), Spain

Nanoplasmonics deals with the manipulation of light using materials of size much smaller than the radiation wavelength. This is typically achieved using nanostructured metals, since they can very efficiently absorb and scatter light because of their ability to support coherent oscillations of free (conduction) electrons. The recent development of nanoplasmonics has largely relied on major advancements in fabrication methods, which provide us with an exquisite control over the composition and morphology of nanostructured metals. Both lithography and colloid chemistry have seen a tremendous increase in the control that can be achieved, to a degree that seemed impossible only a decade ago. In particular, Colloid Chemistry has the advantage of simplicity and larger scale production, while offering a number of parameters that can be used as a handle to direct not only nanoparticle morphology but also surface properties and subsequent processing.

This talk will focus on the use of "colloidal nanoplasmonics" to bridge the basic fabrication of nanoplasmonic building blocks, all the way to devices that can be used for real applications. In particular, rationally designed nanostructured platforms will be introduced as plasmonic substrates for the in-situ, label-free surface-enhanced Raman scattering (SERS) detection of bacterial growth. We monitored a metabolite involved in the quorum sensing (GS) communication of the bacteria Pseudomonas aeruginosa, during the formation of biofilms and microcolonies. Our study pioneers non-invasive, plasmonic imaging of QS providing a powerful analytical approach for studying intercellular communication based on secreted molecules as signals.

INVITED TALK
NEW AND NOTABLE

THE CRYSTAL STRUCTURE OF HUMAN ALDEHYDE OXIDASE AND IMPLICATIONS TOWARDS DRUG METABOLISM

Catarina Coelho[1], Alessandro Foti[2], Teresa Santos-Silva[1], Silke Leimkuehler[2] and Maria João Romão[1]
[1]UCIBIO@REQUIMTE – Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal;
[2]Department of Molecular Enzymology, Institute of Biochemistry and Biology,University of Potsdam, Potsdam, Germany

Aldehyde Oxidase (AOX; EC1.2.3.1) is a cytosolic enzyme with a major role in the metabolism of drugs and xenobiotic compounds. It belongs to the Xanthine Oxidase (XO) family of Mo-containing enzymes and is a homodimer. Each 150 kDa monomer consists of three different domains: the small N-terminal domain (20 kDa) with two spectroscopic distinct [2Fe-2S] clusters; the central FAD domain (40 kDa); and the C-terminal catalytic domain (90 kDa) which contains the molybdenum cofactor (Moco). The cofactor disposition in AOX is related with the electron transfer chain: electrons generated at the Moco active site pass through the two FeS centers until the FAD cofactor, from where they are released to molecular oxygen, which is the terminal electron acceptor. The true physiological function of AOX is unclear. It is known to be responsible for the failure of several clinical trials Phase I, mainly due to its broad substrate specificity oxidizing not only aldehydes but also aromatic N-heterocycles, commonly present in drug molecules. The very recent elucidation of the 3D crystal structure of the human AOX enzyme in the substrate-free form (PDB ID: 3UHW) and in complex with the substrate phthalazine and the noncompetitive inhibitor thioridazine (PDB ID: 4UHX) [1] revealed novel and important structural features. The substrate and the inhibitor molecules were found to be simultaneously bound to the protein at different binding sites. Thioridazine is an antipsychotic drug that was used in the treatment of schizophrenia and psychosis before discontinuation due to severe secondary side effects. Structural analysis of the AOX complex revealed a new and totally unexpected thioridazine binding site structurally conserved among mammalian AOX and XO. These results are of great interest for studying the AOX clinical drug interactions and for the rationale design of future AOX stable putative drugs.

[1] Coelho et al, NATURE CHEM BIOL, 2015

Keywords: Aldehyde Oxidase; Drug Metabolism; Protein-Ligand Complex

INVITED TALK
NEW AND NOTABLE

VISUALIZING GENOME RELEASE DURING THE MECHANICAL UNPACKING OF SINGLE VIRUSES

Pedro J. de Pablo[1]
[1]Universidad Autónoma de Madrid, Madrid, Spain

Viruses package their genome inside a robust protein capsid to protect it during transmission between cells and organisms. In a reaction termed uncoating, the capsid is progressively weakened during entry into cells. The genome separates, becomes transcriptionally active, and thereby initiates the production of progeny. Here, we triggered the uncoating of single human adenovirus capsids with atomic force microscopy, and followed genome exposure by single molecule fluorescence microscopy. By comparing immature (non-infectious) with mature (infectious) adenovirus particles we observed two condensation states of the genome which is a new feature of infectivity. Beyond virus genome unpacking this method may find application in testing the cargo release of bio-inspired delivery vehicles.

INVITED TALK
NEW AND NOTABLE

ATOMIC FORCE MICROSCOPY AS A TOOL TO EVALUATE THE RISK FOR CARDIOVASCULAR DISEASES IN PATIENTS

Ana Filipa Guedes[1], Inês Malho[1], Nuno Lousada[2], Luís Sargento[2], Nuno C. Santos[1], Filomena A. Carvalho[1]

[1]Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal

[2]Unidade de Insuficiência Cardíaca, Departamento de Cardiologia, Hospital Pulido Valente, Centro Hospitalar Lisboa Norte, Lisbon, Portugal.

High fibrinogen levels are a relevant cardiovascular risk factor, but the biological mechanisms associated with pathologic alterations are not totally clear. Fibrinogen-erythrocyte binding in chronic heart failure (CHF) patients and its prognostic value were evaluated. Ischemic and non-ischemic CHF patients, as well as healthy donors were enrolled in the study. Fibrinogen-erythrocyte interactions were evaluated by atomic force microscopy (AFM)-based force spectroscopy and the clinical outcome assessed during a 12-months follow-up. Higher fibrinogen-erythrocyte binding forces were observed for patients, comparing with control group. Ischemic patients presented increased fibrinogen-erythrocyte binding forces relative to non-ischemic. Their cell stiffness is also altered. Follow-up data demonstrate that hospitalization was more frequent for patients with higher fibrinogen-erythrocyte binding forces. As fibrinogen-erythrocyte interactions, evaluated by AFM, are modified in CHF patients and associated with short-term outcome, here we demonstrate the power of this nanotechnology-based evaluation as potential biomarker for cardiovascular risk and patients' clinical prognosis evaluation. [Guedes et al. (2016) Nature Nanotechnol., in press].

Keywords: erythrocyte, fibrinogen, cardiovascular diseases, protein-cell interaction

INVITED TALK
NEW AND NOTABLE

MECHANICAL REGULATION OF A MOLECULAR CLUTCH DEFINES CELL SENSING OF MATRIX RIGIDITY

Pere Roca-Cusachs[1]
[1]Institute for Bioengineering of Catalonia and University of Barcelona

Cell function depends on tissue rigidity, which cells probe by applying and transmitting forces to their extracellular matrix, and then transducing them into biochemical signals. Here we show that in response to matrix rigidity and density, force transmission and transduction are explained by the mechanical properties of the actin–talin–integrin–fibronectin clutch. We demonstrate that force transmission is regulated by a dynamic clutch mechanism, which unveils its fundamental biphasic force/rigidity relationship on talin depletion. Force transduction is triggered by talin unfolding above a stiffness threshold. Below this threshold, integrins unbind and release force before talin can unfold. Above the threshold, talin unfolds and binds to vinculin, leading to adhesion growth and YAP nuclear translocation. Matrix density, myosin contractility, integrin ligation and talin mechanical stability differently and nonlinearly regulate both force transmission and the transduction threshold. In all cases, coupling of talin unfolding dynamics to a theoretical clutch model quantitatively predicts cell response.

INVITED TALK
NEW AND NOTABLE

A PROTEIN TRISULFIDE IS THE PRODUCT OF DISSIMILATORY SULFITE REDUCTION

Inês A. C. Pereira[1], André A. Santos[1], Sofia S. Venceslau[1], Fabian Grein[1]
[1] Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal

The dissimilation of sulfur compounds is likely to have been one of the first energy metabolisms on the early Earth. However, many questions remain about how sulfur-metabolizing organisms obtain energy for growth from reducing, oxidizing or disproportionating sulfur compounds. The dissimilatory sulfite reductase (DsrAB) is one of the most important enzymes in the biogeochemical sulfur cycle, being widespread in sulfur metabolizing microorganisms. The mechanism of sulfite reduction by DsrAB has long been the subject of controversy due to the *in vitro* formation of thiosulfate and trithionate, in contrast to the closely-related assimilatory enzyme that produces only sulfide.

Recent studies have identified the small protein DsrC and the DsrMKJOP membrane complex as physiological partners of DsrAB [1]. DsrC is a small, highly abundant protein, which contains two conserved redox-active cysteines in a flexible C-terminal arm. We will report recent *in vivo* and *in* vitro studies that reveal the function of DsrC in sulfite reduction, identifying the mechanism and physiological product of this reaction as the DsrC trisulfide. These results led to a redesign of the sulfate reduction pathway as having four steps, and not three as previously assumed. Furthermore, they implicate the respiratory membrane complex DsrMKJOP in the process, providing a direct link to energy conservation.

[1] Venceslau SS, Stockdreher Y, Dahl C, Pereira IAC, 2014, Biochim Biophys Acta – Bioenergetics 1837, 1148
[2] Santos AA, Venceslau SS, Grein F, Leavitt WD, Dahl C, Johnston DT, Pereira IAC, 2015, Science, 350, 1541

INVITED TALK
NEW AND NOTABLE

AURORA A DRIVES EARLY SIGNALLING AND VESICLE DYNAMICS DURING T-CELL ACTIVATION

Noa Beatriz Martín-Cófreces[1, 2]
[1]Fundación Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain
[2]Servicio de Inmunología, Hospital Universitario de la Princesa-UAM, IIS-IP, Madrid, Spain

Aurora A is a serine/threonine kinase that contributes to the progression of mitosis by inducing microtubule nucleation. We have identified an unexpected role for Aurora A kinase in regulating antigen-driven T cell activation. We find that Aurora A is phosphorylated upon TCR activation and localizes at two different intracellular pools during TCR-driven cell contact; the Immunological Synapse (IS) and the centrosome. Inhibition of Aurora A with pharmacological agents or genetic deletion in human or mouse T cells severely disrupts the dynamics of microtubules and CD3ζ-bearing vesicles at the IS. However, actin dynamics seems unaffected. The absence of Aurora A activity impairs the activation of early signaling molecules downstream of the TCR. Aurora A inhibition causes delocalized clustering of tyrosine kinase Lck at the IS and decreases Lck phosphorylation, thus indicating Aurora A is required for maintaining Lck active. The expression of IL-2, CD25 and CD69 are impaired by Aurora A blockade. These findings involve Aurora A in the propagation of the TCR activation signal.

INVITED TALK
MEMBRANES AND PROTEIN-LIPID INTERACTIONS

EFFECTS OF PHOSPHOINOSITIDES AND THEIR DERIVATIVES ON MEMBRANE MORPHOLOGY AND FUNCTION

Banafshe Larijani[1]
[1]Head of Cell Biophysics Laboratory IKERBASQUE, Basque Foundation for Science: Biofisika Instituto (CSIC UPV/EHU) & Research Centre for Experimental Marine Biology and Biotechnology (PiE)

To suggest and develop intelligent strategies to comprehend the regulation of organelle formation, a deeper mechanistic interpretation requires more than just the involvement of proteins. Our approaches link the formation of endo-membranes with both signalling and membrane physical properties.
Our studies derive from a cross-disciplinary approach undertaken to determine the molecular mechanisms of nuclear envelope assembly in echinoderm and mammalian cells. Our findings have led to the demonstration of a direct role for phosphoinositides and their derivatives in nuclear membrane formation. We have shown that phosphoinositides and their derivatives, as well as acting as second messengers, are modulators of membrane morphology, and their modifying enzymes regulate nuclear envelope formation. We aim to demonstrate that proteins do not solely drive morphology and dynamics (fusion) of subcellular compartments, which should be instead be considered proteo-lipid events.

Keywords: Nuclear Envelope, Phosphoionositide signalling, subcellular compartments, membrane dynamics, chemical biology, echinoderms

INVITED TALK
MEMBRANES AND PROTEIN-LIPID INTERACTIONS

INTERACTION OF BILE SALTS WITH MODEL MEMBRANES MIMICKING THE GASTROINTESTINAL EPITHELIUM: A STUDY BY ISOTHERMAL TITRATION CALORIMETRY

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Bile salts (BS) are bio-surfactants synthesized in the liver and secreted into the intestinal lumen where they solubilize cholesterol and other hydrophobic compounds facilitating their gastrointestinal absorption. Partition of BS towards biomembranes is an important step in both processes. Depending on the loading of the secreted BS micelles with endogeneous cholesterol and on the amount of cholesterol from diet this may lead to the excretion or absorption of cholesterol, from cholesterol saturated membranes in the liver or to gastrointestinal membranes, respectively. The partition of BS towards the gastrointestinal membranes may also affect the barrier properties of those membranes affecting the permeability for hydrophobic and amphiphilic compounds. Two important parameters in the interaction of the distinct BS with biomembranes are their partition coefficient and the rate of diffusion through the membrane. Altogether, they allow the calculation of BS local concentrations in the membrane as well as their asymmetry in both membrane leaflets. The local concentration and, most importantly, its asymmetric distribution in the bilayer are a measure of induced membrane perturbation which is expected to affect significantly its properties as a cholesterol donor and hydrophobic barrier.

In this work we have characterized the partition of several BS, non-conjugated and conjugated with glycine, to large unilamellar vesicles (LUV) in the liquid-disordered phase and with liquid-ordered/liquid-disordered phase coexistence, using isothermal titration calorimetry (ITC). The partition into the liquid-disordered bilayer was characterized by large partition coefficients and favored by enthalpy while association with the more ordered membrane was weak and driven only by the hydrophobic effect. The tri-hydroxy BS partitions less efficiently towards the membranes but shows faster translocation rates, in agreement with a membrane protective effect of those BS. The rate of translocation through the more ordered membrane was faster indicating accumulation of BS at specific locations in this membrane.

Keywords: Biological Membranes; Bile salts; Partition Coefficient; flip-flop

INVITED TALK
COMPUTATIONAL BIOPHYSICS

MAMMALIAN TUBULIN AS A PARADIGM FOR THE BINDING OF NATURAL PRODUCTS TO A RELEVANT ANTICANCER TARGET

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Structural knowledge on microtubule architecture and a number of tubullin-drug complexes has contributed enormously to a better understanding of the affinity determinants for a large number of natural products and can now be advantageously and judiciously used to rationalize numerous structure-activity relationships and to attempt structure-based ligand optimization and/or virtual screening of putative ligands with affinity for this paradigmatic anticancer target.

Keywords: Tubulin-binding agents; anticancer drugs; structure-based drug design

INVITED TALK
COMPUTATIONAL BIOPHYSICS

METABOLIC SYSTEMS BIOLOGY: TOOLS FOR MODEL-BASED ANALYSIS AND DESIGN OF MICROBIAL SYSTEMS

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Industrial Biotechnology has been replacing chemical processes in numerous industrial sectors since it allows the use of renewable raw-materials and provides a more sustainable manufacturing base. The field of Metabolic Engineering (ME) has thus gained a major importance since it allows the design of improved microorganisms for industrial applications. However, in Metabolic Engineering problems, it is often difficult to predict the effects of genetic modifications on the microorganism, owing to the complexity of the underlying biological systems. Consequently, the task of identifying the modifications that will lead to an improved microbe is a quite complex one, requiring robust mathematical and computational tools.

In this presentation I will introduce the main framework of the in silico design of improved microbial strains and will focus in some of our group's efforts in these fields, namely in the development of improved mathematical models of metabolic and regulatory processes and the development of reliable and effective computational methods for the design of rational metabolic engineering strategies Furthermore, I will introduce the open-source software tool developed in house, called OptFlux (www.OptFlux.org), that allows researchers both from industry and academia to simulate, in a user-friendly way, the behavior of microorganisms under a variety of conditions and also indicates which genetic modifications may lead to enhanced strains for a particular application or which are the putative essential genes that can be used as drug targets, in the case of pathogens.

Bridges with established and emergent fields such as structural biology and enzyme engineering will also be explored.

Keywords: Systems Biology. Metabolic Models

INVITED TALK
MEMBRANES AND PROTEIN-LIPID INTERACTIONS

UNDERSTANDING LIPID PROTEIN ADDUCTS USING MASS SPECTROMETRY BASED APPROACHES

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Lipids in membranes are susceptible to oxidative damage in response to redox imbalance. During lipid oxidation a plethora of oxidized species are formed including highly reactive products with terminal electrophilic carbonyl groups, which can covalently react with peptides and proteins in membranes. The formation of lipid –protein adducts, also called lipoxidation adducts, cause a disturbance of membrane properties, and modification or loss of protein function, and that can have negative health effects. The exact mechanism of action of these lipoxidation adducts is yet to be fully unravelled and their Identification and characterization is an essential step in recognizing the biological effects of the modified proteins.

Mass spectrometry (MS) based analytical approaches have been used in recent years to understand the chemistry and biology of lipid –protein adduct in vitro and in vivo. (1,2) Bottom-up proteomics is a common method to study protein-oxoLPP adducts by MS. In a classical bottom-up approach, the protein mixture containing lipid- protein adducts is subjected to enzymatic hydrolysis and resulting lipid -peptides and peptides are subsequently analysed by LC-MS and MS/MS. (1,2) Derivatization or enriched procedures can be used to improve sensitivity. Till date, most of the published studies focused on the identification of protein/peptide adducts with highly reactive free α,β-unsaturated aldehydes, namely 4-hydroxy-nonenal (4-HNE), and to protein adducts with aldehydes esterified to phospholipids. (1) Overall, MS is capable to provide information not only about the identity of modified lipid or protein, but also to specify the sites of modifications and, in several cases, to define the structure and possible mechanism of formation.

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Keywords: Mass Spectrometry, Lipid oxidation, Lipid- protein adducts

INVITED TALK
MEMBRANES AND PROTEIN-LIPID INTERACTIONS

TOWARDS MEMBRANE PROTEIN DE NOVO DESIGN

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The design of integral membrane proteins form first principles represents a great challenge in biochemistry and structural biology. These proteins are inserted into the ER membrane through a continuous ribosome-translocon channel. Once there, the hydrophobicity and thickness of the hydrocarbon core together with the chemical heterogeneity of the membrane interfaces lead to the expectation that the energetics of the interactions of the polypeptide chains with each other and with the various components of their environment should determine structure and function. Using statistical data obtained from integral membrane proteins with known high-resolution structure we have designed and experimentally validated short transmembrane segments with naturally occurring amino acid composition and distribution. In addition, we have implemented an experimental biological assay to determine a complete interfacial hydrophobicity scale. Coupled with known strategies to control membrane protein topology, these findings may pave the way to de novo membrane protein design.

Keywords: Membrane proteins; de novo design; transmembrane; interfacial scale

INVITED TALK
COMPUTATIONAL BIOPHYSICS

MOLECULAR DYNAMICS OF BIOLOGICAL MEMBRANES. WHAT CAN WE GET?

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The availability of powerful clusters has opened an exciting new way to study the biophysics of biological membranes, giving us a detailed view of their structure and dynamics in an unprecedented atomic detail. This has been possible by the use of general force fields for water, lipids, proteins and nucleic acids as well as the availability of simulation computer programs. These computational techniques, in many cases complementary to experimental biophysical methods, provide us with one of the most detailed perspective on the complex molecular processes involved in the biological membrane. What can we anticipate from membrane molecular dynamics ?. A complete detail of the motions of proteins, lipids and other molecules in the system through a trajectory in space and time, i.e., information on thermodynamic properties and spatial interactions. However, there are limitations and questions. Membrane molecular dynamics, represent the truth ?. The observed disorder, is real ?. The behaviour of the systems, can be predicted ?. Which is the time scale to be used ?. Can we control ions, pH, potentials,…?.

In this presentation, we will describe recent results obtained in our laboratory in the simulation of simple and complex membrane systems. For that goal, we use unrestrained all-atom molecular dynamics and the highly mobile membrane-mimetic lipid bilayer model. We will describe the location and orientation of the protonated and unprotonated forms of the antiviral arbidol. We will describe the interaction of a peptide derived from the Dengue C protein with membrane phospholipids as a possible way to obtain antiviral molecules. Finally, we will describe a complex membrane system composed of the TRPV1 channel and its interaction with cannabinoid ligands, paving the way to obtain more and better protein channel modulators.

This research presented is funded by grant BFU2013-43198-P (Ministerio de Economía y Competitividad, Spain).

INVITED TALK
COMPUTATIONAL BIOPHYSICS

COMPUTATIONAL PROTEOMICS COCKTAIL

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During this talk I will summarize several quasi-related developments in computational proteomics that are taking place within our group. The talk will not focus on specific applications but instead in will give an overview of the fields we are working in, with selected examples to illustrate the concepts. The calculation of enzyme reaction mechanisms with QM/MM techniques will be addressed. The role of enzyme flexibility on catalytic rates will be discussed as well [1, 2].

I will also discuss the methodologies that we have been working in to try to solve some pertinent issues in protein structure prediction and energetics. We will expose a methodology for computational mutagenesis with focus on alanine scanning mutagenesis [3]. Its rationale, its performance and its comparison with other more computing-intensive methodologies will be discussed [4]. Applications of the method in structure-based drug discovery will also be highlighted [5]. Afterwards we will move for protein-protein docking, where we will discuss our new scoring function that allows for the identification of native protein-protein complexes with high accuracy [6]. Its merits and limitations, and its dependence on the availability of reliable experimental data will be addressed as well.

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Keywords: Computer simulations; Enzymes; Reaction Mechanisms; Protein Structure

INVITED TALK
BIOENERGETICS

THE HUMAN APOPTOSIS INDUCING FACTOR: KEY MOLECULAR DETERMINANTS IN CELLULAR RESPIRATION, PROGRAMED CELL DEATH AND NEURODEGENERATIVE DISORDERS

Patricia Ferreira[1], Raquel Villanueva[1], Marta Martínez-Júlvez[1], and Milagros Medina[1].
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The human Apoptosis Inducing Factor (hAIF) is a NAD(P)H-dependent flavoreductase initially described as a mitochondrial factor that after cell death induction distributes to the nucleus to mediate chromatinolysis, but later it was also recognized as a key player in mitochondrial bioenergetics of healthy cells. In addition, AIF is present in a monomer-dimer equilibrium influenced by NADH. Substrates and roles of AIF as a putative oxidoreductase in mitochondria have remained elusive for a long time, but recent studies involve it in the redox control of different cellular processes. Moreover, in the last years at least six mutations in AIFM1, the X-chromosomal gene coding for hAIF, have been described coursing from ventriculomegaly to severe muscular atrophy. The understanding of molecular bases of the hAIF activities thus opens new frontiers, in medical applications at both mitochondrial and nuclear levels, to develop its potential as a therapeutic target in cancers, neuropathies and even obesity. In this context we are using a multidisciplinary approach combining biochemical, biophysical and cellular methodologies to describe the AIF behavior at the molecular level, as well as to identify compounds that might modulate the activities of this bifunctional enzyme.

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Keywords: Apoptosis Inducing Factor, mitochondrial bioenergetics, redox control

INVITED TALK
BIOENERGETICS

ATPASE INHIBITORY FACTOR 1 (IF1): A MAIN DRIVER OF METABOLIC REPROGRAMMING AND NUCLEAR SIGNALING

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Mitochondrial dysfunction is involved in the genesis and/or progression of ageing and a plethora of pathologies such as cancer and neurodegeneration. The mitochondrial ATP synthase is a key transducer in energy conservation by oxidative phosphorylation (OXPHOS), in shaping the structure of mitochondrial cristae, in the execution of cell death and in intracellular ROS signaling. We have described that the ATPase Inhibitory Factor 1 (IF1) of the ATP synthase is highly overexpressed in most prevalent carcinomas and plays a prominent role in metabolic reprogramming of tumor cells to an enhanced aerobic glycolysis. In addition, the IF1-mediated inhibition of the ATP synthase triggers a ROS signal that promotes the activation of nuclear programs of proliferation and resistance to death, illustrating that IF1 is part of the molecular strategy adapted by cancer cells to proliferate and escape from death. Transgenic mice that conditionally express IF1 in neurons, liver o colon demonstrated in vivo the role of IF1 in metabolic reprograming and nuclear signaling. Moreover, we have also demonstrated the mechanisms by which IF1 promotes a pro-oncogenic phenotype in liver. The activity of IF1 as an inhibitor of the ATP synthase is blunted by the activity of a mitochondrial cAMP-dependent protein kinase. Dephospho-IF1 binds to the ATP synthase and promotes the inhibition of the enzyme in hypoxia, cell cycle and in cancer. In this presentation we will review the role of IF1 as a relevant mitochondrial protein defining the cellular phenotype.

Keywords: ATP synthase, ATPase Inhibitory Factor 1, Mitochondrial siganaling

INVITED TALK
CELL AND TISSUE BIOPHYSICS

ENDOTHELIAL CELL DYNAMICS IN VASCULAR MORPHOGENESIS

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The vascular network undergoes extensive vessel remodelling to become fully functional. Is it well established that blood flow is a main driver for vascular remodelling. It has also been proposed that vessel pruning is a central process within physiological vessel remodelling. However, despite its central function, the cellular and molecular mechanisms regulating vessel regression, and their interaction with blood flow patterns, remain largely unexplained.

We identified that reorganization of endothelial cells is at the core of vessel regression, representing vessel anastomosis in reverse.

Using network-level analysis of endothelial polarity maps, rheology models and microfluidics, we show that endothelial non-canonical Wnt signalling regulates endothelial sensitivity to shear forces. Our data suggest that non-canonical Wnt signalling stabilizes forming vascular networks by reducing endothelial shear sensitivity.

INVITED TALK
CELL AND TISSUE BIOPHYSICS

CONTRACTING, SPREADING, FOLDING: HOW FORCES SHAPE BIOLOGICAL TISSUES DURING DEVELOPMENT

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During embryogenesis, dramatic tissue reorganization occurs under the control of specific signaling pathways. Mechanical forces are coordinated in order to rearrange biological tissues and shape organs. The mechanisms at the origin of the generation and regulation of these forces during development remain elusive. My group is interested in understanding how fundamental modes of tissue remodeling such as epithelial contraction, tissue spreading or folding are regulated during development. Here, I will present recent works on the generation and regulation of forces during tissue contraction and show an unusual contractile mechanism involving apoptosis in Drosophila dorsal closure. I will also discuss new results on the role of actomyosin contractility during this process.

Keywords: Morphogesis, epithelial mechanics, actomyosin, Drosophila, dorsal closure

INVITED TALK
BIOENERGETICS

NEUROMETABOLIC COUPLING IN HIPPOCAMPUS MEDIATED BY NEURONAL-DERIVED NITRIC OXIDE

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The brain relies on a delicate balance between energy demands imposed by neural activity and substrate delivery by cerebral blood flow (CBF), a process termed functional hyperemia or neurovascular coupling (NVC). Numerous studies have addressed the vasculature effects on brain metabolism but the challenge is the functional analysis at the level of microvasculature where the NVC process is operative. With the advent of PET and fMRI approaches, it became clear that local CBF increases are associated with cellular-activity increases to meet the need for additional oxygen and glucose but, noteworthy, CBF increase in excess of the increase in oxygen consumption, supporting the occurrence of aerobic glycolysis.

We will provide experimental evidences supporting a consistent and integrated interpretation of aerobic glycolysis considering that a) following excitatory activation, glutamate binds to NMDA receptor and triggers nitric oxide (NO) production from neuronal nitric oxide synthase, which co-localizes with NMDAr in a multiprotein complex; b) a transitory increase in NO concentration induces a local increase in CBF, thus increasing local glucose and O2 availability - we have estimated that NO may diffuse hundreds of microns, a distance long enough to travel to nearby arterioles c) NO competes with O2 for Cytochrome c oxidase (CcOx) in mitochondria; using hippocampal slices we have demonstrated the interplay between NO and O2 tensions, upon glutamatergic activation.

Keywords: Neurometabolic coupling, nitric oxide, brain

INVITED TALK
BIOENERGETICS

EXPLORATION OF THE ELECTRICAL CONTACT BETWEEN ELECTROACTIVE BACTERIA AND CONDUCTING SOLIDS RELEVANT FOR THE OPTIMIZATION OF MICROBIAL ELECTROCHEMICAL TECHNOLOGIES

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Microbial electrochemical technologies provide a versatile platform for the development of novel industrial processes of low ecological footprint and with myriads of applications. The key process for the effective implementation of these technologies is the electrical contact between the metabolism of electroactive microorganisms cultivated in the devices and conductive solid electrodes that collect or provide current under operating conditions. This is called extracellular electron transfer and can occur by two broad mechanisms: direct contact via surface exposed redox proteins or conductive cellular appendages, or indirectly mediated by small molecule redox shuttles excreted into the extracellular medium. The molecular mechanisms underpinning indirect extracellular electron transfer were explored by a combination of NMR spectroscopy, electron transfer kinetics measured by stopped-flow and molecular docking simulations. Outermembrane redox proteins were shown to be functionally distinct, discriminating between different chemical classes of redox shuttles. This occurs despite the fact that they share a common genetic origin and structural architecture. Furthermore, even those proteins that are promiscuous in their interaction with redox shuttles appear to have specific recognition sites for each shuttle. A working model will be presented that provides guidance as to the future directions of research and development towards improved electrical contact between electroactive organisms and electrodes in devices that implement microbial electrochemical technologies.

Keywords: Electron transfer; NMR; Stopped flow Kinetics; Cytochrome; Molecular docking

INVITED TALK
CELL AND TISSUE BIOPHYSICS

HYALURONAN – FROM A SIMPLE POLYSACCHARIDE TO BIOLOGICAL MATERIALS/INTERACTIONS WITH REMARKABLE PROPERTIES

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The polysaccharide hyaluronan (HA) is ubiquitous in the extracellular space of vertebrates. It is important in the assembly and remodelling of extracellular matrices, and in cell-matrix and cell-cell communication. In this talk, we will illustrate how nature combines the unique physical properties of HA and the biochemical recognition of HA by matrix/cell-surface proteins to create biological materials/interactions with remarkable properties.

The cumulus cell-oocyte complex (COC) matrix is an extended HA-rich coat that forms around the oocyte a few hours before ovulation and plays vital roles in oocyte biology. Our micromechanical analysis by colloidal probe atomic force microscopy (AFM) revealed that mouse COC matrix is extremely soft yet elastic. Specifically, the Young's modulus of the softest regions of the COC matrix is below 1 Pa. To our knowledge, this is the smallest modulus of any elastic biological material reported to date, and we propose that the unique mechanical properties of COC matrix serve important functions in the reproductive process, such as oocyte transport and sperm penetration.

Many cells express HA receptors on their surfaces, and the engagement with HA is known to modulate physiological and pathological processes, such as the migration of immune cells (in inflammation), stem cells (in tissue repair) and tumour cells (in cancer metastasis), in ways that are not well understood. Studying a well-defined and tuneable synthetic experimental system that reproduces key features of the HA-cell interface, we found that HA targets surfaces 'superselectively', that is, it can sharply discriminate between high and low receptor densities. A theoretical model reveals how this remarkable property emerges from the physical nature and the multivalent receptor-binding of HA polymers, and how superselective targeting can be tuned. We propose that superselective binding serves as a tool to translate inherently fuzzy cell-matrix interactions into on/off switches that guide downstream cellular decision making.

Keywords: Extracellular matrix; hyaluronan; mechanical property; multivalency; superselective targeting

INVITED TALK
CELL AND TISSUE BIOPHYSICS
MECHANICS OF SPROUTING ANGIOGENESIS IN TUMORS

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Sprouting angiogenesis, where new blood vessels grow from pre-existing ones, is a complex process where bio- chemical and mechanical signals regulate endothelial cell proliferation and movement. In this work, we introduce the first phase-field model of sprouting angiogenesis capable of predicting sprout morphol- ogy as a function of the elastic properties of the tissues and the traction forces exerted by the cells. The model is compact, only consisting of three coupled partial differential equations, and has the clear advantage of a reduced number of parameters. This model allows us to describe sprout growth as a func- tion of the cell-cell adhesion forces and the traction force exerted by the sprout tip cell. In the absence of proliferation, we observe that the sprout either achieves a maximum length or, when the traction and adhesion are very large, it breaks. We explore how different types of endothelial cell proliferation regulation are able to determine the shape of the growing sprout. The largest region in parameter space with well formed long and straight sprouts is obtained always when the proliferation is triggered by endothelial cell strain and its rate grows with angiogenic factor concentration. We conclude that in this scenario the tip cell has the role of creating a tension in the cells that follow its lead. On those first stalk cells, this tension produces strain and/or empty spaces, inevitably triggering cell proliferation. The new cells occupy the space behind the tip, the tension decreases, and the process restarts.

Keywords: Biological modelling, VEGF, angiogenesis, diabetic retinopathy

INVITED TALK
RECEPTORS, CHANNELS AND TRANSPORTERS

FLUORESCENCE AND COMPUTATIONAL STUDIES OF ZINC CHANGES IN HIPPOCAMPAL MOSSY FIBER SYNAPSES

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One of the most interesting properties of synaptic plasticity is long term potentiation (LTP), which is considered to represent a cellular model for learning and memory. In particular, the zinc-enriched mossy fiber synapses from hippocampal CA3 area exhibit a special form of LTP that is independent of the activation of the NMDA glutamate receptors.
This work addresses properties of endogenously released zinc through the measurement of electrically evoked presynaptic fluorescence zinc changes associated with mossy fiber LTP. Computer simulations of the dynamics of zinc complexes formed with various receptors and channels in the synaptic cleft region were also performed.
The fluorescence signals were obtained using the permeant zinc indicator N-(6-methoxy-8-quinolyl)-para-toluenesulfonamide, from hippocampal slices (400 μm), in response to single current pulses applied at the mossy fiber tract. The results indicate that presynaptic zinc signals are maintained during the expression of LTP evoked by high frequency stimulation (100 Hz, 1 s). It was also observed the existence of posttetanic zinc depressions that may, in part, be due to zinc binding to presynaptic KATP channels.
Following release zinc may bind to various glutamate receptors (NMDA, AMPA, KA), channels (VDCCs), transporters (GLAST, EAAT4) and molecules (ATP) in the synaptic cleft region, being also reuptaken. The dynamics and relative contribution of zinc clearance processes was evaluated through a computational model using dissociation and rate constants from the literature and assuming peak cleft free zinc values in the range 10 nM - 1 μM. The simulations suggest that for single and multiple types of stimulation the major zinc complexes are formed with the NMDA NR1a-NR2A and GLAST binding sites, respectively, being the concentration of most of the other complexes much smaller or negligible.

Keywords: Hippocampal mossy fibers, synaptic cleft, LTP, zinc, TSQ, modelling

INVITED TALK
RECEPTORS, CHANNELS AND TRANSPORTERS

THE STRUCTURE OF HETEROMERIC AMINO ACID TRANSPORTERS: GETTING THERE

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Heteromeric amino acid transporters (HATs) are relevant targets for structural studies. On the one hand, HATs are involved in inherited and acquired human pathologies (aminoacidurias, cancer, cocaine addiction), being relevant targets for drug development in cancer and schizophrenia. On the other hand, HATs are the unique example known of solute transporters composed of two subunits (heavy-SLC3A members, and light-SLC7A members or LAT transporters) linked by a disulphide bridge. Unfortunately, structural knowledge of HAT is scarce and is limited to the atomic structure of the ectodomain of a heavy subunit (human 4F2hc-ED) and distant prokaryotic homologues of the light subunits (APC transporters AdiC, ApcT and GadC) that share a LeuT-fold. Recent data at nanometer resolution of human 4F2hc (SLC3A2)/LAT2 (SLC7A8) revealed 4F2hc-ED situated on top of the external loops of the light subunit LAT2. Crystal structures of LAT transporters and structural models of HAT at least at subnanometric resolution become essential to stablish the structural bases for light subunit recognition and evaluate the functional relevance of heavy and light subunit interactions on the transport cycle. Advances towards the resolution of the structure of the first close prokaryotic homologue of the light subunits (a prokaryotic LAT transporter) and a vertebrate 4F2hc/LAT1 heterodimer will be presented.

Keywords: Heteromeric Amino acid Transporters; Structure; bacterial LAT; 4F2hc/LAT1

INVITED TALK
SUPRAMOLECULAR COMPLEXES AND BIOSPECTROSCOPIES

STRUCTURAL CHARACTERISATION OF THE CHAPERONE HSP70 FOLDING NETWORK

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In all cell types, molecular chaperones control cell proteostasis by preventing misfolding and aggregation or by directing proteins to degradation. The Hsp70s are a ubiquitous group of molecular chaperones involved in a large number of pathways that include folding, transport across membranes, and remodelling of specific proteins or complexes. These functions are executed by the Hsp70s with the help of a large number of co-chaperones with which they form transient complexes that are well suited for their analysis by electron microscopy and image processing. These studies provide a clear picture of the plasticity of these interactions and reveal in part the working mechanism of these nanomachines.

Keywords: Macromolecular structure, protein folding and degradation, molecular chaperones

INVITED TALK
SUPRAMOLECULAR COMPLEXES AND BIOSPECTROSCOPIES

SURFACE ENHANCED VIBRATIONAL SPECTROSCOPIC VIEW OF DNA REPAIR

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Endonuclease III (EndoIII) is a Fe-S cluster containing bifunctional DNA glycosylase which is involved in the repair of oxidation damaged DNA in the Base Excision Repair pathway. We have investigated the disputed role of the [4Fe-4S] cluster in EndoIIIs, using the enzyme from the highly radiation and desiccation resistant bacterium D.radiodurans (DrEndoIII) as a model and surface enhanced vibrational spectro-electrochemistry(1). DrEndoIII was immobilized on self assembled monolayer (SAM) functionalized metal supports, which provide surface enhancement of the signal and at the same time serve as electrodes. Distinct orientations of DrEndoIII in electrode/SAM/DrEndoIII constructs were achieved employing different biofunctional alkanethiol SAMs (e.g. OH-, COOH-, NTA-, DNA-terminated) which furthermore can mimic interactions with physiological substrate/reaction partners(2). Surface enhanced infrared absorption (SEIRA) and resonance Raman (SERR) spectroscopies were employed for monitoring the enzyme orientation and probing of the structural/redox properties of Fe-S cluster, respectively; simultaneous CV experiments revealed thermodynamic and electron transfer (ET) parameters of DrEndoIII in different electrode/SAM/DrEndoIII constructs. We provide direct evidence that the [4Fe–4S] cluster is responsible for the enzyme redox activity, and that this process is not exclusively DNA-mediated, as currently proposed. Furthermore, we show that heterogeneous ET critically depends on orientation of the immobilized DrEndoIII. Taken together, our results suggest that in vivo the FeS cluster may be redox-activated by charged species other than DNA, such as proteins or ROS. The obtained insights contribute to understanding of the role of [4Fe–4S] clusters in DNA glycosylases, including the enzyme for ROS damage repair in humans, hNTH1, for which DrEndoIII represents a bacterial homologue.

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2.Todorovic, S., Murgida D. 2016 ´Surface Enhanced Raman scattering of biological materials´ Encyclopedia of Analytical Chemistry, Wiley&Sons

Keywords: Vibrational spectroscopy, Fe-S cluster, DNA repair

INVITED TALK
RECEPTORS, CHANNELS AND TRANSPORTERS

ENTROPY–ENTHALPY COMPENSATION AT THE SINGLE CHANNEL LEVEL

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To achieve a highly efficient ion permeation, some biological channels have evolved to create a suitable internal environment where the ions lose part of the hydration shell, replacing the solvation water molecules by binding sites. We present here a thermodynamic analysis of ion conduction through the OmpF channel, a pore forming protein in the outer membrane of E. coli. Temperature-controlled conductance experiments on single channels reconstituted into planar lipid bilayers allow analyzing the different contributions to the free energy of ion conduction as a function of the cation type, the salt concentration, and the solution acidity (pH). The large enthalpy changes required to replace different cations with protons in key binding sites involve small changes in the Gibbs free energy, showing an approximate compensation between the enthalpic and entropic terms. Entropy–enthalpy compensations have been reported for different protein–ligand associations, with significant implications for ligand engineering. In particular, we show that the decomposition of free energy changes into the enthalpic and entropic contributions can be useful in understanding the essential functions like the pH sensitivity at the single protein level. The OmpF channel has a narrow constriction zone lined by ionizable residues (the sites for ion binding) segregated into positively and negatively charged segments. Calculated free energy profiles show a single free energy barrier located in the central constriction acting as the rate-limiting step for ion conduction. By using the Eyring formalism, we demonstrate that the pH sensing mechanism of the OmpF channel operates via ligand modification: increasing acidity induces the replacement of cations with protons in critical binding sites decreasing the channel conductance. Aside from the change in enthalpy associated with the binding, there is also a change in the microscopic arrangements of ligands, receptors and the surrounding solvent.

Keywords: bacterial porins / thermodynamic analysis / ion conduction

INVITED TALK
RECEPTORS, CHANNELS AND TRANSPORTERS

TRANSLOCATION OF PROTEINS ACROSS THE PEROXISOMAL MEMBRANE

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Peroxisomal matrix proteins are synthesized on cytosolic ribosomes, folded by cytosolic chaperones, and transported into the organelle by PEX5, the peroxisomal shuttling receptor. Understanding how these proteins are translocated across the organelle membrane has been the subject of intense research. Our laboratory has been using a cell-free organelle-based in vitro system to dissect the mechanism of this protein sorting pathway. The nature/properties of the protein translocation step, the ubiquitination/deubiquitination cycle of PEX5, as well as the role of the ATP-dependent mechanoenzymes PEX1/PEX6 in this pathway will be discussed.

Keywords: Peroxisomes, protein translocation, PEX5

INVITED TALK
SUPRAMOLECULAR COMPLEXES AND BIOSPECTROSCOPIES

ENGINEERING OF ELECTRON TRANSFER COMPONENTS FOR OPTIMAL E-/H+ ENERGY TRANSDUCTION PROCESSES: A FOUNDATION TO IMPROVE MICROBIAL FUEL CELLS PERFORMANCE

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Geobacter species have received much attention for their potential use for bioremediation of radioactive and toxic metals in subsurface environments or as current producers in microbial fuel cells (MFC). In both cases, electrons are exported from inside to the cell exterior, via a mechanism designated extracellular electron transfer (EET). However, the use of extracellular electron acceptors leads to a considerable decrease of the biomass production. Therefore, understanding the molecular and structural basis of the mechanisms involved in energy transduction would provide important tools to improve bacterial biomass yields and optimize the Geobacter-based applications. A family of triheme cytochromes was identified as important players in the Geobacter sulfurreducens EET pathways. Two members of this family (PpcA and PpcD) were found to couple e-/H+ transfer in the physiological pH range through the redox-Bohr effect, a feature not observed for cytochromes PpcB and PpcE. All these cytochromes are highly homologous in their amino acid sequence and structures. However, residue 6, located in the vicinity of their redox-Bohr centre, is a leucine in PpcA and PpcD, whereas in PpcB and PpcE is a phenylalanine. Trying to understand the molecular control of the redox-Bohr effect in this family of cytochromes, we replaced the Leu6 residue by a Phe in PpcA and observed that, in contrast with the native protein, the mutant had lost the ability to couple e-/H+ transfer. The reverse mutation (F6L) was introduced in PpcB and PpcE, yielding an evident redox-Bohr effect in both cytochromes. These results clearly establish the role of residue 6 in the control of the redox-Bohr effect in this family of cytochromes, a feature that can enable the rational engineering of G. sulfurreducens strains with optimal energy transduction properties for various biotechnological applications, including current production by MFC.

Keywords: Geobacter; Multiheme cytochrome; Electron transfer; Redox-Bohr effect; NMR

INVITED TALK
SUPRAMOLECULAR COMPLEXES AND BIOSPECTROSCOPIES

ANALYSIS OF NANOSCALE COMPARTMENTALIZATION OF PI(4,5)P2 IN LIVING CELLS AND MODEL MEMBRANES THROUGH FLUORESCENCE SPECTROSCOPY AND FRET IMAGING

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Phosphatidylinositol-4,5-bisphosphate (PI(4,5)P2) is the most abundant phosphorylated phosphoinositide (PIP) at the cytosolic face of mammalian plasma membranes and has been associated with a great number of vital cellular functions. The variation of its local concentration, in time and space, has been claimed to be responsible for the spatiotemporal recruitment of proteins with diverse functions, such as endocytosis and cytoskeleton adhesion to the membrane. Among other factors, the presence of cholesterol-enriched domains, elevated concentrations of divalent cations and the cytoskeleton itself have been suggested to be involved in determining PI(4,5)P2 organization and clustering. Using a combination of fluorescence spectroscopy and microscopy techniques, we show that both the number and position of phosphorylations in the inositol ring of phosphoinositides are crucial for defining the extent of PIP clustering and relative cluster size. Additionally, we show that formation of the liquid ordered phase strongly promotes formation of PIP clusters in model membranes. Evidence for the formation of PI(4,5)P2 enriched nanodomains in the plasma membrane of living cells was obtained through FRET microscopy of pleckstrin homology (PH) domains tagged with fluorescent proteins. FRET imaging data was compared with the theoretical expectation for FRET in the case of a homogeneous distribution of PH domains, and results confirm that distinct PI(4,5)P2 local densities are found in different cellular models, suggesting that PI(4,5)P2 organization varies significantly between eukaryotic cells. In HeLa cells, disruption of the cytoskeleton decreased significantly the compartmentalization of PI(4,5)P2, proving that the organization of a pool of PI(4,5)P2 molecules depends on the presence of membrane-cytoskeleton interactions. On the other hand, formation of PI(4,5)P2 plasma membrane nanodomains is shown to be independent of cholesterol levels, suggesting that membrane rafts are not involved.

This work was supported by FCT (FAPESP/20107/2014, RECI/CTM-POL/0342/2012, UID/NAN/50024/2013, SFRH/BD/80575/2011, SFRH/BPD/105651/2015). M.J.S. current address is: Nanoscopy, Nanophysics, Istituto Italiano di Tecnologia, Genoa, Italy.

Keywords: Phosphatidylinositol-4,5-bisphosphate; Fluorescence spectroscopy, Förster Resonance Energy Transfer; FRET Homotransfer

INVITED TALK
PROTEIN STRUCTURE, FOLDING AND DYNAMICS

ENVEILING INTERACTIONS AND THEIR SPECIFICITIES BETWEEN PROTEOLYTIC ENZYMES, INHIBITORS AND PROTEIN LIGANDS

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Nowadays there is a tendency towards an integrated view on proteolytic enzymes (proteases) and their molecular partners in vivo, particularly in the genomics-proteomics-interactomics context. This also happens in one of their most frequent variants as are metalloproteases and, within them, in metallocarboxypeptidases (MCPs). The significant increase in the number of reported MCPs in the last decade evidences that still is not fully understood its simultaneous or specific action over substrates, as well as its restriction by ligands and natural inhibitors. The fact that such substrates and efectors frequently have a protein nature, add more degrees of sophystication to these interactions (i.e. at physico-chemistry levels, of discrimination capability and kinetics), as well as on the modulation of the activities and specificities that promote. In the case of the M14 familly, one of the most extensive and known, more than 30 variants of such MCP enzymes have been identified, classified as M14A, B and C, mostly secretory, as well as the recently emergent cytosolic forms (CCP type) for the M14D subfamily. Given that all of them seem to keep a conserved "canonical" carboxypeptidase domain, and equivalent ligand recognition sites, there is a real challenge about the comprehension of their complex relationships and their discriminative interaction with natural substrates (peptidic or proteic, given they are proteases), and with their environmental protein inhibitors. This is extensive to its focused drug design to generate ligands for their control, of applicative interest given their biological and biomedical implications.

MCPs, as other proteases, have a characteristic property within interactomics: they act in transient way over protein substrates, promoting cleavages that strongly modify its conformation and functionality. To detect such interactions is not an easy but still feasible task. Although of different kinetics, the knowledge of the role played on such enzymes by the numerous protein inhibitors found in nature is also limited and diverse. Some related selected cases will be discussed, as well as dedicated strategies, mainly based on mass spectrometry and structural biology, for their identification and characterization.

Keywords: Protemics-interactomics, structural biology, proteases-carboxypeptidases, inhibitors, ligand and drug discovery

INVITED TALK
PROTEIN STRUCTURE, FOLDING AND DYNAMICS

UNDERSTANDING DENGUE VIRUS CAPSID PROTEIN FOR DRUG DESIGN: COMBINING WET-LAB AND COMPUTATIONAL BIOLOGY

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Dengue virus (DENV) infection affects millions of people and is becoming a major global disease for which there is no specific available treatment. pep14-23 is a recently designed peptide, based on a conserved segment of DENV capsid (C) protein [1-3]. This peptide inhibits the interaction of DENV C with host intracellular lipid droplets (LDs) [2, 3], essential for viral replication [1, 4]. Moreover, pep14-23 also inhibits DENV C interaction with very low-density lipoproteins [5, 6], which may prevent lipoviroparticle formation. Here [7], combining bioinformatics and biophysics, we analyzed pep14-23 structure and ability to bind different phospholipids, relating that information with the full-length DENV C protein. We show that pep14-23 acquires α-helical conformation upon binding to anionic phospholipid membranes, displaying an asymmetric charge distribution structural arrangement. Structure prediction for the N-terminal segment reveals four viable homodimer orientations that alternatively shield or expose the DENV C hydrophobic pocket. Taken together, these findings suggest a new biological role for the disordered N-terminal region, which may function as an autoinhibitory domain mediating DENV C interaction with its biological targets. The results fit with our current understanding of DENV C and pep14-23 structure and function, paving the way for similar approaches to understanding disordered proteins and improved peptidomimetics drug development strategies against DENV and similar Flavivirus infections.

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Keywords: Dengue virus capsid protein; Inhibitor peptide pep14-23; Intrinsically disordered protein region; Peptide-lipid interactions; Conformational changes

INVITED TALK
NANOBIOPHYSICS AND MOLECULAR RECOGNITION

TAILORED SYNTHETIC AFFINITY REAGENTES

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Over the past 40 years monoclonal antibodies and derived structures became the standard binding proteins representing powerful tools in biotechnology and biomedicine, namely on protein purification, in vivo and in vitro diagnostic and targeted therapy. Other protein binding scaffolds, with the robustness and versatility required, are recently being explored. We employed biological and chemical combinatorial libraries supported by computational design tools to develop robust peptidomimetics based on different scaffold molecules. The scaffold molecules ranged from small synthetic ligands based on the triazine and Ugi reactions, to artificial β-hairpin peptides and small protein domains produced chemically. We studied the potential of these scaffold affinity reagents to find binding partners against several targets. In this presentation we will focus on the design and development of affinity reagents against tagged recombinant proteins and GFP fusion proteins (1), phosphorylated peptides (2) and virus-like particles (3).

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Keywords: Molecular Recognition, Biomimetics, Combinatorial Chemistry

INVITED TALK
NANOBIOPHYSICS AND MOLECULAR RECOGNITION

NOVEL CORRELATIVE MICROSCOPY TOOLS TO STUDY BIOLOGY AT THE NANOSCALE

Cristina Flors[1]
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Correlative microscopy is a powerful approach that combines the capabilities of individual microscopy techniques, typically with the aim of obtaining high specificity and detailed structural information of the same sample area. In this talk, I will present our most recent progress in developing novel tools for different modalities of correlative imaging. The first part will deal with the implementation of a novel correlative microscope that allows sequential imaging of the same sample area by atomic force microscopy (AFM) and super-resolution fluorescence microscopy. I will discuss the technical aspects of the correlative microscope, its application to validate and scrutinize super-resolution methods, and its use to characterize hybrid nanomaterials. In the second part of the talk, the screening and characterization of fluorescent proteins as genetically-encoded tags for correlative light and electron microscopy (CLEM) will be presented. These fluorescent proteins produce reactive oxygen species upon irradiation, which locally photooxidize diaminobenzidine to form an osmiophilic precipitate that gives contrast in EM. Both correlative imaging modalities have the power to answer new biological questions at the nanoscale.

Keywords: Correlative microscopy, super-resolution microscopy, photosensitizing fluorescent proteins

INVITED TALK
PROTEIN STRUCTURE, FOLDING AND DYNAMICS

SER OR LEU? STRUCTURAL IMPACT OF GENETIC CODE AMBIGUITY IN CANDIDA ALBICANS

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Candida albicans is an opportunistic polymorphic fungal pathogen normally residing commensally on mucosal surfaces, the skin, and the gastrointestinal and genitourinary tracts. However, in immunocompromised patients it can lead to superficial mucosal infections or to life-threatening disseminated candidemia. In response to changes in pH, temperature, oxygen levels or nutrient sources, a cascade of molecular events is triggered, leading to morphogenetic alterations to promote survival and thwart damage induced by host macrophages. The complex biology of this human pathogen, reflected in its morphological plasticity, allows a perfect adaptation to the human host niches and constant adjustment to microenvironmental fluctuations.

This complexity is broadened by C. albicans ability to ambiguously translate the universal leucine CUG codon predominantly as serine (97%), but also as leucine (3%). CUG ambiguity affects ~66% of C. albicans protein-coding genes, influences all biological processes and generates proteome diversity. Although a significant effort has been put into studying morphogenetic changes, virulence and antibiotic resistance in C. albicans, the role of CUG ambiguity in pathogenesis has been mostly disregarded.

Our previous, large-scale analysis of CUG-encoded residue-containing proteins from C. albicans and other CTG-clade fungi, revealed that 90% of these residues are optimally located to allow ambiguity without inducing protein misfolding. Interestingly, a small number of enzymes have CUG-encoded residues within conserved active sites or in their vicinity, where serine or leucine incorporation can differentially modulate catalytic activity. The structural and functional impact of CUG ambiguity in a group of proteins implicated in signal transduction pathways, cell wall biosynthesis and morphological switching will be discussed.

Keywords: Genetic code, tRNA synthetase, kinase

INVITED TALK
PROTEIN STRUCTURE, FOLDING AND DYNAMICS

SINGLE-MOLECULE ANALYSIS OF PROTEINS WITH NANOPORE TECHNOLOGY

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Nanopore technology measures the ionic current passing through narrow (nanometer wide) holes made in an insulating membrane, and how molecules rectify this current when they traverse the nanopore. This technology is being used as a new tool to sequence DNA or to follow the chemical reaction of a single-molecule in real time. More recent developments aimed to expand its analytic capabilities to study proteins. Its relevance is justified by two reasons: 1) because it mimics natural processes: in vivo protein synthesis and degradation occurs mainly through nanometer sized pores; and 2) because it promises powerful biotechnological applications: from single-molecule protein sequencing to high-throughput drug screening. Here I will explain the basis of the technology and its implementation into proteins. Later I will show our recent advances in the study of vectorial protein unfolding and folding. And I will finish showing how nanopore technology can be used in phosphoproteomics and in drug screening.

Keywords: Single-molecule, nanopore, vectorial folding

INVITED TALK
NANOBIOPHYSICS AND MOLECULAR RECOGNITION

ANTIMICROBIAL EFFECTS OF PEPTIDES ISOLATED FROM AMPHIBIAN SECRETIONS

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Amphibian secretions are a rich source of antimicrobial peptides (AMPs). Such peptides can show activity against different microorganisms including bacteria, fungi and parasites. Most commonly the action of such peptides directly against the cell membrane. In this work we have characterized the structure and antimicrobial action of antimicrobial peptides recently isolated from Brazilian amphibians.

Specifically, we studied a Dermoseptin, DRS01, originally isolated from Phyllomedusa hypochondrialis, and Ocellatins PT1 to PT8, which we recently isolated from the secretion of Leptodactylus pustulatus. Both classes of peptides exhibit action against bacteria, the action of DRS01 being particularly strong. In addition, both classes are effective against the promastigote model of Leishmania.

We study the interactions between PT1 and PT8 and model membranes which mimic mammalian, bacterial, and parasite membranes using surface plasmon resonance (SPR). Furthermore, the effects of these peptides on L. chagasi cell morphology is studied using electron microscopy and atomic force microscopy. Interestingly, the effects of the two classes of peptides on the cell membrane can be easily distinguished by these techniques.

Keywords: Microscopy, leishmania, leismaniosis, membranes, peptides

INVITED TALK
NANOBIOPHYSICS AND MOLECULAR RECOGNITION

DETERMINING THE GEOMETRY OF EGF RECEPTOR OLIGOMERS ON CELLS WITH 6 NM RESOLUTION

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The human epidermal growth factor receptor (EGFR) initiates signals for cell proliferation and transformation. This receptor has an extracellular growth factor-binding domain (ECD), a single-pass transmembrane region, and an intracellular domain that has tyrosine kinase activity.

The activation of the EGFR is triggered by the binding of small growth factor polypeptide ligands and involves the formation of dimers of these receptors. Oligomers are also commonly observed on cell surface, yet little is known about their structures and their functional role in EGFR signalling. This is largely attributable to the lack of methods with sufficient resolution.

We developed a super-resolution method based on fluorophore localisation imaging with photobleaching (FLImP) to investigate the geometry and size of oligomers of the EGFR family on the cell surface with ~ 5-6 nm resolution. By using non-activating peptide markers and combining the FLImP super-resolution method with fluorescence resonance energy transfer to determine intra-receptor conformation, and single particle tracking to measure interaction kinetics we are beginning to determine conformational changes and interactions in oligomers that regulate EGFR signal transduction across the plasma membrane.

Keywords: EGF receptor, super-resolution, cell signalling

SHORT COMMUNICATION
MEMBRAINE AND PROTEIN-LIPID INTERACTIONS

X-RAY DIFFRACTION STUDIES TO STUDY DRUG-MEMBRANE INTERACTIONS

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X-ray diffraction at both small angle (SAXS) and wide angle (WAXS) represents a powerful tool for the study of drug–membrane interactions. The position of the diffraction peaks, their intensity, and their angular distribution can provide information related to the structures within the sample. It is an especially useful technique to obtain information on the localization of drug molecules and on the conformational (phase) changes of membranes. The observed differences provide information on the localization of the drug within the bilayer as well as on the perturbation of the membrane induced by the drug.

In this work the effect of non-steroidal anti-inflammatory (NSAIDs) and anti-cancer drugs on multilamellar vesicles as mimetic models either composed by a single phospholipid or a phospholipid mixture and regrading each lipid state will be presented. The studies were performed using synchrotron radiation.

Overall, the interaction of these drugs with lipid membrane models depends on three factors: the drug-to-lipid concentration ratio, the initial organization of the lipids and the medium pH. Ultimately, the drugs effect on the structure of the bilayers can be related with their therapeutic and/or toxic effect.

Acknowledgments
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Keywords: SAXS, WAXS, lipid membranes, NSAIDs, anti-cancer drugs

SHORT COMMUNICATION
MEMBRAINE AND PROTEIN-LIPID INTERACTIONS

HIGH-AFFINITY COINCIDENT DETECTION OF FRIZZLED 7 AND PIP2 BY THE PDZ2 DOMAIN OF SYNTENIN SUPPORTS FRIZZLED 7-SYNTENIN TRAFFICKING AND SIGNALING.

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PDZ domain containing proteins work as intracellular scaffolds to control spatiotemporal aspects of cell signaling. This function is supported by the ability of their PDZ domains to bind other proteins such as receptors, but also phosphoinositide lipids important for membrane trafficking. No crystallographic data is currently available that addresses how PDZ domains bind to phosphoinositides and integrate peptide and phosphoinositide binding. Here, we report a crystal structure of the syntenin PDZ tandem in complex with the carboxy-terminal fragment of Frizzled 7 and phosphatidylinositol 4,5-bisphosphate (PIP2). The crystal structure reveals a tripartite interaction formed via the second PDZ domain of syntenin. Biophysical and biochemical experiments establish co-operative binding of the tripartite complex and identify residues crucial for membrane PIP2-specific recognition. Experiments with cells support the importance of the syntenin-PIP2 interaction for plasma membrane targeting of Frizzled 7 and c-jun phosphorylation. This study contributes to our understanding of the biology of PDZ proteins as key players in membrane compartmentalization and dynamics.

Keywords: PDZ, phosphatidylinositol 4,5-bisphosphate, syntenin, Frizzled, structure

SHORT COMMUNICATION
MEMBRAINE AND PROTEIN-LIPID INTERACTIONS

CARDIOLIPIN'S IMPORTANCE IN MITOCHONDRIAL MEMBRANE MODEL CONSTRUCTION. MITOCHONDRIA MEMBRANE, CARDIOLIPIN, DYNAMIC LIGHT SCATTERING, FLUORESCENCE ANISOTROPY

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Cardiolipin is a key phospholipid most specifically found in the mitochondria membrane of yeasts, plants, and animals. Cardiolipin nature has not been fully understood and its function may not be the same in prokaryotes and eukaryotes. Nevertheless, in eukaryotes CL is the only phospholipid exclusively localized in mitochondria being essential for the maintenance of its integrity and dynamics. Cardiolipin physical properties point out for a number of interactions that may have implications in the structural organization of biological membranes and alter global membrane properties such as: structure, packing density, and surface charge density. Different mitochondria CL levels, or even its absence, are related with different biological effects/consequences such as heart failure, aging, cell death or diabetes, to name a few examples. In this work, different mitochondrial membrane model systems were studied, by different techniques, in order to study the influence of CL in their thermotropic properties. The results point out that CL incorporation, being a doubly negatively charged four-tailed phospholipid with a polar head group, which is relatively rigid (i.e. it is a mobility-restricted entity) with a relative small cross-sectional area per lipid, in model system membranes has a significant impact on the membrane properties and should be considered when building up model systems of mitochondria membrane.

Keywords: Mitochondria membrane, cardiolipin, dynamic light scattering, fluorescence anisotropy

SHORT COMMUNICATION
MEMBRAINE AND PROTEIN-LIPID INTERACTIONS

SYNERGISTIC ACTION OF ACTINOPORIN ISOFORMS FROM THE SAME SEA ANEMONE SPECIES ASSEMBLED INTO FUNCTIONALLY ACTIVE HETEROPORES

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Among the toxic polypeptides secreted in the venom of sea anemones, actinoporins are pore forming toxins whose toxic activity relies on the formation of oligomeric pores within biological membranes. Intriguingly, actinoporins appear as multigene families which give rise to many protein isoforms in the same individual displaying high sequence identities but large functional differences. However, the evolutionary advantage of producing such similar isotoxins is not fully understood. Here, using sticholysins I and II (StnI and StnII) from the sea anemone Stichodactyla helianthus, it is shown that actinoporin isoforms can potentiate each other's activity.

Through hemolysis and calcein releasing assays, it is revealed that mixtures of StnI and StnII are more lytic than equivalent preparations of the corresponding isolated isoforms. It is then proposed that this synergy is due to the assembly of heteropores since (i) StnI and StnII can be chemically cross-linked at the membrane and (ii) the affinity of sticholysin mixtures for the membrane is increased with respect to any of them acting in isolation, as revealed by isothermal titration calorimetry experiments. These results help to understand the multigene nature of actinoporins and may be extended to other families of toxins that require
oligomerization to exert toxicity.

Keywords: Pore-forming-toxin, sticholysin, erythrocyte, oligomerization, cross-linking, lipid-protein interaction

SHORT COMMUNICATION
COMPUTATIONAL BIOPHYSICS

COUPLED PARTICLES IN A CONFINED GEOMETRY

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Diffusion processes, which determine the transport properties of suspended particles, play an extremely important role in many fields of physics, chemistry, and biology. These properties, which have been extensively studied for free particles, are modified when the particles are subjected to confinement. For Brownian particles, the confinement means that its transport properties are not just controlled by the fluctuation statistics resulting from the medium in which it is suspended, but also by the restricted phase space in which it may evolve. Indeed recently, much effort has gone into understanding exactly how these transport properties are modified in such settings.

In this talk, we discuss an extention of the study of diffusion processes for single particles in confined geometries. In particular, we consider the case of two coupled particles -- dimers, and examine how the transport properties are modified due to the interaction that exists between them. In more detail, by numerical simulations we examine a system consisting of two coupled particles whose Brownian motion is restricted to a corrugated channel by confining walls. We are interested in how the transport properties of the dimer are modified by the harmonic coupling that exists between its monomers. It is shown that both the mobility and effective diffusion respond non-trivially to changes in the coupling strength, a response that is further complicated by the dependence that these properties have on the constant external bias force that drives the particles through channel bottlenecks.

Keywords: Coupled particles; diffusion process; confined geometry

SHORT COMMUNICATION
COMPUTATIONAL BIOPHYSICS

STRUCTURE AND ACTIVITY OF THE INFLUENZA FUSION PEPTIDE: A COMBINED SIMULATION AND EXPERIMENTAL APPROACH

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The emergence of an influenza pandemic is one of the biggest health threats of our time and, therefore, there is an urgent need to develop vaccines and drugs against a broad spectrum of influenza viruses (IV). A promising strategy to combat IV is to inactivate the fusion process between the viral and host membranes, which is mediated by the surface protein hemagglutinin (HA). During this process, the N-terminal region of HA, known as fusion peptide (FP), inserts into the host membrane. Although it has been shown that the FP plays a crucial role in the fusion process, the molecular effect of the peptide remains unclear.

In order the shed light into this problem, we used a combination of state-of-the-art experimental and simulation techniques to analyse the WT influenza FP and four mutants. Fluorescence based methods were used to analyse the partition coefficient of the WT and mutant peptides in model membranes, and their ability to promote lipid-mixing was analysed using a (FRET)-based assay. To rationalize the results obtained in these experiments, we analysed the energy landscape of the peptides by performing bias-exchange metadynamics (BE-META) simulations. This allowed us to characterize the conformational properties of the WT peptide in a model membrane and understand how this structure is affected by the mutations studied. This study also elucidated the factors that explain the reduced activity of the mutants, which contributes to a better understanding of the role of the influenza FP in the fusion process.

Keywords: Influenza virus, fusion peptide, metadynamics simulation

SHORT COMMUNICATION
COMPUTATIONAL BIOPHYSICS

SIMULATION OF A PARTLY ATOMISTC, PARTLY COARSE-GRAIN MEMBRANE CHANNEL

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The time and length scales of computer simulations are frequently limited by the available computer power. Coarse grain (CG) models, in which several atoms are represented by a single super-particle, can provide a computational speedup of several orders of magnitude. However, CG models are not accurate enough for all applications, and one might wish to combine them with finer models. To this end, we have adapted the region-based adaptive resolution scheme (AdResS) to focus on only part of a protein. In specific, we simulate a protein membrane channel partly at atomistic detail (the channel core) and partly at CG detail (the rest of the protein and the membrane; using the Martini model). We show that for this setup the multiscale approach was able to preserve the conformation in and away from the atomistic region, while preserving some of the speedup from the CG approach.

Keywords: Multiscaling AdResS Martini

SHORT COMMUNICATION
COMPUTATIONAL BIOPHYSICS

COMPUTATIONAL METHODS TO CALCULATE PKA VALUES AT THE WATER/MEMBRANE INTERFACE

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pH is a crucial physicochemical property that affects most biomolecules. Changes in protonation equilibrium of susceptible sites will modify the electrostatic environment and, consequently, have an effect on the molecular structure, stability and catalysis (1). The pKa values of the typical titrable amino acids can be significantly influenced by changes in solvent mixture or due to insertion in a lipid bilayer (2-3).

In this work, we present a methodology that allows us to calculate pKa values of both peptides/proteins and lipid headgroups at the water/membrane interface. We take advantage of the recently developed CpHMD-L methodology (4) and apply it to different systems, namely, the model Ala-based pentapeptides that have already been well characterized in water by Pace and co-workers (5), and the pHLIP peptide, a 36 amino acid peptide derived from bacteriorhodopsin that is able to insert the membrane at acidic pH (6).

Our most recent results with these systems will be presented.

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Keywords: pH, membranes, CpHMD, simulations, peptides

SHORT COMMUNICATION
BIOENERGETICS

HOW MOLECULES FORM TRANSIENT COMPLEXES IN PHOTOSYNTHESIS AND RESPIRATION

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Protein complex formation is at least a two-step process in which the formation of a final, well-defined complex entails the initial formation of a dynamic encounter complex. The lifetime of the protein complex is determined by the dissociation rate. Highly transient complexes, with lifetimes on the order of milliseconds, exhibit moderate or low binding affinities, with dissociation constants in the uM–mM range. Electron transfer (ET) reactions mediated by soluble redox proteins exchanging electrons between large membrane complexes in photosynthesis and respiration are excellent examples of transient interactions.

Here, experimental approaches based on dia and paramagnetic NMR spectroscopy are combined with NMR restraint- or charge-driven docking simulations to study the molecular recognition processes in ET complexes, using the cyanobacterial Cf-Cc6 interaction in photosynthesis and the plant Cc1-Cc adduct in respiration, as physiological model systems. Both ET ensembles exhibit optimal coupling between the redox centers although they might differ in their dynamic behavior. Needless to say that such an integrative methodology opens new perspectives in our understanding of the dynamic, transient adducts formed between proteins beyond the model systems herein analyzed.

Keywords: Cytochrome, NMR, photosynthesis, respiration, transient interactions

SHORT COMMUNICATION
BIOENERGETICS

COUPLING BETWEEN PROTONATION AND CONFORMATION IN CYTOCHROME C OXIDASE: INSIGHTS FROM CONSTANT-PH MD SIMULATIONS

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Cytochrome c oxidases (CcOs) are the terminal enzymes of the respiratory chain in mitochondria and most bacteria. These enzymes reduce dioxygen (O2) to water and, simultaneously, generate a transmembrane electrochemical proton gradient. Despite their importance in the aerobic metabolism and the large amount of structural and biochemical data available for the A1-type CcO family, there is still no consensually accepted description of the molecular mechanisms operating in this protein. A substantial number of questions about the CcO's working mechanism remains to be answered, including how the protonation behavior of some key residues is modulated during a reduction cycle and how is the conformation of the protein affected by protonation.

The main objective of this work was to study the protonation–conformation coupling in CcOs and identify the molecular factors that control the protonation state of some key residues. In order to directly capture the interplay between protonation and conformational effects, we have performed constant-pH MD simulations of an A1-type CcO inserted into a lipid bilayer in two redox states (oxidized and reduced) at physiological pH. From the simulations, we were able to identify several groups with unusual titration behavior that are highly dependent on the protein redox state, including the A-propionate from heme a and the D-propionate from heme a3, two key groups possible involved in proton pumping. The protonation state of these two groups is heavily influenced by subtle conformational changes in the protein (notably of R481I and R482I) and by small changes in the hydrogen bond network.

Keywords: Cytochrome c oxidase, Protonation–conformation coupling, proton transfer, redox-dependent change, computer simulation

SHORT COMMUNICATION
BIOENERGETICS

CONTROL OF MITOCHONDRIAL BIOGENESIS BY FREE HEME IN THE YEAST S. CEREVISIAE

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Mitochondrial biogenesis is a key feature for both cell growth and fate, due to the energy demand during the cell biomass formation and function maintenance. It is established that the heme molecule is the precursor for the cytochromes of the respiratory chain, but there is evidence that heme can have the feature of a regulating molecule at the transcriptional level. We investigated the role of heme as a regulatory molecule for the HAP2/3/4/5 transcriptional complex, the yeast master regulator for mitochondrial biogenesis in which the Hap4p acts as the regulatory subunit. We were able to manipulate the cellular amount of free heme in a S. Cerevisiae yeast strain lacking the Aminolevulinic Acid synthase, the enzyme that synthetizes the 5-Aminolevulinic Acid, which is an upstream molecule of the heme biosynthesis. We show a directly proportional relationship between the cellular amount of heme and the Hap4 protein. Moreover, we were able to show that when the cellular amount of free heme decreases, the activity of the transcriptional complex responsible for mitochondrial biogenesis decreases. We measured the activity of the HAP complex through two transformed yeast strains, each one expressing a promotor that has a site for the interaction with the complex using the beta-galactosidase gene as an activity reporter. This is the first time that it has been shown that heme could act as a regulatory molecule for the mitochondrial biogenesis through the regulation of the HAP2/3/4/5 transcriptional complex in yeast.

Keywords: Mitochondria, Heme biosynthesis, Yeast

SHORT COMMUNICATION
BIOENERGETICS

THE ROLE OF THE STRICTLY CONSERVED E172 IN THE REACTION MECHANISM OF TYPE-II NADH:QUINONE OXIDOREDUCTASE OF S. AUREUS

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Multi Resistant Staphylococcus aureus (MRSA) associated infections are responsible for more than 150,000 human deaths per year. New protein targeting antibiotics are increasingly necessary due to the appearance of new resistant strains.

Type-II NADH:quinone oxidoreductases (NDH-2s) are the only enzymes with NADH:quinone oxidoreductase activity expressed in the respiratory chains of many pathogenic organisms and for that, they have been proposed as possible new drug targets for the rational design of antibiotics. In this work we performed a thorough investigation of the role of the strictly conserved Glutamate 172 (E172) residue on the enzymatic mechanism of NDH-2 from Staphylococcus aureus. For this we carried out a complete biochemical characterization of the mutants E172A, E172S, E172Q and E172D. Our steady state kinetic measurements show a clear reduction in the overall reaction rate; the fast kinetics experiments indicate that both the oxidative and reductive reactions were affected; and our substrate interaction studies by fluorescence quenching analysis point out that the binding of the quinone to the protein was also affected by the mutations. Together our results suggest that E172 is of central importance in the catalytic mechanism of the enzyme, having a determinant role on both half reactions, and that the properties of its side chain are important for the reactivity of both substrates.This work aims at contributing to the functional knowledge on the NDH-2 family, whose members are most relevant for the bioenergetic metabolism of the main bacteria causative of infections.

Keywords: NDH-2; Respiratory chain; Staphylococcus aureus; quinone; Enzyme kinetics

SHORT COMMUNICATION
CELL AND TISSUE BIOPHYSICS

CERAMIDE-GEL DOMAINS IN LIVING CELLS: UNDERSTANDING THE ROLE OF MEMBRANE BIOPHYSICAL PROPERTIES IN CELL (PATHO)PHYSIOLOGY

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Ceramides are bioactive sphingolipids with important roles in cell physiology and pathology. Ceramides activity have been related to their unique biophysical properties, namely to their ability to form tightly packed membrane domains 1. Nevertheless, the biophysical impact of ceramides in living cells is still poorly characterized. To tackle this issue we employed multiple methodologies, including steady-state and time-resolved fluorescence spectroscopy, confocal and 2-photon microscopy and fluorescence lifetime imaging (FLIM). Our results show that ceramide formation upon stimulation with TNF-α resulted in an increase in the bulk membrane order and in the formation of intracellular vesicles. Surprisingly, these vesicles displayed biophysical features typical of the gel phase, as shown by the very high lifetime of trans-parinaric acid and Laurdan generalized polarization suggesting that ceramide enriched domains accumulate in these structures. Inhibition of neutral sphingomyelinase blocked the formation of those intracellular vesicles and the increase in membrane order, further showing that the observed alterations are dependent on ceramide formation. Colocalization imaging with different markers suggests that ceramide-derived vesicles are involved in endo-lysosomal trafficking. Overall our results highlight the existence of ceramide-derived biologically-relevant gel domains in cellular membranes upon stress stimulation. Interestingly, a high number of intracellular vesicles with gel phase properties were also observed in several cancer cell lines in the absence of stress stimuli, suggesting that these vesicles might also have a role in cell pathology.

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Keywords: Ceramide; gel domains in living cels, patophysiology of cells, biophysical properties

SHORT COMMUNICATION
CELL AND TISSUE BIOPHYSICS

THE ROLE OF SPATIOTEMPORAL HETEROGENEITY IN THE REGULATION OF CELLULAR FUNCTION

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Molecular organization and diffusion regulate numerous processes underlying biological functions in living cells. In the last decade, advances in single-molecule fluorescence and super-resolution nanoscopy have allowed the visualization of cellular components at unprecedented spatial and temporal resolution, providing novel insights on a variety of cellular processes. These experiments have revealed that the complexity of the cellular environment often produces large heterogeneity both at the structural and dynamical level, whose implications for the cellular function are not fully understood. Based on recent experimental results, I will present two examples of function regulation in living cells influenced by spatial and/or temporal heterogeneity. The first involves the structure of chromatin inside the cell nucleus, controlling the regulation of gene expression and the access of transcriptional factors to genes. By means of stochastic optical reconstruction microscopy (STORM), we have visualized the organization of histone proteins in the nucleus of mammalian cells and found that the nucleosomes are arranged in heterogeneous groups, displaying a broad distribution of composing units, sizes and densities. Importantly, the comparison of this organization in stem and somatic cells shows that these properties are correlated with the degree of pluripotency, i.e. the cell propensity to differentiate.

Second, I will discuss the organization and dynamics of DC-SIGN, a transmembrane pathogen-recognition receptor involved in the capture of viruses, bacteria and parasites. By combining stimulated emission depletion (STED) microscopy and single particle tracking, we have found that DC-SIGN displays a multiscale organization in the cell membrane. In addition, its motion deviates from a purely Brownian behavior, exhibiting anomalous diffusion with signatures of weak-ergodicity breaking and aging. The comparative investigation of receptor mutants allowed us to correlate the receptor's motion with molecular structure and function, thus establishing a link between nonergodicity and DC-SIGN capability in pathogen capture and internalization.

Keywords: Single particle tracking, super-resolution, chromatin, membrane receptors, diffusion

SHORT COMMUNICATION
CELL AND TISSUE BIOPHYSICS

FLEXIBLE NANOFILMS COATED WITH ALIGNED PIEZOELECTRIC MICROFIBERS PRESERVES ENGINEERED CARDIAC TISSUE'S FUNCTION

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Few examples have reported the successful use of engineered cardiac tissue for drug screening/toxicology assessment. This issue is of paramount importance since cardiac toxicity has been implicated in 28% of drug withdrawals over the last 30 years (Gwathmey et al., 2009). The development of tissue engineered cardiac tissue for drug screening requires the development of scaffolds that can be easily produced, flexible and small. Additionally, it should preserve the long-term contractility of cardiomyocytes, ideally in the absence of complex external apparatus. Here we developed a flexible scaffold relatively easy to prepare that reproduces aspects of cardiac ECM, which can preserve the contractility of fetal cardiomyocytes for high-throughput drug screening applications. The scaffold is formed by a nanofilm of poly(caprolactone) (NF) coated by piezoelectric microfibers (PIEZO) composed of poly(vinylidene fluoride–trifluoroethylene) (PVDF-TrFE). When a mechanical force is applied to a piezoelectric material a shift or rotation of the constitutive dipole crystals occurs resulting in the generation of an electric charge. Therefore, PIEZO fibres may act as Purkinje cells, which in the native heart tissue are responsible for initiating and synchronizing cardiac beatings. The studies were performed with rat prenatal cardiac cells and with human cardiomyocytes derived from induced pluripotent stem cells. Our results show that the scaffolds supported the growth of cardiac cells for at least 12 days. The engineered tissue is superior in terms of contractility, morphology and metabolic maturation when compared to the one obtained in standard polystyrene and to other platforms described in the literature for cardiac tissue engineering. Further adaptations of the system are currently being performed, enabling us to use the system for medium to high-throughput cardiotoxicity drug screenings.

Keywords: Piezoelectricity, cardiac tissue, flexible thin films

SHORT COMMUNICATION
CELL AND TISSUE BIOPHYSICS

MICROPATTERNED SUBSTRATES TO ACCELERATE PATHOLOGICAL SMOOTH MUSCLE CELLS AGING

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Hutchinson-Gilford Progeria Syndrome (HGPS) is a rare genetic disorder that causes accelerated aging in children leading to premature death. Smooth muscle cells (SMCs) are the most affected cells in HGPS patients. In this work, we studied whether SMCs, derived from induced pluripotent stem cells with and without HGPS phenotype, responded differentially to ECM, in particular to its topography. Moreover, we studied whether ECM could accelerate SMC aging. Human SMCs with and without HGPS phenotype showed differential cell alignment, nuclear shape and cell apoptosis but not cell cycle or SMCs markers expression when cultured in substrates with different topography. Our results further showed that substrate topography accelerated significantly the aging of SMCs with HGPS during 15 days of cell culture, as confirmed by the increase of progerin and other senescent markers. The aged SMCs showed an over-expression of nuclear envelope proteins SUN1 and Nesprin2, at both nuclear and cytoplasmatic regions. Our results unraveled new possible mechanisms to understand the aging process in SMCs.

Keywords: Hutchinson-Gilford Progeria syndrome, Progeria, iPSCs, Aging, Smooth muscle cells, Topography

SHORT COMMUNICATION
RECEPTORS, CHANNELS AND TRANSPORTERS

EXPLORING THE PH GATING MECHANISM OF HUMAN AQUAPORIN-7

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Aquaporins (AQPs) are a family of membrane channels involved in the bidirectional transfer of water and glycerol across cell membranes in response to osmotic and hydrostatic pressure gradients. In mammals, AQPs (0-12) are expressed in almost every tissue in the body with important roles in physiology and homeostasis and their dysfunction or aberrant expression has been correlated with several diseases. Understanding and controlling AQPs gating mechanisms is crucial to manipulate their activity and to identify new modulators for therapeutic application. In the present study we assessed the function and regulation of the human aquaporin-7 (hAQP7), an aquaglyceroporin that transports glycerol in addition to water, by using a yeast heterologous expression system devoid of endogenous aquaporins, a background where analysis was unlikely to be compromised by the co-expression of other aquaporin isoforms. Using the stopped-flow technique to evaluate the channel permeability for water and for glycerol, we were able to demonstrate for the first time the pH gating of hAQP7 showing that it achieves maximal activity at mammalian physiological pH range (open state) and changes to a closed state with media acidification, for both water and glycerol transport. Additionally, molecular modeling studies allowed us to predict the putative titrable residues involved in pH gating and important monomer-monomer interactions within the hAQP7 tetramer. By site-directed mutagenesis we were able to disclose His165 as a crucial residue for the gating of the pore, affecting water and glycerol permeation through hAQP7. We propose that protonation of His165, a highly accessible residue located in an extracellular loop (loop C) of one monomer, would cause loop C displacement towards the channel entrance with consequent blockage of the pore. This mechanism of regulation can be explored to design new small molecules useful as channel modulators

Keywords: AQP7; pH gating; yeast; water and glycerol permeability

SHORT COMMUNICATION
RECEPTORS, CHANNELS AND TRANSPORTERS

BRADYKININ MODULATION OF TREK-2 CURRENTS IN MOUSE SUPERIOR CERVICAL GANGLION NEURONS

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Bradykinin modulation of the membrane potential of sympathetic neurons has been described and it is classically attributed to KCNQ modulation through bradykinin receptors. In this work we describe the inhibition of a riluzole evoked current mediated by TREK-2 channels (IRIL) in cultured neurons from the mouse superior cervical ganglion. Single-channel recording and voltage- and current-clamp configurations of the perforated patch-clamp technique were used to record from mouse superior cervical ganglion (SCG) neurons in primary culture.

Bath applied bradykinin inhibits IRIL around 40% through B2 receptors and reduced the Po of native TREK-2 channels. Bradykinin B2 receptors are coupled to the Gq/11 – PLC pathway, so we used bisindolylmaleimide and 2-APB to inhibit the PKC and IP3R pathways respectively, nevertheless no significant effect was achieved on the bradykinin mediated inhibition. Finally we modulated the PIP2 membrane content using wortmannin to reduce it and PIP2 coupled to a membrane carrier to avoid the PLC mediated decrease of PIP2 concentration; depletion of PIP2 strongly decrease IRIL while keeping PIP2 levels constant avoided the bradykinin mediated inhibition.

We can conclude that bradykinin inhibits TREK-2 by decreasing PIP2 levels in the membrane. The involvement of KCNQ and TREK channels on the control of the resting membrane potential will be discussed

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Keywords: Resting membrane potential, TREK, K2P, bradykinin, riluzole, patch-clamp

SHORT COMMUNICATION
RECEPTORS, CHANNELS AND TRANSPORTERS

MOLECULAR CHARACTERIZATION OF ADENOSINE AND VEGF RECEPTORS IN SOD1(G93A) MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS (ALS)

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ALS is a motor neuron neurodegenerative disease. Neuromodulation by adenosine is functionally disturbed in ALS. Vascular endothelial growth factor (VEGF) may protect motor neurons in ALS. We now evaluated if there is any changes in the levels of adenosine receptors (A1R and A2AR), VEGF, and VEGF receptors (VEGF-R1 and VEGF-R2) at the cerebral cortex (CTX) and spinal cord (SC) of a model of ALS, SOD1(G93A) mice.

A quantitative Real-time PCR was used to evaluate mRNA levels (n=3-5) and ligand binding assays (n=5-7) were performed to evaluate the expression (Bmax) of A1R protein levels. Pre-symptomatic (PS; 4-6 weeks old) and symptomatic (S; 12-14 weeks old) mice were analysed and data compared with age-matched wild type mice. Significant differences were considered at $p<0.05$.

The expression of mRNA for A1R and A2AR was increased in PS mice, either at CTX or SC. This was accompanied with a decrease in receptor protein levels in CTX of PS and S mice, with no change in the SC. No changes in mRNA levels were detected in S mice at the CTX or SC.

Concerning VEGF and its receptors, at the CTX of PS mice, there was an increase in the expression of mRNA for VEGF with no change in the expression of mRNA for VEGF-R1 and a decrease in VEGF-R2. Interestingly, in CTX of S mice there was an overall decrease of the mRNA expression for VEGF and its receptors. At the SC of PS mice there was an increase in mRNA levels for VEGR-R1, whereas no changes were detected for mRNA of any of the VEGF related molecules in the SC of S mice.

Together the data suggests an early dysfunction at adenosine signaling that occurs before disease onset, and a late dysfunction of VEGF mediated mechanisms at the cerebral cortex that may contribute to disease progression.

Keywords: ALS; Adenosine receptors; VEGF; VEGF rceptors; Neuromodulation

SHORT COMMUNICATION
RECEPTORS, CHANNELS AND TRANSPORTERS

DECIPHERING THE KCNE4 FUNCTION WITHIN THE KV1.3 CHANNELOSOME.

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The voltage-dependent potassium channel Kv1.3 plays an important role in leukocytes. We previously demonstrated that KCNE4, acting as a dominant negative regulatory subunit, physically interacts with Kv1.3 inhibiting K+ currents and retaining the channel intracellular. The interaction of Kv1.3 and KCNE4 is specific, but the molecular determinants participating in the massive intracellular phenotype remain unknown. In the present work we analyzed the KCNE4 motifs which are responsible for both the Kv1.3 interaction and the massive ER retention. Our results identified molecular determinants involved in KCNE4 oligomerizations which compete for the association with Kv1.3. Furthermore, the KCNE4-dependent intracellular retention of the channel complex, which negatively affects the physiological role of Kv1.3, is mediated by two independent and synergic mechanisms. First, KCNE4 association masks the YMVIEE signature at the C-terminal domain of the Kv1.3 that is crucial for the surface targeting of the channel; second, we identify a potent endoplasmic reticulum retention motif in KCNE4 that further limits cell surface expression. Our results pave the way for the understanding of specific KCNE4 molecular determinants which play a crucial role in the channelosome formation in leukocytes.

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Keywords: Channelosome, traffic, KCNE4, Kv1.3, oligomerization

SHORT COMMUNICATION
SUPRAMOLECULAR COMPLEXES AND BIOSPETROSCOPIES

NANOMECHANICAL DATA VALIDATES A MULTILAYER SUPRAMOLECULAR MODEL THAT EXPLAINS THE SHAPE, DIMENSIONS, AND CYTOGENETIC PROPERTIES OF METAPHASE CHROMOSOMES

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Previous studies showed that during mitosis chromatin filaments are folded into multilayer plates. These structures can be self-assembled from chromatin fragments obtained by micrococcal nuclease digestion of chromosomes. Metaphase chromosomes of different animal and plant species show great differences in size (which are dependent on the amount of DNA that they contain), but in all cases chromosomes are elongated cylinders that have relatively similar shape proportions (the length to diameter ratio is approximately 13). It is possible to explain this morphology by considering that chromosomes are self-organizing supramolecular structures formed by stacked layers of planar chromatin having different nucleosome-nucleosome interaction energies in different regions (1). Nucleosomes in the periphery of the chromosome are less stabilized by the attractive interactions with other nucleosomes and this generates a surface potential that destabilizes the structure. Chromosomes are smooth cylinders because this morphology has a lower surface energy than structures having irregular surfaces. The symmetry breaking produced by the different values of the surface energies in the telomeres (4.4 kT per nucleosome) and in the lateral surface (0.34 kT) explains the elongated structure of the chromosomes. The results obtained by other authors in nanomechanical studies of chromatin and chromosome stretching have been used to test the proposed supramolecular structure. It is demonstrated quantitatively that internucleosome interactions between chromatin layers (8.7 kT per nucleosome) can justify the work required for elastic chromosome stretching. Chromosomes can be considered as hydrogels with a lamellar liquid crystal organization. The good mechanical properties of this structure may be useful for the maintenance of chromosome integrity during mitosis. Furthermore, this chromatin organization explains the chromosome structural properties (banding and translocations) which are used in clinical cytogenetics for the diagnosis of hereditary diseases and cancers (2).

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Keywords: Metaphase chromosome structure. Supramolecular complexes. Nanomechanics. DNA packaging. Chromatin structure. Cytogenetics.

SHORT COMMUNICATION SUPRAMOLECULAR COMPLEXES AND BIOSPETROSCOPIES

CHARACTERIZATION OF PROTEINS INVOLVED THE ORP COMPLEX OF DESULFOVIBRIO VULGARIS HILDENBOROUGH

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Cells division mechanism has been mostly studied in Escherichia coli, Bacillus subtilis and Caulobacter crescentus. Recently, a protein complex was proposed to be involved in cell division of anaerobic microorganisms.[1] In Desulfovibrio vulgaris Hildenborough (DvH), this complex is composed by DVU2103, DVU2104, DVU2105 and DVU2109, in addition to the Orange Protein (ORP) (DVU2108), several of which are annotated as metalloproteins. Expression of the genes encoding these proteins is regulated by a σ54-dependent transcriptional regulator, DVU2106.[1] The ORP has been previously isolated from D. gigas[2] as a soluble monomeric protein with an unique mixed metal sulfur cluster [S2MoS2CuS2MoS2]3- and the apo-ORP can be reconstituted either as a Mo-Cu or W-Cu cluster.[3] Here, the homologous expression of DVU2103 encoding a Fe-S cluster ATPase has shown that it co-purifies with ORP. DVU2103 presents a broad absorption band at 400 nm, characteristic of [4Fe-4S] cluster and a molar extinction coefficient per protein of ε400 nm = 32600 M-1 cm-1, with a reduction potential of around -400 mV. This, together with its EPR spectra, suggests the presence of two [4Fe-4S] clusters, which are oxygen sensitive. In addition, the ORP from DvH was heterologously produced and biochemically characterized. UV-visible titrations using (NH4)2MoS4 and CuCl2, in the presence and absence of apo-ORP, have shown that it favors an 2Mo:1Cu stoichiometry and that the metal cluster synthesis is assisted by the protein. The DVU2109 homologue in D. alaskensis G20, Dde3202, was also biochemically characterized as a Fe-S protein. Preliminary data indicates that the ORP system might in fact be involved in Fe-S biosynthesis or repair, with a secondary effect in cell division.

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Keywords: Anaerobic Bacteria, Desulfovibrio, Iron-sulfur Proteins, Cell Division, Orange Protein Complex

SHORT COMMUNICATION SUPRAMOLECULAR COMPLEXES AND BIOSPETROSCOPIES

A STRATEGY TO MIX DIFFERENT BIOPHYSICAL SCORING FUNCTIONS FUSING THE RANKING POWER OF FFT-BASED PROTEIN-PROTEIN DOCKING PROTOCOLS

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Protein-protein interactions are known to play key roles in the most critical cellular and biological processes such as signaling, metabolism, and trafficking. We can gain a full understanding of these interactions only through consideration of their atomic details. Currently, only 7% of the known human interactome is structurally characterized (either experimentally or modeled), but computational protein-protein docking could increase up to 50% the structural coverage. Protein-protein docking consists in the generation of different poses or sampling, and the identification of the correct structures with a scoring function. However, these algorithms generate a significant number of incorrect predictions. Therefore, the predictive success strongly depends on the accuracy of the scoring function used to evaluate the models produced.To characterize the models the scoring functions are usually based on empirical, statistical or energy-based potentials, defined at different resolution levels (atomic, residue), and then return a ranked list that aims to place the near-native solutions as close to the top as possible. We present here a procedure to select from a large pool of biophysical descriptors the best Scoring functions and how they can be combined to generate a new ranking scheme.We present here a procedure to select from a large pool of biophysical descriptors the best scoring functions for the pyDock, Zdock and Sdock docking programs, and how they can be combined to generate a new ranking scheme. A simple combination of pairs of scoring functions shows an improvement of 5-10% in success rates. Moreover, by combining the best pairs of scoring functions of the three methods, the success rates increased an additional 30%. This suggests that the best strategy is to identify the best scoring function for each method, and then combine the best predicted models.

Keywords: Protein-protein docking, Scoring functions,combinations

SHORT COMMUNICATION SUPRAMOLECULAR COMPLEXES AND BIOSPETROSCOPIES

NEISSERIA GONORRHOEAE CYTOCHROME C PEROXIDASE AND THE ELECTRON DONOR, LAZ, A LIPID-MODIFIED AZURIN

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Neisseria gonorrhoeae is an obligate human pathogen that causes the sexually transmitted infection gonorrhea. As other pathogenic bacteria, it has multiple enzymes to deal with reactive oxygen species originated both endogenously and exogenously, in particular from the host immune system defenses(1). One of these enzymes is the bacterial cytochrome c peroxidase (NgBCCP), a dihemic enzyme that reduces hydrogen peroxide to water. These enzymes require as electron donors small redox proteins, either small c-type cytochromes or Type 1 copper proteins. The lipid-modifiez azurin (Laz) has been proposed to be the electron donor of NgBCCP(2). We heterologously expressed and purified NgCCP and Laz successfully for further biochemical characterization. NgBCCP shows catalytic activity, with Laz as electron donor, dependent on reduction activation and calcium ions. The NgBCCP UV-visible and EPR spectra were acquired and the formal redox potentials determined. It is a 38 kDa protein that exhibits a monomer/dimer equilibrium in solution dependent on concentration and calcium ions, similar to other dihemic bacterial peroxidases studied up-to-date, though this equilibrium is not dependent on ionic strength. The solution structure of Laz has been determined recently(2, 3), which can be used to identify the interaction surface with NgBCCP through a protein titration. Better understanding of NgBCCP functional mechanism is essential as it is a potential target for new compounds against gonorrhea, since this enzyme is highly conserved in the species and a ccp knockout strain has shown to be more sensitive to the immune response system(4)

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Keywords: Neisseria gonorrhoeae, bacterial cytochrome c peroxidase, lipid-modified azurin, oxidative stress, electron-transfer complexes

SHORT COMMUNICATION
PROTEIN STRUCTURE, FOLDING AND DYNAMICS

STRUCTURE, FUNCTION AND STABILITY OF A MONOMERIC ADENYLATE CYCLASE (CYAA) TOXIN FROM BORDETELLA PERTUSSIS

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Adenylate cyclase (CyaA), one of the major virulence factors produced by Bordetella pertussis, the causative agent of whooping cough, is a large and complex multi-domain protein (1706 aa) (Ladant and Ullmann 1999). It is part of the RTX (Repeat-In-Toxin) family and as such it is synthesized as an inactive precursor, which is converted into the active form upon a specific acylation (Nicaud, Mackman et al. 1985). Once secreted across the bacterial cell envelope, it is able to intoxicate eukaryotic cells through a unique mechanism that involves a calcium-dependent translocation (Rogel and Hanski 1992). This mechanism of entry is still poorly understood and the main goal of our research is to unravel this remarkable process.

Recent studies defined the experimental conditions required to refold CyaA into a monomeric and functional state (Karst, Ntsogo Enguene et al. 2014).

Here, we extensively characterized the stability properties and its functional activity in vitro and in cellula. We found that the refolded and monomeric toxin is able to preserve its structure and functions even in a milieu depleted of calcium, although the overall stability is calcium dependent. We observed that the cytotoxicity as well the efficiency of permeabilization and haemolytic activity, respectively on vescicles and erythrocytes, critically depend upon the state of CyaA. Interestingly, the monomeric toxin was also able to efficiently permeabilize vescicles and induce hemolysis in erythrocytes even in a milieu depleted of calcium, whereas the cytotoxicity is critically calcium dependent. Moreover, SAXS analysis allowed us to provide the first low-resolution structural model of the toxin in solution. This model suggests that the toxin is a rather compact and globular multi-domain protein. Taken together, our results lead to a better understanding of the behaviour of the toxin when in solution and upon membrane interaction, insertion and translocation.

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Keywords: Structure-function, bacterial toxin, calcium-binding protein

SHORT COMMUNICATION
PROTEIN STRUCTURE, FOLDING AND DYNAMICS

NANOMECHANICAL PHENOTYPES IN FAMILIAL HYPERTROPHIC CARDIOMYOPATHY

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Hypertrophic cardiomyopathy (HCM), the most common cause of sudden cardiac death in the young, is a disease of the heart that causes the left ventricle to thicken. This anatomical feature is accompanied by hypercontractility and impaired relaxation during diastole. HCM is caused by mutations in sarcomeric genes that code for structural proteins with mechanical roles. However, the molecular mechanisms leading from mutations in sarcomeric genes to development of HCM are not well understood, which limits both the interpretation of genetic testing for diagnosis and the development of therapies. We are exploring the connection between dysregulation of protein mechanics and development of HCM. Using single-molecule atomic force microscopy, we measure the mechanical properties of mutant polypeptides that cause HCM. We have obtained preliminary evidence that mutations induce nanomechanical phenotypes in the proteins without perturbing their thermodynamical stability. To get a broader understanding on the molecular mechanisms inducing disease phenotypes, we are planning to examine several other parameters (e.g. myocardial RNA and protein levels) that may be altered by the mutations. For these determinations, we will analyze heart tissue from affected patients and/or animal models of HCM.

Keywords: Protein mechanics, AFM, HCM, stability, folding, cardiomyopathy

SHORT COMMUNICATION
PROTEIN STRUCTURE, FOLDING AND DYNAMICS

REPC NICKING ACTIVITY IS PASSIVE AND FORCE AND TWIST DEPENDENT

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Many bacterial plasmids replicate by an asymmetric rolling-circle mechanism that requires sequence-specific recognition for initiation, nicking of one of the template DNA strands, and unwinding of the duplex prior to subsequent DNA synthesis. Nicking is performed by a replication-initiation protein (Rep) that directly binds to the plasmid double-stranded origin and remains covalently bound to its substrate 5'-end via a phosphotyrosine linkage. It has been proposed that the inverted DNA sequences at the nick site form a cruciform structure that facilitates DNA cleavage. However, the role of Rep proteins in the formation of this cruciform and the implication for its nicking and religation functions is unclear. Here, we have used magnetic tweezers to directly measure the DNA nicking and religation activities of RepC, the replication initiator protein of plasmid pT181, in plasmid sized and torsionally-constrained linear DNA molecules. Nicking by RepC occurred only in negatively supercoiled DNA and was force- and twist-dependent. Comparison with a type IB topoisomerase in similar experiments highlighted a relatively inefficient religation activity of RepC. Based on the structural modeling of RepC and on our experimental evidence, we propose a model where RepC nicking activity is passive and dependent upon the supercoiling degree of the DNA substrate.

Keywords: Rep proteins, RepC, Magnetic Tweezers, Plasmid Rolling-Circle Replication

SHORT COMMUNICATION
PROTEIN STRUCTURE, FOLDING AND DYNAMICS

COMPUTER MODELING OF PROTEIN FOLDING FROM FLEXIBLE NMR STRUCTURES

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Simulation has become a fundamental tool to complement the experimental biophysical techniques devoted to the investigation of the protein folding problem. To tackle this process, and taking into account its very long time scale and the huge number of degrees of freedom involved, researchers have introduced coarse-grained models, which simplify the geometrical representation of the system and its energetic interactions. Among them, native structure-based models have emerged as a powerful technique to analyze the folding process. They define the interactions in the system from the contacts present in the folded state, whose structure has to be experimentally known. Nuclear magnetic resonance (NMR) is able to obtain the structure in solution, where thermal fluctuations allow observing its structural flexibility. Moreover, this flexibility is of paramount importance to explain the function of proteins, either in their natural environment or in an increasing number of biotechnological applications. Each NMR determined structure is recorded into the Protein Data Bank as a set of conformations, termed "models", which taken together mirror this flexibility. However, in present structure-based simulation models the flexibility is ignored, defining the native contacts just from one single "model", usually the first one. Here we try to change this situation, by including in the simulation Hamiltonian information from the full set of NMR "models" present in the experimental structure, i.e., considering the experimentally determined flexibility for the native state. The most challenging task in this project is to define an interaction potential which does not eliminate, by definition, the information already present in the experimental structure. This novel way to employ structure-based potentials has not been seriously tackled before. Comparison between the results we have obtained with this new simulation model and those computed from a single structure are compared in order to assess the novelties our approach can provide

Keywords: Modeling; flexibility; coarse-grained; structure-based potentials

SHORT COMMUNICATION
NANOBIOPHYSICS AND MOLECULAR RECOGNITION

TARGETING THE BRAIN: ENHANCED BRAIN ACCUMULATION OF NANOPARTICLES BY FINE-TUNING SURFACE CHEMISTRY.

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The blood-brain barrier (BBB) is the major barrier of the central nervous system and is responsible for the neuronal microenvironment homeostasis. Due to the tight selectivity of this barrier, the delivery of drug to the brain is very limited. Nanoparticles (NP) are one of the most promising and versatile vehicles for drug delivery to the brain, since it can protect drugs from degradation and also deliver them into desired areas within potential therapeutic values. However, so far, no nanoformulations have reached the market due to their inefficiency in penetrating the BBB. Recently we have generated a stable and reproducible human in vitro BBB model derived from cord blood hematopoietic stem cells. The cells were initially differentiated into endothelial cells followed by the induction of BBB properties by co-culture with bovine pericytes. The brain-like endothelial cells expressed tight junctions and transporters typically observed in brain endothelium and maintained the expression of most properties for at least 20 days. With our co-culture model, we can screen and predict suitable nanoformulations that can also efficiently cross BBB in animal models. Therefore, we have developed a set of nanoformulations (spherical (AuNPs) and rod (AuNRs)) with different density of transferrin to target the BBB. Gold nanoparticles were initially synthetized and followed by functionalization with transferrin peptide. Our in vitro model demonstrates that a large amount of transferrin conjugated AuNPs or AuNRs can cross the endothelial layer compared to bare NPs/NRs. Similar trend of nanoformulation accumulation is also observed in animal's brain after intravenous administration. In conclusion, we are able to prepare gold nanoparticles capable of crossing the BBB. Importantly our co-culture model can predict in a reliable manner the in vivo outcome.

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Keywords: Blood-brain barrier; Nanoformulations; Brain targeting.

SHORT COMMUNICATION
NANOBIOPHYSICS AND MOLECULAR RECOGNITION

DEVELOPMENT AND CHARACTERIZATION OF A HYBRID APPARATUS COMBINING MAGNETIC TWEEZERS AND TIRF MICROSCOPY TO STUDY SINGLE DNA:PROTEIN INTERACTIONS

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Atomic Force Microscopy (AFM), Magnetic Tweezers (MT) and Optical Tweezers (OT) are single-molecule techniques that can be employed to study DNA-protein interactions. There is an increasing interest in combining these single-molecule devices with fluorescence because mechanical measurements could eventually be correlated with the presence of a protein. For instance, other groups have combined AFM with STED super-resolution imaging. In our group, we have developed a hybrid setup incorporating total internal reflection fluorescence (TIRF) microscopy to our MT. This is a powerful approach because it allows us to correlate biological activity with precise positioning and stoichiometries (directly observed by fluorescence) of the proteins. We have implemented an objective-type TIRF, where the excitation beam is directed to the sample surface and the fluorescence emission is collected by the same objective. This implementation allows facile switching between TIRF and epi-illumination, if desired. The setup is combined with a new multistream laminar flow microfluidics device, which permits to control in a precise way the addition of proteins and reagents of interest. We have characterized the apparatus performing several proof of concept experiments, and we have also started testing it with proteins, such as AddAB, a bacterial helicase-nuclease complex involved in DNA repair, and ParB, a bacterial centromere binding protein involved in DNA condensation.

Keywords: Magnetic Tweezers, TIRF microscopy, microfluidics, DNA, proteins

SHORT COMMUNICATION
NANOBIOPHYSICS AND MOLECULAR RECOGNITION

WEST-NILE VIRUS CAPSID PROTEIN BINDS TO HOST LIPID SYSTEMS REQUIRING POTASSIUM IONS

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West-Nile and dengue viruses are closely related flaviviruses, originating mosquito-borne viral infections for which there are no effective and specific treatments. Their capsid proteins sequence and structure are particularly similar, forming superimposable α-helical homodimers. The measurement of protein-ligand interactions at the single-molecule through nanobiophysics techniques yields detailed information of use to nanomedicine and nanotechnology. In this work, such an approach enabled measuring at the nanoscale the West-Nile capsid protein biologically relevant interactions with host lipid structures. Since we already reported that dengue virus capsid protein binds intracellular lipid droplets (an essential step of viral replication) and blood plasma very-low density lipoproteins (which may prompt the formation of highly infectious lipoviroparticles), the related West-Nile virus capsid protein could be expected to also interact with these host lipid systems. Here, we directly tested West-Nile virus capsid protein interaction with these lipid systems. Zeta potential studies, dynamic light scattering measurements and single-molecule atomic force microscopy-based force spectroscopy show that the interactions with lipid droplets and lipoproteins are strong, specific and require K+ ions, as well as surface proteins of these host lipid systems. West-Nile virus capsid protein binds very-low density lipoproteins but not low-density lipoproteins. The previously proposed model of dengue virus capsid protein interaction with host lipid systems seems thus to be similar in West-Nile virus and, possibly, in other closely related flaviviruses.

Keywords: West-Nile Virus; Lipid droplets; Lipoproteins; Atomic force microscopy; Zeta potential

SHORT COMMUNICATION
NANOBIOPHYSICS AND MOLECULAR RECOGNITION

REPEAT PROTEINS AS TEMPLATES TO ORGANIZE PHOTOACTIVE MOLECULES

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The development of application-oriented innovative materials requires methods for control of structures along different size scales. Bottom-up self-assembly that relies on highly specific biomolecular interactions of small defined components, is an attractive approach for biomaterial design and nanostructure templating. In this work, we used modular designed consensus tetratricopeptide repeat proteins (CTPRs) for the generation of functional nanostructures and nanostructured materials. CTPR arrays contain multiple identical repeats that interact through a single inter-repeat interface to form elongated superhelical structures. We have characterized the self-assembly properties of long consensus repeat protein arrays in self-standing films to use them as templates for the creation of functional materials. We show the use of the proteins and generated structures as a template to organize photoactive organic molecules.We propose to use CTPR proteins in order to template donor-acceptor pairs for electro-active materials. In order to achieve an efficient electron transfer the arrays of molecules need to be ordered at defined distances. We show the data in which we explore the potential of CTPR protein scaffolds for nanometer-precise arrangement of the molecules. We are able to form photo-active films using the conjugates where we obtain photo-induced current transport.

Keywords: Protein design, self-assembly, bio conjugation, functional structures, bio-hybrid materials, electron-donor systems

POSTERS

P01: EFFECT OF SPHINGOSINE ON MEMBRANE PERMEABILITY AND BIOPHYSICAL PROPERTIES OF LYSOSOMAL-MIMICKING VESICLES

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Sphingosine (Sph) is one of the simplest lipids and one of the most prevalent backbones of sphingolipids in mammals. This lipid plays important bioactive roles in different cellular processes and has been implicated in Niemann Pick type C1 (NPC1), a complex lysosomal storage disease. To understand how the accumulation of this lipid in NPC1 impacts lysosomal membrane structure and biophysical properties, we developed lysosomal-mimicking vesicles displaying internal acidic pH and external neutral pH. Moreover, the lipid composition of the vesicles was modified in order to resemble physiological- or NPC1-like lysosomes. To this end, ternary 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC)/Sphingomyelin (SM)/Cholesterol (Chol) mixtures with, respectively, low and high Chol/SM levels were prepared. The effect of Sph on the membrane permeability and biophysical properties was then evaluated by fluorescence spectroscopy, electrophoretic and dynamic light scattering. Our results showed that Sph has the ability to cause a shift in vesicle surface charge, increase the packing properties of the membrane and promote a rapid increase in membrane permeability. These effects are enhanced in NPC1-lysosomal-mimicking vesicles, i.e., containing higher levels of Chol and SM. These results suggest that lysosomal accumulation of these lipids, as observed under pathological conditions, might significantly affect lysosomal membrane structure and integrity, and therefore contribute to the impairment of cell function.

Acknowledgments: This work was supported by Fundação para a Ciência e Tecnologia (FCT), Portugal: PTDC/BBB-BQB/0506/2012, PTDC/BBB-BQB/3710/2014, SFRH/BD/88194/2012 to ACC and Investigador FCT to RFMA and LCS.

Keywords: lysosomal-mimicking compartments, membrane permeability, sphingosine, lipid domains

POSTERS

P02: FIBRINOGEN-ERYTHROCYTE BINDING AS A POTENTIAL PROGNOSIS BIOMARKER IN HEART FAILURE PATIENTS

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High fibrinogen levels are a relevant cardiovascular risk factor, but the biological mechanisms associated with pathologic alterations are not totally clear. Fibrinogen-erythrocyte binding in chronic heart failure (CHF) patients and its prognostic value were evaluated. 15 ischemic and 15 non-ischemic CHF patients, as well as 15 healthy donors were enrolled in the study. Fibrinogen-erythrocyte interactions were evaluated, at the single-molecule level, by atomic force microscopy (AFM)-based force spectroscopy. These measurements were performed in buffer, with the protein covalently attached to the silanized AFM tip, and the erythrocytes on a poly-L-lysine coated-glass slide. Clinical outcome was assessed during a 12-months follow-up. AFM stiffness studies were also performed on erythrocytes from these patients and compared with the control.

Force spectroscopy data showed that CHF patients presented higher fibrinogen-erythrocyte binding forces than the control group, despite a lower binding frequency. According to etiology, ischemic patients had higher binding forces than donors and lower binding frequency. Ischemic patients presented increased fibrinogen-erythrocyte binding forces relative to non-ischemic. Non-ischemic patients also had a lower binding frequency than donors. Their cell stiffness is also altered. These variations were statistically significant for the median, in which we observed an increased cell stiffness (or decreased elasticity) on both groups of CHF patients, with the larger variation being observed for the non-ischemic patients. Follow-up data demonstrated that patients presenting higher fibrinogen-erythrocyte binding forces at the beginning of the study had a higher probability of being hospitalized due to cardiovascular complications on the subsequent year.

As fibrinogen-erythrocyte interactions, evaluated by AFM, are modified in CHF patients and associated with short-term clinical outcome, here we demonstrate the power of this nanotechnology-based evaluation as potential biomarker for cardiovascular risk and patients' clinical prognosis evaluation [Guedes et al. (2016) Nature Nanotechnol., in press].

Keywords: Fibrinogen, Erythrocyte, Binding, Prognosis Biomarker, Atomic Force Microscopy

POSTERS

P03: EFFORT IN NEUROPSYCHIATRIC DISORDERS

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Motivation and effort are crucial for adaptive behavior in everyday life. However, despite their importance, the neural mechanisms that regulate them are still far from well-understood.

Evidences suggest that there are two key neuromodulators involved in these processes: dopamine (DA) and serotonin (5-HT). The former has a wealthy amount of studies, showing how the modulation of DA influences the vigor and willingness to make effort towards a reward. The significance of the serotonin is less clear but it has been featured as having an opposite role to DA. So, while DA may regulate situations where subjects have to balance the costs and benefits of reward, 5-HT might play a similar role in aversive processing and behavioral inhibition (avoiding punishments).

Since attention-deficit/hyperactivity disorder (ADHD) and obsessive-compulsive disorder (OCD) have clinical features that suggest anomalies on how effort is processed, and are known for their deficits in the dopaminergic and serotonergic circuits, we hope that by assessing the performance of patients diagnosed with these disorders in a novel computational task (related to effort and to the cost-benefit tradeoff), we can improve the current knowledge about effort in these two disorders.

The work done so far is mostly related to the optimization of the task. Through an iterative process with several phases of data collection – first in healthy adults, and then in healthy children with different ages – key parameters like task's duration, difficulty or instructions were optimized for the statistical analysis. The results from this pilot phase show that the task is robust enough for our purposes, allowing us to find variability even between heathy subjects. From our preliminary results, subjects significantly exert more effort in order to avoid punishments than to receive rewards.

The next phase will consist on collecting data from healthy control, ADHD and OCD children, together with complementary neuropsychiatric and personality questionnaires.

Keywords: Effort, cost, computational task

P04: PROBING THE INTERACTION BETWEEN SEROTONIN RECEPTORS AND MODEL LIPID BILAYERS CONTAINING ISOMERIC FORMS OF CHOLESTEROL USING COMPUTER SIMULATION

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Despite the ubiquity of cholesterol within the cell membrane, the mechanism by which it influences embedded proteins remains elusive. Numerous GPCR's have been shown to be functionally inactive in the absence of cholesterol; additionally, the serotonin1A receptor ceases function in the presence of cholesterol isomers differing by the orientation of a single bond. However, the molecular determinants of this are unknown. Our current study investigates the specific binding of cholesterol molecules to the newly resolved crystal structures of the serotonin1B and serotonin2B receptors, in addition to a homology model of the serotonin1A receptor, using a combination of docking calculations and flooding molecular dynamics simulations. Up to ten high occupancy sites are identified on each receptor surface, with a conserved non-annular site in the extracellular leaflet between helices I and VII. Key residues constituting these binding pockets are variable between receptors, suggesting cholesterol in this site may modulate protein behaviour in a subtype specific manner. Differential behaviour of isomeric forms of cholesterol, suggests specific interactions are required for functionality in the 5-HT1 receptor family. This data will contribute to our understanding of how lipids modulate receptor function, and aid the development of targeted therapies of GPCR's.

Keywords: Membrane proteins; computer simulation; protein-lipid interactions; GPCRs

POSTERS

P05: VEHICULIZING DRUGS AND LIPOSOMES WITH PULMONARY SURFACTANT ALONG THE RESPIRATORY SURFACE

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The respiratory surface of the mammalian lung is covered by a thin aqueous layer and, on top of it, by a lipid-protein surface active material, the pulmonary surfactant (PS). Apart from preventing pulmonary collapse during breathing, PS is able to adsorb very rapidly (in few seconds) into the air-liquid interface and, once there, to spread efficiently along it. Therefore, it offers novel opportunities to vehiculize different drugs and nanocarriers, while hiding and protecting them from clearance in the lung. As PS is mainly composed by lipids, hydrophobic drugs can be directly vehiculized into PS membranes while hydrophilic drugs need to be encapsulated into proper containers prior to their integration into pulmonary surfactant. In the present work we have evaluated the possibilities of vehiculizing both hydrophobic and hydrophilic drugs, and the possible effects on pulmonary surfactant integrity. We have used tacrolimus as a hydrophobic model drug and isoniazid as a hydrophilic one.

When we analysed structural and functional changes associated with the presence of the drug into PS, we observed that isoniazid has no effects on structure nor functionality of PS. This suggests that the hydrophilic drug does not interact with PS and cannot be transported directly into it. Consequently, we explored the encapsulation approach using model liposomes containing calceine as a model carrier for delivering hydrophilic molecules.

In the case of tacrolimus, we observed that it affects the lateral structure of DPPC and surfactant interfacial films, as well as their functionality. It inhibits the compression-driven segregation of domains associated with expanded-to-condensed lateral phase transitions. Interestingly, after some compression-expansion cycles, this effect is apparently reverted, suggesting that surfactant films can be progressively refined and depurated from tacrolimus during interfacial dynamics. Experiments performed in a modified Wilhelmy balance show that only in the presence of PS, the tacrolimus travels along the air-liquid interface. Therefore, we suggest that, once the drugs or nanocarriers are transported by surfactant along the respiratory surface to the distal airways, breathing dynamics could facilitate the progressive drug release.

Keywords: Pulmonary surfactant, drug delivery, respiratory nanomedicine, nanocarriers, liposomes, air–liquid interface

P06: DAUNORUBICIN-MEMBRANE INTERACTIONS WITH MODEL MEMBRANES: RELEVANCE FOR THE DRUG'S BIOLOGICAL ACTIVITY

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Daunorubicin is extensively used in chemotherapy for diverse types of cancer. Even after more than 50 years of its discovery, daunorubicin continues to be widely studied because its mechanisms of action are still a matter of substantial controversy. Furthermore, strong evidence suggests that the cytotoxic effects caused by daunorubicin are also associated with interactions at the membrane level. The present work aimed, therefore, to study the interplay between daunorubicin and mimetic membrane models composed of different ratios of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DMPC), sphingomyelin (SM) and cholesterol (Chol).

In order to accomplish that, several biophysical parameters were assessed using liposomes as mimetic model membranes. Thereby, the ability of daunorubicin to partition into lipid bilayers, its apparent location within the membrane and its effect in membrane fluidity were investigated. The gathered data supports that daunorubicin permeates all types of membranes in different degrees, interacts with phospholipids through electrostatic and hydrophobic bonds and causes alterations in the biophysical properties of the bilayers, namely in membrane fluidity.

Ultimately, such outcomes can be correlated with daunorubicin's biological action, where membrane structure and lipid composition have an important role. In fact, the results indicate that the intercalation of daunorubicin between the phospholipids can also take place in rigid domains, such as rafts that are known to be involved in different receptor processes important for cellular function.

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Keywords: Drug-membrane interactions, daunomycin, liposomes, partition, location, fluidity, lipid raft

POSTERS

P07: INFLUENCE OF UNSATURATED SPHINGOMYELIN AND CERAMIDE IN THE FORMATION OF SEGREGATED LATERAL DOMAINS IN LIPID BILAYERS

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Sphingomyelins (SM) are important phospholipids in plasma membranes of most cells. Due to their mostly saturated nature (commonly 16:0, 18:0 and 24:0 N-linked acyl chains), they affect the lateral structure of membranes. These SM can interact with cholesterol (Chol) and ceramide (Cer) and give rise to the formation of highly ordered lipid domains, either fluid or gel1. However, SM containing 24:1 N-linked acyl chains are also common in most tissues and have been described as unable to form ordered domains in the presence of Chol, apparently because of their unsaturated chain. Thus, unsaturated SM could act as a natural tool for preventing lateral phase separation in cell membranes2.

The aim of this study was to determine the influence of unsaturated SM and Cer in the formation of SM-Chol-Cer segregated lateral domains. Membrane models (liposomes or supported planar bilayers) of dioleoylphosphatidylcholine, SM, Chol and Cer were built, using SM and Cer containing either 16:0, or 24:1, or a mixture of both. The samples were analyzed by differential scanning calorimetry (DSC), confocal microscopy and atomic force microscopy (AFM). Contrary to what was expected, 24:1SM did not systematically prevent the formation of Cer-rich domains. The results obtained using both 24:1 Cer and 16:0 Cer in the same sample depended on the SM: (i) 16:0 SM induced the formation of homogeneous bilayers, whose properties were close to a 24:1 Cer domain, (ii) 24:1 induced lateral phase separation, with formation of domains whose physical properties differed from those of the continuous phase.

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[2]Mate et al. (2014) Biophys. J. 106:2606.

POSTERS

P08: ERYTHROCYTE-ERYTHROCYTE ADHESION PROMOTED BY FIBRINOGEN IN ESSENTIAL ARTERIAL HYPERTENSION PATIENTS

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Erythrocyte aggregation has been suggested as indicator of cardiovascular risk, in part due to its conditioning by high plasma fibrinogen levels. In this work, our main goals were to understand how fibrinogen-erythrocyte interaction influences erythrocyte aggregation and how it constitutes a cardiovascular risk factor for Essential Arterial Hypertension (EAH). 31 EAH patients and 15 healthy donors (control group) were enrolled on the study. Atomic Force Microscopy (AFM) was used to measure fibrinogen-erythrocyte interaction and erythrocyte-erythrocyte adhesion, at different fibrinogen concentrations. The stiffness of the erythrocytes was also assessed. Force spectroscopy measurements, at the single-molecule level, showed that the average fibrinogen-erythrocyte binding forces are increased in EAH patients relative to healthy blood donors, despite a lower binding frequency. Our cell-cell adhesion data showed that increasing the fibrinogen levels, the work and the maximum force necessary to detach one erythrocyte from another also increases. Moreover, we found that EAH patients presented at all plasma fibrinogen concentrations higher values of work and detachment force than healthy donors. Elasticity studies revealed an increase of the cell penetration depth and an increase of erythrocytes' stiffness of EAH patients relative to the control.

We conclude that the interaction between fibrinogen and erythrocyte is increased on EAH patients, which could be an important cardiovascular risk factor to be considered. Our findings also evidence that fibrinogen promotes erythrocyte-erythrocyte adhesion, probably by transient simultaneous binding of the protein to two cells, bridging them. This is expected to lead to the increase of erythrocyte aggregation on EAH patients. Understanding the role of fibrinogen on erythrocyte aggregation may be relevant for potential future drug interventions to reduce aggregation and enhance microcirculatory flow conditions in patients with cardiovascular risk.

Keywords: Atomic force microscopy; fibrinogen-erythrocyte interaction; erythrocyte-erythrocyte adhesion

P09: STRUCTURE OF A DIMERIC FRAGMENT OF THE PLAKIN DOMAIN OF PLECTIN BY HYBRID METHODS

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Plectin is a member of the plakin family of high molecular weight cytolinkers. Plectin has a tripartite structure with N- and C-terminal regions separated by a central rod domain. The N-terminal region contains an actin binding domain (ABD) and a plakin domain. The ABD binds to integrin α6β4 in hemidesmosomes and to the nuclear envelope protein nesprin-3. The plakin domain is formed by nine spectrin repeats (SR1-SR9) and an SH3 domain. Each SR consists of three α-helices (A-B-C) arranged in a left-handed bundle. Helix C of a SR is fussed to helix A of the downstream repeat; hence, the plakin domain has an elongated shape. Downstream of SR9, the rod domain forms a parallel coiled-coil that mediates dimerization. Here, we have combined hybrid methods to elucidate the structure of a dimeric fragment of plectin that includes the SR7-SR9, and the initial region of the rod domain. The crystal structure revealed contacts along the plakin domain between two protomers. Analysis by small angle X-ray scattering (SAXS) supports that the closed arrangement of the dimer also occurs in solution. This was further confirmed by measuring eight inter-monomer distances combining site directed spin labelling and double electron-electron resonance spectroscopy (EPR-DEER).

Collectively, our data suggest that the plakin domain contributes to the stabilization of the plectin dimer. Moreover, the rigid structure of the SR3-SR9 constraints the maximum spacing between the binding-sites for integrin β4 and nesprin-3 in the dimer, suggesting that avidity-driven recruitment of plectin might depend on the density of the interaction partners.

POSTERS

P10: STRUCTURAL ORGANIZATION OF THE GUANINE NUCLEOTIDE EXCHANGE FACTOR C3G

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C3G is a guanine nucleotide exchange factor (GEF) that activates the small GTPases Rap1 and R-Ras. C3G is involved in multiple cellular functions including adhesion, migration, cytoskeletal remodeling, cell proliferation, differentiation, transformation, and apoptosis. C3G (120 kDa) has a tripartite structure. The N-terminal region (N-C3G) mediates binding to E-cadherin at nascent adherent junctions. The structure of N-C3G is unknown and bears no similarity to other proteins. The central region contains five Pro-rich sequence motifs that mediate the interaction with protein that contain SH3 domains, such as Crk, p130Cas, Grb2, Hck and c-Abl. The central region also mediates binding to the TC-PTP phosphatase and to β-catenin, which do not contain SH3 domains. The C-terminal catalytic region consists of a REM (Ras exchange motif) and a Cdc25H domain. Deletion of the N-terminal half of C3G increases the GEF activity, suggesting that this N-terminal half acts as a regulatory element. Yet, the mechanisms of autoinhibition of C3G remain unknown. Here we have identified and characterized an intramolecular interaction between the N-C3G and the catalytic region. We have identified residues important for maintaining the close conformation. This head-tail interaction in C3G resembles autoinhibitory conformations of other GEFs of the Cdc25H family, such as Sos1, Epac2, and RasGRP1. Finally, using recombinant N-C3G fragments we show that this region has a high content of α-helical structure. Sequence analysis suggests the presence of amphipathic helices that could fold in a helical bundle.

POSTERS

P11: UNVEILING THE ROLE OF THE MUTATION F508DEL IN CYSTIC FIBROSIS

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Cystic fibrosis is a genetic disease that causes the accumulation of mucus in epithelia, mainly affecting the airways of the lungs. These secretions not only cause obstruction, but also inflammation and infections by Pseudomonas and Staphylococcus organisms. (1)(2) The mutated gene in this disease encodes for the cystic fibrosis transmembrane conductance regulator (CFTR) protein, which is a chloride channel. (1) Although there are over 1500 mutations related to cystic fibrosis, the most common is the deletion of phenylalanine 508 (F508del). F508del causes the misfolding of the CFTR polypeptide leading to its degradation in the ER. Nevertheless, a small amount of defective channels is still able to reach the cell membrane, but display an impaired function leading to minimal chloride transport. (3)

The CFTR channel belongs to the class of ABC transporters. These proteins have been extensively studied using computational methods in our lab (5-7) in order to clarify their mechanism and mode of action.

In the present work, we derived a new model for the NBD1-NBD2 association of human CFTR based on existing data using comparative modelling techniques (8), and performed molecular dynamics simulations of the CFTR protein in both mutant and wild-type forms with the goal of studying the conformational consequences of ATP hydrolysis in the mutant and wild-type forms.

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Keywords: ABC Transporters CFTR F508del MD simulation

P12: THE ANTI-EPILEPTIC DRUG LEVETIRACETAM INACTIVATES N-TYPE, BUT NOT L-TYPE, CALCIUM CHANNELS IN CULTURE HUMAN NEUROBLASTOMA SH-SY5Y CELLS AND RAT CEREBELLAR GRANULE NEURONS.

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Levetiracetam is a widely used antiepileptic drug in human therapy. However, the molecular mechanism by which levetiracetam exerts its antiepileptic effect and the effects of chronic treatment with this drug on neuronal excitability are poorly known. Levetiracetam binds to synaptic vesicle protein SV2A, which has been proposed to be involved in the regulation of vesicle exocytosis, and inhibits presynaptic calcium channels in superior cervical ganglion neurons [Vogl et al (2012) Mol Pharmacol 82, 199-208]. In this work we have used SH-SY5Y human neuroblastoma cells and rat cerebellar granule neurons (CGN) cultures to identify which types of neuronal calcium channels are more sensitive to inhibition by levetiracetam. SH-SY-5Y cells, which mainly express L-, N- and T-type calcium channels [Sousa et al (2013) PLOs One 8, e59293], and CGN cultures, whose steady state cytosolic calcium is largely controlled by L-type calcium channels [Marques-da-Silva et al (2014) Cell Calcium 56, 108-123], were prepared and loaded with Fura-2/acetoxymethyl esther as in previous works [Berrocal et al (2009) FASEB J. 23, 1826-1834; Marques-da-Silva et al (2014) Cell Calcium 56, 108-123]. Nifedipine, ω-conotoxin MVIIC , ω-conotoxin GVIA and ω-agatoxin MIVA have been used as blockers of L-type, (N,P and Q)-type, N-type, and P-type calcium channels, respectively. Results showed that treatment of these cells with 100-300 μM levetiracetam for up to 5 hours led to a potent inactivation of N-type calcium channels. In contrast, this treatment did not significantly alter the calcium entry through L-type calcium channels, their response to partial plasma membrane depolarization induced by extracellular K+, or the steady-state cytosolic calcium in CGN cultures. Acknowledgements: This work has been supported by Grant BFU2014-53641-P of the Spanish Ministerio de Economía y Competitividad and by Grant GR15139 of the Junta de Extremadura to research group BBB008, with FEDER co-financing].

Keywords: Levetiracetam, N-type and L-type calcium channels

P13: THE PH EFFECT ON MARINOBACTER HYDROCARBONOCLASTICUS DENITRIFICATION GENES AND NITROUS OXIDE REDUCTASE

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The only known biological system capable of detoxify nitrous oxide in vivo, a very potent greenhouse gas, is catalysed by nitrous oxide reductase, in a metabolic pathway named denitrification, during which nitrate is converted, via nitrite, to nitric oxide, nitrous oxide, and to the inert dinitrogen. Most of the atmospheric nitrous oxide is due to incomplete denitrification or the last reaction, catalysed by nitrous oxide reductase, is not efficiently performed, mostly due to a drop in pH in the environment.

In this work, we investigated the pH effect on the growth of Marinobacter hydrocarbonoclasticus, as a model of marine organisms. For growths carried out at pH 6.5, 7.5 and 8.5, the gene expression profile, the denitrification intermediates and the reduction rate of nitrous oxide reductase by the cells were analysed during 48h in a batch culture. The results indicate an accumulation of nitrite and a very low rate of nitrous oxide reduction by the whole cells at low pH.

Furthermore, the effect of growth pH on nitrous oxide reductase was investigated. The enzyme was isolated and biochemically characterized from growths performed at each pH. The correlation of steady-state kinetics with the spectroscopic data allow us to identify different features of the catalytic center of the enzyme isolated from growth performed at low pH compared to pH 7.5 and 8.5. In order to understand which proteins are involved in maturation or maintaining an active nitrous oxide reductase, protein-protein interactions in the denitrification apparatus were also investigated. This study reveals a clue to identify the active form of nitrous oxide reductase in vivo, as well as, pin-points possible molecular mechanisms involved in the release of nitrous oxide due to environment acidification.

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Keywords: Denitrification, nitrous oxide reductase, Marinobacter hydrocarbonoclasticus, CuZ center, proteomics/transcriptomics/spectroscopy

POSTERS

P15: CISPLATIN-INDUCED CHANGES ON MEMBRANE BIOPHYSICAL PROPERTIES OF COLON CANCER CELLS: CHARACTERIZATION OF THE INITIAL STEPS OF CISPLATIN MECHANISM OF ACTION

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Cisplatin is a platinum-based drug used in chemotherapy. Cisplatin mode of action includes the activation of acid sphingomyelinase and ceramide production at the plasma membrane prior to its interaction with DNA. These initial steps of cisplatin mechanism of action, particularly its effects on membrane structure and biophysical properties are still poorly characterized. To further elucidate this issue, fluorescence spectroscopy and microscopy methodologies were employed to characterize membrane biophysical properties of colon cancer cells with different degrees of pathogenicity (sensitive vs resistant cells) upon treatment with varying concentrations of cisplatin (16 to 300 μM). Plasma membrane order of cancer cells was found generally higher when comparing to non-cancer cells. In addition, under cisplatin treatment the membrane order of sensitive cells (HEK and SW480 cell lines) changed over time, while almost no variation was found for resistant cells (HT29 and SW620 cell lines). In accordance, after cisplatin treatment sensitive cells experienced morphological alterations suggestive of cell death with higher impact than in resistant cells. Our work shows that cisplatin-sensitive cells present significant biophysical and morphological alterations upon cisplatin treatment, in contrast to the observed for resistant cells. These results suggest that cisplatin-mediated cytotoxicity might depend on its ability to promote an initial disruption of membrane structure and organization.

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Keywords: Cisplatin, colon cancer, fluorescence spectroscopy, membrane biophysical properties, 2-photon microscopy

P16: EXPLORING THE CONFORMATIONAL AND STRUCTURAL PROPERTIES OF THE INFLUENZA FUSION PEPTIDE IN MEMBRANE BILAYERS: A COMPUTATIONAL STUDY

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One of the key players involved in the entry of the influenza virus in the host cells is the hemagglutinin protein. After the viral uptake by endocytosis and consequent lowering of the pH, this protein suffers major conformational changes, which enables the fusion of the viral envelope and host cell membrane, allowing the entry of the genetic material that will resume the infection process[1]. One particular region of the hemagglutinin protein, called the fusion peptide (FP), has a major role in the membrane fusion process. However, the molecular determinants behind the action of the FP in this process are yet to be elucidated, and experimental studies have not been able to determine the conformation of the FP inside the membrane. Recently, our group has published results that bring new insights on this subject, revealing that the FP can adopt two different stable conformations [2]. In the present work, we expand our knowledge on the conformational and structural properties of the FP in a model membrane using enhanced sampling computational methods, such as well-tempered bias-exchange metadynamics simulations.
The results of this work will contribute with a better understanding of the mechanisms of membrane fusion that occur during influenza virus infection.

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Keywords: Influenza, hemagglutinin, fusion, peptide, membrane

P17: PH-DEPENDENT INSERTION OF PHLIP PEPTIDE INTO LIPID BILAYERS: PKA VALUES OF KEY RESIDUES

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The pH (low) insertion peptide (pHLIP) (1-3) is a family of peptides that are able to insert into a lipid bilayer at acidic pH. These peptides are based in a transmembranar sequence of bacteriorhodopsin that is unstructured in solution (stage I), interacts with lipid bilayers remaining unstructured at neutral pH (stage II) and inserts into the bilayer with a significant α-helical content at acidic pH (stage III). This family of peptides have already been used to target tumor cells in vivo since acidosis is an hallmark of these tissues (4). These events are difficult to study at the molecular level, in particular, the relation between the pK of insertion of pHLIP peptides and the pKa of some key residues is yet to be clarified. In this work, we used a linear response approximation to determine the pKa of these residues. We studied four different pHLIP variants to understand the importance of the ASP positions and its mutation to GLU. For these variants, there are experimental data available that we used to validate our approach. Finally, we also propose the addition of a HIS residue to the sequence, which we expect to turn off the pHLIP peptide insertion into the membrane (stage III) at too low pH values.

We acknowledge the financial support from FCT (PTDC/QEQCOM/5904/2014 and UID/MULTI/00612/2013).

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Keywords: pH, membranes, pHLIP, simulations, pKa

POSTERS

P18: UNRAVELING THE ANTICARCINOGENIC EFFECTS OF RESVERATROL THROUGH A MEMBRANE BIOPHYSICAL APPROACH

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Resveratrol is a natural polyphenol compound with great potential in the cancer therapy. The mechanism of action is still uncertain and this work brings a new membrane approach to understand the anticarcinogenic effects of this compound.

Unilamellar liposomes composed of phosphatidylcholine, cholesterol and sphingomyelin were chosen as membrane mimetic systems. The partition coefficient of resveratrol in lipid bilayers was assessed by derivative spectrophotometry using liposomes/water systems. The membrane location of resveratrol was studied by fluorescence quenching of probes by steady-state and time-dependent measurements. Anisotropy studies were applied to infer about the effect of resveratrol on membrane fluidity. The molecular interactions between resveratrol and lipid rafts in cell membranes were assessed by means of Förster resonance energy transfer. Finally, the structural modifications of the lipid bilayers by the presence of resveratrol were studied by small-angle and wide-angle X-ray scattering.

The results indicate that resveratrol is able to incorporate in lipid bilayers, penetrating into the acyl chain region but also positioning its polar hydroxyl groups near the membrane interface. Resveratrol either fluidizes or stiffens the membranes depending on the organizational state and order. Resveratrol rigidify the more fluid tumor cell membranes, contributing to the cancer therapy. The compound induces phase separation, stabilizing and promoting the formation of ordered domains (lipid rafts) which can act as organizing centers for the assembly of apoptotic molecules. By interfering with cell membranes, resveratrol is able to inhibit protein kinase C and cyclooxygenase activity, which in turn are involved in cell signaling and tumor promotion.

The biophysical interaction studies of resveratrol with model membranes provide a rational approach to better understand the anticarcinogenic effects of this compound.

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Keywords: Resveratrol, liposomes, drug-membrane interactions, lipid rafts, FRET, X-ray diffraction

P19: THE IMPACT OF RESVERATROL IN LIPID BILAYERS: A NEUTRON REFLECTOMETRY STUDY

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The natural antioxidant resveratrol, contained in the skin of red grape and accordingly in their wines, is hold liable for health impacts such as cardiovascular protection and anti-oxidative effect [1-3]. Clinical trials of resveratrol as prophylactic or even therapeutic drug are ongoing [4, 5]. However, basic knowledge on its probable working mechanism is rare.
In this biophysical study, neutron reflectometry was used to investigate the direct impact of resveratrol on lipid membranes with solid supported bilayers [6]. When interacting with di-palmitoyl-phosphatidyl-choline (DPPC) bilayers, resveratrol accumulates in between the headgroups but is absent in the hydrophobic core. Without a biogenic removal mechanism, the headgroup region may host up to ~25 mol% of resveratrol. The average thickness and the interfacial roughness of the headgroup layer are increased. From the structural results, the average tilting of the PC headgroups is calculated. Upon the presence of resveratrol, the PC headgroups are turned to a more upright orientation, thus reducing their projected area. This effect is propagated into the hydrophobic core, changing the chain packing there despite of the absence of resveratrol. When being adsorbed onto a binary DPPC/cholesterol membrane, the structural results suggest that resveratrol most probably locates on top of the hydroxyl group of the cholesterol in between the PC headgroups. The ordering effect of cholesterol on the hydrophobic core is reversed when both, cholesterol and resveratrol, are present [6].

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Keywords: Resveratrol; Cholesterol; DPPC model membrane; Neutron reflectometry; Membrane structure; Membrane dynamics

P20: STABILITY OF NANOCOMPOSITES OVER IONIC FORCE

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Silk fibroin nanoparticles (SFN) are a promising alternative in nanomedicine. However, the high ionic strength of biological media leads to self-aggregation. Chitosan (CS), a linear polycationic polymer, represents a good option to stabilize nanoparticles either by electrostatic or by steric way. We have studied the best proportion between CS and SFN in order to obtain nanocomposites stable when the ionic strength of the medium is increased. Also, we have used the "in silico" blind docking technique to obtain details regarding how CS molecules interact with fibroin, which areas parts of the protein surface interact, and the nature and intensity of these interactions.

The stabilizing effect of the CS on the SFN was developed at ionic strength equal to 0.024 M because of the strong aggregation that SFN show in this condition. Thus, CS prevents aggregation when the proportion CS:SFN in suspension was from 1:1.6 mg:mg to 1:12.7 mg:mg. Nevertheless, to obtain nanocomposites which show a narrower size distribution, proportions CS:SFN between 1:6.35 mg:mg and 1:12.7 mg:mg must be used. It is noteworthy that the lower CS present in the sample, the lower Z potential of the nanocomposites.

Results obtained from blind docking calculations show that a maximum of 5 sites in the protein can interact with this polysaccharide. They also suggest that there is a preferred site for docking of CS in the fibroin monomer model with a good steric fit, which is mainly due to electrostatic interactions. Additional positive binding interaction are due to a network of hydrogen bonds.

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Keywords: Fibroin, chitosan, nanocomposites, ionic force, stability

POSTERS

P21: A NMR-BASED METHOD TO QUICKLY PROBE DISORDERED PROTEIN REGIONS VIA N-H SOLVENT ACCESSIBILITY

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Understanding proteins structure and dynamic properties governing cellular processes is crucial for basic and applied research. Of particular interest are intrinsically disordered protein (IDP) regions that display alternative transient conformations, enabling a multitude of functions. IDPs are particularly hard and time-consuming to study by classical approaches, developed for globular proteins with well-defined conformations. New methods are required, given IDPs role in disease mechanisms. Here, employing dengue virus capsid protein (that possesses both structured and disordered regions), we developed a fast NMR method to immediately determine N-H groups' solvent accessibility with amino acid residue resolution. The protein regions dynamics and the first residues of α-helices are also readily determined. The method is based on minimal pH changes, using the well-established 1H-15N HSQC pulse sequence in a simple to interpret manner. This approach can thus complement other data, being easily implemented in current protein NMR routines, providing structural and dynamics information.

Keywords: NMR; Protein structure and dynamics; Dengue virus capsid protein; Intrinsically disordered proteins.

POSTERS

P22: CHARACTERIZATION OF THERMO-STABLE KTRA MUTANTS

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Animals have a tight control over their extracellular fluid composition. In contrast, prokaryotes have little or no control over these fluids and are frequently subjected to osmolarity changes in their external medium. Therefore, these organisms developed regulatory mechanisms that allow them to cope with osmotic stress and maintain turgor pressure stable. One of these mechanisms is the regulation of K+ concentration.

Potassium transporters, particularly the Trk and Ktr systems, play an essential role in the regulation of K+ uptake. The KtrAB potassium transporter is a K+/Na+ transporter. This system is composed by the KtrB membrane protein and the KtrA cytosolic regulatory protein. KtrA is able to bind ATP and ADP and adopt different conformations that result in a high or low activity state of the transporter, respectively.

The structures of the KtrAB complex bound to ATP or ADP have revealed many molecular details of this protein complex, however the low resolution of these structures (3.5Å and 6Å, respectively) limits our understanding of the structural properties of the transporter. We have created KtrA C-terminal mutants that show improved thermal stability in both ADP and ATP bound states and are still capable of forming an octameric ring. One mutant in particular, KtrA CysLHDN, also showed improved crystal diffraction quality, resulting in an ATP bound KtrA structure with better resolution than before. Importantly, this construct also assembles with KtrB forming a stable complex and will be an interesting tool to obtain better KtrAB structures.

Keywords: Potassium, transporter, KtrA, structure, stability

POSTERS

P23: AQUAPORIN INVOLVEMENT IN OXIDATIVE STRESS RESPONSE

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Aquaporins (AQPs) are membrane channels that mediate rapid movement of water across plasma membrane, which is extremely important for cells adaptation to the external environment. In addition to water, a subset of the AQP family is also permeable to small-uncharged solutes like glycerol. AQPs have important roles in physiology and their dysfunction or abnormal expression has been associated with several pathologies related water and energy homeostasis (kidney disorders, brain edema, obesity and cancer).

Recently, some mammalian AQPs were reported to transport hydrogen peroxide (H2O2) which has been considered as a possible mechanism explaining their involvement in cell redox status and tumorigenesis. To explore the involvement of AQPs in cell response to oxidative stress we used a yeast model for lipid peroxidation transformed with Δ12 desaturase (BYdesa). This strain shows increased levels of polyunsaturated fatty acids (PUFAs) and induction of AQP expression, suggesting its interplay in cell redox status. Thus, to investigate the correlation between PUFAs production and AQP-mRNA levels, we assessed AQP activity. Elevated water permeability (Pf) and lower activation energy (Ea) for water transport of BYdesa strain compared to control confirmed that AQP-induced expression has a functional outcome. Additionally, we found inhibition of AQP activity by the product of lipid peroxidation, 4-hydroxynonenal (HNE) that accumulates intracellularly.

To confirm AQP involvement in oxidative stress, we used a yeast strain overexpressing endogenous aquaporin (AQY1). Sensitivity assay showed that AQY1-yeast strain was more sensitive to H2O2 and to HNE. By measuring ROS production, a significantly higher influx of H2O2 was detected for the AQY1-strain. Regarding cellular antioxidative defense system, the glutathione level was similar, but the catalase activity was significantly higher for AQY1-strain compared to control. Although these data needs further clarification, overall our data indicate that aquaporins are important players in oxidative stress resistance.

Keywords: Aquaporins; Oxidative Stress; Hydrogen Peroxide; 4-hydroxynonenal (HNE)

POSTERS

P24: USE OF VOLTAGE-CLAMP FLUOROMETRY TO DETERMINE THE RELATIVE POSITIONING OF ION CHANNEL CYTOPLASMIC DOMAINS-LOCATED FLUORESCENT TAGS TOWARD THE PLASMA MEMBRANE

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As for other plasma membrane-located ion channels, the overall architecture and the function-related reorganizations of the cytoplasmic domains of the KCNH voltage-dependent potassium channels remain largely unknown. Both the non fluorescent dye dipicrylamine (DPA) and the fluorescent oxonol DiBAC4(5) are translocated through the lipid bilayer in response to membrane potential changes in a very fast (few milliseconds) time scale. Furthermore, their spectroscopic properties allow them to act as effective FRET quenchers of cyan, green and yellow fluorescent proteins (FPs), when these are incorporated as fluorescent tags at some positions of host proteins. We used a fast voltage-clamp fluorometry approach with HEK293 cells expressing FPs with plasma membrane-anchoring modifications and incubated with DPA or DiBAC4(5), to characterize the voltage-dependent translocation of the dyes and the subsequent FRET-triggered FP quench. Under these conditions in which only the plasma membrane potential of the patch-clamped cell is modified, we also studied the dye-induced quench of several KCNH2 (hERG) channel constructs labeled with CFP and/or YFP in different positions. We directly demonstrated for the first time that the distance between an amino-terminal FP tag and the intracellular plasma membrane surface is much shorter than that between the membrane surface and a C-terminally-located tag. The distances are also different when the attachment point of the FP was changed to other positions along the cytoplasmic domains of the channel. Additionally, since the fast voltage-dependent dye translocations precede the time course of conformational rearrangements associated to channel operation, we anticipate that using some tagged channel variants, the late fluorometric responses could allow to detect function-dependent movements of the labels orthogonal to the plasma membrane.

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Keywords: Voltage-dependent potassium channel, hERG, voltage-clamp fluorometry, FRET quenchers, fluorescent protein tags

P25: LANTHANIDE DO3A-TYPE COMPLEXES CONJUGATED TO PITTSBURGH COMPOUND B AS OPTICAL AND MRI PROBES FOR TARGETING Aβ AMYLOID AGGREGATES INVOLVED IN ALZHEIMER'S DISEASE

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Alzheimer's disease is one of the most frequent disorders in elderly individuals, characterized by progressive neural degeneration, causing devastating cognitive disorders. The diagnosis is only possible at an advanced stage of the disease when symptoms characteristic of the disease are observed, except in its familiar form, where mutations have already been identified. The definitive diagnosis of sporadic Alzheimer's disease is only obtained post-mortem, because of the need for a histological section from the brain to assess the presence of amyloid plaques. Thus, there is a need to identify these amyloid plaques during the life of the patient. We have developed a multimodal probe consisting of two parts 1) the Pittsburg compound B (PiB), which is a phenyl-benzothiazole, (with proven affinity to the amyloid aggregates and will also work as antenna sensitizing the metal ion for optical imaging), and 2) a DO3A derivative, offering thermodynamic stability and kinetical inertness, for the coordination of a specific metal ion depending on the imaging modality. In our group we already have developed other probes for this purpose, especially for use in Magnetic Resonance Imaging (MRI)1–4. In this work, we studied not only the possibility of using this ligand complex with Gd3+ as contrast agent for MRI with the determination of the relaxivity and others relevant parameters, but also the potential of using the Eu3+ complex for Optical Imaging with the determination of the luminescence quantum yield and other relevant photophysical properties. The interaction of the Gd3+ complex with the Aβ1-40 peptide shows a moderate affinity towards the Aβ amyloid aggregates (KD= 19.5 ± 3.0 μM), higher than the affinity towards the monomeric form of the peptide, which is fundamental since it is intended to be used to detect its aggregated form.

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P27: UNRAVELING THE ACEMETACIN EFFECTS ON PHOSPHATIDYLCHOLINE BILAYERS

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Acemetacin is a nonsteroidal anti-inflammatory drug (NSAID) used in the treatment of rheumatoid arthritis, osteoarthritis and for pain relief. Despite its remarkable therapeutic properties, the long-term therapy with acemetacin is associated with gastrointestinal (GI) toxicity. (1) The topical action of NSAIDs on the phospholipid layers of GI mucosa is a key toxicity mechanism of these anti-inflammatory drugs. (2) In this context, the objective of this work is to assess the interaction of acemetacin with phospholipid bilayers at acidic and neutral media. Large unilamellar vesicles made of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) were prepared by the film hydration method followed by extrusion. The drug partition in the bilayer was determined by derivative spectrophotometry and isothermal titration calorimetry. The acemetacin effect on the thermal behavior of DMPC vesicles was assessed by differential scanning calorimetry and on the bilayer structure was studied by electron paramagnetic resonance.

The partition coefficient of acemetacin is pH-dependent and higher at acidic media, since the drug molecules become neutral and more efficiently interact with phosphatidylcholine bilayers. The most pronounced effect of acemetacin on the main phase transition of DMPC is observed at pH 3.0 with a decrease on the temperature and on the cooperativity and an increase on the transition enthalpy. Lastly, the insertion of acemetacin increases the structural organization of the phosphatidylcholine bilayer at both the gel and fluid phases. The acemetacin-induced alterations on the structure and dynamics of phosphatidylcholine bilayers may facilitate the entrance of noxious agents and lead to the occurrence of adverse effects in the GI tract.

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Keywords: Acemetacin, phosphatidylcholine, derivative spectrophotometry, isothermal titration calorimetry, differential scanning calorimetry, electron paramagnetic resonance

P28: AN ADDITIONAL PHASE TRANSITION OF DPPC MONOLAYERS AT HIGH SURFACE PRESSURE CONFIRMED BY GIXD STUDIES

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Pulmonary surfactant forms the alveolar monolayer at the air/aqueous interface within the lung. During the breathing process, the surface pressure periodically varies from ~40mN/m up to ~70mN/m. The film is mechanically stable during this rapid and reversible expansion. The monolayer consists of ~90% of lipid with 10% integrated proteins. Among its lipid compounds, di-palmitoyl-phosphatidylcholine (DPPC) dominates (~45wt%). No other lipid but DPPC was so far reported to be compressible to very high surface pressure (~70mN/m [1]) before its monolayer collapsed. Its liquid-expanded/liquid-condensed (LE/LC) phase transition at ~10mN/m is well known [2].

Here we present results from Langmuir isotherm measurements that evidence a so far not documented second phase transition at elevated surface pressure Π (~50mN/m). The varying lateral structures of the monolayer at 8mN/m, 20mN/m, 30mN/m, 40mN/m, 50mN/m, 60mN/m, 70mN/m were investigated by grazing incidence X-ray diffraction (GIXD). The results report on the 2D packing lattice with the inter-chain distance d-xy. Moreover, the tilt angle of the palmitoyl chains was calculated combining the lattice parameters and the geometrical boundary conditions. The course of the inter-chain distance versus surface pressure exhibits three regimes, separated by the LE/LC transition and the second phase transition at the higher pressure. This feature may assign a functional task to DPPC in the lung surfactant since it contributes to the mechanical stability of the alveolae monolayer and at the same time allows reduction of the interfacial tension to ~0mN/m.

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Keywords: Lung surfactant; DPPC monolayer phases; mechanical stability; Langmuir isotherm; GIXD;

P29: CHARACTERIZATION OF MEK1 PATHOLOGICAL MUTATIONS BY ENHANCED MOLECULAR DYNAMICS

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Protein kinases are key regulators of eukaryotic living cells, since they are involved in crucial biochemical functions and signaling networks. These enzymes share a common fold and, in response to specific cellular signals, switch between distinctive inactive and active states undergoing large conformational changes. The exhaustive description of these large conformational changes is highly challenging. One relevant example within the protein kinase family is MEK1 (Mitogen-activated protein kinase kinase 1), involved in the MAPK/ERK pathway [1]. Given its regulatory role in many important cell processes (such as gene expression, cell differentiation and apoptosis), over-activating mutations on MEK1 are known to cause serious pathologies, such as different congenital anomaly disorders, like the Cardio-Facio-Cutaneous (CFC) syndrome [2,3], or cancer. Nevertheless, the mechanism underlying the boost in MEK1 inactive-to-active transition led by these mutations is not yet understood at atomic level.

Here we report a comprehensive study on the pathological effects of two selected pathological mutations related to CFC syndrome: Q56P, lying on the regulatory helix (Q56P), which is known to cause a significant increase of the kinase activity in vitro [4]; Y130C, falling within the MEK1 kinase domain boundaries and facing αA-helix, which was reported to lead to a slight MEK1 upregulation [5]. Thus, in order to understand the effect of such mutations at molecular level as well as their impact on the intrinsic propensity for MEK1 inactive-to-active transition, 1-μs-long conventional MD simulations were initially performed on different biologically relevant states of MEK1 protein kinase. Moreover, four additional 1-μs-long simulations were run using PTmetaD-WTE protocol [6], an enhanced sampling approach that combines parallel tempering with well-tempered metadynamics.

The combination of these two computational methods can help to rationalize the activating effects induced by these pathological mutations, and can be used to propose a mechanistic explanation to the different levels of MEK1 over-activation that were observed experimentally.

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POSTERS

P30: NMR SOLUTION STRUCTURE OF THE EXCALIBUR DOMAIN OF CBPL FROM S. PNEUMONIAE

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Choline-binding proteins (CBPs) are involved in a wide rage of physiological functions. CbpL from S. pneumoniae is a CBPs of 332 residues-long protein. It has 3 well-differentiated domains connected by linker regions that provide flexibility to the general structure. The N-terminal domain includes an Excalibur domain (from extracelular calcium-binding region) of unknown structure and function, although in some cases these domains have been proposed to be involved in adhesion and colonization processes. Here we describe for the first time, the solution 3D structure of the Excalibur domain by using heteronuclear multidimensional NMR spectroscopy. In the absence of Ca2+, the NMR spectra showed that the protein exists as a random coil or an unfolded polypeptide. Addition of Ca2+ dramatically changed the distribution and the dispersion of the NMR signals indicating that the protein was folded upon Ca2+ binding. According to this, we have structurally characterized the folded entity in the presence of saturation concentrations of Ca2+.

The global fold of the domain shows an intricate globular shape (16 x 20 x 27 Å) resembling a wool ball. The structure has a disordered N-terminal short tail, and a globular packed domain containing a short α-helical region, a calcium-binding site, and a disordered C-terminal region. All the residues in the DxDxDGxxCE motif are involved in calcium binding in a canonical disposition. The two Cys residues establish a disulphide bridge that connects the short α-helical region with the Ca-binding site.

The structural details of the Excalibur domain will be discussed in the context of the full-length CbpL protein, which is expected to remain strongly anchored to peptidoglycan layer exposing the Excalibur domain to the capsule or the extracellular medium.

Keywords: NMR, Excalibur domain, CbpL, structure-function relationships

P31: GASTRIC TRANSPORT STUDIES AND BREAST CANCER APOPTOSIS MEDIATED CELL-DEATH BY MALVIDIN-3-O-GLUCOSIDE AND THEIR DERIVATIVES.

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Breast cancer, still is, nowadays, the most common cancer afecting women around the world. Until now, a considerable number of research have been done using estrogen dependent MCF-7 cancer cell line to evaluate the effect of several food components on the progression of breast cancer. However, it is crucial to know the transport properties of the compounds, and also the effects of food matrix on their bioavailability [1].

Anthocyanins are an important example of natural compounds involved on this kind of studies. These pigments are present in several foodstuffs, including red wine [2]. These changes are generally attributed to several different chemical reactions, resulting in new pigments responsible for colour changing and longevity of wines, such as carboxy-pyranoanthocyanins (type A vitisins) and methyl-pyranoanthocyanins, both present in aged Port wines [3].

In this study, the antiproliferative and anti-apoptotic properties of Malvidin-3-O-glucoside flavilium and non-flavilium derivatives, in MCF-7 breast cancer cell line were assayed. Antiproliferative capacities were evaluated by SRB assay, while apoptotic properties wereassayed by TUNEL assay and modulation of caspases 3 and 9 and bcl-2 proteins. The results showed a correlation between the chemical structure of the compounds and the biological properties. Overall the results suggest that the differences on the newly formed pyrano ring moiety of the derivatives may be the main reason for the different bioactivities.

This study confirms the importance of the natural micro-oxidative processes that occur during food ageing, to their bioactive properties in vivo against breast cancer.

Keywords: Bioavailability, Anthocyanins, Gastric Transport, Breast Cancer

P32: AQUAGLYCEROPORINS EXPRESSION IN ENDOTHELIAL CELLS AND IMPLICATIONS IN CARDIOVASCULAR DISEASE

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Aquaporin (AQPs) are a family of protein channels that facilitate the transport of water and/or glycerol through the plasma membrane of all kinds of organisms. The aquaglyceroporins (AQP3, 7, 9, 10) facilitate glycerol permeation and are involved in glycerol metabolism being crucial for energy production and homeostasis in different organs (liver, adipose tissue, muscle and heart). Aquaglyceroporin dysfunction was reported in several disease states involving fat accumulation, such as obesity, liver steatosis and metabolic syndrome.

In endothelial tissue that mediates fluid and metabolite distribution from blood to all body, AQP1 was reported to be highly expressed and important for water permeation; however, less is known about aquaglyceroporins expression and function.

In this study we used human umbilical vein endothelial cells (HUVECs), an established study model for hypomethylation, atherosclerosis and cardiovascular disease (CVD), to evaluate the participation of aquaglyceroporins in glycerol permeation and their possible involvement in pathology. Thus, AQP3, AQP7, AQP9 and AQP10 expression were screened by RT-qPCR using AQP1 as a positive control. S-Adenosyl homocysteine (SAH) negatively regulates cell methylation and is an atherogenic metabolite. Thus, we reevaluated the expression levels of the aforementioned AQPs after triggering SAH intracellular accumulation.

Our data confirmed a high expression level of AQP1 and revealed, for the first time, that AQP3 is highly expressed in HUVEC. AQP7 and AQP10 were also detected, whereas AQP9 was absent.

Under SAH accumulation, the expression levels of AQP1, AQP3 and AQP10 were decreased. Interestingly, AQP7 was 4-fold increased. While the expression/function relationship of aquaglyceroporins in HUVEC and their involvement in glycerol transport in CVD conditions are still under investigation, our data suggest the AQP3 has a physiological role in glycerol permeation in healthy conditions while AQP7 may be involved in the development of disease.

Keywords: Aquaporin, glycerol, permeability, cardiovascular disease

P33: PKA VALUES OF TITRABLE AMINO ACIDS AT THE WATER/MEMBRANE INTERFACE

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The study of biological membranes has been for quite some time a challenge for researchers. A detailed description of the water/membrane interface has to take in consideration all important factors and pH is recognizably one of them, even though it is usually ignored due to its high complexity in terms of modelling. The pKa values of typical titrable amino acids can be significantly influenced by changes in the environment, i.e., peptide insertion into a lipid bilayer (1-2) The main objective of this work is a comprehensive study of how a membrane environment can shift the pKa values of common pH-sensitive amino acids (Asp, Glu, His, Lys, Cys, Tyr, and the N- and C-termini). For this, we used our recently developed CpHMD-L methodology (3) with a DMPC membrane and the model Ala-based pentapeptides that have already been well characterized in water by Pace and co-workers (4). With this approach, we intend to capture the coupling between conformation/configuration/insertion and protonation at the membrane interface, taking into consideration that if complete insertion occurs, the peptides will probably no longer be able to exchange protons with the solvent.

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Keywords: Peptides, pKa, pH, membrane, insertion

P34: PROOF OF PORE FORMATION THROUGH A 2D AMOXICILLIN-MEMBRANE INTERACTION STUDY

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Amoxicillin is a commonly used antibiotic, being classified as a first-line drug against different bacterial infections, such as *Helicobacter pylori*. However, it has also been associated with gastrointestinal and renal side effects, with higher toxicity when the pH is lower [1,2]. Given this association and the well-known pH gradient of the gastric mucosa, where *Helicobacter pylori* is located in vivo, this work aimed to evaluate the influence of pH on amoxicillin-membranes interactions.

Monolayers of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) were used, due to their highly frequent existence in biological membranes. Three different pH (pH 1.2, pH 5 and pH 7.4) were evaluated and several techniques were employed, namely isotherm measurements, infrared reflection-absorption spectroscopy, Brewster angle microscopy and grazing incident X-ray diffraction.

Amoxicillin's effect on the biophysical properties of DPPC monolayers was dependent on the pH. At physiological pH, the higher effect was visualized at lower pressures. However, at acidic pH, a higher perturbation was shown where pores were visualized by Brewster angle microscopy. These perturbations may ultimately be related with amoxicillin toxicity.

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Keywords: Amoxicillin, interfaces, monolayers, pores

POSTERS

P35: EQUILIBRUM AND DYNAMIC INTERFACIAL PROPERTIES OF PULMONARY SURFACTANT STUDIED BY NEW MICROPIPETTE TECHNIQUES

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Mechanical stabilization of the lungs is achieved by a surface-active material, the so-called lung surfactant, which minimizes the energy expenditure for breathing and avoids alveolar collapse. The present study used a new platform for lung surfactant research, the micropipette technique, for a microscopic characterization of a series of lung surfactants: the clinical formulations Curosurf, Infasurf and Survanta; native surfactant (NS) from porcine lungs; and a new synthetic formulation containing the SP-B analog Super Mini-B (SMB) peptide. The motivation is the understanding of the mechanisms behind the good surface activity in vitro and in vivo that these surfactants have shown.

Here, we have addressed the characterization of lung surfactant adsorption to microscopic air-water interfaces (~100 um diameter, comparable to the alveolar scale) under physiological conditions of temperature, pH and salinity, including: (1) measurement of equilibrium surface tensions, obtaining values of 21-24 mN/m for all formulations; (2) dynamic adsorption using two different methods, a single-pipette and a dual-pipette technique for microinjection, measuring fast rates (~1-4 mN/m/s); and (3) visualization of microscopic membrane structures beneath the surface. The presence of SMB promoted vesicle condensation as thick membrane multilayers, the nucleation and growth of microtubes emanating from these lamellae (2-15 um diameter, growth rates of 2-3 um/s and 20-30 um3/s), and in some cases their subsequent transformation into helices. A direct correlation was found between the number of tubes and SMB concentration, suggesting that SMB molecules are the promoters of tube nucleation. Microtube formation was also observed in Infasurf, and in NS only after subsequent expansion and compression, but neither in the other clinical surfactants nor in protein-free preparations.

The versatility of the micropipette technique to combine accurate surface tension measurements with microscopic structural information is expected to be important for future lung surfactant research.

Keywords: Micropipette technique; lung surfactant; membrane proteins; synthetic peptides; lipid microtubes; myelin figures

POSTERS

P36: AMYLOID-FORMATION IN FOOD ALLERGENS ENDORSE EPITOPE MULTIVALENCY FOR OPTIMAL IGE BINDING

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Amyloids are highly cross-β-sheet-rich aggregated states that confer protease resistance, membrane activity and multivalence properties to proteins, all essential features for food proteins causing IgE-mediated hypersensitivity. In fish allergies the small calcium-binding protein β-parvalbumin was identified as the major allergen. Stabilization of its apo form caused the formation of amyloids and the enhancement of IgE-reactivity, suggesting a role in epitope architecture. Peptide arrays, sera from 4 fish-allergic patients and anti-amyloid antibodies show that the relevant antigenic regions of Gad m1 are flanked by segments with cross β-sheet forming capacity. Impairment of adhesive segments by mutagenesis yield chains with preserved epitope sequence and native conformation but prevented IgE-binding. These data support that in food allergens amyloid formation drives the epitope multivalence required for best IgE-binding.

Keywords: Amyloids, food allergens, β-parvalbumin, IgE binding.

P37: IN BACTERIAL FAD SYNTHETASES LITTLE STRUCTURAL VARIATIONS PRODUCE BIG CATALYTIC DIFFERENCES

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Bacterial FAD synthetases (FADS) are bifunctional proteins that perform the flavin adenine dinucleotide (FAD) synthesis from riboflavin (RF) in two reactions (Frago, 2008). First, a riboflavin kinase activity (RFK) phosphorylates RF to FMN, and then a FMN adenylyl transferase activity (FMNAT) transforms FMN into FAD. Bacterial FADSs share function and show homologous structures organized in two domains: the C-terminus with RFK activity and the N-terminus with the transferase one. Despite that, FADSs from different organism present important differences. For instance, FADS from the non-pathogenic bacteria C. ammoniagenes does not require reducing conditions to perform its activities and the RFK one is inhibited by RF. On the contrary, the FMNAT activity of FADS from the human pathogen S. pneumoniae needs a reducing environment while the RFK one does not show inhibition.

FMN and FAD are involved in processes of crucial relevance for life, playing a key role in the energetic metabolism, cellular homeostasis and apoptosis, and their deficiency directly leads to death. For this reason—and due to the differences between bacterial FADSs and the human homologous—these proteins appear as potential drug targets, so their deep characterization determines a main issue of central relevance (Serrano et al., 2013).

In this work we biophysically characterize the interaction of the FADS from Streptococcus pneumoniae with its ligands, which allows proposing a model for its catalytic cycle. Additionally, we compare the results with those obtained for Corynebacterium ammoniagenes, which is the best known member of the family (Frago et al., 2009) .

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Keywords: FAD synthetase

P38: IMMUNOTARGETING OF E.COLI TOLC OUTER MEMBRANE CHANNEL

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TolC is an outer-membrane channel of Gram-negative bacteria, responsible for extrusion of antibiotics and other toxic compounds from the cell and plays a determinant role in bacterial resistance.

TolC is coupled with two other proteins, an inner membrane efflux pump and a periplasmic fusion protein, assembling a functional complex. TolC works as a non-specific extrusion duct but can be associated with various efflux pumps with different compound specificity, thus appearing as a relevant target for modulation of bacterial resistance.

TolC is a 428-residue protein, natively occurring as a homotrimer and presents two distinct structural regions: a β-barrel outer membrane region and a helical periplasmic region. At the extracellular region the channel presents a loop involved in channel gating. In this work we generated antibodies intended to bind the extracellular loop region of TolC and block or modulate the extrusion activity across the channel.

Herein we present the expression and purification of 1) E.coli TolC and 2) a recombinant peptide corresponding to the extracellular region of the channel and their biochemical characterization by SDS-PAGE, size exclusion chromatography, western blot (WB) and differential scanning calorimetry (DSC).

Both proteins were used for immunization of avian hosts and subsequent generation of anti-TolC specific antibodies. These were further characterized in terms of titer, specificity and cross-reactivity using ELISA and WB assays. The antibodies produced by both antigens revealed great robustness and specificity against TolC and in particular those produced against the extracellular region of the channel presented high specificity against the full-length protein. This work demonstrates that the E.coli TolC channel can be targeted by avian antibodies and the data supports future work on the functional characterization to validate antimicrobial potential of such antibodies. Ultimately this will allow the development of therapeutic monoclonal anti-TolC antibodies to be used to fight bacterial resistance.

Keywords: Membrane protein, Multidrug resistance, Modulation, Antibody

P39: UBIQUITIN-MEDIATED ENDOCYTOSIS OF THE VOLTAGE-GATED POTASSIUM CHANNEL KV1.3

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The voltage-dependent potassium channel Kv1.3 is expressed mostly in the nervous and immune systems, where participates in the sensory discrimination and leukocyte physiological responses. An altered function as well as an exacerbated expression or surface mistargeting are related with autoimmunitary diseases. Regulation of this transmembrane protein is therefore essential. The turnover of Kv1.3 is highly dynamic and, upon insults, the balance between the number of channels located at the membrane and the internalization is crucial for an appropriate signaling. Therefore, endocytosis is an essential mechanism for the regulation of Kv1.3 abundance on the cell surface. Ubiquitinanion has emerged as a crucial mechanism for membrane protein turnover. In this study we investigated ubiquitination-mediated endocytosis and the lysosomal sorting of Kv1.3. To that end several Kv1.3 lysine mutants were created, in which we examined ubiquitination, endocytosis and membrane targeting. We observed ubiquitination and ubiquitin-mediated endocytosis by inducing PKC and PKA activation. Our results indicate that more than one lysine are involved in the ubiquitination of the channel. Furthermore, we have mapped the most relevant for the channel internalization and degradation. Our results indicate that Kv1.3 undergoes specific ubiquitination in residues which participate in the endocytosis and the turnover of the channel.

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Keywords: Kv1.3, ion channels, ubiquitination, endocytosis

P40: ANALYSIS OF NANOSCALE COMPARTMENTALIZATION OF PI(4,5)P2 IN LIVING CELLS AND MODEL MEMBRANES THROUGH FLUORESCENCE SPECTROSCOPY AND FRET IMAGING

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Phosphatidylinositol-4,5-bisphosphate (PI(4,5)P2) is the most abundant phosphorylated phosphoinositide (PIP) at the cytosolic face of mammalian plasma membranes and has been associated with a great number of vital cellular functions. The variation of its local concentration, in time and space, has been claimed to be responsible for the spatiotemporal recruitment of proteins with diverse functions, such as endocytosis and cytoskeleton adhesion to the membrane. Among other factors, the presence of cholesterol-enriched domains, elevated concentrations of divalent cations and the cytoskeleton itself have been suggested to be involved in determining PI(4,5)P2 organization and clustering. Using a combination of fluorescence spectroscopy and microscopy techniques, we show that both the number and position of phosphorylations in the inositol ring of phosphoinositides are crucial for defining the extent of PIP clustering and relative cluster size. Additionally, we show that formation of the liquid ordered phase strongly promotes formation of PIP clusters in model membranes. Evidence for the formation of PI(4,5)P2 enriched nanodomains in the plasma membrane of living cells was obtained through FRET microscopy of pleckstrin homology (PH) domains tagged with fluorescent proteins. FRET imaging data was compared with the theoretical expectation for FRET in the case of a homogeneous distribution of PH domains, and results confirm that distinct PI(4,5)P2 local densities are found in different cellular models, suggesting that PI(4,5)P2 organization varies significantly between eukaryotic cells. In HeLa cells, disruption of the cytoskeleton decreased significantly the compartmentalization of PI(4,5)P2, proving that the organization of a pool of PI(4,5)P2 molecules depends on the presence of membrane-cytoskeleton interactions. On the other hand, formation of PI(4,5)P2 plasma membrane nanodomains is shown to be independent of cholesterol levels, suggesting that membrane rafts are not involved.

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Keywords: Phosphatidylinositol-4,5-bisphosphate; Fluorescence spectroscopy, Förster Resonance Energy Transfer; FRET Homotransfer

POSTERS

P41: CONFORMATIONAL TRANSITIONS AND MEMBRANE BINDING PROPERTIES OF HUMAN AND SALMON CALCITONINS: A TIME-RESOLVED FLUORESCENCE STUDY

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The conversion of peptides and proteins from their native soluble states into extracellular beta-sheet-rich fibrillar assemblies, generally termed as amyloid fibrils, is the distinctive feature of a number of human diseases. The HiLyte Fluor 488-labeled variants of both human and salmon calcitonin (hCT-HL488 and sCT-HL488, respectively) were used here to study the role that accessory cellular components like biological membranes can play in modulating amyloid pathologies. The fluorescence properties of this recently commercialized dye (free HL488) and both HL488-labelled CT variants were first extensively characterized in trifluoroethanol (TFE)/buffer binary mixtures. Both the fluorescence intensity and anisotropy decays of the N-terminal fluorescently-labelled peptides were sensitive reporters of the conformational transitions undergone by both peptides upon increasing the TFE content of the binary solvent mixtures. In agreement with the data obtained from parallel CD studies, a two-step alfa-helical folding was detected for hCT over the whole TFE/buffer range, whereas sCT displayed a single conformational transition, with the alfa-helical structure promoted almost completely in a single step at 0-30% TFE. The interaction of both HL488-labelled calcitonin variants with POPC liposomes prepared with variable anionic phospholipid (POPS) content was also investigated by performing a multi-parametric fluorescence intensity/anisotropy study. The partition coefficient of monomeric sCT-HL488, contrary to hCT-HL488, varied exponentially with the POPS content of the anionic lipid vesicles confirming the importance of electrostatic interactions in CT binding to the lipid vesicles. When higher concentrations of sCT-HL488 were used, excitonic interactions between the chromophores were detected in their absorption spectra revealing the formation of parallel aggregates (H-type) at high peptide membrane coverage. Accordingly, a coupled partition/oligomerization model was needed to describe the biphasic fluorescence intensity data obtained for sCT-HL488 in interaction with anionic liposomes.

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Keywords: Amyloidogenic peptides; Trifluoroethanol; Anionic lipid membranes; Fluorescence spectroscopy.

POSTERS

P42: STUDY OF INTERACTION OF THE RIFAMPICIN WITH LIPID BILAYERS

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The pathway of a drug from oral administration towards its target involves interaction and/or permeation through distinct biological membranes. Also, the efficacy once at the target site involves frequently interactions with biological membranes; either directly in the case of drugs active in the membrane, or indirectly through an increase or decrease in the local concentration available to interact with the therapeutic target.

Biological membranes are formed by a continuous lipid bilayer, with proteins embedded in it, or adsorbed at its polar surface; with the lipid bilayer being the diffusion barrier that limits the permeability of drugs. It is therefore important to characterize the interactions established between the drug and model systems that mimic the lipid bilayer portion of the biological membrane, as well as the effect of the drug in the properties of the bilayer.

Large Unilamelar Vesicles (LUVs) are excellent models systems, as they are stable and can be prepared with distinct lipid compositions to mimic the properties of the relevant biological membrane. In this work we have characterized the interaction of Rifampicin with LUVs prepared from pure POPC (mimicking membranes in general) and from a mixture of POPE/POPC/POPG at 50:30:20 molar % (mimicking bacterial membranes). Rifampicin inhibits RNA synthesis and is an antibiotic widely used in bacterial infections such as tuberculosis.

The interaction of Rifampicin with the lipid bilayers was studied by isothermal titration calorimetry; with the characterization of the partition coefficient and associated enthalpy variation at distinct local concentrations of the drug, changes in the ionization state of Rifampicin when interacting with the lipid bilayer, and qualitative information regarding the rate of permeation. Variations in the properties of the lipid bilayers due to the presence of distinct local concentrations of Rifampicin were evaluated by Dynamic Light Scatter and through the effect on the rate of carboxyfluorescein permeation.

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P43: PHASE COEXISTENCE IN DMPC/CHOL BILAYERS AND LATERAL PARTITIONING OF DPH

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Cholesterol (Chol) interacts with saturated lipids such as 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) [1,2], as Chol aligns with the methylenic chains, modulating their conformational dynamics [2]. Lipid bilayers composed of DMPC/Chol mixtures have been used to elucidate the coexistence between liquid-ordered/liquid-disordered (lo/ld) phases [3,4,5]. This study grounds on 1,6-diphenyl-1,3,5-hexatriene (DPH) fluorescence in multi-lamellar vesicles (MLV) composed of DMPC/Chol, using steady-state anisotropy (r), normalized for the absorption at the excitation wavelength and for the fluorescence intensity and quantum yield at the emission wavelength. The partition constants (Kp) consider that DPH is in equilibrium between lo/ld phases [6].

For a probe to lipid ratio of 1:1000, the results suggest well-defined partitioning between lo/ld phases at 35 and 37 °C, for 10 mol % Chol, and at 45 °C for 5, 10 and 15 mol % Chol, indicating that DPH partitions preferentially to the lo phase. The results are not unequivocal concerning the Chol proportion for which there is lo/ld coexistence. According to the literature, at 45 °C and for 5, 10 and 15 mol % Chol, the bilayers only display the liquid-crystalline (Lα) phase, and the lo/ld coexistence occurs between about 10 and 30 mol % Chol above the phase transition temperature (Tm) of DMPC. Ongoing experiments improving the experimental resolution will offer a suitable quantification of the DPH lateral partitioning and lo/ld coexistence in model membranes.

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Keywords: Cholesterol; DMPC; DPH; Partition; Phase coexistence

POSTERS

P44: CELLECTAB – ENGINEERING THE NEXT GENERATION OF ANTIBODY DISCOVERY

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Antibodies are nowadays the fastest growing therapeutics in the pharmaceutical market and represent already one third of the pipeline of the largest pharmaceutical companies. Despite this, there are still many problems concerning the discovery and selection of antibodies. The current technologies are time consuming, require multiple steps and have a low success rate. Furthermore commonly used technologies include animal use, the antibodies are selected in non native environments and need optimization after selection.

Here we present a new technology that integrates all the discovery steps of antibodies in a single cell platform to optimize, reduce cost and increase productivity of new biotherapeutics. CellectAb uses an engineered human T-cell line to create intracellular diversity of any antibody format, mimicking B-cell diversification mechanisms, and allows the interaction of antigen-antibody to occur in the native format. This technology is being designed for selection of either binders or neutralizing antibodies against membrane or soluble proteins, including difficult target such as GPCRs or ion channels.

We are now performing the proof-of-concept for this platform, creating variability in antibodies using our knowledge on DNA specific recognition sites, and selecting the antibodies against a membrane protein. The final goal is to bring all these assumptions together in a unique single-cell system.

This novel technology can drastically improve the biophysical and functional properties of new immunotherapies increasing effectiveness and reducing the time to enter in clinical trials, and consequently the time-to-market.

Keywords: Antibody, membrane target, immunotherapy

POSTERS

P45: PYRENE PROBES IN QUANTIFYING DIELECTRIC CONSTANTS AND EXCIMER FORMATION IN UNSATURATED LIPID BILAYER: THE IMPORTANCE OF THEIR MOLECULAR PROPERTIES

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Pyrene probes are advantageous to study biophysical phenomena in lipid bilayer due to high quantum yields, long lifetimes, and precise solvatochromic effects [1]. Although often used, their molecular and fluorescence properties are frequently overlooked. The physical-chemical characteristics of probes (apolar and bulky group) and their locations allow obtaining correct information about model membranes. Pyrene displays distinct localizations within lipid bilayers as free molecule [2] or linked to phospholipids [3]. The free molecule location defines the values of dielectric constants of bilayers, averaged transversally in space and laterally in time. The hydrophobic interactions of the rigid pyrenyl with acyl chains increases the methylenic chain ordering leading to vacant analysis of excimer formation at moderate probe proportions. The bilayer polarity of pure POPC or DOPC and their binary mixtures with cholesterol were monitored using the pyrene Ham Effect. Pure DOPC exhibits higher dielectric constants than POPC, due to larger free volume enabling higher hydration within lipid bilayers. The mixtures display similar variation of bilayer polarity, pointing to related features in their thermal phase diagrams.

The excimer formation using pyrenyl-labeled phosphatidylcholines (py10-PC, py6-PC) in fluid POPC was quantified by steady-state and lifetime fluorescence. Very good agreement with the theoretical predictions of a kinetic formalism for self-quenching processes occurring in two-dimensional (2D) media [4] is observed. However, significant divergences occur in 2D Förster-Kasper plots above 2 mol % for py10-PC and 4 mol % for py6-PC content, revealing deviations to the presumed constancy of the probes' lateral diffusion dynamics.

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Keywords: Pyrene, excimer formation, lipid bilayers, 2D kinetics, fluorescence, quenching

POSTERS

P46: PULMONARY SURFACTANT: A HETEROGENEOUS MIXTURE OF MEMBRANES WITH DIFFERENT STRUCTURAL AND FUNCTIONAL PROPERTIES.

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Pulmonary surfactant is a lipo-protein complex localized in the lung and essential to maintain respiratory dynamics. Surfactant used as reference or in clinical applications has usually been isolated from bronchoalveolar lavages of porcine lungs. However, as this material has been subjected to respiratory mechanics and oxidation processes in the lung, it could not maintain the properties of newly synthesized lung surfactant, as it is stored and secreted as lamellar bodies by the specialized pulmonary cells involved in this process, the type II pneumocytes. Therefore, it is essential to understand the structural and functional relationships between freshly secreted surfactant complexes and the final surface active films maintaining a functional air-liquid pulmonary interface. The study of the different structures involved could aid to understand the molecular mechanisms that sustain surfactant system from its synthesis and packaging to its recycling once it has been used. Moreover, it would allow the improvement or development of new therapeutic surfactant preparations for the treatment of respiratory diseases with better functional properties.

In this work, we have analysed the structural and biophysical properties of different fractions obtained by density gradient ultracentrifugation of pulmonary surfactants purified from bronchoalveolar lavages of animal lungs. The results obtained indicate that surfactant from lavage is actually composed by a heterogeneous mixture of membranes that differ in composition, structure and biophysical activity. Likewise, this work suggests that a relationship could exist between these fractions and the different stages of synthesis, packaging, adsorption to the interface and recycling of pulmonary surfactant. Nevertheless, no one of these fractions have the properties of newly synthesized and secreted pulmonary surfactant as exhibited by lamellar bodies. This suggest that, once pulmonary surfactant spreads onto the air-liquid interface and is subjected to compression-expansion cycles, it probably loses its initial properties and it is somehow at least partially spent.

Keywords: Pulmonary surfactant biogenesis, Laurdan, electron microscopy, captive bubble surfactometer.

POSTERS

P47: STUDYING THE INTERACTION OF THE INACTIVE FORM OF TOPOTECAN WITH CELL MEMBRANES USING 3D AND 2D BIOMIMETIC MODELS

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Topotecan is an anticancer drug whose activity is driven by the inhibition of Topoisomerase I, hindering therefore DNA replication. Topotecan stability is largely dependent on the pH in aqueous solutions. At pH below 4 the lactone ring, essential for the Topotecan pharmacologic activity, is intact. However, as the pH increases, a reversible opening of the lactone ring occurs resulting in the formation of a non-active carboxilate derivative. Furthermore, the aqueous solubility of Topotecan decreases with the increase of the pH. At physiologic pH and temperature the hydrolisation of Topotecan has a half-life around 20 min. Therefore, one can expect that most of the administered drug will end interacting with the cells in its inactive form.
In this study, biomimetic 3D and 2D membrane models, liposomes and phospholipid monolayers respectively, were used to evaluate the interaction of the carboxilated Topotecan form with the membranes of healthy and cancer cells. Phospolipid composition and pH chosen to mimic the healthy and cancer cells were, respectively, dimyristoilphosphatidilcholine (DMPC) at pH7.4 and dimyristoilphosphatidilserin (DMPS) and DMPC at pH 6.5. Partition coefficients were calculated in the 3D models using derivative spectroscopy and isothermal titration calorimetry. Surface pressure-molecular area isotherms coupled either with Brewster angle microscopy or with infrared reflectance-absorbance spectroscopy were used to assess the influence of the drug on the monolayers.

The obtained results show that composition of the membranes and pH of the medium influenced the interaction of the carboxilated form of Topotecan with these biological membranes.

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Keywords: Topotecan, liposomes, phospholipid monolayers, drug-membrane interactions

POSTERS

P48: CHARACTERIZATION OF THE CALCIUM BINDING MODE OF RABPHILIN 3A

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C2 modules are most commonly found in enzymes involved in lipid modifications and signal transduction and in proteins involved in membrane trafficking. They consist of 130 residues and share a common fold composed of two four-stranded β-sheets arranged in a compact β-sandwich connected by surface loops and helices. Many of these C2 domains have been demonstrated to function in a Ca2+-dependent membrane-binding manner and hence act as cellular Ca2+ sensors. Calcium ions bind in a cupshaped invagination formed by three loops at one tip of the β-sandwich where the coordination spheres for the Ca2+ ions are incomplete. This incomplete coordination sphere can be occupied by neutral and anionic phospholipids, enabling the C2 domain to dock at the membrane.

In this work we have characterized the Ca2+-binding mode of the C2AB domain of Rabphilin 3A. We have measured the Ca2+-binding affinities of the C2AB, C2A and C2B domains of Rabphilin 3A by Isothermal Titration Calorimetry. The C2A domain exhibits a very low affinity for Ca2+ the calorimetric data suggest a three sequential binding mode with dissociation constants of 117, 524 and 710 uM. The C2B domain fitted to a one set of sites model with a dissociation constant of 2 uM, indicating that the C2B domain has a higher affinity for Ca2+ than the C2A domain. Strikingly, when the calorimetric study was performed with the C2AB domain, the results fitted into a two set of sites, being the dissociation constants 1.4 uM and 3.8 mM for the first and second components, respectively. Site-directed mutagenesis of the residues involved in calcium binding has confirmed that the C2B domain confers Rabphilin 3A with a quick response to Ca2+ and the C2A with a slow response due to its low affinity.

These results indicate that Rabphilin 3A might play different roles at several states of the vesicle fusion cycle, depending on the Ca2+ and target lipids available at each moment at the plasma membrane and transport vesicles.

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Keywords: Rabphilin 3A; C2 domain; Ca2+

POSTERS

P49: ANCHORING OF A PLANT PROTEIN, REMORIN 1.3 TO LIPID DOMAINS: ROLE OF PHOSPHOINOSITIDES

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The function of Remorins, a diverse family of plant-specific proteins (1) is far to be fully elucidated. One of them, StREM1.3 (for Solanum tuberosum Remorin from group 1, homolog 3) has been reported to regulate cell-to-cell propagation of the potato virus X (2). It was also shown to be localized to the inner leaflet of plasma membranes (PMs) in raft domains and along plasmodesmata, bridges connecting neighbor cells essential for cell-to-cell communication in plants (3). The mechanisms driving StREM1.3 association with PM is still an open question. We have recently shown that a domain of 28 residues at the C-terminus of the potato (RemCA) is required and sufficient for anchoring to the PM (4).

Here we combined experimental and in silico biophysics to unravel the molecular bases of RemCA membrane binding with a special emphasis on lipid specificity. Biomimetic membrane models of plant PM such as monolayers and liposomes were used with various biophysical techniques (Langmuir monolayer technique, Fourier-transformed infrared spectroscopy, circular dichroïsm) and modeling tools (home-made methods and molecular dynamics) (5) to answer to three questions: (i) What is the conformation adopted by RemCA within a membrane?, (ii) Is there any membrane lipid specificity in the RemCA-membrane binding? (iii) What is the role of the two different RemCA domains in the interaction?

Results show that RemCA displays a preference for plant phosphoinositide-enriched inner leaflet plasma membrane rafts. Within the membrane, the C-terminal and the N-terminal domains of RemCA adopt an extended and a helical conformation respectively. The C-terminal domain acts as a driver to bind RemCA to the membrane while the N-terminal domain stabilizes the peptide at the membrane. Lysine residues have a crucial importance in this interaction.

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Keywords: Lipid specificity, biomimetic plant lipid, molecular modelling

POSTERS

P50: GLUCOSYLCERAMIDE-INDUCED BIOPHYSICAL CHANGES IN ARTIFICIAL AND CELL MEMBRANES

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Glucosylceramide (GlcCer) is an active player in the regulation of different cellular events. Moreover, GlcCer is also a key modulator of membrane biophysical properties, which might be linked to the mechanism of its biological action. With the aim to analyze the impact of GlcCer in membranes that are compositionally closer to a cell membrane, we studied the interplay between GlcCer and complex artificial membranes containing 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), Sphingomyelin (SM) and Cholesterol (Chol). Using an array of biophysical methodologies we showed that at lower GlcCer-to-Chol ratios, GlcCer stabilizes the SM/Chol-enriched liquid-ordered domains. However, upon decreasing the Chol content of the membrane, GlcCer significantly increased membrane order through the formation of gel domains. Changes in pH acidification disturbed the packing properties of GlcCer- containing membranes, leading to an increase in membrane fluidity and reduced membrane electronegativity, which consequently increase vesicle aggregation. To address the biophysical impact of GlcCer in biological membranes, studies were performed in wild-type fibroblasts and fibroblasts with GBA mutation for type I Gaucher Disease. The results showed that decreased membrane fluidity occurred in cells containing higher levels of GlcCer, such as the cells from patients with Gaucher Disease. This suggests that pathological elevated levels of GlcCer change membrane biophysical properties and might compromise membrane-associated cellular events.

Keywords: Lipid domains, Membrane organization, Membrane fluidity, Gaucher Disease

POSTERS

P51: COMBINING ANTIMALARIAL DRUGS WITH THE CELL-PENETRATING PEPTIDE TP10: BETTING ON INTRACELLULAR DELIVERY

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Malaria remains a worldwide threat, and despite all efforts against this infection, it killed almost half a million people and infected over 200 million in 2015 [1]. Although malaria cases and deaths have declined over the years, antimalarial drug (AM) resistance poses a huge burden for controlling this infection and the development of new drugs is a pressing matter. A cost-effective reduced-risk strategy for developing new drugs is recycling classical drugs, a suitable approach for antimalarial chemotherapy, as this is mostly addressed to low-income countries. Targeted delivery of known AM may be a promising strategy, as it may increase drug's concentration at site of action - reducing side effects associated to high doses. More importantly, drug masking with a suitable carrier may elude parasite resistance.

Cell-penetrating peptides (CPP) are becoming prominent shuttles for intracellular drug delivery as a consequence of their low toxicity, ability to be uptaken by diverse cell types, and compatibility with different cargo sizes or types [2]. CPP uptake into Plasmodium-infected erythrocytes (PiRBC) is usually higher than healthy erythrocytes (hRBC), as RBC undergo significant changes upon infection, including acquired adhesion properties, improving their permeability towards cationic amphipathic CPP. Moreover, amphipathic peptides specifically targeting PiRBC have been identified. Carefully chosen peptides may act as selective shuttles for intracellular delivery of antimalarials into PiRBC [3]. The approach herein proposed, unprecedented in the literature, will address this hypothesis, toward a paradigm shift in antimalarial chemotherapy.

In this work, classical AM – chloroquine and quinacrine, were conjugated through different strategies to TP10, a CPP active against blood-stage Plasmodium falciparum, for subsequent assessment of conjugates' (i) in vitro antimalarial activity, (ii) interactions with model membranes, and (iii) cell-penetration ability. Results thus far obtained will be presented.

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Keywords: Cell-Penetrating Peptides; Antimalarial Drugs

POSTERS

P52: STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF THE CATIONIC ANTIMICROBIAL PEPTIDE ECAMP1R2

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The thriving of conventional antibiotic-resistant bacteria is a worldwide problem and one of the most concerning threats that health services have to confront nowadays. Antimicrobial peptides (AMPs) are among the most promising alternatives to conventional antibiotics. The main target of these molecules is the negatively charged membranes of bacteria, which, after interaction are eventually killed through diverse modes of action.
A recently discovered AMP, EcAMP1R2, has showed antimicrobial activity against Escherichia coli at 11.7 μM. In order to shed some light on the mechanisms of action at the molecular level of this peptide, studies using biomembrane models that mimic bacterial composition, and E. coli cells were assessed. Large unilamellar vesicles (LUVs) with different lipid compositions were used for this purpose, namely POPC/cholesterol (characteristic of the outer leaflet of mammalian cell membranes) and POPC/POPG (Gram negative bacteria) mixtures. Changes on membrane packing, fluidity and membrane potential were followed by the extrinsically fluorescence of molecular probes: Laurdan, TMA-DPH, DPH and di-8-ANEPPS, upon membrane binding/insertion. Both structural (based in in silico predictions and circular dichroism studies) and functional analysis (based in fluorescence and light scattering spectroscopy techniques) have been performed. EcAMP1R2 displayed higher partition constant values (Kp) when interacting with negatively charged membranes (POPC:POPG). Interestingly, the studies using the fluorescent probe di-8-ANEPPS have revealed that the peptide produces a stronger depolarization in E. coli compared to the lipid mixtures analyzed. The results obtained suggest that the initial activity exerted by EcAMP1R2 is highly dependent upon electrostatic interactions. Based on this, we suggest that the bacterial growth inhibition effect might be exerted by the disruption of the bacterial cell electric homeostasis.

Keywords: EcAMP1R2, Antimicrobial peptides, Fluorescence spectroscopy

POSTERS

P53: TERNARY COMPLEXES OF FLUOROQUINOLONES AS NEW METALLOANTIBIOTICS: A BIOPHYSICAL AND BIOLOGICAL APPROACH

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Fluoroquinolones are antibiotics that have a large spectrum of action against Gram negative and some Gram positive bacteria. Nevertheless, the misuse and overuse of antibiotics has triggered the development of bacterial resistance mechanisms against these drug (1). One of the strategies to circumvent this problem is the complexation of fluoroquinolones with divalent metal ions and phenanthroline, since it forms stable complexes (known as metalloantibiotics) with different activity and enhanced pharmacological behaviour (1), possibly due to their alternative translocation pathway through the bacterial membranes. Partition studies previously performed with these metalloantiotics suggest that their influx route can be strongly dependent on lipid interaction. To try to clarify the translocation pathways, the location of the drugs in the bacterial membrane was assessed through the study of their interaction with probes with known location (2-3). In this work we evaluated the interaction of four pure fluoroquinolones and their metalloantibiotics with two probes of the series n-(9-anthroyloxy) stearic acids (n-AS) - 2 AS and 12-AS probes - incorporated in E. coli total lipid extract liposomes, by steady-state and time resolved fluorescence spectroscopy. Additionally, we determined the changes in the thermotropic properties of the E. coli total lipid extract liposomes in the presence of the fluoroquinolones and their metalloantibiotics by steady-state fluorescence anisotropy, using DPH and TMA-DPH probes (4). All studies were performed under physiological conditions of pH and temperature. Furthermore, biological studies, cell viability and cell proliferation assays were also performed to assess the safety of these new compounds. The biophysical and biological results obtained show a much more hydrophobic location of the metalloantibiotics than of the pure fluoroquinolones and suggest that metalloantibiotics have a promising ability to bypass, at least, one of the mechanisms of the bacterial resistance to fluoroquinolones (based on alterations on the permeability of the bacterial membrane).

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Keywords: Fluoroquinolones; Metalloantibiotics; Gram negative bacteria; Bacterial resistance; Fluorescence spectroscopy; in vitro cytotoxicity.

P54: STUDY OF THE MOLECULAR INTERACTIONS OF ANTIMICROBIAL PEPTIDES WITH BACTERIA

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In the last decades, the increase number of multi-resistant pathogens has become a major health problem worldwide. The indiscriminate conventional antibiotic dosage that has been used in therapeutics was one of the factors that accelerated this resistance. It is then urgent to find new therapeutics that could be applied to fight these pathogens. Antimicrobial peptides (AMPs) have risen as one the most promising alternatives. AMPs are, usually, short amphipathic cationic peptides that act at the membrane level of the pathogens, with a broad-spectrum of targets (bacteria, fungi, cancer cells). Additionally, some AMPs act at the immune system, with modulation actions, but more details are needed in order to understand how these peptides act against the target cells.

The aim of this work was to understand how the AMPs Pa-MAP2 and 1.9 act against the target cells. The modes of action of these two AMPs, specific for Gram-negative bacteria, were studied using liposomes, as model membrane systems, and two strains of Escherichia coli (ATCC 25292 and KPC, a clinical multi-resistant isolate). Firstly, the interaction with negatively charged membranes and bacteria was demonstrated by surface plasmon resonance and live/dead studies using flow cytometry. By fluorescence spectroscopy and flow cytometry, it was possible to observe that both peptides interfere with membrane surface and dipole potentials, without changing the fluidity or the lipid packing of the membrane. Also, with dynamic light scattering and zeta-potential studies, it became clear that the peptides tested have different modes of action at the membrane level, corroborated by atomic force microscopy imaging of E. coli after different times of incubation. With these studies, it was possible to conclude that even very similar AMPs, both leading to bacteria cell death, may achieve this through different mechanisms, both being promising molecules to be used in healthcare.

Keywords: Antimicrobial peptides; Pa-MAP2; Pa-MAP 1.9; Atomic force microscopy

P55: BIOPHYSICAL ANALYSIS OF LIPID-CONJUGATED PEPTIDE FUSION INHIBITORS THAT TARGET PARAMYXOVIRUSES

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Respiratory paramyxoviruses are a major source of severe respiratory infections. Acute respiratory infections are the leading cause of mortality in children under 5 years of age, accounting for nearly one fifth of childhood deaths worldwide and no effective treatment is available. A membrane-targeted fusion inhibitory peptide derived from the C-terminus heptad repeat (HRC) region of paramyxovirus fusion proteins (F), with broad-spectrum activity has been previously generated and assessed in vivo. Inclusion of a lipid group has been suggested to improve membrane binding and thus elevating the local concentration of the peptide at the target site. We have investigated four lipid-conjugated variants of this broad-spectrum peptide to search for properties that may correlate with efficacy and broad-spectrum activity. We analyzed several paramyxovirus F proteins and a set of multiple alignments was carried out. Using several bioinformatics tools, we examined several physiochemical parameters to predict the secondary structure of the peptide. Fluorescence spectroscopy was used to study the interaction of the fusion inhibitor with biomembrane model systems. Additionally, the binding to the membrane of human blood cells, namely PBMC and erythrocytes, was evaluated using the dipole potential probe di-8-ANEPPS. The characterization of the mechanism of action at the molecular level of peptides and their lipid-conjugates as viral fusion (entry) inhibitors is expected to contribute to the design of effective fusion inhibitors for paramyxoviruses.

Keywords: Paramyxovirus, broad-spectrum antivirals, fluorescence

POSTERS

P56: CHOLESTEROL-CONJUGATED PEPTIDE INHIBITORS OF INFLUENZA A VIRUS: BIOPHYSICAL CHARACTERIZATION

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Influenza viruses are major human pathogens responsible for respiratory diseases affecting millions of people worldwide and characterized by high morbidity and mortality. Infections by influenza can be controlled by vaccines and antiviral drugs. However, this virus is constantly under mutations, leading to growing resistance to influenza antivirals currently in use. It is urgent to develop new strategies for therapeutics. The influenza hemagglutinin (HA) is a potential target for antiviral drugs, because it is a key protein in the initial stages of infection. This protein is involved in receptor binding and promotes the (pH-dependent) fusion of virus and cell membranes after endocytosis. HA-targeted peptides are expected to lead to novel anti-influenza drugs. Cholesterol conjugated HA-derived peptides with anti-fusion activity against influenza have been previously studied on live virus. In this study, we evaluated three HA-derived peptides using fluorescence spectroscopy. Membrane partition assays were performed at two different pH values to assess the interaction with biomembrane model systems. Human blood cells were used to evaluate the extent of cell membrane binding, using the dipole potential probe di-8-ANEPPS. Preferential localization of tryptophan in lipid bilayers using aqueous-soluble and lipophilic quenchers was also assessed. Our results will provide new insight into molecular interactions between HA-derived peptides and cell membranes, that contribute toward the development of new influenza A virus inhibitors.

Keywords: Cholesterol-tagging, influenza, anti-fusion peptides, biomembranes

POSTERS

P57: HUMAN SP-A1 ENHANCES INTERFACIAL PROPERTIES OF LUNG SURFACTANT AND RESTORES A PROPER BEHAVIOR IN THE PRESENCE OF INHIBITORY AGENTS

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Pulmonary surfactant is a lipid-protein complex secreted by type II alveolar cells. This surface-active agent prevents alveolar collapse by reducing surface tension at the respiratory air-liquid interface during expiration and contributes to the protection of the respiratory surface from the entry of pathogens. Surfactant protein A (SP-A) is a hydrophilic glycoprotein of the collectin family and its main function is related to host defense. However, it has been shown that SP-A participates in the formation and biophysical properties of surfactant films at the interface and to reestablish surfactant function in presence of some inhibitors. There are two SP-A genes in humans, SFTPA1 and SFTPA2, which products differ in structure and function.

With the aim of investigating the differences in the biophysical properties of surfactant containing human SP-A1, SP-A2 or both, we have studied for the first time, 1) pulmonary surfactant from individual humanized transgenic mice expressing human SP-A1, SP-A2, or both SP-A1/2, in a Captive Bubble Surfactometer (CBS), and 2) the capability of this two variants of the protein to restore the biophysical activity of a reconstituted organic extract (OE) from native porcine surfactant in the presence of serum as inhibitory agent, mimicking a situation of lung injury and inflammation in the lungs.

The experiments revealed consistent functional differences in surfactant containing SP-A1 or SP-A2. Surfactant from mice containing SP-A1 and the OE reconstituted upon addition of SP-A1, achieved lower surface tension after post-expansion interfacial adsorption than surfactants containing no SP-A or SP-A2. Under interfacial compression-expansion cycling conditions, surfactant films containing SP-A1 also showed a better performance, particularly with respect to the reorganization of the films that take place during compression, which was also more efficient than films containing SP-A2 in the presence of serum.

We conclude that the presence of hSP-A1 allows surfactant to adopt a particularly favorable structure with optimal biophysical properties and helps to restore a proper behavior in the presence of an inhibitory agent as serum.

Keywords: Pulmonary-surfactant; SP-A; interface; inhibition

P58: TOXIN-INDUCED PORE FORMATION IS HINDERED BY INTERMOLECULAR HYDROGEN BONDING IN SPHINGOMYELIN BILAYERS

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Sticholysin I and II (StnI and StnII) are pore-forming toxins that use sphingomyelin (SM) for membrane binding. We examined how hydrogen bonding among membrane SMs affected the StnI- and StnII-induced pore formation process, resulting in bilayer permeabilization. We compared toxin-induced permeabilization in bilayers containing either SM or dihydro-SM (lacking the trans Δ4 double bond of the long-chain base), since their hydrogen bonding properties are known to differ greatly. We observed that whereas both StnI and StnII formed pores in unilamellar vesicles containing palmitoyl-SM or oleoyl-SM, the toxins failed to similarly form pores in vesicles prepared from dihydro-PSM or dihydro-OSM. In supported bilayers containing OSM, StnII bound efficiently, as determined by surface plasmon resonance. However, StnII binding to supported bilayers prepared from dihydro-OSM was very low under similar experimental conditions. The association of the positively charged StnII (at pH 7.0) with unilamellar vesicles prepared from OSM led to a concentration-dependent increase in vesicle charge, as determined from zeta-potential measurements. With dihydro-OSM vesicles, a similar response was not observed. Benzyl alcohol, which is a small hydrogen-bonding compound with affinity to lipid bilayer interfaces, strongly facilitated StnII-induced pore formation in dihydro-OSM bilayers, suggesting that hydrogen bonding in the interfacial region originally prevented StnII from membrane binding and pore formation. We conclude that interfacial hydrogen bonding was able to affect the membrane association of StnI- and StnII, and hence their pore forming capacity. Our results suggest that other types of protein interactions in bilayers may also be affected by hydrogen-bonding origination from SMs.

Keywords: Actinoporin - sticholysin - permeabilization - surface plasmon resonance

P59: EXPLORING THE STRUCTURAL FEATURES OF ANTIBACTERIAL RHODAMINE LABELLED 3,4-HPO CHELATORS THAT FAVOUR PERMEATION THROUGH LIPOSOMES AND MACROPHAGES

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We previously reported that the antimycobacterial effect of rhodamine-labelled 3-hydroxy-4-pyridinone chelators with N-ethyl substituents on the xanthene ring of rhodamine and a thiourea linkage between rhodamine and the chelating unit was distinct from that of compounds with methyl substituents and an amide linkage. To quantify chelator´s interaction with membranes and to correlate their affinity for lipid phases with its intracellular distribution we calculated: (a) partition constants in DMPC and DMPG liposomes used here as model membranes and (b) performed confocal microscopy studies using several fluorescent markers to gain insight on the cellular distribution of the chelators and identify the compartments accessible to each type of chelator. The results show that: (i) all chelators interact with liposomes; (ii) the partition constant (Kp) values are indicative that rhodamine B isothiocyanate (F7) derivatives interact strongly with liposomes and such interaction is higher with the negatively charged liposome thus indicating the importance of the chelator's interaction with the liposome surface; (iii) fluorescence anisotropy results reinforce the influence of the structure of the fluorophore on surface interactions; (iv) confocal microscopy shows that all chelators have access to the macrophage; (v) comparison of the chelator concentration required to produce similar intracellular confocal microscopy signal is indicative that a six times higher amount of F7 derived chelators crosses the macrophage membrane; (vi) the distribution and interaction of chelators with different cellular compartments is similar for all tested chelators. According to our overall results we hypothesize that the "strength" of the interaction of a rhodamine labeled chelator with membranes is determinant for the final antimycobacterial effect and therefore rhodamine B isothiocyanate derivatives appear to be better candidates for controlling intramacrophagic growth of M. avium than those derived from carboxytetramethylrhodamine. The information provided by the present studies is relevant to design new antimicrobials with improved biological activity.

Keywords: Iron Chelator, Membrane, Liposome, DMPC, DMPG, Confocal microscopy, Mycobacteria, Infection

P60: QUANTIFYING BIOMOLECULAR-LIPID MEMBRANE INTERACTIONS USING SURFACE PLASMON RESONANCE EQUILIBRIUM AND KINETIC DATA

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Molecular interactions with lipid membranes are ubiquitous to most biological processes and have been increasingly studied for their potential biomedical and biotechnological applications. These range from drug delivery vectorization to antimicrobial development, through the use of membrane active molecules such as peptides. Quantifying molecular partition towards the lipid phase, through adequate parameterization, is thus fundamental when studying these processes.

Surface Plasmon Resonance (SPR) is a powerful technique to study molecular association, but is limited in its quantitative interpretation of molecular membrane partition data. We have developed and applied a novel analytical method for SPR data treatment which enables the experimental determination of equilibrium and kinetic membrane partition parameters. This methodology makes use of two complementary mathematical fitting models for SPR sensorgram association and dissociation data. The partition constants (Kp) determined for of single-domain antibody F63, the anti-HIV peptide enfuvirtide, and the endogenous neuropeptide kyotorphin towards POPC membranes were comparable to literature data obtained with alternative techniques. Both models were further applied to the interaction of HRC4, a novel Measles Virus fusion-inhibitor peptide dimer, revealing its increased partition and retention in cholesterol-rich membranes.

Our work offers an alternative data treatment approach for SPR membrane interaction data and expands the applicability of the technique to the quantitative analysis of lipid phase partition phenomena.

Keywords: Biomolecules, Lipids, Interaction, SPR, Partition, Models

P61: DEOXYCHOLIC ACID MODULATES CELL DEATH SIGNALING THROUGH CHANGES IN MITOCHONDRIAL MEMBRANE PROPERTIES

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Cytotoxic bile acids, such as deoxycholic acid (DCA), are responsible for hepatocyte cell death during intrahepatic cholestasis. The mechanisms responsible for this effect are unclear, and recent studies conflict, pointing to either a modulation of plasma membrane structure or mitochondrial-mediated toxicity through perturbation of mitochondrial outer membrane (MOM) properties. We conducted a comprehensive comparative study of the impact of cytotoxic and cytoprotective bile acids on the membrane structure of different cellular compartments. We show that DCA increases the plasma membrane fluidity of hepatocytes to a minor extent, and that this effect is not correlated with the incidence of apoptosis. Additionally, plasma membrane fluidity recovers to normal values over time suggesting the presence of cellular compensatory mechanisms for this perturbation. Colocalization experiments in living cells confirmed the presence of bile acids within mitochondrial membranes. Experiments with active isolated mitochondria revealed that physiologically active concentrations of DCA change MOM order in a concentration- and time-dependent manner, and that these changes preceded the mitochondrial permeability transition. Importantly, these effects are not observed on liposomes mimicking MOM lipid composition, suggesting that DCA apoptotic activity depends on features of mitochondrial membranes that are absent in protein-free mimetic liposomes, such as the double-membrane structure, lipid asymmetry, or mitochondrial protein environment. In contrast, the mechanism of action of cytoprotective bile acids is likely not associated with changes in cellular membrane structure.

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Keywords: Apoptosis, bile acids, membrane fluidity, mitochondria

POSTERS

P62: TYPE-II NADH:QUINONE OXIDOREDUCTASE FROM STAPHYLOCOCCUS AUREUS HAS TWO DISTINCT BINDING SITES AND IS RATE LIMITED BY QUINONE REDUCTION

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NDHs-2 are membrane proteins involved in respiratory chains and recognized as suitable targets for novel antimicrobial therapies, since they seem to be the only enzymes with NADH:quinone oxidoreductase activity expressed in many pathogenic organisms, both bacteria and protozoa. We determined the crystal and solution structures of NDH-2. We performed titrations of the protein with its substrates, monitored by fluorescence and STD-NMR spectroscopies and observed that NADH and the quinone bind to different sites. We report fast kinetic analyses of the protein and detected a charge-transfer complex formed between NAD+ and the reduced flavin, which is dissociated by the quinone. This indicates that a ternary complex may be involved in the catalytic mechanism. We observed that the quinone reduction is the rate limiting step and also the only half-reaction affected by the presence of HQNO, an inhibitor.

Keywords: Quinone, respiration, fast kinetics, STD-NMR, flavin

POSTERS

P63: PROTEIN-BASED BIOMATERIALS MIMICKING MUSCLE TISSUE

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Proper function of striated muscle is dependent on its mechanical properties. Single-molecule approaches have shown that the passive elasticity of muscle is determined by the mechanics of specific cytoskeletal proteins in cardiomyocytes, such as titin. However, due to the complexity of muscle architecture, there are no models that can predict macroscopic mechanical behaviour directly from the mechanical properties of constituent proteins.

In this work, we propose a new bottom-up approach to produce protein-based hydrogels that recapitulate the basic passive properties of muscle. The advantage of our approach is that we can examine direct correlations between the mechanical properties of the constituent proteins, measured at the single-molecule level, and those of the resulting hydrogels. To generate the hydrogels, we use engineered polyproteins based on titin domains whose mechanical properties can be probed by single-molecule Atomic Force Microscopy (AFM). The production of the hydrogels is achieved via a photocatalytic reaction that crosslinks tyrosine residues exposed in the different domains of the polyproteins 1. Hydrogel stiffness is then measured using custom-built stretching devices 2. Working with single-point mutants of the constituent polyproteins, we will be able to determine how changes in the nanomechanical properties of the protein building blocks translate into macroscopic changes in hydrogel stiffness.

Thanks to our new strategy it will be possible to translate the mechanical properties of proteins to higher order arrangements, let them be protein hydrogels or muscle fibres. In addition, these protein-based hydrogels represent an initial step in the establishment of versatile biomaterials that mimic the adjustable mechanical properties of muscle, with promising potential for applications in biomedical and tissue engineering.

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