XVth CONGRESS OF THE SPANISH BIOPHYSICAL SOCIETY

Granada, 10-12 June, 2015

Facultad de Ciencias. Campus Fuentenueva

SBE

BOOK OF ABSTRACTS

Presentation

On behalf of the Organising Committee, it is a great honour to invite you to the 15th Congress of the Spanish Biophysical Society in Granada, a world heritage city by UNESCO.

The congress will be held at the "Facultad de Ciencias", an excellent facility in the centre of the city, as a part of the University of Granada, which will allow for an easy access to all scientific sessions.

Dra. Ana Isabel Azuaga Fortes
(in the name of the Organizing Committee)

The University of Granada (UGR), founded in 1531, continues a long teaching tradition, the roots of which can be traced back to the madrasahs of the last Nasrid Kingdom. The University is a vibrant presence in the city of Granada, benefiting from the distinctive beauty of its environment and a privileged geographical location due to its proximity to the Sierra Nevada and the Mediterranean coast.

The commitment to high-quality research has placed the UGR in a prominent position in terms of national rankings. The Postgraduate School offers 68 master's and 116 doctorate programmes. For many years, the UGR has promoted a significant international activity. It is the leading European university in terms of receiving students and the second Spanish university in terms of the mobility of its own students.

The congress will be structured in parallel symposiums/symposia with several plenary lectures, conducted by outstanding scientists in their areas. All participants will be able to attend the scientific program, which has been carefully designed to promote debating and enriching discussions, with especial "care" given to the poster sessions. The wide variety of areas within Biophysics to be discussed during the conference will provide to the participants with an unique vision of the state-of-the-art at an international level in this attractive and highly developing field.

Any important news regarding to the organization as well as registration, accommodation, etc., will be conveniently posted and periodically updated on the website of the Conference (**www.sbe.es/granada2015**), as well as useful tourist/social information about the City of Granada, which may be of participants´ interest.

We specially encourage young scientists to participate in the 15th Spanish Biophysical Congress, where they should expect not only an enriching scientific training during the Congress but also a wonderful personal experience in the city.

Organizing Committees

Local Organizing Committee

Departamento de Química Física (UGR)

Ana Isabel Azuaga Fortes
Salvador Casares Atienza
Francisco Conejero Lara
Beatriz Ibarra Molero
Irene Luque
Pedro Luis Mateo Alarcón
Jose Cristóbal Martínez Herrerías
Javier Ruiz Sanz
Eva Sánchez Cobos
Obdulio López Mayorga

Departamento de Física Aplicada (UGR)

Mª Jose Gálvez
Alberto Martín Molina
Francisco Galisteo

Departamento de Biología Molecular y Bioquímica (UMA)

Antonio Heredia Bayona

Scientific Committee

José López Carrascosa
Antonio Heredia Bayona
Jesús Salgado Benito
Francisco Barros de la Roza
Fernando Moreno
Francisco Galisteo
Antonio V. Ferrer Montiel
Marta Bruix Bayés
Antonio Rey Gayo
Miquel Pons
María García Parajo
Juan Carmelo Gómez
Ana Isabel Azuaga Fortes
Francisco Conejero Lara

Scientific Program

Wednesday, 10 June 2015

Time		
15:00-15:30	**OPENING CEREMONY (AULA MAGNA)**	
15:30-16:15	**Plenary Lecture 1 (AULA MAGNA)** Chairperson: Irene Luque	
	Ernesto Freire (Dept. of Biology. The Johns Hopkins University) *Protein Thermodynamics and the Biopharmaceutical Industry*.	
16:30-18:00	**Symposium S1: Supramolecular complexes** **SALÓN DE GRADOS** Chairperson: José López Carrascosa	**Symposium S2: Cell and tissue biophysics** **EDIFICIO MECENAS** Chairperson: Antonio Heredia
	Rafael Giraldo (Department of Cellular & Molecular Biology, CIB-CSIC, Madrid) *Untangling amyloid proteinopathies with a synthetic bacterial model system: the RepA-WH1 prionoid*.	**Victoria Fernández** (School of Forest Engineering, Technical University of Madrid) *Analyzing the properties of plant surfaces: a biophysical approach*.
	Maria. J. Macias (Structural and Computational Biology, IRB Barcelona) *Smad proteins during the last 500 million years: evolution, human SNP variations and somatic mutations. An structural approach*.	**Iván López Montero** (Physical Chemistry, Universidad Complutense de Madrid) *Cell mechanics as a biophysical biomarker*.
Oral communications:		
	Pedro M. Nieto (Glycosystems Laboratory, Chemical Research Institute (CSIC-US) *Molecular recognition of glycosaminoglycan oligosaccharides by langerin*.	**Elías Herrero-Galán** (Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), *A mass-spectrometry-based method to study the redox state of cysteines in titin*.
	Maria Adell-Morunol (Molecular biology Institute of Barcelona, IBMB-CSIC) *New insights into the chaperones system in Mycoplasma genitalium*.	**Elena Beltrán-Heredia** (Departamento de Física Atómica, Molecular y Nuclear, Universidad Complutense de Madrid) *Mechanics of the cell constriction during division*.
18:00-18:30	**Coffee break and visit to posters (HALL)**	
	José R. Castón (Department of Structure of Macromolecules, CNB-CSIC) *Cryo-electron microscopy at atomic resolution of macromolecular machines*.	**María García-Parajo** (ICFO-Institute of Photonic Sciences, Barcelona) *Nanophotonic approaches for live cell research: from nanoimaging to spectroscopy*.
	Aitor Hierro (CIC bioGUNE, Bizkaia) *Escape from death,strategies for intracellular survival by Legionella pneumophila*	**María Luisa García Martín** (Andalusian Centre for Nanomedicine and Biotechnology, BIONAND) *Imaging the tumor microenvironment*.
Oral communications:		
	María del Mar Collado-González (Department of Physical Chemistry, University of Murcia) *Chitosan-gold nanocomposites: controlled size synthesis and stability*.	**Juan A. Torreno-Pina (**ICFO – Institut de Ciencies Fotoniques) *The actin cytoskeleton modulates the activation of invariant NKT cells by segregating CD1d nanoclusters on antigen presenting cells*.
	Christina S. Kamma-Lorger (ALBA Synchrotron Light Source) *Role of decorin core protein in collagen organisation in congenital stromal corneal dystrophy (CSCD)*.	
20:00-22:00	**Wellcome reception: CARMEN DE LA VICTORIA (ALBAICÍN)**	
22:00	**Visit to the ALHAMBRA**	

Thursday, 11 June 2015

Time	Symposium S3	Symposium S4
9:00-10:30	**Symposium S3: Protein folding, misfolding and stability** **EDIFICIO MECENAS** Chairperson: Francisco Conejero Lara	**Symposium S4: Lipid and membrane biophysics.** **SALÓN DE GRADOS** Chairperson: Jesús Salgado
	José Manuel Sánchez Ruiz (Departamento de Química Física, Universidad de Granada) *Using ancestral resurrection to probe evolutionary protein biophysics*.	**Victor Lórenz-Fonfría** (Institute of Experimental Physics, Department of Physics, Freie Universität Berlin) *Insights into the dynamics and function of membrane transporters and ion channels by vibrational spectroscopy*.
	Juan Salvador Jiménez (Universidad Autónoma de Madrid, Applied Physical Chemistry Department) *A surface plasmon resonance study of the DNA interaction with Tau protein and amyloid peptides, involved in the molecular basis of Alzheimer's disease*.	**Juan R. Granja** (Department of Organic Chemistry and Center for Research in Biological Chemistry and Molecular Materials (CIQUS), University of Santiago de Compostela *Membrane targeting self-assembling peptides*.
Oral communications:		
	Encarnación Medina (Department of Physical Chemistry t, University of Granada) *Natural ligands restore the function of a cancer-associated polymorphism in NQO1*.	**Ángel Pérez-Lara** (Department of Neurobiology, Max Planck Institute for Biophysical Chemistry) *New insights into synaptotagmin-1 binding*.
	Nunilo Cremades (Institute for Biocomputation and Physics of Complex Systems (BIFI)) *Understanding protein misfolding and amyloid fibril formation: sequential folding upon self-assembly*.	**Marisela Vélez** (Instituto de Catálisis y Petroleoquímica, CSIC) *Induction of a proton gradient across a gold-supported biomimetic membrane by electroenzymatic H2 oxidation*.
10:30-11:00	**Coffee break and visit to posters (HALL)**	
	Luis Alberto Campos (Centro Nacional de Biotecnología CNB-CSIC) *Engineering Macromolecular Assemblies from Monomeric Proteins: Learning from Molecular Evolution*.	**Fèlix Campelo** (Cell and Developmental Biology, Centre for Genomic Regulation (CRG), Barcelona) *The Biophysical Basis of Transport Carrier Biogenesis at the Golgi Complex*.
	Fernando Moro (Departamento de Bioquímica y Biología Molecular, UPV/EHU; Unidad de Biofísica, CSIC-UPV/EHU) *Chaperoning protein aggregates: the disaggregase machinery*.	**Gabriel Gomila** (Institut for Bioengineering of Catalonia (IBEC)) *Dielectric polarization properties of supported biolayers measured with electrostatic force microscopy*
Oral communications:		
	Fátima Herranz-Trillo (Department of Drug Design and Pharmacology, University of Copenhagen) *Towards an improved decomposition of time-dependent SAXS data from fibrillating proteins*.	**Zehra Kahveci** (Instituto de Biología Molecular y Celular, Universidad Miguel Hernández de Elche) *Interaction of new fluorescent conjugated polyelectrolytes with model membranes: their potential use as fluorescent membrane markers*.
	Jörg Schönfelder (IMDEA Nanociencia, Nanobiosystems, Madrid) *Mechanical force modulates the unfolding pathways of the cold-shock protein B from Thermotoga Maritima*.	**Sara García-Linares** (Departamento de Bioquímica y Biología Molecular I, Universidad Complutense, Madrid) *The effect of cholesterol on the long-range network of interactions established among sea anemone sticholysin II residues at the water-membrane interface*.
12:45-13:15	**Plenary Lecture 2 (AULA MAGNA)** Chairperson: María García-Parajo	
	Laura Fumagalli (School of Physics and Astronomy, University of Manchester, UK) *Probing electric polarization in biology with scanning probe microscopy: from single bacteria and viruses to DNA*	

Time		
13:15-15:00	**Lunch and visit to posters**	**EXHIBITOR WORKSHOP**: **(SALA AUDIOVISUALES)** **Marco Marenchino** (Iesmat-Malvern) Microcalorimetry as a versatile tool for characterization of biomolecules and biomolecular interactions.
15:00-16:30	**Symposium S5: Protein Structure, dynamics and function** **AULA MAGNA** Chairperson: Beatriz Ibarra Molero	**Symposium S6: Receptors, channels and transporters** **SALÓN DE GRADOS** Chairperson: Francisco Barros
	Jerónimo Bravo (Instituto de Biomedicina de Valencia, Department of Genomics and Proteomics) *Role of the carboxy-terminal domain of Erb1 during ribosome biogenesis in the formation of the Nop7/PeBoW complex.*	**Félix Viana** (Instituto de Neurociencias de Alicante UMH-CSIC) *Molecular dissection of cold temperature sensing in mammalian neurons*
	Paola Fucini (CIC bioGUNE, Structural Biology Department) *Structural studies on co-translational protein folding and sorting.*	**Mario Mellado** (Dpt. Immunology & Oncology, CNB/CSIC, Madrid) *Studying chemokine receptor conformations and dynamics at the cell membrane*
Oral communications:		
	Julio Bacarizo (*Department of Chemistry and Physics, Research Centre for Agricultural and Food Biotechnology (BITAL), University of Almería)* *Structure of the human TSG101-UEV domain in complex with the PTAP motif of viral L-domains.*	**Antonio Felipe** (*Molecular Physiology Laboratory, Departament de Bioquímica i Biologia Molecular, Institut de Biomedicina (IBUB), Universitat de Barcelona)* *Unconventional EGF-induced ERK1/2-mediated Kv1.3 endocytosis.*
	Cristina Balcells (Department of Physical Chemistry and Research Institute of Theoretical and Computational Chemistry (IQTCUB) of Barcelona University) *In-vivo-like study of the excluded volume effects on the kinetics of enzymatic reactions.*	**Carlo Manzo** (ICFO – Institut de Ciències Fotòniques, Castelldefels (Barcelona) *The role of membrane heterogeneity on receptor diffusion and function: are diffusion constants constant?*
16:30-17:00	**Coffee break and visit to posters (HALL)**	
	Irene Luque (Department of Physical Chemistry and Institute of Biotechnology. University of Granada, Granada) *Structural and thermodynamic studies of viral Late domain interactions: towards the development of host-oriented therapeutics.*	**Pilar de la Peña Cortines** (Department of Biochemistry and Molecular Biology, University of Oviedo) *Covalent link between the voltage-sensing module and the pore domain is not required for voltage-depending gating in hERG K^+ channels.*
	Xavier Daura (Institute of Biotechnology and Biomedicine – UAB) *Exploiting protein flexibility to predict the location of allosteric sites.*	**Javier García-Sancho** (Instituto de Biología y Genética Molecular (IBGM), Universidad de Valladolid y CSIC, Valladolid) *Gap, a new family of fluorescent protein probes for imaging calcium signals in intracellular organelles*
Oral communications:		
	Helton J. Wiggers (Instituto de Física de São Carlos, Universidade de São Paulo) *Identification of compounds that inhibits bacterial diguanylate cyclases involved in biofilm formation from theraputics drugs.*	**Álvaro Ortega** (Estación Experimental del Zaidín-CSIC. Granada) *Global characterization of the bimodular ligand binding domains of PP2249 and PP1228: two paralogous chemoreceptors from Pseudomonas putida*
	María Sebastián (Departamento de Bioquímica y Biología Molecular y Celular, Facultad de Ciencias, and Instituto de Biocomputación y Física de Sistemas Complejos (BIFI), Universidad de Zaragoza) *Revisiting the riboflavin kinase catalytic cycle of bacterial FAD synthetase.*	
18:30-19:15	**Poster Party (HALL) (Sponsors GRONTAL**	

19:15-19:45	**SBE General Assembly (AULA MAGNA)**
20:00-21:30	**SBE and Society: "Biofísica gastronómica:** "De la cocina al laboratorio y viceversa" **(AULA MAGNA)** **Francisco Galisteo Gonzalez** Departamento de Física Aplicada, Facultad de Ciencias de la Universidad de Granada

Friday, 12 June 2015

9:00-10:30	**Symposium S7: Biophysics of nucleic acids** **EDIFICIO MECENAS** Chairperson: Fernando Moreno	**Symposium S8: Biocolloids and biointerfaces** **SALÓN DE GRADOS** Chairperson: Alberto Martín Molina
	Felix Ritort (Small Biosystems Lab, Universitat de Barcelona) *Measuring binding affinities using force methods*.	**Elena Junquera González** (Department of Physical Chemistry I, Universidad Complutense of Madrid) *What May Colloidal and Supramolecular Chemistry Provide in Gene Therapy?*
	Silvia Hernández Aínsa (Department of Physics (Cavendish Laboratory), University of Cambridge, United Kingdom *DNA origami nanopores for single molecule detection*.	**Verónica Salgueiriño** (Departamento de Física Aplicada, University of Vigo) *Synergy effects of magnetic silica nanostructures for drug and heat delivery applications*.
Oral communications:		
	Irene Gutiérrez (Instituto Madrileño de Estudios Avanzados en Nanociencia (IMDEA Nanociencia), *Single-stranded RNA interaction with long human telomeric RNA unveiled at the single-molecule level*.	**Teresa del Castillo-Santaella** (Department of Applied Physics, University of Granada) *Natural extracts inhibit the lipolysis using a single drop method*.
	Julene Madariaga-Marcos (Department of Macromolecular Structures, CNB, CSIC, Madrid) *Towards visualizing DNA repair at the single molecule level combining magnetic tweezers and TIRF microscopy*.	**Paola Sánchez Moreno** (Centro Europeo di Nanomedicina (CEN) and Dipartimento di Chimica, Materiali e Ingegneria Chimica Giulio Natta, Politecnico di Milano) *Functionalized nanoparticles in a biological environment: impact of protein corona*.
10:30-11:00	**Coffee break and visit to posters (HALL)**	
	Pablo Huertas (CABIMER/University of Seville) *High resolution methods to analyze the repair of broken DNA*.	**Pablo Taboada** (University of Santiago de Compostela, Condensed Matter Physics Department) *Amyloid fibrils: Not only a marker of disease but also an exciting nanomaterial*.
	Álvaro Somoza (IMDEA Nanociencia, Madrid) *Oligonucleotides and Nanostructures in Nanomedicine*.	**Julia Maldonado-Valderrama** (Applied Physics Department. University of Granada) *Impact of interfacial structure on digestibility of food emulsions*.
Oral communications:		
	Jesús I. Mendieta-Moreno (Departamento de Física Teórica de la Materia Condensada, Universidad Autónoma de Madrid) *Ultrafast photochemical reactions in DNA: a QM/MM study*.	**Elia Grueso** (Department of Physical Chemistry, Faculty of Chemistry, University of Seville) *Cationic gemini surfactants induce abnormal DNA conformational change at high surfactant-DNA molar ratios*.
	Cesar L. Pastrana (Department of Macromolecular Structures, CNB, CSIC, Madrid) *Understanding the mechanisms of DNA condensation by the bacterial protein ParB/Spo0J*.	**Germán Luque Caballero** (University of Granada) *Lipid-cation-DNA complexes: interfacial characterization and modeling*.
12:45-13:15	**Plenary Lecture 3 (AULA MAGNA)** Chairperson: María José Gálvez	

Pedro Tarazona (IFIMAC Institute for Condensed Matter Physics, Dept. Física Teórica de la Materia Condensada, Universidad Autónoma de Madrid)
Modelling of FtsZ protein filaments from AFM images.

13:15-15:00 **Lunch and visit to posters**

EXHIBITOR WORKSHOP: (SALA AUDIOVISUALES)
Peter Vikegard (TA Instruments, Microcalorimetry Manager)
The Affinity-(Auto)-ITC: A new improved system for automated and manual operation

15:00-15:30 **Plenary Lecture 4. Sociedad Argentina de Biofísica (AULA MAGNA)**
Chairperson: Ana Isabel Azuaga Fortes

Gerardo D. Fidelio (Dep. Química Biológica, CIQUIBIC, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina).
Surface properties of amphiphilic peptides.

15:30-17:00 **SBE prizes. Annual Awards Ceremony (AULA MAGNA)**
Chairperson: Antonio Ferrer- Montiel

Bruker Prize 2015: Juan A. Hermoso (Instituto de Física-Química Rocasolano. Dept. of Crystallography and Structural Biology, Madrid)
Structural Insights into Bacterial Pathogenesis

Enrique Pérez Payá Prize (SBE-40): Irene Díaz Moreno (IBVF CSIC-Universidad de Sevilla)
Biointeractomics of Cytochrome c: From Transient Life to Stable Death

SBE-33 Prize: Cecilia Artola (Instituto Química-Física "Rocasolano", CSIC. Department of Crystallography and Structural Biology)
Structural biology of macromolecular machines involved in Peptidoglycan recycling and their implications in antibiotic resistance

---

17:00-18:00 **Coffee break (HALL)**

18:00-18:45 **Plenary Lecture 5 (AULA MAGNA)**
Chairperson: Juan Carmelo Gómez-Fernández

Reinhard Jahn (Department of Neurobiology, Max-Planck-Institute for Biophysical Chemistry)
Neuronal exocytosis: mechanisms and energy barriers.

18:45-19:00 **Posters prizes and closing ceremony (AULA MAGNA)**

21:00 **Congress dinner: CARMEN DE LOS FAVORES**

About the invited speakers

Plenary lecturers

Dr. Ernesto Freire

Department of Biology. The Johns Hopkins University.

Protein Thermodynamics and the Biopharmaceutical Industry

ERNESTO FREIRE is the Henry Walters Professor at the Johns Hopkins University. He has been a member of the Department of Biology and Biophysics since 1986. He also holds a joint appointment in the Department of Biophysics and Biophysical Chemistry at the Johns Hopkins Medical School. Dr. Freire obtained his doctorate in Biophysics from the University of Virginia.

Dr. Freire is a world recognized expert in biological thermodynamics. He performs research in the thermodynamics of protein stability, protein-protein interactions, and protein-ligand binding. Dr. Freire has pioneered the development of drug design and optimization strategies using thermodynamics techniques. Dr. Freire has been associated with several startups including Fulcrum Pharmaceuticals, Ception Therapeutics and currently AVIA Biosystems. Dr. Freire is the author or co-author of over 250 publications and several patents. Dr. Freire is on the editorial board of several journals and has served on many scientific advisory committees for the National Institutes of Health and the National Science Foundation. Dr. Freire is an Honor Member of the Spanish Society of Biochemistry and Molecular Biology, and a member of the Academy of Sciences of Latin America.

Dr. Laura Fumagalli

School of Physics and Astronomy, University of Manchester (UK).

Probing electric polarization in biology with scanning probe microscopy: from single bacteria and viruses to DNA.

Dr. Laura Fumagalli did her undergraduate studies at Polytechnic of Milan (Italy) and École Superior d'Electricité (Paris, France). She obtained her PhD in Information Technology (specialization in Electronics) at Polytechnic of Milan (Italy) in 2006. She then joined as a Post-Doc the newly formed Institute for BioEngineering of Catalonia (IBEC, Barcelona) and then in 2009 the University of Barcelona (UB) as a Juan de la Cierva Young Researcher. During her PhD and post-doctoral research, she developed novel instrumentation for Scanning Probe Microscopy that achieved sub-attoFarad capacitance resolution and enabled to quantitatively determine the dielectric constant of nano-objects and macromolecules. Her studies have opened up an exciting sub-field of research dedicated to the measurement of the dielectric properties of matter at the nanoscale. From 2010 to 2014, she was full-time Lecturer Professor of several courses - electronics, informatics, scanning probe microscopy, nanobiotechnology among the others - at the Electronic Department of the University of Barcelona and Senior Researcher at IBEC. Currently, she is Lecturer in Condensed Matter Physics at the School of Physics and Astronomy of the University of Manchester (UK) and Researcher of the new National Graphene Institute (UK).

Dr. Pedro Tarazona

IFIMAC Institute for Condensed Matter Physics, Dept. Fisica Teorica de la Materia Condensada, Universidad Autonoma de Madrid.

Modelling of FtsZ protein filaments from AFM images

Pedro Tarazona is professor at the Theoretical Condensed Matter Physics Dept. and coordinator for the Soft-Matter and Biophysics area of IFIMAC institute at UAM. He has a long experience in the statistical physics of liquids, liquid-crystals and other soft condensed matter phases, their phase transitions and interfaces. In 1991, a sabbatical stay in Vienna was the starting point for his work in the application of those theoretical tools to biological systems, with the analysis of the RNA folding and the quasi-species model of Eigen and Schuster under complex replication landscapes. Later, he has also addressed problems related to the stability and fluctuations of bilayer membranes. Over the last decade, and in close collaboration with the experimental group of M. Vélez, he has worked on the theoretical description of the FtsZ protein filaments from the experimental information gathered from AFM images.

Dr. Gerardo D. Fidelio

Departamento de Química Biológica, CIQUIBIC, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina.

Surface properties of amphiphilic peptides

Dr. Fidelio received his first degree of Biochemist in 1979 at the Faculty of Chemistry Science, National University of Cordoba (the oldest University in Argentina); and his Ph.D. in Biochemistry in 1984 at the same University. Since then, he was involved in teaching and research in the field of Biophysics. He has spent a period as posdoct at the London University from 1985 to 1987 (as Wellcome Trust Fellow, England). In his return to Argentina in 1987, He joined the Scientific Research Career of CONICET (from our National Research Council). Currently, he is in the category of Principal, the penultimate step of the scientific ladder in the Research Council in Argentina. He is also Full Professor at Cordoba University. He has supervised many graduate and Ph.D. students, posdocts and graduate students. He has published over 80 papers.

He was frequently acting as evaluator from the National Research Council (CONICET) and National Agency for Science and Technology Promotion in Argentina (ANPCYT). At the University, he has served firstly as Dean of the Faculty of Chemistry Science from 1999 to 2007 and, afterwards he was Vice Chancellor of the National University of Cordoba in the period 2007-2009. Also, he was president of the Argentinean Biophysical Society (2012-2014). At the moment, he serves as Head of Biological Chemistry Department in the School of Chemistry Science, National Córdoba University. His research interest is in the field of protein and membrane biophysics. The main research topics are protein stability, protein-ligand stability, protein-protein interaction, protein-lipid interaction (including enzymes such as phospholipase A2) and the interaction of amphiphilic peptides with organized interfaces.

Dr. Reinhard Jahn

Max Planck Institute for Biophysical Chemistry, Department of Neurobiology

Neuronal exocytosis: mechanisms and energy barriers

Reinhard Jahn studied biology and chemistry at the Universities of Freiburg and Göttingen (Germany) and finished his studies in 1981 with a PhD at the University of Göttingen. After a postdoc with Paul Greengard at Rockefeller University he became Assistant Professor at Rockefeller and then re-located in 1986 as junior group leader to the Max-Planck-Institute for Psychiatry in Munich. In 1991, Reinhard Jahn was appointed as tenured Associate Professor for Pharmacology and Cell Biology at Yale University, with a joint appointment at the Howard Hughes Medical Institute. 1995 he was promoted to Professor. In 1997, he returned to Germany where he holds the position of a director and scientific member at the Max Planck Institute for Biophysical Chemistry and of a professor of the University of Göttingen.

Reinhard Jahn won several awards such as the Max-Planck Research Prize (1990), the Gottfried-Wilhelm Leibniz Prize (2000), the Ernst-Jung Prize for Medicine (2006), the Sir Bernhard Katz Award (2008), the Eduard Buchner Prize (2013), and the Heinrich Wieland Prize (2014). Since many years, Reinhard Jahns' research interests focus on the molecular mechanisms of neuronal exocytosis, on SNARE-mediated membrane fusion, and on the structure and function of synaptic vesicles.

In addition to his scientific activities, Reinhard Jahn has served the scientific community as Panel member/chair of the ERC, as member and chair of scientific advisory boards (e.g. EMBL), and in numerous peer evaluations. Furthermore, Reinhard Jahn is the Dean of the Göttingen Graduate School for Neurosciences, Biophysics, and Molecular Biosciences.

Simposia and workshop speakers (in alphabetical order)

Dr. Francisco J Blanco

CIC bioGUNE. Structural Biology Unit.

Structure of the p15^{PAF}/PCNA complex and implications for clamp sliding on the DNA during replication and repair.

Francisco Blanco obtained his Bachelor and Doctorate degrees in Chemistry at the Complutense University of Madrid, and did his Ph.D. thesis as supervised by J.L. Nieto at the Instituto de Estructura de la Materia (CSIC, Madrid) in 1992. Using NMR he characterized for the first time peptides folded into beta-hairpins. In 1993 he started as Postdoctoral Fellow at the European Molecular Biology Laboratory (EMBL, Heidelberg) in L. Serrano's Group studying the structure and folding of the spectrin SH3 domain as a model protein, showing that the appearance of a new fold from an existing one is unlikely to occur by evolution through folded intermediate sequences. In 1997 he moved to R. Tycko's Lab at the National Institute of Diabetes, Digestive and Kidney Diseases (NIH, Bethesda), as Visiting Fellow. Solid state NMR analysis of the HIV-Rev protein fibers supported a helix-loop-helix structural model. In 2000 he returned to Spain to work with M. Rico at the Instituto de Química Física Rocasolano (CSIC, Madrid). He determined the structure of an archaeal protein revealing a novel fold and a possible role in cell division. He was awarded a Ramón y Cajal contract in 2002 and joined the CNIO to establish the NMR group. There he characterized native and engineered endonucleases as tools for gene repair in human cells with low citotoxicity. In 2007 he joined the Structural Biology Unit at the CIC bioGUNE as an Ikerbasque Research Professor.

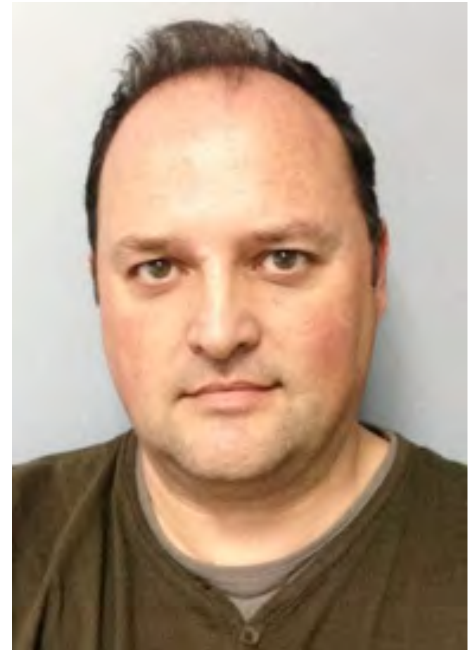
His current interest is the structure-function of proteins involved in chromatin remodelling and DNA repair. He studies the ING family of tumor suppressors, which recognize methylated histone tails in nucleosomes, and PCNA, a DNA sliding clamp essential for DNA replication and repair through interactions with many proteins.

He has coauthored 90 scientific publications, has an h-index of 33, and has supervised 6 PhD Thesis.

Dr. Jerónimo Bravo

Instituto de Biomedicina de Valencia, Department of Genomics and Proteomics

Role of the carboxy-terminal domain of Erb1 during ribosome biogenesis in the formation of the Nop7/PeBoW complex.



Jerónimo Bravo graduated at the Universidad Autónoma de Barcelona, Spain. He obtained an MSc degree from the same University focusing on the search for tumour markers in pancreatic cancer. He then joined I. Fita's group in 1991 at the Universitat Politècnica de Catalunya, Barcelona. His thesis involved the study of the mechanism of Reactive Oxygen Species detoxification - the 3D structure of catalases in particular.

In 1996 he obtained a postdoctoral fellowship from the Spanish Ministry of Science and Education followed by a European Community research grant to join Y. Jones at the Laboratory of Molecular Biophysics, University of Oxford, UK where he solved the structure of the cytokine-binding homology region of gp130, which is required for signal transduction by a set of cytokines (leukaemia inhibitory factor or oncostatin).

In 1998 Dr. Bravo switched from Oxford to Cambridge to join R. Williams at the Molecular Biology Laboratory. Jerónimo was the first to report a structure of a PX domain in complex with a phosphoinositide, providing key evidence for the lipid specificity and membrane targeting mechanism of PX domains. Work on transcription factors in collaboration with T. H. Rabbitts also contributed to the understanding of the molecular aspects of genetic abnormalities present in acute leukaemia.

Returning home to Spain in April 2002 he was appointed as Group Leader of the CNIO Signal Transduction Group were he focused on the molecular mechanisms of tyrosine kinase receptors downregulation and metastasis. In 2009 he obtained a position at the Instituto de Biomedicina de Valencia (CSIC) were he was appointed head of department and later deputy director until 2015 focusing on the structure and function of abnormal cell proliferation and control of gene expression.

Dr. Fèlix Campelo

Cell and Developmental Biology, Centre for Genomic Regulation (CRG), Barcelona.

The Biophysical Basis of Transport Carrier Biogenesis at the Golgi Complex

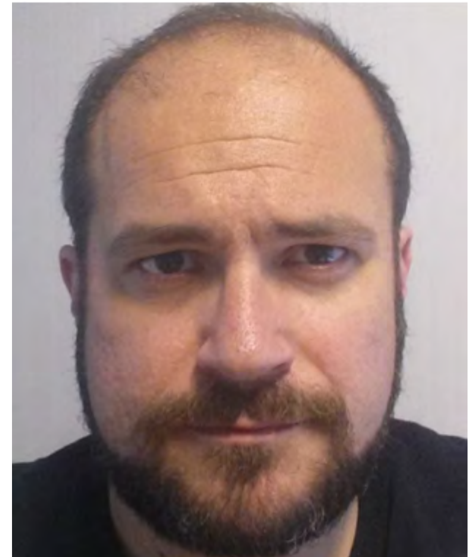
Felix Campelo did his Ph.D. thesis in theoretical biophysics on the physical mechanisms controlling the shapes of biological membranes under the supervision of Dr. Aurora Hernández-Machado at the University of Barcelona. During this time, he also worked with Dr. Martine Ben Amar at the LPS-ENS Paris, and at Tel Aviv University under the supervision of Dr. Michael Kozlov. In 2009, he received the "Award for Outstanding Doctoral Thesis Research in Biological Physics" to the best Ph.D. thesis in physical biology given by the American Physical Society.

After working on theoretical aspects of the morphological changes in cellular membranes and membrane mechanics, Felix switched into an in-depth experimental study of the cell biology of these processes, by doing a post-doc in Vivek Malhotra's lab at the Centre for Genomic Regulation (CRG) in Barcelona, where he is still currently working. During this period, membrane fission, the mechanisms of transport carrier formation, and the interplay between lipids and proteins in the regulation of Golgi membrane dynamics have been the main research topics he has been studying.

Dr. Luis Alberto Campos

Centro Nacional de Biotecnología CNB-CSIC

Engineering Macromolecular Assemblies from Monomeric Proteins: Learning from Molecular Evolution



Luis Alberto Campos graduated in Organic Chemistry at the University of Zaragoza and obtained his Ph.D. (2004) at the Department of Biochemistry and Molecular Biology, University of Zaragoza under the supervision of Prof. Javier Sancho. During this period he focused his research on protein folding and, in particular, describing a three state process through spectroscopic techniques and the way to manipulate it and stabilize conformations using mutations.

Later on he completed a 3-year postdoctoral stage at the University of Maryland, USA, where he collaborated with Dr. Victor Muñoz group in the development of a new method to obtain information from single molecule FRET at the microsecond timescale using new photoprotection cocktails. In 2008 he came back to Spain (to the Biological Research Center (CIB) in Madrid) with a Marie Curie postdoctoral fellowship, where he continued working on single molecule techniques, applied to fast folding proteins with small or no energy barrier.

Nowadays, he is a recent Ramón y Cajal researcher (2014) working in the Spanish National Biotechnology center (CNB) in Madrid, where he is working on the conversion of a protein from two-state to downhill no barrier behavior and on the formation of oligomers from monomeric proteins in a controlled way by mutations.

Dr. José R. Castón

Department of Structure of Macromolecules. CNB-CSIC

Cryo-electron microscopy at atomic resolution of viral macromolecular machines

Our studies address to elucidate structure-function-assembly-evolution relationships of viral macromolecular complexes, also known as viral nanomachines, which control many fundamental processes in virus life cycle. Our model systems of viral molecular machines are the viral capsid and other viral macromolecular complexes, such as helical tubular structures and ribonucleoprotein complexes.

Structural analysis of these assemblies at the highest achievable resolution is therefore essential to understand their properties. To reveal the three-dimensional structure of such complex assemblies under close-to-native conditions we use cryo-electron microscopy (cryo-EM) and image processing techniques combined with high-resolution X-ray structures (the hybrid approach). Recent advances have allowed us to yield atomic models from cryo-EM two-dimensional images.

Dr. Xavier Daura

Institute of Biotechnology and Biomedicine – UAB

Exploiting protein flexibility to predict the location of allosteric sites

I studied Biological Sciences at Autonomous University of Barcelona (UAB), where I graduated in 1991. I had never thought of becoming a scientist, but was offered the chance to do a PhD in biomolecular simulation, a still young field that the labs of F. X. Avilés and E. Querol at the Institute of Biotechnology and Biomedicine (IBB) of UAB where starting to explore. I obtained the PhD in 1996 and after this initial formative period I moved to the lab of Wilfred van Gunsteren (Computer-Aided Chemistry) in the Department of Physical Chemistry of the Swiss Federal Insitute of Technology - ETH Zürich, a main reference in the field of computational modelling and simulation of biomolecular systems. These were extraordinary years that marked my career. In 2002 I was appointed Research Professor by the Catalan Institution for Research and Advanced Studies (ICREA) and returned with this position to the Institute of Biotechnology and Biomedicine of UAB, starting a new group in Computational Biology. In 2005 I was also appointed Associate Professor at UAB to combine my research activities with teaching at the Master's level. Since January 2011 I serve as Director of IBB.

Research keywords: multidrug-resistance, synthetic vaccinology, bioinformatics, biomolecular modelling, biomolecular simulation, pathogen proteomics.

Dr. Victoria Fernández

Forest Genetics and Ecophysiology Research Group. School of Forest Engineering. Technical University of Madrid (U.P.M.)

Analysing the properties of plant surfaces: a biophysical approach

Victoria Fernández gained a Bachelor of Science in Agriculture at University College Dublin (Ireland) and a PhD at Humboldt University of Berlin (Germany). For 5 years, she worked as postdoctoral researcher at Aula Dei experimental Station-CSIC (Zaragoza, Spain), and joined the Forest Genetics and Ecophysiology Research Group (Technical University of Madrid) with a "Ramón y Cajal" research tenure in 2011. Since 15 years, Victoria Fernández has been implementing applied and fundamental research approaches for analyzing plant surface-liquid interactions and permeability with especial regard to water, solutes and foliar fertilizers. She is author of 45 scientific contributions and held several keynote lectures in international scientific and technical conferences. Her research efforts are currently focused on analysing the physico-chemical properties and permeability of plant surfaces from an ecophysiological and agronomic viewpoint.

Dr. Vadim A. Frolov

Unidad de Biofísica, UPV

Nanomechanics of energy transduction in protein-driven membrane fission

Milestones:

- Graduated from Moscow Institute of Physics and Technology, Moscow, 1993
- PhD in Biophysics from Moscow State University, 1997
- Post-Doc training in A.N. Frumkin Institute of Electrochemistry (Moscow) and National Institutes of Health, Bethesda, USA
- Ikerbasque Research Professor, Biophysics Unit, University of the Basque Country

Research lines:

- Mechanics and dynamics of biomimetic membranes at nanoscale
- Shape and topological transformations of membrane
- Proteo-lipid interaction, creation and regulation of membrane curvature and shape by proteins

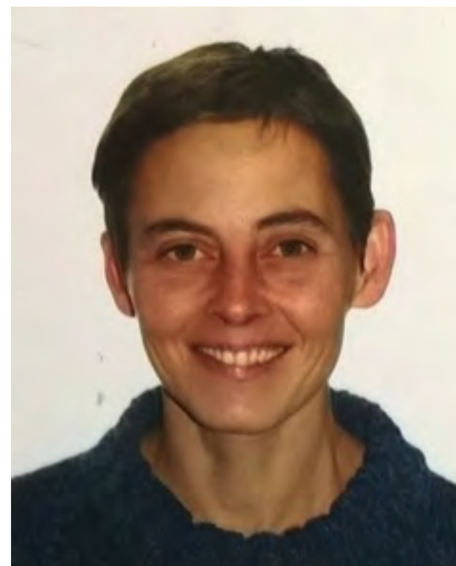
Selected publications:

1. Geng J., Kim K., Zhang J., Escalada A., Tunuguntla R., Comolli R.L., Allen F.I., Shnyrova A.V., Cho K.R., Munoz D., Wang Y.W., Grigoropoulos C.P., Ajo-Franklin C.M., Frolov V.A., Noy A. Stochastic transport through carbon nanotubes in lipid bilayers and live cell membranes. Nature 2014 514:612-5.
2. Shnyrova A.V., Bashkirov P.V., Akimov, S.A. Pucadyil T.J., Zimmerberg, J., Schmid S.L., Frolov V.A. Geometric catalysis of membrane fission driven by flexible dynamin rings. Science 2013 339:1433-6.
3. Schmid S.L. and Frolov V.A. Dynamin: Functional design of a membrane fission catalyst Ann. Rev. Cell Dev. Biol. 2011 27:79-105.
4. Bashkirov P.V., Akimov S.A., Evseev A.I., Schmid S.L., Zimmerberg J., Frolov V.A. GTPase cycle of dynamin is coupled to membrane squeeze and release, leading to spontaneous fission. Cell 2008, 135:1276-86.

Dr. Paola Fucini

CIC bioGUNE, Structural Biology Department

Structural studies on co-translational protein folding and sorting



Paola Fucini obtained her PhD degree from the Ludwig-Maximilians-Universität in München, for a thesis conducted at the Max-Planck-Institute for Biochemistry, in Martinsried (Germany), under the supervision of Prof. Angelika Noegel and Tad Holak. With this first study she started her career in structural biology becoming interested in tackling the fascinating process of protein folding. During her postdoctoral studies, as a Research Associate in the group of Prof. Chris Dobson and Carol Robinson, first at the Oxford Center for Molecular Science (University of Oxford) and later in the Chemical Laboratory at Cambridge University, she developed an in vitro transcription/translation system for the preparation of nascent chain ribosomal complexes, suitable for Mass Spectrometry, NMR and Cryo-EM analysis. The system allowed pioneering studies on co-translational protein folding which she later pursued as an independent Group Leader at the Max-Planck-Institute for Molecular Genetics, AG Ribosomen, in Berlin. There, after acquiring further expertise in ribosome X-ray crystallography, she started to develop three main research lines, namely (i) the mode of action of antibiotics and translational factor, (ii) the process of co-translational protein folding and sorting, (iii) ribosome biogenesis. These studies, consolidated as Professor in X-ray Crystallography for RNA and Protein complexes at the Cluster of Excellence for Macromolecular Complexes at the University of Frankfurt, are currently continued as Ikerbasque Research Professor in the ideal environment offered by the Centro de Investigación Cooperativa en Biociencias, CIC bioGUNE, en Derio, Bizkaia.

Dr. María Luisa García Martín

Andalusian Centre for Nanomedicine and Biotechnology (BIONAND)

Imaging the tumor microenvironment

Dr. María Luisa García-Martín has worked in the field of Magnetic Resonance Imaging and Spectroscopy applied to biomedical research since the beginning of her scientific career. She obtained her Ph.D. under the direction of Prof. Sebastián Cerdán at the Biomedical Research Institute of Madrid (CSIC/UAM) in 2001. During this period she complemented her training with short stays at different institutions in Europe and the United States. Then she moved to the US and joined the group of Prof. Robert Gillies at the Arizona Cancer Center (University of Arizona) as Research Associate. Her work focused on the characterization of the tumor microenvironment, particularly on the development of new MRI methods to measure the extracellular pH of tumors. In 2005 she came back to Spain as “Ramón y Cajal” Investigator and joined the group of Prof. Cerdán, at the Biomedical Reseach Insitute in Madrid, where she focused on the multiparametric characterization of the tumor microenviroment by magnetic resonance imaging and spectroscopy. In 2008 she undertook a position as Clinical Scientist in the MRI facility of Hospital Ntra. Sra. del Rosario, in Madrid, and worked on the application of MR techniques to early diagnosis of cancer. Since 2011 she holds a position in the new Andalusian Centre for Nanomedicine and Biotechnology (BIONAND), in Málaga, as principal investigator and head of the Nano-Imaging facility, and since the beginning of 2014 she is also deputy director of Bionand.

Dr. Maria F. Garcia-Parajo

ICFO-Institute of Photonic Sciences, Barcelona

Nanophotonic approaches for live cell research: from nanoimaging to spectroscopy

Garcia-Parajo obtained her PhD in Physical Electronics in 1993 at Imperial College, London, UK, working on the fabrication and photoluminescence spectroscopy of quantum structures based on GaAs/AlGaAs. After acquiring extensive expertise in scanning probe microscopy at the L2M-CNRS, Bagneux, France (1993-1995) and University of Barcelona, Spain (1995-1996) she moved in 1996 to the Applied Optics group of the University of Twente, the Netherlands. In 1998 she obtained the prestigious award from the Dutch Academy of Sciences for her research on molecular optical sources for biological applications. In 2005 se moved to Barcelona as ICREA Research Professor, first hosted at the IBEC - Institute for Bioengineering of Catalonia and since July 2011 at ICFO - The Institute of Photonic Sciences, leading the Single Molecule Biophotonics group.

Garcia-Parajo research focuses on the development of advanced optical techniques to the study of biological processes at the single molecular level on living cells. The group develops near-field optical approaches and photonic antennas to provide simultaneous nanoimaging and nanospectroscopy on living cells, in combination with other far-field nanoscopy methods. Fluorescence correlation spectroscopy in ultraconfined volumes, and multi-color single particle tracking are exploited to gain access to dynamic processes down to the µs time resolution. In parallel, the group brings biophysical insight into fundamental biological questions that have important implications for health and disease, including cell biology and immunology, in close collaboration with biologists.

Dr. Javier García-Sancho

Instituto de Biología y Genética Molecular (IBGM), Universidad de Valladolid y CSIC, Valladolid, Spain

GAP, a new family of fluorescent protein probes for imaging calcium signals in intracellular organelles.

M.D. and Ph.D. Professor of Physiology in the Medical School, University of Valladolid (UVa), and Group Leader of the Cell Activation Unit of the Institute for Molecular Biology & Genetics (IBGM), a joint venture of the UVa and the Spanish Research Council (CSIC). Member of the Academia Europaea.

Our group's main focus is on Cell Activation, especially on aspects related to the role of Ca^{2+} as a second messenger. During the last 25 years our group has studied Ca^{2+} signaling in relation to physiological functions and pathophysioly of blood cells, anterior pituitary, pancreatic islets, chromaffin cells and neurons. Recently, we have developed a new family of fluorescent Ca^{2+} probes based on aequorin, which are specially suitable for monitoring calcium signaling into intracellular organelles of living cells. During the last few years we have interested on the possibility of restoring lost function by Cell Therapy procedures and we have promoted translational research in this field through the Spanish Cell Therapy Network.

For further details see: http://www.ae-info.org/ae/User/Garc%C3%ADa-Sancho_Javier

Dr. Rafael Giraldo

Department of Cellular & Molecular Biology, CIB-CSIC, Madrid

Untangling amyloid proteinopathies with a synthetic bacterial model system: the RepA-WH1 prionoid

I got a Ph. D. Biol. Sci. in 1991 (Complutense U., Madrid) on the genetics and biochemistry of plasmid DNA replication initiation, under the supervision of R. Díaz-Orejas (CIB-CSIC). Then, I spent a postdoctoral (1992-94) in the group of D. Rhodes at the MRC Laboratory of Molecular Biology (Cambridge, UK), where we studied the role of Rap1 protein in packing telomeric dsDNA, which led to the crystal structure of the first telomeric nucleoprotein complex. We also found that Rap1 promoted the assembly of parallel DNA quadruplexes by the G-rich strand of yeast telomeres, an early example of a protein chaperoning a DNA structure.

Back to CIB-CSIC, first as a postdoctoral (1995-1999) and since then as staff scientist, my main focus was on how sequence-specific DNA binding elicits substantial conformational changes in the winged-helix (WH) domains of plasmid-encoded bacterial replication (Rep) proteins. Besides this, we studied the assembly of yeast ORC initiator. We found in 2007 the way to tailor WH domains to become DNA-modulated amyloidogenic devices, having recently developed synthetic prion-like modules recapitulating essential features of mammalian amyloid proteinopathies (e.g., toxicity, chaperone modulation of conformational strains), albeit confined to a bio-safe bacterial host. Since 2010, I am a CSIC Research Professor and a member of Academia Europaea.

Web pages:
http://www.cib.csic.es/en/grupo.php?idgrupo=61
http://www.researchgate.net/profile/Rafael_Giraldo

Dr. Gabriel Gomila

Institut for Bioengineering of Catalonia (IBEC)

Dielectric polarization properties of supported biolayers measured with electrostatic force microscopy

I have a PhD in Physics from the University of Barcelona (1997) with a thesis based on the theoretical modelling of electron transport at semiconductor interfaces. Later on, I was postdoctoral researcher at three different universities in Italy, France and Spain where I specialized in the theoretical modelling of nanoscale electronic devices. In 2001 I moved to the Department of Electronics at the University of Barcelona thanks to a Ramon y Cajal fellowship, where I expanded my research interests towards the merge of electronics and biological fields, thus focusing on microsystems for biological applications on-a-chip and on Atomic Force Microscopy for the electrical study of biological samples. Since 2005 I have been Associate Professor at the University of Barcelona and since 2007 Group Leader at the Institut for Bioengineering of Catalonia (IBEC). My current research interests are centred on the understanding of bioelectrical phenomena at the nanoscale. I combine the research activities with teaching on Nanobiotechnology, Scanning Probe Microscopy, Bioelectricity and Nanomedicine at the University of Barcelona.

Dr. Juan R. Granja

Department of Organic Chemistry and Center for Research in Biological Chemistry and Molecular Materials (CIQUS)

Membrane targeting self-assembling peptides

Prof. Juan R. Granja received the PhD in chemistry from the University of Santiago de Compostela in 1988, under the guidance of Profs. Antonio Mouriño and Luis Castedo, working on the synthesis of main metabolites of vitamin D2. After twenty one months of postdoctoral studies in the group of Prof. Barry M. Trost at the Chemistry Department of Stanford University working on the synthesis of macrolides using Pd chemistry, he returned to the University of Santiago as Assistant Professor (Oct-1991). In 1992 he spent six months in the group of, at that time, Assistant Professor M. Reza Ghadiri at The Scripps Research Institute in La Jolla, starting a long and productive scientific collaboration, including several visits at The Scripps. As a consequence of this scientific collaboration, novel studies on peptide chemistry were developed, such as self-assembling peptides, peptide nanotubes, supramolecular antimicrobial agents or self-replicating processes. In 1995 he was promoted at the University of Santiago de Compostela to Associate Professor and in 2006 to Full Professor after a national habilitation in 2005. His research interest is devoted to the synthesis of complex structures by efficient methods. One of these programs is seeking for the synthesis of functional nanotubes by self-assembling process of cyclic peptides. Specially, he is interested on peptide nanotubes based on cyclic peptides that contain cyclic gamma-amino acids. These supramolecular entities are designed to achieve efficient transmembrane transport.

Dr. Silvia Hernández-Ainsa

Department of Physics (Cavendish Laboratory), University of Cambridge, United Kingdom

DNA origami nanopores for single molecule detection.

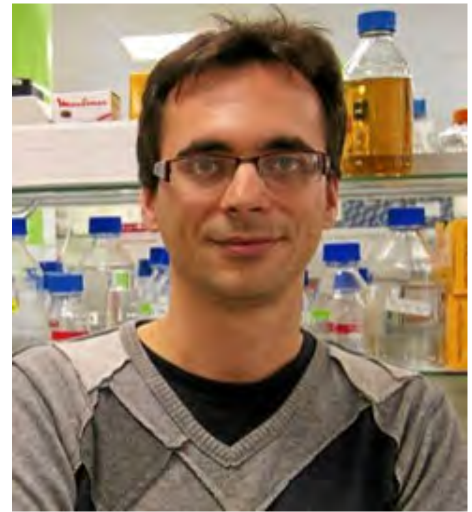
Silvia Hernández-Ainsa obtained her PhD in Chemistry in February 2011 from the University of Zaragoza (Spain), where she worked at the Liquid Crystals and Polymer group at the Department of Chemistry. Her PhD thesis was focused on the development of novel functional materials based on ionic liquid crystalline dendrimers. These systems showed interesting properties for applications in diverse scientific fields including material science and nanomedicine. In March 2011 she joined the group of Dr. Ulrich Keyser at the Department of Physics (Cavendish Laboratory), University of Cambridge (UK), as a research associate working on nanopore technology for single molecule detection. She developed different chemical methodologies to improve the capabilities of these devices.

Since February 2014, she is a Herchel Smith Research Fellow at the Department of Physics, University of Cambridge (UK). Her current research involves the fabrication of new nanopores based on DNA origami nanostructures for several applications in biotechnology and nanomedicine.

Dr. Aitor Hierro

CIC bioGUNE

Escape from death, strategies for intracellular survival by *Legionella pneumophila*



Dr. Aitor Hierro got his PhD degree in biochemistry in 2002 at the University of the Basque Country (UPV/EHU). Between 2002 and 2007, he conducted his postdoctoral research under the supervision of Prof. James H. Hurley at the National Institutes of Health (Bethesda, USA) where he specialized on the structural characterization of multiprotein complexes involved in membrane trafficking. In 2008, Dr. Hierro joined CIC bioGUNE (Bilbao) and began his independent research work on structural aspects of endosomal trafficking regulation and its role in physiological malfunction and disease. This research concentrates on the mechanisms and specific interactions during selective recruitment of cargo molecules from endosomes, and how the trafficking routes in this compartment are exploited by toxins and pathogens.

Dr. Pablo Huertas

CABIMER/University of Seville

High resolution methods to analyze the repair of broken DNA.

Pablo Huertas studied Biology at the University of Seville, where he also obtained his PhD in Molecular and Cellular Biology in 2004 under the supervision of Andrés Aguilera. From 2004 until mid 2010 he worked as a postdoc in the laboratory of Stephen P. Jackson in The Gurdon Institute, University of Cambridge, United Kingdom. Since June 2010 he is a group leader at the Andalusian Center of Molecular Biology and Regenerative Medicine (CABIMER) and he is also part of the Department of Genetics of the University of Seville.

During his career, his research topics have evolved from the relationship of RNA metabolism with genomic instability in the budding yeast *Saccharomyces cerevisiae*, through the regulation of homologous recombination by the cell cycle in yeast and human cellular systems, to the repair of DNA double strand breaks in human cells. Nowadays, he is focused in understanding the complex regulatory network that control the choice between different DNA repair pathways, both at the cellular and organismal level, and the repercussions of altering such choice in terms of human disease.

Dr. Borja Ibarra

IMDEA Nanociencia

Understanding how biological molecular motors work: identification of the translocation step of a replicative DNA polymerase

Borja Ibarra received his Ph.D. in Molecular Biology from Universidad Autónoma Madrid. He made the 'leap' to molecular biophysics as a postdoctoral fellow at University of California, Berkeley, where he used optical tweezers to study at single-molecule level the dynamics of molecular motors involved in DNA replication. Back in Spain, he applied the optical tweezers technology at IMDEA Nanoscience to study the physical-chemical and mechanistic principles that govern the operation of biological molecular motors at the nanoscale.

Recent Publications

- J.A. Morin, F.J. Cao, J.M. Lázaro, J.R. Arias-Gonzalez, J.M. Valpuesta, J.L. Carrascosa, M. Salas, **B. Ibarra** (2015). Mechano-chemical kinetics of DNA replication: identification of the translocation step of a replicative DNA polymerase. Nucleic Acid Research DOI: 10.1093/nar/gkv204
- J.A. Morin, F.J. Cao, J.M. Lázaro, J.R. Arias-Gonzalez, J.M. Valpuesta, J.L. Carrascosa, M. Salas, **B. Ibarra** (2012). Active DNA unwinding dynamics during processive DNA replication. PNAS, USA 109(21): 8115-20.
- Morin J.A., Cao F.J., Valpuesta J.M., Carrascosa J.L., Salas M., **Ibarra B.** (2012). Manipulation of single polymerase-DNA complexes: A mechanical view of DNA unwinding during Cell Cycle 11(16): 2967- 2968.
- Ibarra B., et al. (2009). Proofreading Dynamics of a Processive DNA Polymerase. EMBO J., 28: 2794 – 2802.

Dr. Juan S. Jiménez

Universidad Autónoma de Madrid. Applied Physical Chemistry Department

A surface plasmon resonance study of the DNA interaction with Tau protein and amyloid peptides, involved in the molecular basis of Alzheimer´s disease.

Juan S. Jiménez graduated from Murcia University. He received his PhD at the Granada University. Along 1978 and 1979 he joined the group of Prof. Shmuel Shaltiel as a postdoctoral fellow at the Weizmann Institute of Science, Israel. In 1982 he moved to Autonomous University of Madrid. At present he is a Physical Chemistry Professor at the Applied Physical Chemistry Department of this University.

Research in the Prof Jiménez's laboratory has been focused on the structure-function relationship of enzymes and proteins. Over the last ten years they have been particularly interested in the molecular basis of Alzheimer's Disease. This severe disorder is characterized by the presence in the brain of two types of aberrant structures: intraneuronal neurofibrillary tangles and extracellular senile plaques. The former are composed mainly of tau protein, while the main component of senile plaques is amyloid peptide, Aβ. The Prof Jiménez's group has developed a Surface Plasmon Resonance set-up which allows the study of DNA-peptide/protein interactions. Results obtained by means of this technique have led them to pose the hypothesis that an alteration of transcription provoked by an interaction of amyloid peptides with nucleic acids, in which tau protein is somehow implicated, may represent an important contribution to the origin of Alzheimer's Disease.

Dr. Elena Junquera González

Department of Physical Chemistry I, UCM. (Universidad Complutense of Madrid)

What May Colloidal and Supramolecular Chemistry Provide in Gene Therapy?

Elena Junquera is co-leader of the Group of Colloidal and Supramolecular Chemistry, recognized by CAM as an excellent group, with a scientific trajectory of more than 25 years involved in the physicochemical characterization of colloidal and supramolecular systems as vectors or solubilizing agents of substrates with biological interest. She got the PhD degree in 1992 (UCM) and afterwards she was involved in two post-doctoral stays, the first in 1994-95 in the Carbohydrates Group (CSIC, Madrid) working on the characterization of the carbohydrate-carbohydrate interaction in aqueous media from a physicochemical stand point, and the second one at the University of California Irvine (UCI) in 1997-98, involved in the study of the folding of artificial proteic βeta-sheets in competitive media (peptide-mimic chemistry). Since then, she has opened different researching lines in her group, all having in common a clear multidisciplinary approach to biophysical events. Nowadays, her most active research is focused on searching efficient and safe non-viral gene (DNA and siRNA) vectors, with better outcomes than the viral ones, an important challenge in the field of gene therapy. For that purpose, rigorous and well designed biophysical and biochemical studies are necessary, reason for what Prof. Junquera has well established collaborations with a number of prestigious national and international groups of organic chemists, theoretical physics and biochemists.

Dr. Melike Lakamyali

ICFO – Institut de Ciències Fotòniques, Barcelona

Super-resolution imaging of nucleosome organization

Dr. Lakadamyali received her PhD in 2006 from Harvard University. She carried out her postdoctoral training in the Center for Brain Science at Harvard University. She started her independent group at ICFO-Institute of Photonic Sciences in 2010. Her research focuses on the development of advanced light microscopy methods that provide high spatial and temporal resolution and the application of these methods to studies of fundamental questions in cell biology and neuroscience. In particular, her goal is to adress a central question in biology: how does the organization of proteins in space and in time impact their cellular function. To study this key question she combines cutting-edge optical and molecular-biology tools such as super-resolution nanoscopy, single molecule biophysics, microfluidics, genetic manipulation and labeling, biophysical modeling and quantitative statistical analysis. Since she joined ICFO, she has published several original papers in top peer-reviewed journals including PNAS, Nature Methods, Cell. She has received multiple European research grants. Of note is the European Research Council Starting Grant, ERC-StG. She has also received the European Molecular Biology Organization (EMBO) Young Investigator award.

Dr. Iván López Montero

Physical Chemistry, Universidad Complutense de Madrid

Cell mechanics as a biophysical biomarker

Iván López Montero completed his B.Sc. in Condensed Matter Physics at Universidad Autónoma de Madrid (UAM) in 2001. Supervised by Prof Philippe F. Devaux at Institut de Biologie Physico-Chimique (CNRS) and Marisela Vélez (UAM); his PhD thesis (2006, Université Paris 7) focused on lipid asymmetry, the flip-flop of ceramides as well as the biological implications of the enzymatic conversion of sphingomyelin into ceramide. He joined the group of Prof. Francisco Monroy at the Universidad Complutense de Madrid (UCM) as a Juan de la Cierva fellow. His research efforts focused on the mechanics of model lipid membranes, with the long-term goal being to infer the mechanical role of lipid membranes in different biological processes such as apoptosis and bacterial cell division. In 2013, he was awarded with an ERC Strating Grant from the European Research Council to go deep into the fabrication of membrane-based bioinspired artificial systems able to interact with cultured cells, with the final goal to improve the treatments currently available for mitochondrial diseases. From 2014, Iván López Montero is a Ramón y Cajal researcher at UCM.

Dr. Víctor A. Lórenz-Fonfría

Institute of Experimental Physics, Department of Physics, Freie Universität Berlin

Dynamics and function of membrane transporters and ion channels by vibrational spectroscopy

Degree in chemistry (1997) by the Universitat de València and PhD in biochemistry (2003) by the Universitat Autònoma de Barcelona (UAB), the latter under the supervision of Prof. Padrós. Postdoctoral stage for over 3 years in the group of Prof. Kandori (Department of Material Science, Nagoya Institute of Technology, Japan), with the support of a Fellowship from the Japanese Society for the Promotion of Science. In 2008, moved to the Center for Biophysical Studies (UAB, Spain), awarded by a Marie Curie Reintegration Grant. From 2011 in the group of Prof. Heberle, at the Institute of Experimental Physics of Freie Universiät Berlin. In 2014 awarded by the Ramon y Cajal program to continue his research from the end of 2015 at the Universitat de València.

His interests include the dynamics and function of membrane proteins from a molecular perspective. His research mostly relies on state-of-the-art steady-state and time-resolved spectroscopies, mostly vibrational techniques, combined with advance data analysis. The main systems he studied so far include the light-gated ion channels channelrhodopsin-1 and channelrhodopsin-2; the proton/Cl^- pumps bacteriorhodosin and halorhodopsin; the GPCR photoreceptor visual rhodopsin; the Na^+-coupled sugar transporter melibiose permease and the mitochondrial ADP/ATP exchanger.

Author of 38 original research publications, 2 reviews and 1 book chapter, he is the first author in 24 publications and corresponding author in 14 publications.

Dr. Irene Luque

Department of Physical Chemistry and Institute of Biotechnology. University of Granada, Granada, Spain

Structural and thermodynamic studies of viral Late domain interactions: towards the development of host-oriented therapeutics.

Irene Luque Fernández obtained her PhD at the University of Granada in 1998. She worked at the Department of Biology and Biocalorimetry Center at The Johns Hopkins University from 1998-2001 on the development of structural-thermodynamic parameterizations for the prediction of binding energetics and its application to the study of molecular basis of protein cooperativity and resistance to HIV-1 protease inhibition. In 2002 she enrolled back to the University of Granada as a Ramón y Cajal Researcher. Currently, she is Professor of Physical Chemistry and research fellow at the Institute of Biotechnology in the University of Granada. Her work is devoted to the structural and thermodynamic analysis of the molecular determinants of proline-rich sequence recognition by protein-protein interaction domains. Specifically, during the last years her research has been focused on the identification and development of inhibitors of viral Late domain interactions with their cellular partners (Tsg101-UEV and Nedd4-WW3 domains) with potential as wide-spectrum host oriented therapeutic agents.

Dr. Maria. J. Macias

Structural and Computational Biology, IRB Barcelona

Evolution and Structural analysis of Smad domains

Maria J. Macias es doctora en Ciencias Químicas por la Universidad de Salamanca 1993. Comenzó su carrera como investigadora independiente gracias a una posición de "Staff-Scientist" que ganó en el año 1998, en el EMBL-Heidelberg. En ese periodo avanzó en el estudio estructural de proteínas involucradas en procesos de señalización celular. Obtuvo la posición de profesora de investigación en la primera convocatoria ICREA, y se trasladó al IRB Barcelona en 2002. Hasta la actualidad es la investigadora principal del grupo de Complejos macromoleculares del departamento de estructuras y biocomputación en el IRB Barcelona.

Recientemente su grupo ha descrito los determinantes estructurales que controlan la regulación de los R-Smads en el núcleo. Descubrimos que las parejas de domínios WW presentes en las proteínas que interaccionan con los Smads funcionan como controladores de información. En colaboración con el grupo del Dr. J. Massagué (Sloan Kettering Institute, NY) resolvimos el patrón de fosforilaciones en la región interdominios que representa un código de lectura, que marca a los Smads para activar al máximo su función de transcripción o para su eliminación.

En la actualidad planeamos descifrar un nuevo motivo de interacción de los Smads y el promotor goosecoid, para poder clarificar el papel de TGF beta en los pasos que determinan el comienzo de la diferenciación celular. Para ello utilizaremos un enfoque funcional y estructural combinando RMN y cristalografía de rayos X. A más largo plazo estamos interesados en descubrir y caracterizar las interacciones de complejos de Smads, otros cofactores y promotores, para determinar los puntos vulnerables de esta cascada de señalización y su implicación en cáncer.

Dr. Julia Maldonado-Valderrama

Applied Physics Department. University of Granada.

Impact of interfacial structure on digestibility of food emulsions.

I am a senior lecturer (Ramon y Cajal Reintegration Programme) at the Department of Applied Physics at the University of Granada. My current research line deals with the physicochemical characterization and colloidal modelisation of new materials with biotechnological interest (food colloids, proteins and DNA). My research is multidisciplinary and applied and in this line I have coordinated 4 research projects. As a researcher I have participated in 8 national research projects and 2 EU-projects as well as in 4 contracts with companies (Petróleos de Venezuela SA and BIOSEARCH LIFE). I have coauthored 38 articles in peer-reviewed journals and 1 patent (P201001588, submitted) which have provided to date 654 citations and an H index of 16 (WOS). I have worked in research laboratories in Germany, France and UK, where I worked for 4 years at the Institute of Food Research in Norwich, UK, funded with a Marie Curie Intra European Fellowship. Therein I started the research line of digestion of food structures which I have continued to develop at the University of Granada. Currently, I impart lectures in the Biochemistry, Biotechnology, Food Science and Physics Degrees and I have co-supervised 3 PhD students at the UGR. I have two sons born in 2012 and 2014.

Dr. Mario Mellado

Dpt. Immunology & Oncology, CNB/CSIC

Studying chemokine receptor conformations and dynamics at the cell membrane

Dr. Mario Mellado (University of Alcalá, 1990) initiated his scientific career in 1991 at Pharmacia Iberia, SL. In 1993, he joined the Department of Immunology and Oncology (DIO) at the National Center for Biotechnology/CSIC and initiated his independent research group studying the chemokine/chemokine receptor biology. He described for the first time that the chemokines activate the JANUS kinase family and that the chemokine receptors form homo and heterodimers at the cell surface. In this period stand out more than 50 publications, 4 patents and he supervised 4 PhD theses. In 2005, he obtained a permanent position at the CSIC and he incorporated to the chemokine receptor analysis, new image techniques based on resonance energy transfer (FRET and BRET). He thus confirmed the existence of dynamic chemokine receptor complexes. At this second stage he supervised 7 PhD theses, published 50 manuscripts in international peer-review journals and wrote 1 patent. He then became interested in more translational aspects of his research allowing him to be part of two European consortia funded in the context the 7th framework program and two national consortia for studying rheumatic diseases that brings him closer to clinical research.

He is now interested in TIRF-M technology (total internal reflection microscopy) as allows him to analyze the dynamics of chemokine receptors at the cell membrane and to define new therapeutic tools based on chemokine receptor oligomerization.

Personal web site.

Dr. Fernando Moro Pérez

Unidad de Biofísica (CSIC/UPV-EHU) y Departamento de Bioquímica y Biología Molecular, Facultad de Ciencia y Tecnología, Universidad del País Vasco (UPV/EHU)

Chaperoning protein aggregates: the disaggregase machinery

I obtained my degree in Chemistry in 1990 at the University of the Basque Country UPV/EHU. Between 1991-1996, I worked in the group of Professor Felix Goñi, characterizing a membrane protein from the conjugative plasmid R388 that encodes a type IV secretion system of bacterial DNA. After obtaining my PhD in Biochemistry in 1996, I joined the laboratory of Professor Walter Neupert in the Ludwig Maximilians Universität in Munich, as a Marie Curie postdoctoral. My work focused on characterization of the protein translocation machinery in the mitochondrial inner membrane. One of the essential components of this machinery is a chaperone; the mitochondrial Hsp70 that builds the molecular motor that pulls protein precursors across the membrane. Hsp70 proteins, as well as other chaperones, are required to maintain protein homeostasis in the cells. This turned my scientific interest into the study of the mechanism of molecular chaperones. In 2001, I returned to the UPV/EHU and joined the group of Professor Arturo Muga as a postdoctoral researcher until 2007, studying the chaperone system formed by DnaK, the main bacterial Hsp70, and its cochaperones DnaJ (Hsp40) and GrpE. In 2007, I obtained a Ramón y Cajal research position, and in 2011 a permanent researcher position in UPV/EHU. The line of research developed over these years has continued the characterization of the functional mechanism of bacterial and yeast Hsp70 and Hsp40 chaperones, and their association to Hsp100 disaggregases that allows solubilization and refolding of proteins aggregates formed after exposure to stress conditions. Currently, I have started a new line of research to study the reactivation of protein aggregates by the human chaperones Hsc70 (Hsp70), Hdj1 (Hsp40) and Apg2 (Hsp110).

Dr. Pilar de la Peña Cortines

Department of Biochemistry and Molecular Biology. University of Oviedo, Edificio Santiago Gascón. Campus de El Cristo. Oviedo, Spain

Covalent link between the voltage-sensing module and the pore domain is not required for voltage-depending gating in hERG K^+ channels

Full Professor of Biochemistry and Molecular Biology. University of Oviedo.

Research lines: G-protein-coupled receptors. TRH receptor. Hormonal regulation and structure-function relationships of hERG K^+ channel. Molecular architecture of hERG K^+ channel in vivo. Intramolecular interactions and functional coupling of hERG K^+ channel.

Representative articles published in the last years:

-P. Miranda, D. G. Manso, F. Barros, L. Carretero, T. E. Hughes, C. Alonso-Ron, P. Domínguez, P. de la Peña (2008). "FRET with multiply labeled hERG K^+ channels as a reporter of the in vivo coarse architecture of the cytoplasmic domains". Biochim. Biophys. Acta – Mol. Cell. Res. 1783:1681-1699.

-P. de la Peña, C. Alonso-Ron; A. Machín, J. Fernández-Trillo, L. Carretero; P. Domínguez, F. Barros (2011). "Demonstration of physical proximity between the amino terminus and the S4-S5 linker of the hERG potassium channel". J. Biol. Chem. 286:19065-19075

-P. de la Peña, A. Machín, J. Fernández-Trillo, P. Domínguez, F. Barros (2013). "Mapping of interactions between the amino and carboxy termini and the channel core in hERG potassium channels". Biochem. J. 451:463-474.

-P. de la Peña, A. Machín, J. Fernández-Trillo, P. Domínguez, F. Barros (2014). "Interactions between the N-terminal tail and the gating machinery of hERG K^+ channels during conformational rearrangements between closed and open/inactive states". Pflugers Arch-Eur J. Physiol. DOI 10.1007/s00424-014-1612-1.

Dr. Felix Ritort

Small Biosystems Lab, Universitat de Barcelona

Measuring binding affinities using force methods.

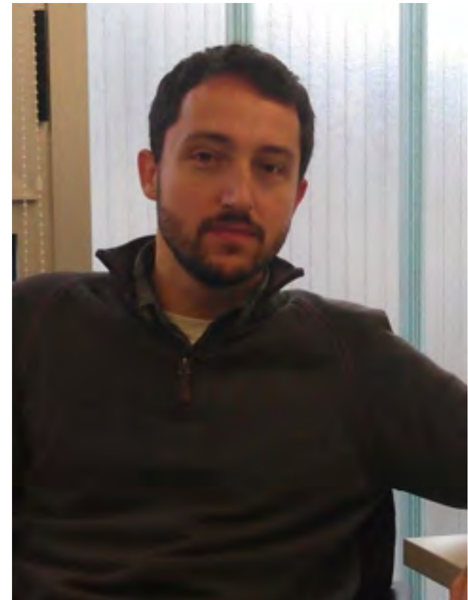
Dr. Felix Ritort carried out his PhD during the years 1989-1991 in theoretical physics in the area of statistical physics. During the years 1992-2002 he made several contributions to the field of disordered systems and nonequilibrium physics. Since 2002 he worked in single-molecule biophysics by manipulating individual nucleic acids and proteins to investigate energy processes in the molecular world. Ritort's group is recognized worldwide as leader in applying the finest and most powerful methods to extract accurate quantitative information about thermodynamics and kinetics of molecular interactions. Dr. Ritort has been awarded several prices for his research: the Distinció de la Generalitat de Catalunya in 2001 for his theoretical research during the years 1991-2000; ICREA Academia Award 2008 for his research as scholar at the University of Barcelona; Premio Bruker 2013 from the Sociedad de Biofísica de España for his contributions to molecular biophysics research in Spain. He is also chaor of the Division of Physics for Life Sciences of the European Physical Society.

My scientific research is highly multidisciplinary at the frontiers of physics, chemistry and biology. My lab is a worldwide reference in combining theory and experiments to investigate the thermodynamics and nonequilibrium behavior of small systems using single molecule methods. I apply the finest concepts and tools from statistical physics to extract valuable information about a wide range of molecular processes: from the energetics of nucleic acids to the kinetics of formation of molecular aggregates induced by drugs or the elasticity of antigen-antibody bonds in the immune system. A recurring theme in my research is the understanding of how molecular systems embedded in highly noisy environments outperform the efficiency of macroscopic systems: being small has key advantages that nature has fruitfully exploited. Recently I have directed my interest to the discovery of the principles that govern the emergent complexity of evolutionary ensembles in the molecular and cellular world.

Dr. Pere Roca-Cusachs

Institute for Bioengineering of Catalonia (IBEC), University of Barcelona

Understanding the cell-extracellular matrix mechanical link: from molecular roles to emerging behaviors.



Pere Roca-Cusachs obtained his PhD in cellular biophysics in 2007 from the Medical School at the University of Barcelona. He then worked in the lab of Prof. Michael Sheetz (Department of Biological Sciences, Columbia University) as a post-doctoral researcher until 2011. In 2011, He joined the University of Barcelona as a tenure-track lecturer. In 2012, he obtained a position as junior group leader at the Institute for bioengineering of Catalonia (IBEC). His research focuses on unraveling the physical and molecular mechanisms by which cells detect and respond to mechanical force.

Dr. Verónica Salgueiriño

Departamento de Física Aplicada, Universidade de Vigo

Synergy effects of magnetic silica nanostructures for drug and heat delivery applications.

Verónica Salgueiriño obtained her Ph.D. degree in 2003. Her work, supervised by Luis M. Liz-Marzán, focused on silica-coated gold nanoparticles in particular ordered arrays, which allowed the study of the optical properties displayed. After two years of post-doc, first in Universität Duisburg-Essen (Germany) (group of M. Farle), then in Ira A. Fulton School of Engineering - Arizona State University, (USA, group of R. Díaz), she joined the Nanomag Group (Universidade de Santiago de Compostela, group of J. Rivas / M. A. López-Quintela) as a IPP researcher to work on incorporating diverse functionalities into a single nanoparticle. In 2008 she was awarded the L'Oréal-UNESCO (Women in Science) National Award. In March 2009 she joined the Universidade de Vigo as a Ramon y Cajal fellow leading the Magnetic Materials group she initiated. She is assistant professor (tenured) at the department of Applied Physics, since March 2014. Her group research targets the development and physical and chemical characterization of new magnetic nanoparticles and nanocomposites, with particular focus on interfaces between transition metal oxides.

Dr. José M. Sánchez-Ruiz

Departamento de Química Física, Universidad de Granada

Using ancestral resurrection to probe evolutionary protein

Degree in Chemistry. University of Granada (Spain). 1979. Ph.D. in Physical Chemistry. University of Granada, 1983. Fulbright Scholar: 1984-1986. The Medical College of Virginia (USA). Current position: Professor of Physical Chemistry, University of Granada. Member of the Editorial Board of Biophysical Chemistry (2004-2010). Member of the Editorial Board of Biochemical Journal (2008- in active). Academic Editor of PLOS ONE (2012- in active).

Research_interests:
Relation structure-energetics in proteins. Protein folding. Solvent effects on protein stability. Electrostatic contributions to protein stability. Rational design of thermostable proteins. Residual structure in denatured states. Protein evolution. Protein design.

Industrial experience: Collaboration agreements with Novozymes and Noricum (a Spanish company devoted to the development of applications of bone morphogenetic proteins).
-13 invited talks in scientific meetings in the period 2010-2014).

Selected publications from the last five years:
-Risso et al. (2014). Molecular Biology and Evolution, doi: 10.1093/molbev/msu310.
-Zou et al. (2014) Molecular Biology and Evolution, doi: 10.1093/molbev/msu281.
-Ingles-Prieto et al. (2013). Structure 21, 1690-1697.
-Risso et al. (2013). Journal of the American Chemical Society 135, 2899-2902.
-Kosuri et al. (2012). Cell 151, 794-806.
-Garcia-Seisdedos et al. (2012). PLoS Computational Biology 8(6):e1002558.
-Perez-Jimenez et al. (2011). Nature Structural and Molecular Biology 18, 592-596.
-Sanchez-Ruiz (2011) Annual Review of Physical Chemistry 62, 231-255.
-Naganathan et al. (2010). Journal of the American Chemical Society 132, 11183-11190.
-Sanchez-Ruiz (2010). Protein kinetic stability. Biophysical Chemistry 148, 1-15.
-Pey, D. et al. (2010). Modulation of buried ionizable groups in proteins with engineered surface charge. Journal of the American Chemical Society 130, 7489-7495.

Dr. Álvaro Somoza

IMDEA Nanociencia

Oligonucleotides and Nanostructures in Nanomedicine.

Álvaro Somoza did his PhD at the Universidad Autónoma de Madrid in the group of Prof. Carmen Carreño focused on the total synthesis of natural compounds. Then, he joined the group of Prof. Eric T. Kool at Stanford University where he worked on the preparation of modified ribonucleosides to study the role of hydrogen bonding interactions between RNA strands in RNA interference. Later on, he moved back to Spain and joined the group of Prof. Ramón Eritja at the Institute for Research In Biomedicine Barcelona. There, he studied the interaction of hydrophobic moieties in RNA derivatives with protein complexes involved in the RNA interference process. In 2009 he joined IMDEA Nanociencia and in 2014 was promoted to Senior Researcher. His research projects are focused on the use of modified oligonucleotides and nanostructures in diverse applications such as the preparation of structured materials as well as systems to detect and treat different diseases.

Dr. Pablo Taboada

University of Santiago de Compostela, Condensed Matter Physics Department

Amyloid fibrils: Not only a marker of disease but also an exciting nanomaterial.

Born in 1974, Pablo Taboada obtained his PhD in Physics at the University of Santiago de Compostela in 1999 where he is nowadays an Associate Professor with Professorship Habilitation at the Condensed Physics Department. He is author of more than 130 papers in reputed international scientific journals, has leaded/participated in more than 30 international and national research projects and is recipient of several grants and awards. His current main research interest are focused on the obtention of hybrid nanoparticles for simultaneous diagnosis and therapy (theranostics), development of hybrid thin film nanostructures for sensing and energy applications, and the analysis of the self-assembly process of (bio)polymers (for example, protein fibrillation) and their prospective applications.

Dr. Félix Viana

Instituto de Neurociencias de Alicante UMH-CSIC

Molecular dissection of cold temperature sensing in mammalian neurons

Tenured Investigator CSIC, is co-Director of the Sensory Transduction and Nociception Group at the Instituto de Neurociencias de Alicante.

Trained with Albert Berger and Bertil Hille (University of Washington, USA) and Bernd Nilius (KULeuven, Belgium) on various aspects of the physiology and pharmacology of cationic and anionic channels in excitable and non-excitable cells.

His research group is interested in the transduction of somatosensory stimuli, specially pain and temperature, by peripheral sensory neurons. Recently they have focused on the phenotypic diversity of mammalian cold thermoreceptors, the role of TRPA1 channels in neuroinflammation and structure-function relationships of TRPM8 channels.

SBE 2015 Awards

Bruker Prize 2015

Dr. Juan A. Hermoso

Instituto de Física-Química Rocasolano. Dept. of Crystallograpy and Structural Biology

Structural advances in the pneumococcal divisome

Research Professor CSIC at Department of Crystallography and Structural Biology (CBE) of Instituto de Química-Física "Rocasolano". CSIC. Madrid. Born in León 10 October 1964.

Research lines: The research interests in my group encompass studies in the mechanisms of virulence and pathogenesis and the means of circumvent them. We focus on the structural biology of the bacterial surface proteins involved in key functions, such as host-pathogen interactions, division, antibiotics resistance, pathogenesis mechanisms or the remodeling of the peptidoglycan framework, which represent important mechanisms in the process of disease. More information.

Author of more than 140 publications in international journals and co-author of 5 books. According to ISI's Web of Science (on Sept 2014) there were 2811 citations (H-index = 30) citations to my work. Councilor of the Spanish Synchrotron Radiation Users Organization Committee (AUSE) 2003-2011; Councilor of the Spanish Group of Crystallography (GEC) 2000- 2010; Scientific Comity of the Spanish beamline BM25 at the ESRF (Grenoble) 2007-2014; Scientific Committee (Sub-Committee 8 (Biology)) of ILL (Grenoble) 2009-2010, among others.

Selection of most recent articles (since 2005):

- Bartual et al., ***Nature Communications***, (2014) 5.
- Fishovitz et al., ***JACS*** (2014) 136, 9814-9817.
- Otero et al., ***Proceedings of National Academy of Sciences USA***, (2013) 110, 16808-16813.
- Saleh et al ***EMBO Molecular Medicine***, (2013) 5, 1852-1870.
- Lee et al., ***JACS*** (2013) 12605-12607.
- Martínez-Caballero et al. ***JACS*** (2013) 135, 10318-10321.
- Pérez-Dorado et al., ***Nature Structural & Molecular Biology***, (2010) Vol.17, N 5; 576-581.
- Molina et al., ***EMBO reports***. (2009). 10, 246-251.
- Hermoso et al., ***Current Opinion in Microbiology***, (2007), 10:1-2.
- Hermoso et al., ***Nature Structural & Molecular Biology***, (2005) Vol.12, N 6; 533-538.

Enrique Pérez Payá Prize (SBE-40) 2015

Dr. Irene Díaz-Moreno

Institute of Biochemistry and Molecular Biology, cicCartuja, University of Seville - CSIC

Biointeractomics of Cytochrome c: From Transient Life to Stable Death

Dr. Irene Díaz-Moreno is Associate Professor of Biochemistry and Molecular Biology at the Institute of Plant Biochemistry and Photosynthesis – IBVF of the Scientific Research Centre Isla de la Cartuja – cicCartuja, in Seville (Spain).

She was awarded with her Ph.D. with European mention from the University of Seville, Spain in 2005. Dr. Irene Díaz-Moreno has worked in collaboration with groups at the Universities of Göteborg (Sweden) and Leiden (The Netherlands), on molecular recognition between metalloproteins involved in electron-transfer processes. She was an EMBO postdoctoral fellow (2006 – 2008) at the NIMR-MRC in London (UK), working on the regulatory mechanisms of mRNA decay by RNA-binding proteins. In 2010, she won a permanent position at the University of Seville, where she is developing research projects on Biointeractomics field, as well as on the post-translational regulation of biological macromolecules.

More information

The significance of all her work has been published in high impact journals such as *Nature Structural & Molecular Biology*, *Nucleic Acids Res.*, *Chemistry and Biology*, *Structure* and *J. Biol. Chem.*, among others.

SBE-33 Prize 2015: Dr. Cecilia Artola Recolons

Instituto Química Física "Rocasolano", CSIC. Department of Crystallography and Structural Biology

Structural biology of macromolecular machines involved in Peptidoglycan recycling and their implications in antibiotic resistance

I have worked as a Postdoctoral fellow in the Spanish National Research Council, and I did my Ph.D. with Prof. Dr. Juan A. Hermoso, in the Department of Crystallography and Structural Biology. My Ph.D project was in collaboration with Prof. Dr. Shahriar Mobashery from the University of Notre Dame (Indiana, USA), where I had the opportunity to learn science during my research stay in 2012.

I have a B.S. in Chemistry, by the University of Oviedo (2009) and in 2008 I worked as a research assistant with Dr. Jason Halfen (University of Wisconsin, USA).

The relationship between cell wall recycling and antibiotic resistance has been proved essential to look for new antibiotic targets in bacteria. My Ph.D. thesis research is focused on understanding the mechanism of key proteins involved in the recycling process of the bacterial cell wall, analyzing their structural biology and different functions, using X-ray crystallography and bioinformatics techniques.

In Gram-negative bacteria, the recycling process serves to control induction of the expression of beta-lactamases. Although there are many enzymes involved in this process, there are some key steps that need of certain proteins. The first step is the cleavage of the saccharide bonds that conform the PG. This work is done by the lytic transglycosylases (LTs).

This work presents the crystallographic structure of two essential Lytic transglycosylases. MltE (*Artola-Recolons* et al. **Biochemistry**, 2011) was the first endoactive lytic transglycosylase ever discovered, and we have proposed it to be the enzyme starting the recycling process. The crystallographic structure shows how this protein is attached to the inner leaflet of the outer membrane and how that MltE can accommodate up to eight sugars unlike the rest of LTs.

The other LT studied, MltC (*Artola-Recolons* et al. **ACS Chemical Biology**, 2014), is able to react, in a processive way, over both cross-linked and non-cross-linked peptidoglycan chains due to its additional module, described for the first time and highly conserved in bacteria. MltC is attached to the inner leaflet of the outer membrane and has a mobile region that allows the enzyme to move around the peptidoglycan chains, which could have crucial implications in flagellar mobility and biofilm formation.

Pseudomonas aeruginosa is a human pathogen that causes infection and generalized inflammation and sepsis. It has been shown that the penicillin resistance mechanism of *P. aeruginosa* is substantially different from the rest of Gram-negative organisms. Analysis of the genes of revealed P. aeruginosa to posses three paralogous amidases: AmpD, AmpDh2 and AmpDh3.

As in the rest of bacteria, AmpD is placed in the cytoplasm. However, AmpDh2 and AmpDh3 only appear in *P. aeruginosa*, and have a periplasmic localization. Both enzymes appear to be involved in virulence of this organism. This work has shown a whole mechanism of action for both AmpDh2 (*Martínez-caballero* et al.

JACS, 2013) and AmpDh3 (*Lee* et al. **JACS**, 2013), explaining their specificity for the soluble or insoluble fraction of the Peptidoglycan.

The crystallographic structure and the complexes with cell-wall analogs were a key factor for determining the mechanism of these two enzymes. Although the structure of the monomer is very similar between AmpDh2 and AmpDh3, their quaternary structure has been proved essential to understand their role in recycling. What is more, both proteins AmpDh2 and AmpDh3 complement each other in the turnover and maturation of the cell wall.

This work tries to correlate the activity of lytic transglycosylases, with that of AmpDh2 and AmpDh3 working altogether in the periplasm of the bacteria, and performing unique activities in the PG recycling process.

Abstracts Index

Plenary Lectures

Protein thermodynamics and the biopharmaceutical industry. L1
Ernesto Freire

Probing electric polarization in biology with scanning probe microscopy: from single bacteria and viruses to DNA. L2
Laura Fumagalli and Gabriel Gomila

Modelling of FtsZ protein filaments from AFM images. L3
Pedro Tarazona, Pablo González de Prado-Salas and Marisela Vélez

Surface properties of amphiphilic peptides. L4
Gerardo D. Fidelio

Neuronal exocytosis: mechanisms and energy barriers. L5
Reinhard Jahn

Symposium Lectures

Symposium S1. **Supramolecular Complexes**

Untangling amyloid proteinopathies with a synthetic bacterial model system: the RepA-WH1 prionoid. S1-1
Rafael Giraldo, Cristina Fernández, María Moreno-del Álamo, Laura Molina-García

Smad proteins during the last 500 million years: evolution, human SNP variations and somatic mutations. An structural approach. S1-2
Maria. J. Macias

Cryo-electron microscopy at atomic resolution of macromolecular machines. S1-3
José R. Castón

Escape from death, strategies for intracellular survival by Legionella pneumophila. S1-4
Aitor Hierro

Symposium S2. **Cell and tissue biophysics**

Analysing the properties of plant surfaces: a biophysical approach. S2-1
Victoria Fernández, Antonio Heredia, and Mohamed Khayet

Cell mechanics as a biophysical biomarker. S2-2
Ruddi Rodríguez-Garcí, **Iván López-Montero**, Michael Mell, Gustavo Egea, Nir S. Gov and Francisco Monroy

Nanophotonic approaches for live cell research: from nanoimaging to spectroscopy. S2-3
María García-Parajo, Mathieu Mivelle, Thomas S. van Zanten and Carlo Manzo

Imaging the tumor microenvironment. S2-4
María Luisa García Martín

Symposium S3. **Protein folding, misfolding and stability**

Using ancestral resurrection to probe evolutionary protein biophysics. S3-1
José Manuel Sánchez Ruiz

A surface plasmon resonance study of the DNA interaction with Tau protein and amyloid peptides, involved in the molecular basis of Alzheimer's disease. S3-2
Juan Salvador Jiménez, Sergio Camero, Alejandro Barrantes, María J. Benítez, Félix Hernández, Jesús Ávila, Pedro Navarro, Ángel García-Lucas, José M. Ayuso, Raquel Cuadros.

Engineering macromolecular assemblies from monomeric proteins: learning from molecular evolution. S3-3
Luis A. Campos Prieto, Beatriz Ibarra-Molero, Federico M. Ruiz, Sara Alvira, Mourad Sadqi, Rajendra Sharma, Jörg Schönfelder, Carlos Alfonso, Germán Rivas, José M. Valpuesta, Antonio Romero, José M. Sánchez-Ruiz & Victor Muñoz

Chaperoning protein aggregates: the disaggregase machinery. S3-4
Alejandra Aguado, José Angel Fernández-Higuero, Yovana Cabrera, **Fernando Moro** and Arturo Muga

Symposium S4. **Lipid and membrane biophysics**

Insights into the dynamics and function of membrane transporters and ion channels by vibrational spectroscopy. S4-1
Victor Lórenz-Fonfría

Membrane targeting self-assembling peptides. S4-2
Juan R. Granja, Nuria Rodríguez-Vázquez, Alberto Fuertes, Juan M. Priegue, Eva González-Freire, Manuel Amorín and Javier Montenegro

The biophysical basis of transport carrier biogenesis at the golgi complex. S4-3
Fèlix Campelo and Vivek Malhotra

Dielectric polarization properties of supported biolayers measured with electrostatic force microscopy. S4-4
Gabriel Gomila

Symposium S5. **Protein Structure, dynamics and function**

Role of the carboxy-terminal domain of Erb1 during ribosome biogenesis in the formation of the Nop7/PeBoW complex. S5-1
Marcin Wegrecki, Jose Luis Neira and **Jerónimo Bravo**

Structural studies on co-translational protein folding and sorting. S5-2
Paola Fucini, Neha Dhimole, Andreas Schedlbauer, Borja Ochoa-Lizarralde, Tatsuya Kaminishi, Tammo Diercks and Sean Connell

Structural and thermodynamic studies of viral Late domain interactions: towards the development of host-oriented therapeutics. S5-3
Irene Luque

Exploiting protein flexibility to predict the location of allosteric sites. S5-4
Alejandro Panjkovich and **Xavier Daura**

Symposium S6. **Receptors, channels and transporters**

Molecular dissection of cold temperature sensing in mammalian neurons. S6-1
Félix Viana

Studying chemokine receptor conformations and dynamics at the cell membrane. S6-2
Mario Mellado

Covalent link between the voltage-sensing module and the pore domain is not required for voltage-depending gating in hERG K^+ channels. S6-3
Pilar de la Peña, Luis A. Pardo and Francisco Barros

GAP, a new family of fluorescent protein probes for imaging calcium signals in intracellular organelles. S6-4
María Teresa Alonso, Jonathan Rojo, Paloma Navas and **Javier García-Sancho**

Symposium S7. **Biophysics of nucleic acids**

Measuring binding affinities using force methods. S7-1
J. Camunas-Soler, A. Alemany and **F. Ritort**

DNA origami nanopores for single molecule detection. S7-2
Silvia Hernández-Ainsa, K. Göpfrich, V.V.Thacker, E. Hemmig, T. Zettl, N. A. W. Bell, K. Misiunas, U. F. Keyser

High resolution methods to analyze the repair of broken DNA. S7-3
Pablo Huertas, Andrés Cruz-García and Fernando Mejías-Navarro

Oligonucleotides and nanostructures in nanomedicine. S7-4
Álvaro Somoza

Symposium S8. **Biocolloids and biointerfaces**

What may colloidal and supramolecular chemistry provide in gene therapy? S8-1
Elena Junquera, Ana L. Barrán Berdón and Emilio Aicart

Synergy effects of magnetic silica nanostructures for drug and heat delivery applications. S8-2
Verónica Salgueiriño

Amyloid fibrils: Not only a marker of disease but also an exciting nanomaterial. S8-3
Pablo Taboada, Silvia Barbosa

Impact of interfacial structure on digestibility of food emulsions. S8-4
Julia Maldonado-Valderrama

Satelite Workshop "New & Notable in Biophysics" Lectures

Understanding how biological molecular motors work: identification of the translocation step of a replicative DNA polymerase W1
José A. Morin, Francisco J. Cao, José M. Lázaro, José M. Valpuesta, José L. Carrascosa, Ricardo J Arias-González, Margarita Salas and **Borja Ibarra**

Super-resolution imaging of nucleosome organization W2
Melike Lakadamyali, Maria Aurelia Ricci, Carlo Manzo, Maria F. Garcia-Parajo, Maria Pia Cosma

Nanomechanics of energy transduction in protein-driven membrane fission W3
Vadim Frolov, Eva Rodriguez Hortelano, Sandra Schmid and Anna Shnyrova

Understanding the cell-extracellular matrix mechanical link: from molecular roles to emerging behaviors. W4
Pere Roca-Cusachs

Structure of the p15PAF/PCNA complex and implications for clamp sliding on the DNA during replication and repair. W5
Alfredo De Biasio, Alain Ibáñez de Opakua, Gulnahar B. Mortuza, Rafael Molina, Tiago N. Cordeiro, Francisco Castillo, Maider Villate, Nekane Merino, Sandra Delgado, David Gil-Cartón, Irene Luque, Tammo Diercks, Pau Bernadó, Guillermo Montoya and **Francisco J. Blanco**

Awards Lectures

Structural insights into bacterial pathogenesis A1
Juan A. Hermoso (Bruker Prize 2015)

Biointeractomics of cytochrome c: from transient life to stable death A2
Irene Díaz Moreno (Enrique Pérez Payá Prize (SBE-40))

Structural biology of macromolecular machines involved in Peptidoglycan recycling and their implications in antibiotic resistance A3
Cecilia Artola-Recolons (SBE-33 Prize) and Juan A. Hermoso

Oral Communications

Symposium S1. **Supramolecular Complexes**

Molecular recognition of glycosaminoglycan oligosaccharides by langerin. O1-1
Jesús Angulo, Juan C. Muñoz-García, Eric Chabrol, José L. de Paz, Javier Rojo, Franck Fieschi, **Pedro M. Nieto** P1-1

New insights into the chaperones system in Mycoplasma genitalium. O1-2
Maria Adell-Morunol, Luca Martinelli, Martha Brennich, Ignacio Fita and Bárbara M. Calisto P1-2

Chitosan-gold nanocomposites: controlled size synthesis and stability. O1-3
María del Mar Collado-González, MG Montalbán, R Trigo, V Fernandez Espin, JG Hernández Cifre, J García de la Torre, G Villora, and F Guillermo Díaz Baños. P1-3

Role of decorin core protein in collagen organisation in congenital stromal corneal dystrophy (CSCD). O1-4 P1-4
Christina S. Kamma-Lorger, Christian Pinali, Juan Carlos Martínez, Jon Harris, Robert D. Young, Cecilie Bredrup, Eva Crosas, Marc Malfois, Eyvind Rødahl, Carlo Knupp, Keith M. Meek

Symposium S2. **Cell and tissue biophysics**

A mass-spectrometry-based method to study the redox state of cysteines in titin. O2-1
Elías Herrero-Galán, Carmen Suay-Corredera, Cristina Sánchez-González, Elena Bonzón-Kulichenko, Jesús Vázquez and Jorge Alegre-Cebollada P2-1

Mechanics of the cell constriction during division. O2-2
Elena Beltrán-Heredia, Víctor Almendo-Vedia, Francisco Monroy and Francisco J. Cao P2-2

The actin cytoskeleton modulates the activation of invariant NKT cells by segregating CD1d nanoclusters on antigen presenting cells. O2-3 P2-3
Juan A. Torreno-Pina, Carlo Manzo, Mariolina Salio, Michael Aichinger, Dawn Shepherd, Gurdyal S. Besra, Vincenzo Cerundolo and Maria F. Garcia-Parajo

Symposium S3. **Protein folding, misfolding and stability**

Natural ligands restore the function of a cancer-associated polymorphism in NQO1. O3-1
Encarnación Medina Carmona, Rogelio Palomino Morales, Julian F. Fuchs, David J. Timson, Ángel Luis Pey. P3-1

Understanding protein misfolding and amyloid fibril formation: sequential folding upon self-assembly. O3-2 P3-2
Nunilo Cremades, Serene W. Chen , Srdja Drakulic, Myriam Ouberai, Rocío Arranz, Carlos Alfonso, Germán Rivas, José María Valpuesta and Christopher M. Dobson

Towards an improved decomposition of time-dependent SAXS data from fibrillating proteins O3-3
Fátima Herranz-Trillo*,* Romà Tauler, Bente Vestergaard & Pau Bernadó P3-3

Mechanical force modulates the unfolding pathways of the cold-shock protein B from Thermotoga Maritima. O3-4 P3-4
Jörg Schönfelder, Raúl Pérez-Jiménez and Victor Muñoz

Symposium S4. **Lipid and membrane biophysics**

New insights into synaptotagmin-1 binding. O4-1
Ángel Pérez-Lara, Anusa Thapa, Sarah Nyenhuis, Partho Halder,Michael Tietzel, Kai Tittmann, David S. Cafiso, Reinhard Jahn. P4-1

Induction of a proton gradient across a gold-supported biomimetic membrane by electroenzymatic H2 oxidation. O4-2 P4-2
Marisela Vélez, Oscar Gutiérrez-Sanz, Cristina Tapia, Marta C. Marques, Sonia Zacarias, Inés A. C. Pereira, and Antonio L. De Lacey

Interaction of new fluorescent conjugated polyelectrolytes with model membranes: their potential use as fluorescent membrane markers. O4-3 P4-3
Zehra Kahveci, Maria José Martínez-Tomé, Rebeca Vázquez, Amalia Mira, Ricardo Mallavia and C. Reyes Mateo

The effect of cholesterol on the long-range network of interactions established among sea anemone sticholysin II residues at the water-membrane interface. O4-4 P4-4
Sara García-Linares, Ida Alm, Terhi Maula, José G. Gavilanes, J. Peter Slotte and Álvaro Martínez-del-Pozo

Symposium S5. **Protein Structure, dynamics and function**

Structure of the human TSG101-UEV domain in complex with the PTAP motif of viral L-domains. O5-1
Julio Bacarizo, Montserrat Andujar-Sánchez, Emilia Ortiz-Salmerón, Marina Plaza-Garrido and Ana Cámara-Artigas P5-1

In-vivo-like study of the excluded volume effects on the kinetics of enzymatic reactions. O5-2
Cristina Balcells, Claudia Hernández, Mireia Via, Isabel Pastor, Josep Lluís Garcés, Sergio Madurga, Eudald Vilaseca, Marta Cascante and Francesc Mas P5-2

Identification of compounds that inhibits bacterial diguanylate cyclases involved in biofilm formation from theraputics drugs. O5-3 P5-3
Helton J. Wiggers, Éverton E. D. Silva, Juliana Cheleski, Naiara U. Torres, Ederson Crusca, Marcos V. A. S. Navarro

Revisiting the riboflavin kinase catalytic cycle of bacterial FAD synthetase. O5-4
María Sebastián, Ana Serrano, Beatriz Herguedas and Milagros Medina P5-4

Symposium S6. **Receptors, channels and transporters**

Unconventional EGF-induced ERK1/2-mediated Kv1.3 endocytosis. O6-1
Ramón Martínez-Mármol, Núria Comes, Mireia Pérez-Verdaguer, Jesusa Capera, Katarzyna Styrczewska, Lluís Pujadas, Eduardo Soriano, Alexander Sorkin, **Antonio Felipe** P6-1

The role of membrane heterogeneity on receptor diffusion and function: are diffusion constants constant? O6-2 P6-2
Carlo Manzo, Juan A. Torreno-Pina, Pietro Massignan, Gerald J. Lapeyre, Jr., Maciej Lewenstein, and Maria F. Garcia-Parajo

Global characterization of the bimodular ligand binding domains of PP2249 and PP1228: two paralogous chemoreceptors from Pseudomonas putida. O6-3 P6-3
Álvaro Ortega, Andrés Corral-Lugo, Bertrand Morel and Tino Krell

Symposium S7. **Biophysics of nucleic acids**

Single-stranded RNA interaction with long human telomeric RNA unveiled at the single-molecule level. O7-1 P7-1
Irene Gutiérrez, Miguel Garavís, Santiago Casado, Carlos González, Alfredo Villasante and J. Ricardo Arias-González

Towards visualizing DNA repair at the single molecule level combining magnetic tweezers and TIRF microscopy. O7-2 P7-2
Julene Madariaga-Marcos, Mark S. Dillingham and Fernando Moreno-Herrero

Ultrafast photochemical reactions in DNA: a QM/MM study. O7-3
Jesús I. Mendieta-Moreno, Paulino Gómez-Puertas, Jesús Mendieta and José Ortega P7-3

Understanding the mechanisms of DNA condensation by the bacterial protein ParB/Spo0J. O7-4
Cesar L. Pastrana, James A. Taylor, Gemma L. Fisher, Mark S. Dillingham and Fernando Moreno-Herrero P7-4

Symposium S8. **Biocolloids and biointerfaces**

Natural extracts inhibit the lipolysis using a single drop method. O8-1
del Castillo-Santaella Teresa, Maldonado-Valderrama Julia, Rivadeneira-Ruiz Ceferino, Rondon-Rodriguez Deyanira, Cabrerizo-Vílchez Miguel Ángel and Gálvez-Ruiz MªJosé. P8-1

Functionalized nanoparticles in a biological environment: impact of protein corona. O8-2
Paola Sánchez Moreno, Daniele Maiolo, Claudia Pigliacelli, Ilaria Tirotta, Pierangelo Metrangolo,2, Giuseppe Resnati, Francesca Baldelli Bombelli P8-2

Cationic gemini surfactants induce abnormal DNA conformational change at high surfactant-DNA molar ratios. O8-3 P8-3
Elia Grueso, Edyta Kuliszewska, Emilio Roldan, Pilar Perez-Tejeda, Rafael Prado-Gotor and Brecker Lothar

Lipid-cation-DNA complexes: interfacial characterization and modeling. O8-4
Germán Luque Caballero, Teresa del Castillo Santaella, Julia Maldonado Valderrama, Manuel Quesada Pérez and Alberto Martín Molina P8-4

Posters

Functional and structural characterization of the Human mTOR Complex II P1-5
Elena Aranda Serrano, David Gil Cartón, Nuria Roldán, Jesús Pérez-Gil and Begoña García-Alvarez

The role of molecular structure in the interaction between maslinic acid derivatives and albumin P1-6
F. Galisteo-González, J.A. Molina-Bolívar, C. Carnero Ruiz, M. Medina-O´ Donnell, A. Parra

The mechanochemistry of a structural Zinc finger P1-7
Judit Perales-Calvo, Ainhoa Lezamiz and Sergi Garcia-Manyes

Towards the characterization of monoclonal gammopathy of undetermined significance by DSC and MALDI-TOF MS analyses of blood serum proteome P2-4
Francisca Barceló, Rosa M. Gomila, José L. Merino, Regina Alemany, Oliver Vogler, Ivan de Paúl; Jaume Segura, Albert Pérez-Montaña, Bernardo López, Antonia Sampol, Joan Besalduch, Teresa Gutierrez and José Portugal

Structural and Functional Characterization of MeCP2, a Protein Target Associated with Rett Syndrome P3-5
Rafael Claveria-Gimeno, Pilar Maria Lanuza, Olga de la Caridad Jorge, Sonia Vega, Olga Abian, Manel Esteller, Adrian Velazquez-Campoy

The effect of N-terminal acetylation on SDS-induced α-Synuclein amyloid aggregation. P3-6
David Ruzafa, Yuriko S. Hernández-Gómez, Giovanni Bisello, Bertrand Morel, Francisco Conejero-Lara.

Single molecule studies of the first amyloidogenic step P3-7
Fabio Castello, Salvador Casares, Maria J. Ruedas-Rama and **Angel Orte**

Comparative mutational studies reveal conservation of site-specific amino acid preferences over billions of years P3-8
Fadia Manssour-Triedo, Valeria A. Risso, Alvaro Inglés-Prieto, Raquel Godoy-Ruiz, Jose A. Gavira, Beatriz Ibarra-Molero and Jose M. Sanchez-Ruiz.

Unfolding from the N- or C- termini? Copper tells the story P3-9
Amy E.M. Beedle, Ainhoa Lezamiz, Guillaume Stirnemann and **Sergi Garcia-Manyes**

Single substitutions in the β2-α2 loop region sculpture the PrP amyloid state P3-10
Javier A. Martínez, Rosa M. Sánchez, Milagros Castellanos, Natalia Makarava, Ilia V. Baskakov, Adriano Aguzzi and María Gasset

End-product diacylglycerol enhances activity of phosphatidylinositol phospholipase C through changes in membrane lipid domain structure. P4-5
Hasna Ahyayauch, Jesús Sot, M. Isabel Collado, Nerea Huarte, José Requejo-Isidro, Alicia Alonso, and Félix M. Goñi

Effect of hyaluronan pre-treatement on the lateral structure of pulmonary surfactant interfacial films P4-6
Raquel Arroyo, Mercedes Echaide, Elena Lopez-Rodriguez, Jesús Pérez-Gil

Interactions of Cationic Peptides Derived from Galleria mellonella Decropin D-like with Membrane Models and Antimicrobial Activity P4-7
José Oñate, Marcela Manrique-Moreno, Steven Trier, Chad Lady, Rodrigo Torres, Edwin Patiño

Dynamic behaviour of tacrolimus, an immunosuppressive drug, in pulmonary surfactant films P4-8
Alberto Hidalgo, Francesca Salis, Guillermo Orellana, Jesús Perez-Gil and Antonio Cruz

Steroid molecular properties influence the biophysical state of steroid-containing membranes P4-9
Jorge J. Wenz

Localization of idebenone and idebenol in membranes by using solid-state NMR P4-10
Victoria Gómez-Murcia, Ana M. de Godos, Monika Schneider, Senena Corbalán-García and **Juan C. Gómez-Fernández**

Coordinating curvature scaffolding and membrane insertion during dynamin-mediated membrane fission P4-11
Anna V. Shnyrova, Eva Rodriguez Hortelano, Juha-Pekka Mattila, Sandra L. Schmid, Vadim A. Frolov

New "fast and easy" method of making giant unilamellar vesicles for studying membrane processes under physiological conditions P4-12
Ariana Velasco del Olmo, Vadim Frolov and Anna Shnyrova

Intrinsic oligomerization capacity and pore-formation in membrane -active peptides P4-13
Edel Cunill, Orlando L. Sánchez-Muñoz and Jesús Salgado

Fungal Ribotoxins as tools for the study of ribosome biogenesis in yeast P5-5
Miriam Olombrada, Álvaro Martínez del Pozo, Vikram G. Panse, José G. Gavilanes Franco and Lucía García-Ortega.

A single mutation rescues cancer-associated polymorphic NQO1 by targeting native state dynamics P5-6
Encarnación Medina Carmona, Rogelio Palomino Morales, Julian F. Fuchs, David J. Timson, **Angel Luis Pey**

Replication dynamics of the human mitochondrial DNA polymerase P5-7
Fernando Cerrón, Laurie S. Kaguni, Borja Ibarra

Characterization of a new family of Broad-spectrum racemases involved in production of noncanonical D-amino acids and cell-wall regulation P5-8
Noelia Bernardo-García, Cesar Carrasco-López, Akbar Espaillat, Natalia Pietrosemoli, Lisandro H. Otero, Laura Álvarez, Miguel A. de Pedro, Florencio Pazos, Brigid M. Davis, Mathew K. Waldor, Felipe Cava and Juan A. Hermoso

Effect of a mutation linked to chronic lymphocytic leukemia on the substrate specificity of the export receptor CRM1 P5-9
Igor Arregi, Marián Alonso-Mariño, Iraia García-Santisteban, Juan Jesús García-Vallejo, Yvette van Kooyk, María Ángeles Urbaneja, José Antonio Rodríguez, **Sonia Bañuelos**

Structural and Thermodynamic basis for cellulosome high-affinity protein-protein interaction P5-10
Juliana Cheleski, Naiara Torres, Helton J. Wiggers, Richard C. Garratt and Marcos V. A. S. Navarro

Structure and biophysical characterization of α mating pheromone from the fungus Fusarium oxysporum P5-11
Soraya Serrano, Stefania Vitale, David Turrà, Álvaro Martinez-del-Pozo,
Antonio Di Pietro and **Marta Bruix**

Allostery in the tau-Hsp70 complex P5-12
Javier Oroz, B. A. Nordhues, J. Biernat, E. Mandelkow, C. A. Dickey and M. Zweckstetter

Single-molecule characterization of the interaction between human Rad54 protein and double-stranded DNA P5-13
Kateryna Mykolayivna Lemishko, Humberto Sánchez and Borja Ibarra

Studies of interactions between drug and macromolecule by different spectroscopic techniques P5-14
Andrés Garzón, Iván Bravo, Pedro J. Pacheco, Carlos Alonso, and José Albaladejo

Optimization of phenyilalananine hydroxilase stabilizers by a tested 'alchemichal' free-energy approach P5-15
Galano-Frutos, J.J., Conde-Giménez, M., Galiana, M., Victor, B. L., Brito, R. M. and Sancho, J.

Brownian motion simulations of reaction-diffusion processes of proteins in intracellular media P5-16
Mireia Via, Pablo M. Blanco, Sergio Madurga, Josep Lluís Garcés, Eudald Vilaseca and Francesc Mas

Crowding effects on oligomeric enzymes: kinetic analysis of the ALKP-catalyzed hydrolysis P5-17
Claudia Hernández, Cristina Balcells, Mireia Via, Isabel Pastor, Josep Lluís Garcés, Sergio Madurga, Marta Cascante and Francesc Mas

Host Oriented Inhibitors of Late Domain Interactions as Broad-Spectrum Antivirals P5-18
Pedro Buzón, Francisco Castillo, Manuel Iglesias-Bexiga, Andrés Palencia, Bastien Cautain, Francisca Vicente, Javier Ruiz-Sanz, Jose C. Martínez, Ana Camara-Artigas and Irene Luque

Biophysical characterization of the association between In and IC from N. Equitans P5-19
Verónica Gordo, Javier Murciano, Antoni Benito, Maria Vilanova, Jose C. Martínez and Marc Ribó

The Kv1.3 carboxy terminal domain is involved in the KCNE4 interaction P6-4
Sara R. Roig, Laura Solé, Albert Vallejo-Gracia, Ramón Martínez-Mármol, Antonio Serrano-Albarrás, Anna Oliveras, Antonio Ferrer-Montiel, Gregorio Fernández-Ballester, Michael M. Tamkum, Antonio Felipe.

Characteristic transport mechanisms of a protein ion channel investigated using current fluctuations analysis P6-5
María Queralt-Martín, Lidón M. López and Antonio Alcaraz

Modelization of binding of SSB proteins to ssDNA P7-5
Javier Jarillo, José A. Morin, Elena Beltrán-Heredia, Juan P. G. Villaluenga, Borja Ibarra and Francisco J. Cao

Formation of interstrand cross-link (ICLs) in DNA by nitrous acid P7-6
J. Ortega-Castro, N. Hernández-Haro, J. Frau1, F.Muñoz, J. Donoso and A. Grand

Reaction initiation of individual molecular motors in single-stream laminar flow cells P7-7
Benjamin Gollnick, Carolina Carrasco, Alberto Marín, Neville S. Gilhooly, Mark S. Dillingham and Fernando Moreno-Herrero

Interactions of allergenic proteins and big hydrophobic ions P8-5
Leonor Pérez-Fuentes, Carlos Drummond, Jordi Faraudo and Delfi Bastos-González

Cellular uptake mechanims of lipid nanocapsules P8-6
P. Sánchez-Moreno, A. Salvati, H. Boulaiz, J.A. Marchal, J.L. Ortega-Vinuesa and **J.M. Peula-García**

Influence of the hydrophobic moieties of poly(amidoamine)s on the condensation process and on the morphology of polymer/plasmid DNA complexes P8-7
Azahara Rata-Aguilar, Julia Maldonado-Valderrama, Juan Luis Ortega-Vinuesa, Antonio Martín-Rodríguez, Ernst Wagner and **Ana Belén Jódar-Reyes**

Fluorescence lifetime dendrimeric sensors based on tris(phenylenevinylene)benzene with polyamine and polyamidoamine branches P8-8
Pedro J. Pacheco, Iván Bravo, Andrés Garzón, Julian Rodriguez López, Juan Tolosa, Joaquín C. García, Jesús Canales-Vázquez, and José Albaladejo

In-vitro digestibility of bacteriocin AS-48 P8-9
del Castillo-Santaella Teresa, Cebrián Rubén, Eva Valdivia, Manuel Martínez-Bueno, Gálvez-Ruiz MªJosé, Maqueda Abreu Mercedes, **Maldonado-Valderrama Julia**

Plenary Lectures

L1

Protein Thermodynamics and the Biopharmaceutical Industry

Ernesto Freire

Johns Hopkins University, Baltimore, MD 21218, USA ef@jhu.edu

Protein therapeutics (biologics) is the fastest growing segment in the pharmaceutical industry. Last year, seven of the top ten selling drugs were biologics. The development of proteins as therapeutic drugs poses unique challenges. Conformational stability, protein aggregation and solution viscosity are critical issues in the development of protein pharmaceuticals, especially high concentration formulations that can be as high as 200mg/mL. Achieving optimal formulation conditions is a difficult balancing act, as excipients used to minimize aggregation or to lower viscosity often have an adverse effect on conformational stability, thus compromising long term stability. Unraveling the thermodynamic linkage between these quantities allows implementation of novel strategies aimed at formulation optimization and developability. These topics and the synergism between basic and translational research will be discussed.

L2

Probing electric polarization in biology with scanning probe microscopy: from single bacteria and viruses to DNA

Laura Fumagalli[1] and Gabriel Gomila[2]

[1] School of Physics and Astronomy, University of Manchester, Manchester, United Kingdom laura.fumagalli28@gmail.com

[2] University of Barcelona and Institut de Bioenginyeria de Catalunya (IBEC), Barcelona, Spain

Electric polarization, represented by the dielectric constant, e_r, is an intrinsic property of matter that plays a fundamental role in biology. It modulates the electrostatic interaction between biomolecules and it influences their shapes. In particular, it is key in DNA interaction with effector proteins and DNA bending and packaging. Yet, quantifying local dielectric properties of macromolecules such as DNA has been a long-standing challenge because the dielectric signal is extremely weak, mostly dominated by non-local contributions and shape/size artefacts.

In this talk we will review our results achieved in recent years in probing dielectric constant of biological samples using scanning probe microscopy, namely, current-sensing atomic force microscopy (C-AFM) [1,2] and electrostatic force microscopy (EFM) [3-6]. By combining low-noise detection with quantitative numerical analysis of the tip-sample electrostatic interaction, we showed that both techniques are able to *quantitatively* determine the dielectric constant of biological samples, from single bacteria [5] and viruses [3] down to condensed DNA [4] in air environment.

In particular, the long-range nature of polarization forces enabled us to experimentally resolve the dielectric constant of DNA [3,4], remained unknown owing to the lack of tools able to access it, in its natural condensed state inside single viruses. In contrast to the common assumption of low-polarizable behavior like proteins ($e_r \sim 2$–4), we found that the DNA dielectric constant is $e_r \sim 8$, considerably higher than the value of $e_r \sim 3$ found for capsid proteins. Atomistic molecular dynamic simulations confirmed our experimental findings, predicting $e_r \sim 8$ for DNA, which results in sensibly decreased DNA interaction free energy and lowered affinity to the ligand binding than normally predicted by Poisson–Boltzmann methods [4].

Finally, we showed that dielectric constant quantification can be extended to liquid environment [6] at high frequencies (MHz), which will enable to study biological systems in their natural environment.

References:

[1] Fumagalli, L., Ferrari, G., Sampietro, M., Gomila, G. *Appl. Phys. Lett. 91*, 243110, 2007
[2] Fumagalli, L., Ferrari, G., Sampietro, M., Gomila, G. *Nano Lett. 9*, 1604, 2009
[3] Fumagalli, L., Esteban, D. Cuervo, A, Carrascosa, J.L., Gomila,G. *Nature Mater. 11*, 808, 2012
[4] Cuervo, A., Dans, P.D., Carrascosa, J.L., Orozco, M., Gomila, G., Fumagalli, L. *PNAS* 3624, 2014
[5] Esteban-Ferrer, D, Edwards M.A., Fumagalli, L, Juárez, A, Gomila, G. *ACS Nano* 9843, 8 2104
[6] Gramse, G.; Edwards, M. A.; Fumagalli, L, Gomila, G. *Appl. Phys. Lett. 101*, 213108, 2012

L3

MODELING OF FtsZ PROTEIN FILAMENTS FROM AFM IMAGES

Pedro Tarazona[1], Pablo González de Prado-Salas[1] and Marisela Vélez[2]

[1]Depto. Física Teórica de la Materia Condensada, IFIMAC Center for Condensed Matter Research, Universidad Autónoma de Madrid, Madrid 28049 , Spain. pedro.tarazona@uam.es
[2]Institution of the second author, city, country

Atomic Force Microscopy (AFM) images of structures formed by FtsZ have provided a wealth of experimental information on the collective behavior of this bacterial protein [1]. Filaments are observed to form, to break, to fluctuate and to assemble in polymorphic bundles under controlled experimental conditions, either for the protein adsorbed on mica, and for the protein anchored to lipid bilayers through specific links. Simple models have been developed to reproduce, in Monte Carlo simulations, the structures and textures similar to those observed in the AFM images [2]. The models are based on a minimal set of effective interaction terms between protein monomers, described at a much coarser level than atomistic of force-field representations, so that the collective properties may be explored for the biologically relevant scales of size and time, and still with enough resolution to take full advantage of the AFM images. The refinement of the models, based on new experimental results, has been very successful to identify and to characterize the relevant aspects of these systems, including the coupling between the torsion of the filaments and their preferential orientation on the substrate [3], and the formation of lipid rafts associated to structures formed by the FtsZ filaments [4].

Acknowledgements: This work has been sponsored by the Ministerio de Economía and Competitividad of Spain under grant FIS2013-47350-C5-1-R

References:

[1] Jesus Mingorance et al. *"Visualization of single Escherichia coli FtsZ filament dynamics with atomic force microscopy"*, Journal of Biological Chemistry, Volume: 280, Number: 21, pages: 20909-20914, 2005.
[2] Alfonso Páez, et al., *"Simple modeling of FtsZ polymers on flat and curved surfaces: correlation with experimental in vitro observations"* PMC biophysics Volume: 2 Number: 1, page 8 2009
[3] Pablo González de Prado Salas, et al. , *"Torsion and curvature of FtsZ filaments"*, Soft matter, Volume 10, Number 12, pag.1977-86, 2014.
[4] Pablo González de Prado Salas, et al. , "Modeling the interplay between protein and *lipid aggregation in supported membranes"*, Chemistry and physics of lipids Volume: 185, pages: 141 52, 2015.

L4

SURFACE PROPERTIES OF AMPHIPHILIC PEPTIDES

Gerardo D. Fidelio

Departamento de Química Biológica, CIQUIBIC, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina. gfidelio@fcq.unc.edu.ar

Langmuir monolayers at the air/water interface is practically the unique technique allowing the study of the surface properties of peptides and proteins and their interaction with lipids in a confined *"crowded"* condition similar to the that found in natural biomembranes. The surface compactness and surface covered proportion of the peptide compared with the lipid are quite controlled. Using Langmuir monolayer many studies can be performed such us: ability of peptides to absorb to clean air/water interfaces (tensio-active properties), to interact with organized lipid monolayers (penetration), surface stability of spread protein/peptide sample, peptide/lipid lateral miscibility, surface rheology, conformation and secondary structure by PM-IRRAS and lateral topography by using the Brewster Angle Microscopy technique (BAM).

Even when the proteins and short peptides are so diverse in sequence (and in amphiphilicity and structure) some generalities can be achieved regarding to their surface behavior at water/air interface. Amphipathic proteins and peptides acquire similar surface properties independently they absorbed from bulk aqueous phase or spread from aqueous or from an appropriate organic solvent solution; a higher stability upon lateral compression is observed with hydrophobic peptides with a higher tendency to adopt a β-sheet conformation at the interface compared with those with a higher tendency to adopt an α-helix and, in turn, this higher stability confers a greater tendency to remain miscible in mixed lipid-peptide systems; some representative pure peptide monolayers have similar properties than lipids when their surface characteristics are compared (lateral stability and surface potential) and, a higher liquid-expanded character of the lipid at the interface confers a more adequate lateral environment for bidimensional miscibility.

We observed that the inversion in the sequence of peptides with the same global hydrophobicity has a central role on the lateral stability of peptide monolayers and on the ability of the molecules to partition into the air-water interface, whereas the rheological properties of all molecules are similar.

Finally, amyloidogenic peptides such as Aβ1-42 amyloid peptide with a higher tendency to adopt β-sheet conformation has a remarkable shear elasticity modulus when compared to lytic melittin compatible with a fiber-like topography at the surface found for the former.

Acknowledgements: This work has been sponsored by CONICET, FONCYT and SECyT-UNC, from Argentina.

L5

MEMBRANE FUSION MEDIATED BY SNARE PROTEINS

Reinhard Jahn

Department of Neurobiology, Max-Planck-Institute for Biophysical Chemistry, 37077 Göttingen, Germany, email: rjahn@gwdg.de

Eukaryotic cells are compartmentalized into membrane-enclosed organelles. Most of them are connected with each other by the regulated exchange of transport vesicles that bud from the precursor membrane and are transported to their destination membrane where they dock and fuse. In most (but not all) cases, fusion is carried out by SNAREs that represent an evolutionarily conserved superfamily of small and mostly membrane-anchored proteins. SNAREs are distinguished by a conserved stretch of 60-70 amino acids, termed SNARE-motifs, that are located adjacent to the membrane anchor domain. During fusion, four of such SNARE motifs, each belonging to a different subfamily, align with each other to form a highly stable coiled-coil of α-helices. Complex formation proceeds from the N-terminal end towards the C-terminal membrane anchors, thus pulling the membranes together and initiating fusion (“zipper” hypothesis of SNARE function). The steps of SNARE assembly are controlled by members of conserved protein families such as the SM- and CATCHR-proteins. Further regulation is imposed by specialist proteins, such as those responsible for calcium dependent exocytosis in neurons such as the calcium sensor synaptotagmin and complexins (for review see [1]).

In our own work, we have focused on understanding the mechanisms of SNARE assembly and SNARE-induced fusion using structural and biochemical approaches and *in-vitro* fusion reactions with native and artificial membranes. Our recent results lend strong support to the zipper hypothesis, showing that during SNARE complex formation the helical bundle extends into the membrane [2] and that only few SNARE complexes may suffice for effective fusion of bilayers [3]. Furthermore, we have studied the organization of SNAREs in the plasma membrane [4]and intermediate states of the SNARE-dependent fusion pathway involving techniques such as cryo-electron microscopy [5], resulting in novel insights into the structure of fusion intermediates.

Acknowledgements: This work has been sponsored by grants from the Deutsche Forschungsgemeinschaft (Germany) and the National Institutes of Health (USA)

References:

[1] Jahn, R., Fasshauer, D. (2012) Molecular machines governing exocytosis of synaptic vesicles. *Nature* 490, 201-207.

[2] Stein, A., Weber, G., Wahl, M.C., Jahn, R.(2009) Helical extension of the neuronal SNARE complex into the membrane. *Nature* 460, 525-528.

[3] Hernandez, J.M., Kreutzberger, A.J., Kiessling, V., Tamm, L.K., Jahn, R. (2014) Variable cooperativity in SNARE-mediated membrane fusion. Proc. Natl. Acad. Sci. U S A. 111,12037-12042.

[4] van den Bogaart, G., Meyenberg, K., Risselada, J.H., Amin, H., Willig, K.I., Hubrich, B.E., Dier, M., Hell, S.W., Grubmüller, H., Diederichsen, U., Jahn, R. (2011) Membrane protein sequestering by ionic protein-lipid interactions. *Nature* 479, 552-555.

[5] Hernandez, J.M., Stein, A., Behrmann, E., Riedel, D., Cypionka, A., Farsi, Z., Walla, P.J., Raunser, S., Jahn, R. (2012) Membrane fusion intermediates via directional and full assembly of the SNARE complex. *Science*, 336, 1581-1584.

Simposium Lectures

S1-1

UNTANGLING AMYLOID PROTEINOPATHIES WITH A SYNTHETIC BACTERIAL MODEL SYSTEM: THE RepA-WH1 PRIONOID

Rafael Giraldo[1], Cristina Fernández[1], María Moreno-del Álamo[1], Laura Molina-García[1]

[1]Dept. of Cellular & Molecular Biology, CIB-CSIC, Madrid, Spain, rgiraldo@cib.csic.es

Protein amyloids arise from the conformational conversion and templated assembly of a soluble protein into fibrillar aggregates with a crossed b-sheet backbone, leading to human neurodegenerative and systemic proteinopathies. In bacteria, amyloids assemble as functional extracellular scaffolds but no natural proteinopathic amyloidosis has been found in microorganisms yet [1].

In some bacterial plasmids, RepA-type proteins initiate DNA replication by undergoing a complex structural transformation coupled to dimer dissociation [2]. *In vitro*, the 'winged-helix' N-terminal domain of RepA (WH1) can assemble into amyloid fibres upon binding to short plasmid-specific DNA sequences [3,4]. We have engineered tandem repeats of the amyloid stretch in RepA-WH1 in replacement of the Q/N-rich oligopeptide repeats in yeast Sup35p/[*PSI*$^{+}$], thus building functional chimeric [*REP-PSI*$^{+}$] prions [5]. As assessed through EM and AFM, RepA-WH1 amyloid fibres are bundles made of double, intertwined tubular protofilaments, which are assembled by structurally distorted monomers of the protein [6]. Upon incubation of RepA-WH1 with vesicles having the lipidic composition of bacterial membranes, the protein aggregates on the bilayer, leading to the leakage of the vesicle contents, thus mimicking a well-established route for cytotoxicity in human amyloidosis [7].

RepA-WH1 causes in *E. coli* an amyloid proteinopathy, which is *vertically* transmissible from mother to daughter cells, but not infectious, enabling conformational templating by cross-seeding *in vitro* [8] and *in vivo* [9], i.e. RepA-WH1 is a 'prionoid'. Through microfluidics, we have directly assessed the dynamics of the RepA-WH1 prionoid in *E. coli*. Bacterial lineages maintain two mutually exclusive types (strains) of RepA-WH1 amyloids: either multiple globular particles that inhibit cell division, or a single elongated aggregate, mildly detrimental to growth. The bacterial Hsp70 chaperone DnaK modulates RepA-WH1 amyloidogenesis and the vertical propagation of amyloid strains [10].

The results presented here empower the bacterial RepA-WH1 prionoid as a synthetic minimalist model system for amyloid proteinopathies.

Acknowledgements: This work has been sponsored by MINECO grants BIO2012-30852 and CSD2009-00088

References:

[1] Rafael Giraldo. "Amyloid assemblies: Protein Legos at a crossroads in bottom-up synthetic biology". ChemBioChem, 11(17), 2347-2357, 2010.

[2] Rafael Giraldo, Carlos Fernández-Tornero, Phil R. Evans, Ramón Díaz-Orejas, Antonio Romero. *"A conformational switch between transcriptional repression and replication initiation in the RepA dimerization domain"*. Nat. Struct. Biol. 10(7), 565-571, 2003.

[3] Rafael Giraldo. "Defined DNA sequences promote the assembly of a bacterial protein into distinct amyloid nanostructures". Proc. Natl. Acad. Sci. USA 104(44), 17388-17393, 2007.

[4] Fátima Gasset-Rosa, María J. Maté, Cristina Dávila-Fajardo, Jerónimo Bravo, Rafael Giraldo. *"Binding of sulphonated indigo derivatives to RepA-WH1 inhibits DNA-induced protein amyloidogenesis"*. Nucleic Acids Res. 36(7), 2249-2256, 2008.

[5] Fátima Gasset-Rosa, Rafael Giraldo. "Engineered bacterial hydrophobic oligopeptide repeats in a synthetic yeast prion, [REP-PSI^{+}]". Front. Microbiol. 6, 311, 2015.
[6] Eva Torreira , María Moreno-del Álamo, María E. Fuentes-Perez, Cristina Fernández, Jaime Martín-Benito, Fernando Moreno-Herrero, Rafael Giraldo, Oscar Llorca *"Amyloidogenesis of bacterial prionoid RepA-WH1 recapitulates dimer to monomer transitions of RepA in DNA replication initiation"*. Structure 23(1), 183-189, 2015.
[7] Cristina Fernández, Mercedes Jiménez, Germán Rivas, Rafael Giraldo. "RepA-WH1, the agent of an amyloid proteinopathy in bacteria, targets and leaks lipidic vesicles". Submitted.
[8] María E. Fernández-Tresguerres, Susana Moreno-Díaz de la Espina, Fátima Gasset, Rafael Giraldo. *"A DNA-promoted amyloid proteinopathy in Escherichia coli"*. Mol. Microbiol. 77(6), 1456-1469, 2010.
[9] Laura Molina-García, Rafael Giraldo. *"Aggregation interplay between variants of the RepA-WH1 prionoid in Escherichia coli"*. J. Bacteriol. 196(14), 2536-2542, 2014.
[10] Fátima Gasset-Rosa, Anne S. Coquel, María Moreno-del Álamo, Peipei Chen, Xiaohu Song, Ana M. Serrano, María E. Fernández-Tresguerres, Susana Moreno-Díaz de la Espina, Ariel B. Lindner, Rafael Giraldo. *"Direct assessment in bacteria of prionoid propagation and phenotype selection by Hsp70 chaperone"*. Mol. Microbiol. 91(6), 1070-1087, 2014.

S1-2

SMAD PROTEINS DURING THE LAST 500 MILLION YEARS: EVOLUTION, HUMAN SNP VARIATIONS AND SOMATIC MUTATIONS. AN STRUCTURAL APPROACH

María Macías

Structural and Computational Biology, IRB Barcelona, Spain

We will present a combined view of sequences and structures of Smad proteins, which are the main players of the TGF beta signaling pathway. These proteins are highly conserved in metazoans. We have used the available structures to analyse the regions in the structures (determined by NMR and also by X-ray) that concentrate the differences along evolution and also the mutations identified in tumours and the variations in human populations. Mutations in tumors tend to accumulate at the interfaces of protein interactions. These mutations are not conservative while differences in evolution accumulate in loops and are conservative.

S1-3

CRYO-ELECTRON MICROSCOPY AT ATOMIC RESOLUTION OF MACROMOLECULAR MACHINES

José R. Castón

Department of Structure of Macromolecules, Centro Nacional de Biotecnología/CSIC, Madrid, Spain. jrcaston@cnb.csic.es

Three-dimensional structures of macromolecular assemblies or biological nanomachines are crucial for advancing our understanding of key biological processes. X-ray crystallography and nuclear magnetic resonance (NMR) are methods used to characterize the overall structural organization of individual protein complexes. Macromolecular complexes are often too large or heterogeneous to be crystallized or diffract poorly when crystallized. In the case of NMR, complex size is a limitation. Cryo-electron microscopy (cryo-EM) combined with single-particle reconstruction techniques is a well-established approach for obtaining high-resolution structural information for macromolecular assemblies in native conditions. Cryo-EM allows dynamic study of these assemblies by trapping "transient" or intermediate states that show induced conformational changes.

Our studies address structure-function-assembly relationships of viral macromolecular complexes, using the viral capsid and other assemblies as model systems. Three studies will be presented in which we obtained atomic resolution details from two-dimensional cryo-EM images. The first is a hybrid approach that docked X-ray/NMR atomic models into low/medium resolution cryo-EM maps. In studies of helical assemblies of the VP4 viral protease, which occurs naturally in infectious bursal disease virus-infected cells, the dimeric structural unit showed conformational changes that block the catalytic site located on a surface crevice. The second, a classical approach using digitized cryo-micrographs of Penicillium chrysogenum virus acquired in a 300 kV FEG electron microscope, generated a 4.2 Å resolution map suitable for a nearly complete trace of the 982-amino-acid capsid protein. A full-atom model of the capsid showed contacts among subunits and RNA-protein interactions. The capsid protein is a structural duplication of a single domain that is preserved in the dsRNA virus lineage. Finally, we analyzed Rosellinia necatrix quadrivirus 1 (RnQV1) with the new generation of direct electron detectors. Data were acquired in a Titan Krios electron microscope equipped with this detector and processed using RELION software. The 3D structure of the RnQV1 capsid, built of heterodimers with two proteins of 1,356 (P2) and 1,059 (P4) residues, was determined at 3.73 Å resolution. Although P2 and P4 sequence similarity is very low, their shell domains share the a-helical core preserved in the dsRNA virus lineage.

Acknowledgements: This work has been sponsored by grants BFU2011-25902 from the Spanish Ministry of Economy and Competitivity and S2013/MIT-2807 from Comunidad Autónoma de Madrid

References:

[1] X Bai, G McMullan, SHW Scheres, "*How cryo-EM is revolutionizing structural biology*", TiBS 40 (1): 49-57.

[2] D Luque, J Gómez-Blanco, D Garriga et al., "Cryo-EM near-atomic structure of a dsRNA fungal virus shows ancient structural motifs preserved in the dsRNA viral lineage", PNAS 111 (21): 7641-7646.

[3] http://www.cnb.csic.es/~jrcaston/Caston-lab/Home.html

S1-4

ESCAPE FROM DEATH, STRATEGIES FOR INTRACELLULAR SURVIVAL BY *Legionella pneumophila*.

Aitor Hierro[1]

[1]CIC bioGUNE, Derio (Bilbao), Spain. *ahierro@cicbiogune.es*

A crucial step in the elimination of invading microbes is phagosomal maturation. Intravacuolar pathogens deliver virulence proteins, so-called effectors, into the cytosol of the infected cell. Many of the translocated effectors studied to date alter cellular processes such as vesicle trafficking, apoptosis, autophagy, protein ubiquitylation, or protein synthesis among others, thereby creating conditions that support intracellular survival and replication of the microbe.

Rab proteins are small Guanosine triphosphatases (GTPases) that act as molecular switches by simply alternating between an active GTP-bound form and an inactive GDP-bound conformation. These structural differences control the association with downstream effectors to integrate both membrane trafficking and intracellular signaling in a temporally and spatially sensitive manner. Not surprisingly, Rab GTPases have become the target of many pathogens that selectively exploit their activity in order to establish conditions supportive for infection and disease development. The Gram-negative bacterium *Legionella pneumophila*, the causative agent of Legionnaires' pneumonia, delivers more than 200 effectors into the infected host cell to elude endo/lysosomal degradation. Yet, only a few of these effectors have been characterized in detail.

Two examples of such strategies will be presented: (I) The modification of Rab GTPases with adenosine monophosphate (AMPylation - de-AMPylation) to establish a temporal control of their switching activity[1], and (II) how a virulence phospholipase is activated by endosomal GTPases to block the early-endosome to late-endosome transition[2].

Acknowledgements: This work has been sponsored by the Carlos III Health Institute grant PI11/00121 and the Basque Government grant PI2011-26. This study made use of the Diamond Light Source (Oxfordshire, UK) part funded by the BioStruct-X program, the European Synchrotron Radiation Facility (ESRF, Grenoble, France) and ALBA synchrotron beamline BL13-XALOC.

References:

[1] Chen Y, Tascón I, Neunuebel MR, Pallara C, Brady J, Kinch LN, Fernández-Recio J, Rojas AL, Machner MP, Hierro A. Structural basis for Rab1 de-AMPylation by the Legionella pneumophila effector SidD. PLoS Pathog. 9(5):e1003382, 2013.

[2] Lucas M, Gaspar AH, Pallara C, Rojas AL, Fernández-Recio J, Machner MP, Hierro A. Structural basis for the recruitment and activation of the Legionella phospholipase VipD by the host GTPase Rab5. Proc Natl Acad Sci U S A. 26;111(34):E3514-23, 2014.

S2-1

Analysing the properties of plant surfaces: a biophysical approach

Victoria Fernández[1], Antonio Heredia[2], and Mohamed Khayet[2]

[1]Forest Genetics and Ecophysiology Research Group, School of Forest Engineering, Technical University of Madrid, Spain v.fernandez@upm.es
[2]Molecular Biology and Biochemistry Department, Instituto de Hortofruticultura Subtropical Mediterránea (IHSM) La Mayora. CSIC-UMA, Málaga, Spain
[3]Department of Applied Physics I, Faculty of Physics, University Complutense of Madrid, Spain

Plant surfaces have a major degree of physical and chemical heterogeneity that will influence their interaction with surface-deposited water, particles, agrochemical drops, insects or microorganisms. The combined effects of surface chemistry and roughness may lead to variable rates of plant surface wettability and water drop adhesion or repellence [1]. Most aerial plant parts such as leaves, fruits, flowers and stems are covered with an extra-cellular, lipid-rich layer named cuticle, which is the interface between plant organs and the surrounding environment. The physical and chemical complexity of the plant cuticle poses obstacles for the development of permeability models that generally consider its internal structure and interactions with surface-applied agrochemicals. For this purpose, we estimated the solubility parameter of model plant surface chemical constituents commonly present in plant surfaces. Following the van Krevelen and Hoftyzer method, we calculated theoretically and empirically the solubility parameter of plant surfaces and also assessed their affinity for selected agrochemicals [2]. By estimating the solubility parameter of plant surfaces, theoretically (i.e., as derived from the molecular properties of cuticle chemical constituents) or empirically (after contact angle measurements of 3 liquids with different polarity), we were able to distinguish the physical effect of surface roughness from the effect of the chemical nature of the epicuticular waxes. A solubility parameter model for plant surfaces is proposed on the basis of an increasing gradient from the cuticular surface towards the underlying cell wall. It is concluded that the calculation of the solubility parameter of plant surfaces can be an useful tool for improving our understanding of biological surface interactions.

References:

[1] Fernández V., Sancho-Knapik D, Guzmán P, Peguero-Pina JJ, Gil L, Karabourniotis G, Khayet M, Fasseas C, Heredia-Guerrero JA, Heredia A, Gil-Pelegrin E, "*Wettability, polarity and water absorption of Quercus ilex leaves: effect of leaf side and age*", Plant Physiology 166, 168-180, 2014.

[2] Khayet M, Fernández V., "Estimation of the solubility parameter of model plant surfaces and agrochemicals: a valuable tool for understanding plant surface interactions". Theoretical Biology and Medical Modelling 9, 45, 2012.

S2-2

CELL MECHANICS AS A BIOPHYSICAL BIOMARKER

Ruddi Rodríguez-García[1], Iván López-Montero[1,2], Michael Mell[1,2], Gustavo Egea[3], Nir S. Gov[4] and Francisco Monroy[1,2]

[1]Department of Physical Chemistry, Universidad Complutense, Madrid, Spain *ivanlopez@quim.ucm.es*
[2]Instituto de Investigación Hospital Doce de Octubre (i+12), Madrid, Spain;
[3]Departament of Cell Biology, Immunology and Neurosciences, University of Barcelona School of Medicine and Institut d'Investigacions Biome`diques August Pi i Sunyer (IDIBAPS) and Nanocie`ncies i Nanotecnologia (IN2UB), Barcelona, Spain
[4]Department of Chemical Physics, Weizmann Institute of Science, Rehovot, Israel

Structural integrity of eukaryotic cells is provided by plasma membrane and cytoskeleton. The cytoskeleton is a crowded network of structural proteins that exhibit a wide variety of rheological behavior according to different timescale deformations [1]. Coupled to cytoskeleton and the cell body, plasma membrane is characterized by elastic and frictional properties that can correlate with the metabolic state of the cell [2] and influence a wide range of cell functions. For example, erythrocytes are flexible cells specialized in the systemic transport of oxygen in vertebrates. This physiological function is connected to their outstanding ability to deform in passing through narrow capillaries. Here [3], we show experimental evidence of the presence of temporally correlated forces superposed over the thermal fluctuations of the erythrocyte membrane. These forces are ATP-dependent and drive enhanced flickering motions in human erythrocytes. Such metabolically regulated active forces cause global membrane softening, a mechanical attribute related to the functional erythroid deformability. The mechanical analysis of cell stiffness is becoming a powerful tool to detect pathological cells from normal ones, cancerous cells among them [4].

Acknowledgements: This work was supported by grants FIS2009-14650-C02-01, FIS2012-35723, and CSD2007-0010 (Consolider-Ingenio Nanociencia Molecular) from the Ministerio de Economía y Competitividad (MINECO); S2013/MIT-2807 (NanoBIOSOMA), S2009MAT-1507 (NOBIMAT) from CAM; BFU2012-33932 from MINECO; International Studies Fund (grant no. 580/12); the grant ERC-StG-338133 and the Ramon y Cajal Program (RYC-2013-12609)

References:

[1] Linhong Deng, Xavier Trepat, James P. Butler, Emil Millet, Kathleen G. Morgan, David D. Weitz and Jeffrey J. Fredberg, *Fast and slow dynamics of the cytoskeleton*, Nat Mat, 6, 636-640, 2006.
[2] Ming Guo, Allen J. Ehrlicher, Mikkel H. Jensen, Malte Renz, Jeffrey R. Moore, Robert D. Goldman, Jennifer Lippincott-Schwartz, Frederick C. Mackintosh and David A. Weitz, *Probing the Stochastic, Motor-Driven Properties of the Cytoplasm Using Force Spectrum Microscopy*, Cell, 158, 822–832, 2014.
[3] Ruddi Rodríguez-García, Iván López-Montero, Michael Mell, Gustavo Egea, Nir S. Gov and Francisco Monroy, *Direct Cytoskeleton Forces Cause Membrane Softening in Red Blood Cells*, Biophys J, 108, 1-13, 2005
[4] A. Raman, S. Trigueros, A. Cartagena, A. P. Z. Stevenson, M. Susilo, E. Nauman and S. Antoranz Contera, *Mapping nanomechanical properties of live cells using multi-harmonic atomic force microscopy*, Nat Nanotechnology, 6, 809-814, 2011.

S2-3

NANOPHOTONIC APPROACHES FOR LIVE CELL RESEARCH: FROM NANOIMAGING TO SPECTROSCOPY.

Maria F. Garcia-Parajo[1,2], Mathieu Mivelle[1], Thomas S. van Zanten[3] and Carlo Manzo[1]

[1]ICFO-Institute of Photonic Sciences, Castelldefels (Barcelona), Spain. Maria.garcia-parajo@icfo.es
[2]ICREA- Institució Catalana de Recerca i Estudis Avançats, Barcelona, Spain
[3]NCBS-National Center for Biological Sciences, Bangalore, India

The quest for optical imaging of biological processes at the nanoscale has driven in recent years a swift development of a large number of microscopy techniques based on far-field optics. These, so called, super-resolution methods are providing new capabilities for probing biology at the nanoscale by fluorescence. While these techniques conveniently use lens-based microscopy, the attainable resolution and/or localization precision severely depend on the sample fluorescence properties. True nanoscale optical resolution free from these constrains can alternatively be obtained by interacting with fluorophores in the near-field. Indeed, near-field scanning optical microscopy (NSOM) using subwavelength aperture probes is one of the earliest approaches sought to achieve nanometric optical resolution[1]. More recently, photonic antennas have emerged as excellent alternative candidates to further improve the resolution of NSOM by amplifying electromagnetic fields into regions of space much smaller than the wavelength of light. In this contribution, I will describe our efforts towards the fabrication of different nanoantenna probe configurations as well as 2D antenna arrays for applications in nano-imaging and spectroscopy of living cells[2-6]. I will show examples on how these devices can be exploited to detect individual molecules at micro-molar concentrations[4,5], as well as our efforts towards the study of cell membrane lipids in living cells with unprecedented resolution and sensitivity[6].

Acknowledgements: This work has been sponsored by the European Commission's Seventh Framework Programme under Grant agreements 288263 (NANO-VISTA) and 284464 (LaserLab Europe), HFSP (Grant agreement RGP0027/2012) and Spanish Ministry of Science (MAT2011-22887)

References:

[1] P. Hinterdorfer, M.F. Garcia-Parajo, Y. Dufrene, *"Single-molecule imaging of cell surfaces using near-field nanoscopy",* Acc. Chem. Res. **45**, 327-336, 2012.
[2] M. Mivelle, T. S. Van Zanten, C. Manzo, M. F. Garcia-Parajo, *"Nanophotonic approaches for nanoscale imaging and single-molecule detection at ultrahigh concentrations",* Microsc. Res. Techniq. **77**, 537–545, 2014.
[3] M. Mivelle, T. S. van Zanten, L. Neumann, N. F. van Hulst, M. F. Garcia-Parajo, *"Ultrabright bowtie nanoaperture antenna probes studied by single molecule fluorescence"*, Nano Lett. **12**, 5972–5978, 2012.
[4] D. Punj, M. Mivelle, S. B. Moparthi, T. S. van Zanten, H. Rigneault, N. F. van Hulst, M. F. García-Parajó, J. Wenger, *"A plasmonic 'antenna-in-box' platform for enhanced single-molecule analysis at micromolar concentrations",* Nature Nanotechnol. **8**, 512-516, 2013.
[5] M. Mivelle, T. S. van Zanten, M. F. Garcia-Parajo, "Hybrid photonic antennas for subnanometer multicolor localization and nanoimaging of single molecules", Nano Lett. 4, 4895-4900, 2014.
[6] V. Flauraud, T. S. van Zanten, M. Mivelle, C. Manzo, M. F. Garcia Parajo, J. Brugger, *"Large-scale arrays of bowtie nanoaperture antennas for nanoscale dynamics in living cell membranes",* Nano Lett. [online DOI: 10.1021/acs.nanolett.5b01335], 2015.

S2-4

Imaging the tumor microenvironment

María L. García-Martín[1]

[1]BIONAND – Centro Andaluz de Nanomedicina y Biotecnología, Málaga, Spain, mlgarcia@bionand.es

The microenvironment of solid tumors is characterized by the presence of regional hypoxia, poorly organized and leaky vasculature, aerobic glycolysis (Warburg effect) and extracellular acidosis. Although it is widely accepted that tumor transformation and progression results from the accumulation of gene mutations, there is also a growing body evidence suggesting that tumor microenvironment plays a crucial role in many of the subsequent events, including tumor proliferation and metastasis [1]. Furthermore, it has been shown that tumor microenvironment influences tumor response to therapy [2, 3]. Therefore, being able to characterize the tumor microenvironment non-invasively can be of great help both for tumor diagnosis and treatment follow up. Magnetic resonance imaging (MRI), the most versatile in vivo imaging technique, allows the interrogation of perfusion, vascular permeability, pH, pO_2 and metabolism in living organisms with relatively high resolution and specificity [4]. An overview of these techniques and their applications will be provided in this talk.

References:

[1] Quail, D.F. and J.A. Joyce, "*Microenvironmental regulation of tumor progression and metastasis*". Nat Med, 19(11): p. 1423-37, 2013.

[2] Klemm, F. and J.A. Joyce, "*Microenvironmental regulation of therapeutic response in cancer*". Trends Cell Biol, 25(4): p. 198-213, 2015.

[3] Junttila, M.R. and F.J. de Sauvage, "*Influence of tumour micro-environment heterogeneity on therapeutic response*". Nature, 501(7467): p. 346-54, 2013.

[4] Gillies, R.J., et al., "*MRI of the tumor microenvironment*". J Magn Reson Imaging, 16(4): p. 430-50, 2002.

S3-1

USING ANCESTRAL RESURRECTION TO PROBE EVOLUTIONARY PROTEIN BIOPHYSICS

Jose M. Sanchez-Ruiz[1]

[1]Facultad de Ciencias, Departamento de Químca Física, Universidad de Granada, 18071-Granada, Spain, sanchezr@ugr.es

The impressive diversity of natural enzyme functions is though to have arisen through gene duplication events, together with the recruitment and evolutionary optimization of weak promiscuous activities linked to pre-existing active sites. Yet, it appears inescapable to assume that, at some early stages of protein evolution, "primordial" active sites capable of catalyzing simple reactions were generated de novo and subsequently served as the origin of evolutionary divergence towards a diversity of related but increasingly complex enzyme functions. The molecular mechanisms that led to these primordial enzyme activities are, however, a mystery. Partially buried ionizable groups in modern enzymes are known to often play fundamental roles in catalysis. It has been suggested, therefore, that de novo active sites could be generated through the random accumulation of mutations that introduce ionizable groups at internal positions, such as, for instance, hydrophobic-to-ionizable residue replacements. Experimental evidence for this proposal is, however, lacking in the published literature. Here, we use ancestral protein resurrection and protein engineering to explore de novo catalysis in an evolutionary context. We find substantial levels of de novo catalysis in resurrected ancestral proteins as the result of single hydrophobic-to-ionizable residue mutations. We further provide evidence that enhanced conformational flexibility contributes to the success of the function generation approach in the ancestral scaffolds. In addition to providing clues to function emergence during early protein evolution, this work has immediate implications for enzyme engineering and design.

S3-2

A SURFACE PLASMON RESONANCE STUDY OF THE DNA INTERACTION WITH TAU PROTEIN AND AMYLOID PEPTIDES, INVOLVED IN THE MOLECULAR BASIS OF ALZHEIMER´S DISEASE

Juan S. Jiménez[1], Sergio Camero[1], Alejandro Barrantes[1], María J. Benítez[1], Félix Hernández[2], Jesús Ávila[2], Pedro Navarro[1], Ángel García-Lucas[1], José M. Ayuso[1], Raquel Cuadros[2].
[1]*Departamento de Química Física Aplicada, Universidad Autónoma de Madrid, Madrid, Spain. E-mail: juans.jimenez@uam.es*

[2]Centro de Biología Molecular Severo Ochoa, Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid, (CSIC/UAM), Madrid, Spain.

Surface plasmon resonance (SPR) yields real-time results concerning the interaction between macromolecules of biological interest. SPR has allowed us to follow the interaction between DNA and the main peptides and protein concerning Alzheimer`s disease: tau protein and b-amyloid peptides. Suffered at present by millions of people, Alzheimer's Disease (AD) is the most common age-related dementia. At present there are no curative treatments for this disease. Intraneuronal tangles composed mainly of tau protein and extraneuronal senile plaques, containing a high percentage of b-amyloid, are the main hallmarks of this disease.
The most widely accepted theory concerning pathogenesis is the amyloid hypothesis which claims that the abnormal processing of amyloid precursor protein plays a central role in the AD pathogenesis. The initial molecular events by which toxicity is exerted remain, however, unclear and controversial. SPR, together with electronic microscopy, UV and Infrared spectroscopies have led us to conclude that aggregates formed by amyloid peptides show a particular proneness to interact with DNA. Similarly to toxicity, this interaction has been observed to be dependent on structure and aggregation state of the amyloid, therefore strengthening the hypothesis that amyloid peptides may, by means of interaction with nuclear DNA, alter transcription, thus contributing to the onset of neurodegeneration.
Our results describe how Tau confers thermodynamic stability properties to DNA which are similar to those provided by histone. Tau binding to DNA is reversible. By means of SPR we measured the free energy, enthalpy and entropy changes associated to the Tau-DNA complex formation. The results suggest that hydrophobicity must represent an important contribution to the stability of the Tau-DNA complex. SPR results show that phosphorylation prevent Tau from binding to DNA. On the other hand, the binding of tau to DNA become irreversible concomitantly with the process of aggregation. Under these conditions Tau would remain irreversibly bound to DNA, losing the possibility of regulating the DNA interaction by phosphorylation. Protein aggregation and hyperphosphorylation are the two main hallmarks found in Tau protein linked to neurodegeneration. One would conclude that both Tau modifications might contribute to the onset of neurological disorders by impairing the reversible Tau-DNA interaction, leading to a Tau-induced DNA deregulation.

S3-3

Engineering Macromolecular Assemblies from Monomeric Proteins: Learning from Molecular Evolution

Luis A. Campos Prieto[1], Beatriz Ibarra-Molero[2], Federico M. Ruiz[3], Sara Alvira[1], Mourad Sadqi[1], Rajendra Sharma[1], Jörg Schönfelder[1], Carlos Alfonso[3], Germán Rivas[3], José M. Valpuesta[1], Antonio Romero[3], José M. Sánchez-Ruiz[2] & Victor Muñoz[1,4]

[1]Centro Nacional de Biotecnología, CSIC. 28049 Madrid, Spain
[2]Departamento de Química Física, Facultad de Ciencias, Universidad de Granada. 18071Granada, Spain
[3]Centro de Investigaciones Biológicas, CSIC. 28040 Madrid, Spain
[4]School of Engineering, University of California Merced. Merced, CA 95343

Proteins carry out most cellular functions, including chemical catalysis, transport, structural scaffolding, energy production, signaling, defense, and replication. To perform such roles, proteins operate as true nanomachines that rely on their ability to spontaneously self assemble onto 3D structures and use thermal and chemical energy to change shape and function. Learning how to engineer protein macromolecular assemblies would thus open a wide avenue of exciting opportunities for developing nanotechnology approaches capable of mimicking and improving nature. Interestingly, inspection of the catalog of natural protein assemblies reveals a hierarchical organization in which single-domain monomeric proteins constitute the building blocks that are successively converted onto either multi-domain proteins or assemblies of monomers. This hierarchical architecture recapitulates the course of evolution in which the interplay of genetic drift, recombination and gene duplication facilitated the progressive emergence of increasingly sophisticated assemblies. From an engineering standpoint the challenge is to define procedures that allow for the transformation of naturally monomeric proteins onto assembly-prone species and their controlled assembly to form complexes of specific size and symmetry (as opposed to non-specific protein aggregates).

As way to undertake this challenge, we have devised a simple engineering strategy that borrows ideas from molecular evolution. Particularly, we use “domain swapping”, a process by which protein molecules partially unfold to exchange one of its structural domains with in kind partners, as basic mechanism for inducing specific macromolecular assemblies. The beauty of domain swapping is that it is, in principle, general because it uses the same interaction surfaces that are already present in the folded monomer. To increase domain-swapping propensity in the monomer, we re-engineer its amino-acid sequence with the goal of decreasing its folding cooperativity. This we do either by simplifying its composition to make it more akin pre-biotic proteins, or by targeted partial deletion. In a final step, we trigger the assembly process by manipulating the stability of the folded structure (e.g. changing temperature) and/or protein concentration. Therefore, this scheme provides a built-in mechanism for controlling formation and dissociation of the macromolecular complex on demand. As proof of concept, we applied this engineering strategy to the chymotrypsin inhibitor 2 (CI2), a monomeric, superstable protein widely used as paradigmatic model of cooperative two-state folding. Our results on CI2 demonstrate the feasibility of the approach and suggest it might be generalizable.

S3-4

CHAPERONING PROTEIN AGGREGATES: THE DISAGGREGASE MACHINERY

Alejandra Aguado, José Angel Fernández-Higuero, Yovana Cabrera, Fernando Moro and Arturo Muga

Unidad de Biofísica (CSIC/UPV-EHU) y Departamento de Bioquímica y Biología Molecular, Facultad de Ciencia y Tecnología, Universidad del País Vasco (UPV/EHU), Aptdo. 644, 48080 Bilbao, Spain.
email: *fernando.moro@ehu.es*

Protein quality control within the cell requires the interplay of many molecular chaperones and proteases. When this quality control system is disrupted, polypeptides follow patways leading to misfolding, inactivity and aggregation, damaging processes that drastically reduced cell life. All cells have evolved a machinery composed of molecular chaperones able to solubilize protein aggregates and refold them to their native state, with broad specificity for protein sequence or fold. In bacteria and yeast, aggregate reactivation requires the concerted action of chaperones belonging to the Hsp70, Hsp40 and Hsp100 families [1]. Hsp100 disaggregases belong to the AAA^+ protein family and assemble into hexameric structures with an axial pore. They use ATP hydrolysis to extract polypeptide chains from aggregates by partial or complete translocation through their central channel [2]. The stability of bacterial ClpB hexamers depends on salt and protein concentration, and the presence of nucleotides [3]. An intriguing feature of ClpB is that the active hexamers are highly dynamic and, even in conditions that stabilize the oligomeric form, they can exchange subunits between different oligomers, indicating that dissociation of hexamers occurs. We have used biochemical and fluorescence tools to explore ClpB dynamics under different experimental conditions [4]. The analysis of ClpB chaperone activity and the kinetics of subunit exchange indicates that: i) ATP favors assembly of the hexamers while ADP induces the dissociation/(re)association events; ii) subunit exchange is at least one order of magnitude slower that the ATP hydrolysis rate, suggesting that ClpB hexamers remain associated during several ATP hydrolysis rounds in which the chaperone can translocate substrates through the central channel; iii) ClpB dynamics and chaperone activity are dependent processes; and iv) DnaK and substrate proteins regulate the ATPase activity and dynamics of ClpB.

Acknowledgements: This work has been sponsored by the Spanish Ministry of Economy and Competitiveness [grant BFU2013-47059] and the Basque Government [IT709-13].

References:

[1] Glover, J.R. and Lindquist, S. "Hsp104, Hsp70, and Hsp40: a novel chaperone system that rescues previously aggregated proteins". Cell 94, 73-82 (1998).

[2] Haslberger, T., Weibezahn, J., Zahn, R., Lee, S., Tsai, F.T.F., Bukau, B. and Mogk, A. *"M domains couple the ClpB threading motor with the DnaK chaperone activity"*. Mol. Cell **25**, 247-260 (2007).

[3] Del Castillo, U., Alfonso, C., Acebrón, S. P., Martos, A., Moro, F., Rivas, G. and Muga, A. *"A quantitative analysis of the effect of nucleotides and the M domain on the association equilibrium of ClpB"*. Biochemistry 50, 1991-2003 (2011).

[3] Aguado, A., Fernández-Higuero, J.A., Cabrera, Y., Moro, F. and Muga, A. *"ClpB dynamics is driven by its ATPase cycle and regulated by the DnaK system and substrate proteins"*. Biochem. J. 466, 561-570 (2015).

S4-1

DYNAMICS AND FUNCTION OF MEMBRANE TRANSPORTERS AND ION CHANNELS BY VIBRATIONAL SPECTROSCOPY

Vìctor A. Lórenz-Fonfría[1]

[1]Experimental Molecular Biophysics, Institute of Experimental Physics, Department of Physics, Freie Universität Berlin, Germany. victor.lorenz@gmail.com

Vibrational spectroscopy, and in particular infrared (IR) spectroscopy, is particularly well suited for the study of the functional mechanism and dynamics of membrane proteins. IR spectroscopy combines the high temporal resolution characteristic of optical spectroscopies with appreciable structural and chemical sensitivity. Furthermore, it is not only sensitive to vibrational modes localized in the protein backbone and side chains, but also in water molecules, lipids, and cofactors. We have used IR spectroscopy to understand the functional mechanism of the melibiose permease (MelB) from *E. coli*, a secondary transporter that couples the accumulation of the disaccharide melibiose to the co-transport of Na^+. We have resolved residues involved in Na^+ binding [1], as well as tilt changes in transmembrane helices during the translocation mechanism of the substrates [2]. Recent efforts focus on time-resolved IR studies to understand the dynamics of substrate binding and translocation.

Channelrhodopsins (ChRs) are sensory microbial rhodopsins, renowned for being the first and so far the only light-gated ion channels known in nature [3]. ChR2, from the unicellular green alga *Chlamydomonas reinhardtii*, has been used for nearly a decade to depolarize the cell membrane of excitable cells by light, making it a prominent tool in the field optogenetics [4]. Using IR spectroscopy with microsecond resolution we resolved proton transfer reactions in ChR2 following excitation with a nanosecond laser pulse [5-6]. Subsequently, we resolved conformational changes in the protein and the retinal in the nanosecond time scale [7]. Recently, we have characterized the temporal evolution of water influx and efflux into the transmembrane region, and show to tightly correlate with the start and end ion permeation. Our results highlight the relevance of water dynamics and protonation changes in the activation mechanism of ChR2, and possible in other ion channels.

References:

[1] Granell, M., Leon, X., Leblanc, G., Padros, E. & Lórenz-Fonfría, V.A. "*Structural insights into the activation mechanism of melibiose permease by sodium binding*", Proc. Natl. Acad. Sci. U. S. A. 107, 22078-83, 2010.
[2] Lórenz-Fonfría, V.A., Granell, M., León, X., Leblanc, G. & Padrós, E. "In-plane and out-of-plane infrared difference spectroscopy unravels tilting of helices and structural changes in a membrane protein upon substrate binding", J. Am. Chem. Soc. 131, 15094-5, 2009.
[3] Nagel, G. et al. "Channelrhodopsin-1: a light-gated proton channel in green algae", Science 296, 2395-8, 2002.
[4] Fenno, L., Yizhar, O. & Deisseroth, K. The development and application of optogenetics. *Annu. Rev. Neurosci.* 34, 389-412 (2011)
[5] Lórenz-Fonfría, V.A. et al. "Transient protonation changes in channelrhodopsin-2 and their relevance to channel gating", Proc. Natl. Acad. Sci. U. S. A. 110, E1273-81, 2013.
[6] Lórenz-Fonfría, V.A. & Heberle, J. "Channelrhodopsin unchained: Structure and mechanism of a light-gated cation channel", Biochim. Biophys. Acta 1837, 626-642, 2014.
[7] Lórenz-Fonfría, V.A. et al. "Pre-gating conformational changes in the ChETA variant of channelrhodopsin-2 monitored by nanosecond IR spectroscopy", J. Am. Chem. Soc. 137, 1850-61 (2015).

S4-2

Membrane targeting self-assembled peptides

Juan R. Granja, Nuria Rodríguez-Vázquez, Alberto Fuertes, Juan M. Priegue, Eva González-Freire, Manuel Amorín and Javier Montenegro

Department of Organic Chemistry and Center for Research in Biological Chemistry and Molecular Materials (CIQUS), University of Santiago de Compostela, Campus Vida, Santiago de Compostela. Spain

Peptide nanotubes are a new class of biomaterials-based supramolecular assemblies formed by stacking of cyclic peptide in a flat conformation.[1] These cyclic peptides are specially designed to adopt a flat conformation bearing all the backbone amide groups (carbonyl and N-H) perpendicular oriented to the plain of the ring. In this conformation all the side-chains are outwards projected modifying the surface characteristics of the tubular ensemble. Among other applications, specially designed peptides subunits effectively interact with the lipid bilayers forming channels and other structures interfering in the membrane isolating properties.[2]
In the last few years we have been working with cyclic peptides that contain cyclic gamma-amino acids that self-assemble into nanotubes under appropriated conditions.[3] These cyclic peptides allow the modification of the outer surface and also their inner cavity. In this communication we will describe our studies toward the design and properties of membrane interacting nanotubes.

Acknowledgements: This work was supported by the Spanish Ministry of Economy and Competitivity (MEC) and the ERDF [CTQ2013-43264-R], by the Xunta de Galicia (GPC2013/039). We also thank the ORFEO-CINCA network. All calculations were carried out at the Centro de Supercomputación de Galicia (CESGA).

References:

[1] García-Fandiño, R.; Amorín, M.; Granja, J. R. “Synthesis of Supramolecular Nanotubes” in *Supramolecular Chemistry: From Molecules to Nanomaterials.* P. A. Gale and J. W. Steed Eds. John Wiley & Sons Ltd, Chichester, UK, **2012**, Vol 5, pp 2149.

[2] Fernández-López, S.; Kim, H.-S.; Choi, E.C.; Delgado, M.; Granja, J.R.; Khasanov, A.; Long, G.; Weinberger, D.A.; Wilcoxen, K. M.; Ghadiri, M. R. *Nature*, **2001**, *412*, 452; M. R. Ghadiri, J. R. Granja, L. K. Buehler, *Nature*, **1994**, *369*, 301; Montenegro, J.; Ghadiri, M. R.; Granja, J. R. Ion Channel Models Based on Self-Assembling Cyclic Peptide Nanotubes. *Acc. Chem. Res.* **2013**, *46*, 2955–2965.

[3] Reiriz, C.; Brea, R. J.; Arranz, R.; Carrascosa, J. L.; Garibotti, A.; Manning, B.; Valpuesta, J. M.; Eritja, R.; Castedo, L.; Granja, J. R. *J. Am. Chem. Soc.* **2009**, *131*, 11335; Montenegro, J.; Vázquez-Vázquez, C.; Kalinin, A.; Geckeler, K. E.; Granja, J. R. Coupling of Carbon and Peptide Nanotubes. *J. Am. Chem. Soc.* **2014**, *136*, 2484–2491.

S4-3

THE BIOPHYSICAL BASIS OF TRANSPORT CARRIER BIOGENESIS AT THE GOLGI COMPLEX

Felix Campelo[1,2] and Vivek Malhotra[1,2,3]

[1]Cell and Developmental Biology Programme, Centre for Genomic Regulation (CRG), Barcelona, Spain. *felix.campelo@crg.eu*
[2]Universitat Pompeu Fabra, Barcelona, Spain.
[3]Insitució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.

Intracellular transport relies on the formation of small membrane-bound transport carriers, which shuttle proteins and lipids between different cell compartments. Various steps are required for transport carrier biogenesis: protein sorting and nucleation of the key machinery in a membrane budding site, membrane curvature generation to form the growing bud, and membrane fission, the process by which a cellular membrane is separated into two physically disconnected membranes. A regulated act of specific lipids and proteins is responsible for the generation of the elastic stresses that deform and eventually pinch off the membrane. Hence, a complete understanding of the mechanisms of membrane curvature generation and fission requires both a deep understanding of membrane mechanics and of how the biochemical players are involved [1].

Although different proteins and lipids have been shown to play a functional role in this process, the ultimate mechanism of membrane fission is not known. In this talk, I will discuss how protein kinase D (PKD) activity, through the regulation of lipid homeostasis at the Golgi membranes is crucial for the biogenesis of transport carrier biogenesis at the Golgi membranes destined to the cell surface [2]. Specifically, I will discuss the role of the structural lipid sphingomyelin on the formation of functional domains required for cargo processing, membrane curvature generation and membrane fission [3,4].

Acknowledgements: This work has been sponsored by grants from Plan Nacional (BFU2008-00414), Consolider (CSD2009-00016), Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR) Grups de Recerca Emergents (SGR2009-1488; AGAUR-Catalan Government), and the European Research Council (268692).

References:

[1] F. Campelo, V. Malhotra, *"Membrane fission: the biogenesis of transport carriers"*, Annu. Rev. Biochem., 81, 407–427, 2012.
[2] V. Malhotra, F. Campelo, *"PKD regulates membrane fission to generate TGN to cell surface transport carriers"*, Cold Spring Harb. Perspect. Biol., 3, a005280, 2011.
[3] J. M. Duran, F. Campelo, J. van Galen, T. Sachsenheimer, J. Sot, M.V. Egorov, C. Rentero, C. Enrich, R.S. Polishchuk, F.M. Goñi, B. Brügger, F. Wieland, V. Malhotra, *"Sphingomyelin organization is required for vesicle biogenesis at the Golgi complex"*, EMBO J., 31, 4535–4546, 2012.
[4] J. van Galen, F. Campelo, E. Martínez-Alonso, M. Scarpa, J. Á. Martínez-Menárguez, V. Malhotra, *"Sphingomyelin homeostasis is required to form funcional enzymatic domains at the trans-Golgi network"*, J. Cell Biol., 206, 5, 609–618, 2014.

S4-4

DIELECTRIC POLARIZATION PROPERTIES OF SUPPORTED BIOLAYERS MEASURED WITH ELECTROSTATIC FORCE MICROSCOPY

Gabriel Gomila

Institut de Bioenginyeria de Catalunya (IBEC) and Universitat de Barcelona (UB), Barcelona, Spain
ggomila@ibecbarcelona.eu

The electric polarization properties of ultrathin biolayers play an important role in various areas of science and technology. For instance, cell membrane dielectric properties are key in bioelectric phenomena such as membrane potential formation, action potential propagation or ion membrane transport. On the other side, changes in the dielectric properties of biolayers constitute the main electro-transducing mechanisms used in electrical and electrochemical capacitance or impedance biosensors. Here I will present a methodology based on electrostatic force microscopy able to quantify the dielectric properties of supported ultrathin biolayers (thickness below 10 nm) at an unprecendented lateral spatial resolution (down to 20 nm) and precision (uncertainties in the dielectric constants below 10%). Examples of application will be shown for bacteriorhodopsin layers [1], lipid bilayers [2] and cholesterol layers [2], on both conducting [1] and insulating [2] substrates and, in both, air [1,2] and liquid [3] environments. Comparison of the results reported with existing theoretical predictions, or existing experimental results obtained with macroscopic techniques or other scanning probe microscopy methods (e.g. Nanoscale Capacitance Microscopy [4]) will be provided and discussed.

Acknowledgements: This work has been partially sponsored by the Spanish project TEC2013-48344-C2-1-P.

References:

[1] G. Gramse, I. Casuso, J. Toset, L. Fumagalli, and G. Gomila, *Quantitative dielectric constant measurement of thin films by DC electrostatic force microscopy*, Nanotechnology 20, 395702 (2009).
[2] A. Dols-Pérez, A. Calo, G. Gramse, G. Gomila and L. Fumagalli (in preparation).
[3] G. Gramse, A. Dols-Pérez, M. A. Edwards, L. Fumagalli and G. Gomila, *Nanoscale measurement of the dielectric constant of supported lipid bilayers in aqueous solutions with electrostatic force microscopy*, Biophysical Journal, 104, 1257-1262 (2013).
[4] L. Fumagalli, G. Ferrari, M. Sampietro and G. Gomila, *Quantitative nanoscale dielectric microscopy of single-layer supported biomembranes*, Nano letters 9, 1604-1608 (2009).

S5-1

Role of the carboxy-terminal domain of Erb1 during ribosome biogenesis in the formation of the Nop7/PeBoW complex

Marcin Wegrecki[1], Jose Luis Neira[2] and Jerónimo Bravo[1]

[1]Instituto de Biomedicina de Valencia, Valencia, Spain. *jbravo@ibv.csic.es*
[2]Instituto de Biología Molecular y Celular, Universidad Miguel Hernández, Elche, Spain.

Erb1 (Eukaryotic Ribosome Biogenesis 1) protein is essential for the maturation of the ribosomal 60S subunit. Functional studies in yeast and mammalian cells showed that altogether with Nop7 and Ytm1 it forms a stable subcomplex, called PeBoW in mammals that is crucial for a correct rRNA processing [1]. Since Ytm1 and Nop7 do not physically interact, Erb1 is considered to be the core of the complex and the ratio of Nop7/Erb1 and Erb1/Ytm1 heterodimers is important in controlling the assembly and function of Nop7 complex (as shown for PeBoW complex in mammals by Rohrmoser [2]). It was shown that in yeast a truncated Erb1 lacking the C-terminal domain would not cause growth arrest but presented only a mild defect in rRNA processing [3]. Despite the fact that the β-propeller domain of Erb1 has been proposed as dispensable for ribosome assembly, it still presents a high degree of conservation in all eukaryotes.
We have solved the crystal structure of the domain. This first structural report on Erb1 from yeast describes the architecture of a seven-bladed β-propeller domain that revealed a characteristic extra motif formed by two α-helices and a β-strand that insert within the second WD repeat. The structure provides some new insights into the role of Erb1. In particular, we have shown a direct interaction of the C-terminal domain of Erb1 with polyuridilic acid.
We will present some new evidences towards the need to re-evaluate the role of the carboxy-terminal domain of Erb1 during PeBoW complex formation and ribosome biogenesis.

Acknowledgements: This work has been sponsored by European Community, Seventh Framework Programme (FP7/2007-2013) under BioStruct-X (grant agreement N°283570) to JB; Ministerio de Economía y Competitividad , SAF2012-31405 (JB), and CSD2008-00005 and CTQ2013-4493 (JLN); Generalitat Valenciana PROMETEO/2012/061 (JB) and 2013/018 (JLN); MW received a JAE-PREDOC fellowship from Consejo Superior de Investigaciones Científicas, Spain

References:

[1] Pestov DG, Stockelman MG, Strezoska Z, Lau LF. "ERB1, the yeast homolog of mammalian Bop1, is an essential gene required for maturation of the 25S and 5.8S ribosomal RNAs." Nucleic Acids Res. 2001;29: 3621–30
[2] Rohrmoser M, Ho M, Grimm T, Malamoussi A, Harasim T, Orban M, et al. Interdependence of Pes1, Bop1, and WDR12 controls nucleolar localization and assembly of the PeBoW complex required for maturation of the 60S ribosomal subunit. Mol Cell Biol. 2007;27: 3682–94
[3] Tang L, Sahasranaman A, Jakovljevic J, Schleifman E, Woolford JL. Interactions among Ytm1, Erb1, and Nop7 required for assembly of the Nop7-subcomplex in yeast preribosomes. Mol Biol Cell. 2008;19: 2844–56

S5-2

Structural studies on co-translational protein folding and sorting

Paola Fucini [1,2], Neha Dhimole [1], Andreas Schedlbauer [1], Borja Ochoa-Lizarralde [1], Tatsuya Kaminishi [1], Tammo Diercks [1] and Sean Connell [1,2]

[1] Structural Biology Unit, CIC bioGUNE, Parque Tecnológico de Bizkaia, Derio 48160, Spain

[2] IKERBASQUE, Basque Foundation for Science, 48011 Bilbao, Spain

E-mail: pfucini@gmail.com

Emerging biochemical and biophysical data strongly suggest that the transformation of the potential set of proteins, encrypted in the genome of the cell, into its actual vital functional set, is a function not just of the genetic code but of an additional layer of information encoded within the newly synthesized polypeptide nascent chain (NC).

To date, we know that immediately upon the first steps of protein synthesis, a variety of signals are formed within the evolving nascent chain (NC). These signals, by acting directly on the ribosome or by recruiting additional cellular machines, have the ability to pause or even abrogate the synthesis itself of the NC or to guide it towards the acquisition of its final functional state and cellular location. Due to its distinct nature, we prefer to refer to this ensemble of signals as THE NASCENT CODE. Despite our knowledge of its existence and importance, today we know very little about its mechanism of action.

The aim of my talk is to provide an overview on the experimental approach we have undertaken to decipher the nascent code where, in particular, the use of liquid state NMR is a powerful tool to investigate the dynamic interplay between the ribosome, the nascent chain, and two chaperone factors, SecA and TF, that are involved in the co-translational protein folding and sorting process. Interestingly, both factors appear to modulate the conformation and dynamics of the NC even before its emergence from the ribosomal tunnel and not yet accessible to direct interaction.

S5-3

Structural and thermodynamic studies of viral Late domain interactions: towards the development of host-oriented therapeutics.

Irene Luque

Departamento de Química Física, Facultad de Ciencias, Universidad de Granada. 18071Granada, Spain

Many enveloped viruses encode Late budding domains (L-domains) in their sequence. These L-domains usually contain highly conserved motifs, such as PPxY and PTAP, known to mediate cellular protein-protein interactions. These motifs are essential for the egress of the virions from the infected cell, which takes place through the recruitment of the ESCRT cellular machinery for sorting and scission. Because viral budding mechanisms are shared by many viruses, blocking Late domain interactions with their cellular targets has been proposed as a promising strategy for the development of broad spectrum antivirals effective against viruses such as HIV, Ebola, Marburg, HTLV or Rabies. We present here a structural and thermodynamic characterization of L-domain binding to the UEV domain of hTSG101 and the WW domains of hNEDD4, which has revealed important features of these interactions, both in terms of binding affinity and specificity, as well as cooperativity between domains. Using phage display techniques we have identified peptide sequences binding to these proteins with nanomolar dissociation constants that efficiently block viral egress in Virus-Like-Particle systems. The thermodynamic and structural analysis of these complexes reveals additional interactions outside the canonical binding pocket, of interest for the identification and optimization of high affinity and specificity inhibitors of Late domain interactions as potential broad spectrum antivirals.

S5-4

EXPLOITING PROTEIN FLEXIBILITY TO PREDICT THE LOCATION OF ALLOSTERIC SITES

Alejandro Panjkovich[1] and Xavier Daura[1,2]

[1]Institut de Biotecnologia i de Biomedicina, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Spain, xavier.daura@uab.cat
[2]Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain

Allostery is one of the most powerful and common ways of regulation of protein activity. However, for most allosteric proteins identified to date the mechanistic details of allosteric modulation are not yet well understood. Uncovering common mechanistic patterns underlying allostery would allow not only a better academic understanding of the phenomena, but it would also streamline the design of novel therapeutic solutions. This relatively unexplored therapeutic potential and the putative advantages of allosteric drugs over classical active-site inhibitors fuel the attention allosteric-drug research is receiving at present. A first step to harness the regulatory potential and versatility of allosteric sites, in the context of drug-discovery and design, would be to detect or predict their presence and location. We will describe a simple computational approach to predict the existence and position of allosteric sites in a given protein structure on the basis of the effect a ligand exerts on the overall flexibility of the protein upon binding.
By querying the literature and a recently available database of allosteric sites, we gathered 213 allosteric proteins with structural information that we further filtered into a non-redundant set of 91 proteins [1]. We performed normal-mode analysis and observed significant changes in protein flexibility upon allosteric-ligand binding in 70% of the cases. These results agree with the current view that allosteric mechanisms are in many cases governed by changes in protein dynamics caused by ligand binding. Furthermore, we implemented an approach that achieves a positive predictive value of 65% in identifying allosteric sites within the set of predicted cavities of a protein by combining the analysis of flexibility with an analysis of local structural conservation [2].
Based on these findings, we have developed a simple and fast web server for the identification of cavities with a potential regulatory function in proteins (http://bioinf.uab.cat/pars) [3], and have exploited this tool for genome-wide detection of putative targets of antimicrobials with new modes of action in gram-negative bacteria (http://bioinf.uab.cat/antibactr) [4].

Acknowledgements: This work has been sponsored by the Seventh Research Framework Programme of the European Union (HEALTH-F3-2009-223101).

References:

[1] A. Panjkovich, X. Daura, "Exploiting protein flexibility to predict the location of allosteric sites", BMC Bioinformatics, 13, 273, 2012.
[2] A. Panjkovich, X. Daura, "Assessing the structural conservation of protein pockets to study functional and allosteric sites: implications for drug discovery", BMC Struct Biol, 10, 9, 2010.
[3] A. Panjkovich, X. Daura. "PARS: a web server for the prediction of Protein Allosteric and Regulatory Sites", Bioinformatics, 30, 9, 1314-1315, 2014.
[4] A. Panjkovich, I. Gibert, X. Daura, "*antibacTR: dynamic antibacterial-drug-target ranking integrating comparative genomics, structural analysis and experimental annotation*", BMC Genomics, 15, 36, 2014.

S6-1

MOLECULAR DISSECTION OF COLD TEMPERATURE SENSING IN MAMMALIAN NEURONS

Félix Viana

Instituto de Neurociencias de Alicante, UMH-CSIC, San Juan de Alicante, Spain, felix.viana@umh.es

Mammals, including humans, maintain a tight control over their internal temperature, even in the face of large temperature fluctuations in the environment. Our current understanding of cold temperature sensing in mammalian neurons implicates two major classes of ion channels: two pore domain, leak, potassium channels (K2P) and non-selective cationic TRP channels. The role of specific channels in the transduction process of cold temperatures and their gating mechanisms is still a matter of debate.

Recently, we found that the excitability of mouse hippocampal networks was highly sensitive to temperature fluctuations, leading to the firing of action potentials during minimal cooling ramps. These effects were independent of thermosensitive TRP channels but mainly dependent on the closure of temperature-sensitive K2P channels of the TREK/TRAAK family [1]. In contrast, cold-evoked activity in peripheral thermoreceptors requires the opening of TRPM8 channels [2]. TRPM8 are modular proteins assembled as tetramers. Our molecular analysis of the cold transduction process has led to a number of discoveries: activity of TRPM8 is modulated by the lipid composition of the plasma membrane [3], by posttranslational modifications of the protein, including the N-glycolsilation of a single Asn residue in the vicinity of the pore loop [4], and by specific residues in the intracellular N-terminal domain of the channel [5]. Finally, our transcriptome analysis of purified mammalian cold thermoreceptors revealed the enrichment of several ion channels and membrane receptors in TRPM8-expressing neurons, and uncovered an unexpected role of TASK3, a temperature-insensitive K2P channel, in the modulation of peripheral temperature sensitivity [6].

My lecture will integrate our efforts over the past decade to understand the function of mammalian cold thermoreceptors, with critical contributions from collaborators, as well as current and past laboratory members.

Acknowledgements: This work has been sponsored by MINECO project SAF2013-45608-R.

References:

[1] de la Peña E, Mälkiä A, Vara H, Caires R, Ballesta JJ, Belmonte C, Viana F. The influence of cold temperature on cellular excitability of hippocampal networks. *PLoS One* 7(12):e52475, 2012.

[2] Almaraz L, Manenschijn JA, de la Peña E, Viana F. TRPM8. *Handb Exp Pharmacol.* 222: 547-579, 2014.

[3] Morenilla-Palao C, Pertusa M, Meseguer V, Viana F. Lipid raft segregation modulates TRPM8 channel activity. *J Biol Chem* 284: 9215-9224, 2009.

[4] Pertusa M, Madrid R, Morenilla-Palao C, Belmonte C, Viana F. The N-glycosylation of TRPM8 channels modulates the temperature sensitivity of cold-thermoreceptor neurons. *J Biol Chem* 287: 18218-18229, 2012.

[5] Pertusa M, González A, Hardy P, Madrid R, Viana F. Bidirectional Modulation of Thermal and Chemical Sensitivity of TRPM8Channels by the Initial Region of the N-Terminal Domain. *J Biol Chem* 289: 21828-21843, 2014.

[6] Morenilla-Palao C, Luis E, Fernández-Peña C, Quintero E, Weaver JL, Bayliss DA, Viana F. Ion channel profile of TRPM8 cold receptors reveals a role of TASK-3 potassium channels in thermosensation. *Cell Reports* 8: 1571-1582, 2014. doi: 10.1016/j.celrep.2014.08.003.

S6-2

STUDYING CHEMOKINE RECEPTOR CONFORMATIONS AND DYNAMICS AT THE CELL MEMBRANE

Mario Mellado[1]

[1]Department of Immunology and Oncology, Centro Nacional de Biotecnología/CSIC, Campus de Cantoblanco, E-28049, Madrid, Spain. *mmellado@cnb.csic.es*

The chemokines, a family of structurally related chemoattractant proteins that bind to specific seven-transmembrane receptors linked to G proteins, trigger a broad array of biological responses ranging from cell polarization, movement, immune and inflammatory responses, as well as tumor metastasis to prevention of HIV-1 infection. Chemokine-mediated cell activation was thought to be due to the binding of a monomeric chemokine to its monomeric receptor. Chemokine biology is nonetheless more complex than was initially predicted. Studies using resonance energy transfer techniques (BRET, FRET) show that chemokine receptors homo- and heterodimerize spontaneously in the absence of ligands. These are dynamic conformations regulated by receptor expression and ligand activation to adapt the cell responses to changes in the milieu.

Using total internal reflection microscopy (TIRF-M), we have observed that the chemokine receptors form oligomers that cluster in arrays in membrane microdomains. These pre-formed clusters are reorganized and activated following ligand binding. Membrane microdomains, interactions between transmembrane proteins and cytoskeleton, and interactions between proteins within the membrane regulate receptor dynamics and clustering, and define receptor function.

Acknowledgements: This work has been sponsored in part by grants from the Spanish Ministry of Science and Innovation (SAF 2011-27370), the RETICS Program (RD12/0009/009; RIER and RD12/0042/0056; RIC), and the Madrid regional government (S2010/BMD-2350; RAPHYME).

References:

[1] Sallusto F, Lanzavecchia A, Mackay CR. Chemokines and chemokine receptors in T-cell priming and Th1/Th2-mediated responses. Immunol Today, 19:568-574 (1998).
[2] Baggiolini, M. *Chemokines and leukocyte traffic*. Nature 392, 565-568 (1998)
[3] Matshshima K, Terashima Y, Toda E, Shand F n Ueha S. Chemokines in inflammatory and immune diseases. *Inflam. Reg.* 31:11-22 (2011).
[4] Homey B, Müller A, Zlotnik A. *Chemokines: agents for the immunotherapy of cancer?* Nat Rev Immunol 2002, 2:175-184 (2002).
[5] Reiche EM, Bonametti AM, Voltarelli JC, Morimoto HK, Watanabe MA. Genetic polymorphisms in the chemokine and chemokine receptors: impact on clinical course and therapy of the human immunodeficiency virus type 1 infection (HIV-1). Cur Med Chem, 14:1325-1334 (2007).
[6] Martínez-Muñoz L, Barroso R, Dyrhaug SY, Navarro G, Lucas P, Soriano SF, Vega B, Costas C, Muñoz-Fernández MÁ, Santiago C, Rodríguez Frade JM, Franco R, Mellado M. *CCR5/CD4/CXCR4 oligomerization prevents HIV-1 gp120IIIB binding to the cell surface.* Proc Natl Acad Sci U S A. 111:E1960-E1969 (2014).
[7] Barroso R, Martínez Muñoz L, Barrondo S, Vega B, Holgado BL, Lucas P, Baíllo A, Sallés J, Rodríguez-Frade JM, Mellado M. EBI2 regulates CXCL13-mediated responses by heterodimerization with CXCR5. FASEB J. 26: 4841-4854 (2012).

S6-3

Covalent link between the voltage-sensing module and the pore domain is not required for voltage-depending gating in KCNH potassium channels

Pilar de la Peña[1], Luis A. Pardo[2] and Francisco Barros[1]

[1] Departamento de Bioquímica y Biología Molecular. Universidad de Oviedo, Spain. pdelapena@uniovi.es
[2] Oncophysiology Group. Max Planck Institute of Experimental Medicine, Göttingen, Germany.

Voltage-gated potassium (Kv) channels open paths for ion permeation following changes in membrane potential but how voltage changes are coupled to gating is not entirely understood. Functional Kv channels are formed by an ensemble of two modules, one responsible for voltage sensing (transmembrane segments S1 to S4) and the other for potassium permeation (S5 and S6). Current knowledge indicates that in Kv channels the intracellular S4-S5 linker is crucial for the so-called "electromechanical coupling" providing physical continuity between the voltage sensor and the pore domain. We tested the relevance of the S4-S5 linker as structural link coupling voltage sensing and channel gating in channels of the KCNH family (Kv10.1 y Kv11.1), by generating truncated proteins interrupted at the S4-S5 linker. Our data demonstrate that at least for KCNH channels, a covalent link between the voltage sensing and the pore modules is not required for voltage-dependent gating, challenging the classical view of a S4-S5 linker acting as a rigid mechanical coupler between them, and opening new questions about the nature of the molecular and functional interactions between the voltage-sensing and pore domains of the protein. Our latest results using split Kv11.1 channels analyzing the functional coupling between the voltage-sensing and the permeation modules point to an "electrointeractional coupling" mechanism probably involving interactions between the S4 helix/S4-S5 loop and the C-terminal region of the S6 helix.

Acknowledgements: This work has been sponsored by the Spanish Ministry of Science and Innovation (MICINN) Consolider-Ingenio programme (Grant CSD2008-00005), the 2013 Principado of Asturias Government (Grant SV-PA-13 ECOEMP-69) and the Max Planck Society.

S6-4

GAP, A NEW FAMILY OF FLUORESCENT PROTEIN PROBES FOR IMAGING CALCIUM SIGNALS IN INTRACELLULAR ORGANELLES

María Teresa Alonso, Jonathan Rojo, Paloma Navas and *Javier García-Sancho*

Instituto de Biología y Genética Molecular (IBGM), Universidad de Valladolid y CSIC, Valladolid, Spain.
jgsancho@ibgm.uva.es

Genetically encoded calcium indicators (GECI) allow monitoring subcellular Ca^{2+} signals inside organelles. Most GECI contain endogenous calcium-binding proteins whose functionality in vivo may be perturbed by competition with cellular partners. We describe here a novel family of fluorescent Ca^{2+} probes based on the fusion of two Aequorea victoria proteins, GFP and apo-aequorin (GAP). Both proteins have been used extensively with no report of interferences with Ca^{2+} homeostasis or other secondary effects. GAP exhibited green fluorescence whose excitation spectrum was shifted by Ca^{2+}. GAP displayed a unique combination of features: dual-excitation ratiometric imaging, high dynamic range, good signal-to-noise ratio, insensitivity to pH and Mg^{2+}. Ca^{2+} affinity could be tuned by mutations in the aequorin EF hands. Ca^{2+} calibration was uncomplicated, with a maximum ratio increase of three to fourfold and a Hill coefficient of 1. We have targeted GAP to five distinct organelles and behaviour was as expected for a selective Ca^{2+} probe. Both, virus-induced expression as well as cell lines stably expressing targeted GAPs were successfully achieved. Transgenic mice for endoplasmic reticulum-targeted GAP exhibited a robust long-term expression and reproducible performance in various neural tissues including hippocampus, cerebral cortex, cerebellum, spinal motor neurons or dorsal root sensory neurons. Expression pattern in other tissues will be advanced. This biosensor fills a gap in the actual repertoire of Ca^{2+} indicators for organelles and is a valuable tool for in vivo Ca^{2+} imaging applications.

Acknowledgements: This work was sponsored by grants from the European Research Area Net (ERA-Net) program and the Spanish Ministerio de Economia y Competitividad (BFU2010-17379).

References:

[1] *"GAP, an aequorin-based fluorescent indicator for imaging Ca^{2+} in organelles"*, Arancha Rodriguez-Garcia, Jonathan Rojo-Ruiz, Paloma Navas-Navarro, Francisco Javier Aulestia, Sonia Gallego-Sandin, Javier Garcia-Sancho, and Maria Teresa Alonso. Proc. Nat. Acad. Sci. USA, 111, 2584-2589, 2014

S7-1

Measuring binding affinities using force methods

J. Camunas-Soler[1], A. Alemany[2] and *F. Ritort*[2]

[1]*Small Biosystems Lab, Universitat de Barcelona and CIBER-BBN*
[2] Small Biosystems Lab, Universitat de Barcelona

Intermolecular binding reactions drive a myriad of processes central to molecular biology such as gene regulation, recombination, ribosome assembly and immune response. We introduce a novel fluctuation theorem for ligand binding to measure binding energies of biomolecular reactions at the single-molecule level. We investigate single oligonucleotides, DNA restriction enzymes, and small ligands binding to DNA hairpins in single-molecule pulling experiments. Binding energies are directly measured as a function of ligand concentration providing a direct experimental verification of the law of mass action both for highly specific interactions and non-specific binding to multiple DNA sites. The possibility of monitoring single binding modes one at a time in single molecule experiments [1,2,3], in combination with the fluctuation theorem for ligand binding, makes now possible to determine the energetics of complex multimolecular assemblies with unprecedented reliability and accuracy.

References:

[1] F. Ritort, Single molecule experiments in biological physics: methods and applications, Journal of Physics C (Condensed Matter),18 (2006) R531-R583
[2] A. Alemany, A. Mossa, I. Junier and F. Ritort, Experimental free-energy measurements of kinetic molecular states using fluctuation theorems, Nature Physics, 8 (2012) 688-694
[3] http://www.ffn.ub.es/ritort

S7-2

DNA ORIGAMI NANOPORES FOR SINGLE MOLECULE DETECTION

Silvia Hernández-Ainsa[1], K. Göpfrich[1], V.V.Thacker[1], E. Hemmig[1], T. Zettl[1], N. A. W. Bell[1], K. Misiunas[1], U. F. Keyser[1]

[1]Cavendish Laboratory, University of Cambridge, JJ Thomson Avenue, Cambridge, CB3 0HE, UK
smh80@cam.ac.uk

DNA origami[1] has emerged as a unique and versatile method to fabricate tailored nanostructures with exciting applications in nanobiotechnology. Precise assembly at the nanoscale level can be easily performed thanks to the programmability of DNA base-pairs interactions. This salient property is extremely important in the construction of artificial systems in which nanometre control of shape and dimensions is required. Synthetic nanopores used for biomolecules sensing represent an important example.[2]

Using the DNA origami method, we combined DNA origami structures with glass nanocapillaries to yield hybrid nanopores with controlled surface chemistry and precise diameters.[3, 4] We showed the possibility to control the folding of the translocating λ-DNA molecules by varying the size of the aperture in the DNA origami structure as well as to identify short DNA sequences by attaching appropriate ssDNA fragments at the DNA nanopore entrance.[3] Besides, the incorporation of stimuli-responsive domains in the DNA origami structure has yielded smart DNA origami nanopores that exerted a control in the passage frequency of λ-DNA molecules by tuning the voltage.[5]

DNA origami is also a promising platform to build constructs that mimic biological nanopores.[6] Our group has shown that DNA nanopores can be inserted into a hydrophobic membrane by attaching hydrophobic groups on the DNA construct.[7,8] We are currently incorporating functional molecules onto these DNA nanopores to expand their range of applications in biotechnology and nanomedicine.[9]

Acknowledgements: S.H.A. acknowledges support from a Herchel Smith Trust fellowship, from an ERC starting grant and from Oxford Nanopore Technologies® (http://www.nanoporetech.com).

References:

[1] P.W.K. Rothemund,"Folding DNA to create nanoscale shapes and patterns", Nature 440, 297, 2006
[2] U.F.Keyser, "Controlling molecular transport through nanopores", J. R. Soc. Interf., 8, 1369, 2011
[3] S. Hernández-Ainsa, N. A. W. Bell, V. V. Thacker, K. Göpfrich, K. Misiunas, M. Fuentes-Perez, F. Moreno-Herrero, U. F. Keyser, "*DNA origami nanopores for controlling DNA translocation*", ACS Nano, 7, 6024, 2013
[4] C.-Y. Li, E. A. Hemmig, J. Kong, J. Yoo, S. Hernández-Ainsa, U. F. Keyser, A. Aksimentiev. "*Ionic Conductivity, Structural Deformation and Programmable Anisotropy of DNA Origami in Electric Field*", ACS Nano, 9, 1420, 2015
[5] S. Hernández-Ainsa, K. Misiunas, V. V. Thacker, E. A. Hemmig, U. F. Keyser. "*Voltage dependent properties of DNA origami nanopores*", Nano Letters, 14, 1270, 2014
[6] S. Hernández-Ainsa, U. F. Keyser, "*DNA origami nanopores: developments, challenges and perspectives*", Nanoscale, 6, 14121, 2014
[7] J. R. Burns, K. Göpfrich, J. W. Wood, V. V. Thacker, E. Stulz, U. F. Keyser, S. Howorka. "*Lipid-Bilayer-Spanning DNA Nanopores with a Bifunctional Porphyrin Anchor*", Ang. Chem. Int. Ed. 52, 12069, 2013
[8] K. Göpfrich, T. Zettl, A.E. C. Meijering, S. Hernández-Ainsa, S. Kocabey, T. Liedl, U.F. Keyser "*DNA-tile structures lead to ionic currents through lipid membranes*", in revision.

[9] S. Hernández-Ainsa and U. F. Keyser. "*DNA origami nanopores: an emerging tool in biomedicine*", Nanomedicine, 10, 1551, 2013

S7-3

HIGH RESOLUTION METHODS TO ANALYZE THE REPAIR OF BROKEN DNA

Pablo Huertas[1], Andrés Cruz-García[2] and Fernando Mejías-Navarro[2]

[1]Centro Andaluz de Biología Molecular y Medicina Regenerativa (CABIMER), 41092 Sevilla, Spain and Departamento de Genética, Universidad de Sevilla, 41080, Sevilla, Spain. pablo.huertas@cabimer.es
[2]Centro Andaluz de Biología Molecular y Medicina Regenerativa (CABIMER), 41092 Sevilla, Spain and Departamento de Genética, Universidad de Sevilla, 41080, Sevilla, Spain

DNA-end resection consists of nucleolytic degradation with a 5′–3′ polarity of a single strand of the DNA at each side of a DNA break [1]. This step is critical for repairing broken DNA molecules, as the single-stranded DNA generated is the substrate of the error-free repair pathway known as homologous recombination [1,2]. Proper DNA repair is essential to avoid cancer development, thus mutations in homologous recombination genes are common in many malignancies [3], including genes coding for proteins such as BRCA1 or CtIP [4,5]. In cancer development, as in many other natural and pathological biological processes, subtle defects can accumulate to yield relevant differences in the final outcome. However, the techniques currently available for studying this have limited the differences that can be measured. This is exemplified by studies to determine the contribution of the complex BRCA1-CtIP to DNA-end resection: traditional techniques have both implicated and discarded a role for BRCA1 in resection [6-8]. We reasoned that these apparent contradictions were mainly caused by such technical limitations. Briefly, DNA resection in higher eukaryotes has been analyzed by the focal accumulation of the single-stranded DNA-protecting complex RPA. However, rather than reflecting differences in the length of the resected DNA, such a method reveals if the ssDNA was long enough to accommodate a sufficient number of RPA complexes to form a visible foci. This inability to discriminate between subtle differences in DNA resection has rendered a pretty simplistic view in the field, with proteins categorized into two discrete groups: essential or irrelevant for resection.
With the application of a novel, high-resolution technique, we have now observed shades of grey in this overly simplified, black-and-white picture [9]. By modifying the DNA combing technique used for high-resolution replication analyses, we have created a new assay that can measure resection progression at the level of individual DNA fibers [9]. We call this new approach SMART (Single Molecule Analysis of Resection Tracks). Applying this technique, we have now begun to observe subtle effects in DNA-end resection that were previously ignored but which are relevant to the repair process in the long term.

Acknowledgements: This work was funded by a R+D+I grant from the Spanish Ministry of Economy and Competitivity (SAF2010-14877). AC-G is funded by a PhD fellowship from the Consejo Nacional de Ciencia y Tecnología (CONACYT), Mexico. FM-N is the recipient of an FPU fellowship from the Ministry of Education, Spain.

References:

[1] Huertas P. DNA resection in eukaryotes: deciding how to fix the break. Nat Struct Mol Biol, 17:11-6, 2010.
[2] Heyer WD, Ehmsen KT, Liu J. *Regulation of homologous recombination in eukaryotes*. Annu Rev Genet, 44:113-39, 2010.
[3] Lord CJ, Ashworth A. *The DNA damage response and cancer therapy*. Nature, 481:287-94, 2012.
[4] Silver DP, Livingston DM. *Mechanisms of BRCA1 tumor suppression*. Cancer discovery, 2:679-84, 2012.
[5] Soria-Bretones I, Sáez C, Ruíz-Borrego M, Japón MA, Huertas P. *Prognostic value of CtIP/RBBP8 expression in breast cancer*. Cancer Medicine , 2(6): 774–783, 2013.

[6] Nakamura K, Kogame T, Oshiumi H, Shinohara A, Sumitomo Y, Agama K, Pommier Y, Tsutsui KM, Tsutsui K, Hartsuiker E, et al. Collaborative action of Brca1 and CtIP in elimination of covalent modifications from double-strand breaks to facilitate subsequent break repair. PLoS genetic, 6:e1000828, 2010.
[7] Reczek CR, Szabolcs M, Stark JM, Ludwig T, Baer R. The interaction between CtIP and BRCA1 is not essential for resection-mediated DNA repair or tumor suppression. The Journal of cell biology, 201:693-707, 2013.
[8] Yun MH, Hiom K. CtIP-BRCA1 modulates the choice of DNA double-strand-break repair pathway throughout the cell cycle. Nature, 459:460-3, 2009.
[9] Cruz-Garcia A, Lopez-Saavedra A, Huertas P. *BRCA1 Accelerates CtIP-Mediated DNA-End Resection*. Cell reports, 9:1-9, 2014.

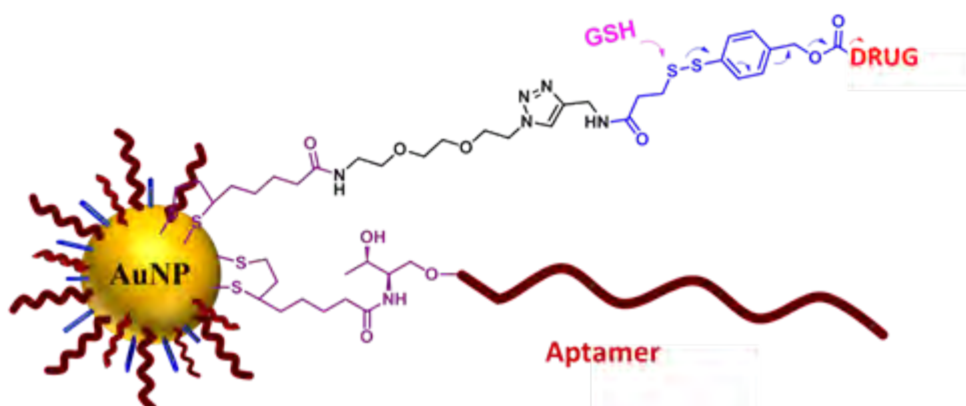
S7-4

OLIGONUCLEOTIDES AND NANOSTRUCTURES IN NANOMEDICINE

Álvaro Somoza

IMDEA Nanociencia, & Nanobiotecnología (IMDEA-Nanociencia), Unidad Asociada al Centro Nacional de Biotecnología (CSIC) Madrid, Spain, alvaro.somoza@imdea.org

In the latest years the combination of oligonucleotides and nanomaterials has attracted the attention of different disciplines due to their great potential in biomedical applications. This is mainly due to the limitations on the use of oligonucleotides in cells or *in vivo*, which can be overcome by the use of biocompatible nanomaterials. A remarkable example of this idea is the systemic delivery of siRNAs to treat tumors that has been successfully achieved in humans using nanoparticles.[1] In this regard we have been working on the use of spherical nucleic acid nanoparticle conjugates to detect and treat different types of cancers, such as Uveal Melanoma (UM). Particularly, we have used gold nanoparticles functionalized with aptamers, non-coding nucleic acids and drugs. This system has shown good selectivity and antitumoral activity against UM cell lines.
Another application of nucleic acids in nanomedicine deals with the preparation of fluorescent silver nanoclusters, which excitation and emission wavelengths can be tuned by the selection of specific oligonucleotide sequences.[2] Currently, there is not a clear correlation between the oligonucleotide sequences and the fluorescent properties, and what is more, the structure of the DNA-silver nanoclusters has not been determined. In this regards, we are studying the structure of different silver nanoclusters stabilized with oligonucleotides using NMR and circular dichroism. In addition, we are evaluating their antimicrobial activity in gram-positive and gram-negative bacteria.



Acknowledgements: This work has been sponsored by Asociación Española Contra el Cancer (*aecc*) and IMDEA Nanociencia.

References:

[1] Mark E. Davis, Jonathan E. Zuckerman, Chung Hang J. Choi, David Seligson, Anthony Tolcher, Christopher A. Alabi, Yun Yen, Jeremy D. Heidel, Antoni Ribas "*Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles*" Nature, 464, 7291, 1067-70, 2010.

[2] Alfonso Latorre, Álvaro Somoza, "DNA-mediated silver nanoclusters: synthesis, properties and applications" ChemBioChem, 13, 951-8, 2012.

S8-1

WHAT MAY COLLOIDAL AND SUPRAMOLECULAR CHEMISTRY PROVIDE IN GENE THERAPY?

Elena Junquera, Ana L. Barrán Berdón y Emilio Aicart

Grupo de Química Coloidal y Supramolecular, Dpto. de Química Física I, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, 28040 Madrid, Spain. junquera@ucm.es

The promise of gene therapy for future medical applications is reflected in the large amount of research in this field.[1] Among the different methods to transfect genetic material to the cellular interior, the colloidal self-aggregated and/or supramolecular systems[2-4] have revealed in the last decade as a plausible alternative to viral gene vectors, normally used in the past. Particularly, the cationic (CLs) or anionic (ALs) lipids, inorganic nanoparticles (NPs), supramolecular macrocycles (as calixarenes CXs or pilararenes PLs) or the polycationic amphiphilic cyclodextrins (paCDs) are able to compact DNA, either linear or plasmid, by means of a strong electrostatic interaction entropically driven, and transfect it to the nucleus of the damaged cells. However, in spite of the large amount of work already done at this respect, there are some problems that still remain unsolved, such as the low levels of transfection efficiency and the relatively high cytotoxicity of these vectors in cellular environments. This talk is focused in what colloidal and supramolecular chemistry may provide to overcome these limitations. Special attention has been paid to the biophysical characterization (electrostatics, structure, size and morphology) of the lipoplexes using a wide variety of experimental methods, and to the biological evaluation (transfection efficiency and cell viability/cytotoxicity) addressed to find the optimum formulations to be used in gene therapy.[3] Moreover, not much is known about the delivery of the genetic material from the vector once the plasmatic membrane has been passed, this fact being beyond the scarce or even non-existing control over the potential of a certain colloidal or supramolecular non-viral vector as an efficient and safe transfection agent *in vitro*, and, finally, *in vivo*. Undoubtedly, reaching the target of being able to cure diseases with new gene therapy protocols relies on acquiring a deeper knowledge and control over the phases of the transfection process: i) the first one, focussed on an adequate compaction and transport of the genetic material through the cell membrane to the cytoplasm; and ii) the second, centered on the proper delivery of the nucleic acid, getting over all the physiological barriers found in its way to the nucleus.[5]

Acknowledgements: This work has been sponsored by MICINN of Spain, Pr. no. CTQ2012-30821.

References:

[1] W. F. Anderson, " *Human gene therapy*", Science, *256*, 808-13, 1992.

[2] E. Junquera, E. Aicart, *"Cationic lipids as transfecting agents of DNA in gene therapy"*, Current Topics in Medicinal Chemistry, 14, 649-663, 2014.

[3] A. L. Barrán-Berdón, S. K. Misra, S. Datta, M. Muñoz-Úbeda, P. Kondaiah, E. Junquera, S. Bhattacharya y E. Aicart, *"Cationic gemini lipids containing polyoxyethylene spacers as improved transfecting agents of plasmid DNA in cancer cells"*, J. Materials Chemistry B, 2, 4640-4652, 2014.

[4] Alejandro Díaz-Moscoso, Dries Vercauteren, Joanna Rejman, Juan M. Benito, Carmen Ortiz Mellet, Stefaan C. De Smedt, José M. García Fernández, J.M. Benito, Carmen Ortiz Mellet, "I*nsights in cellular uptake mechanisms of pDNA–polycationic amphiphilic cyclodextrin nanoparticles (CDplexes)"* Journal of Controlled Release 146, 318–325, 2010.

[5] Ana Lilia Barrán-Berdón, Daniela Pozzi, Giulio Caracciolo, Anna Laura Capriotti, Giuseppe Caruso, Chiara Cavaliere, Anna Riccioli, Sara Palchetti, Aldo Laganà, "Time Evolution of Nanoparticle−Protein Corona in Human Plasma: Relevance for Targeted Drug Delivery", Langmuir, 29, 6485−6494, 2013.

S8-2

Synergy effects of magnetic silica nanostructures for drug and heat delivery applications

Verónica Salgueiriño

Departamento de Física Aplicada, Universidade de Vigo, 36310 Vigo (Spain)
E-mail: vsalgue@uvigo.es

Magnetic nanomaterials for drug delivery and related therapies has been extensively explored. The nanoparticles when bio-functionalized can indeed resemble biomolecules and biomolecular assemblies in terms of size and chemical composition and sometimes even function. This "nano-bio" interface comprises therefore the dynamic physicochemical interactions, the kinetics and the thermodynamic exchanges between nanomaterials surface and biological components. Its ability to get the nanostructures recruited and multivalently bond to surface receptor(s) is crucial to enhance specific affinity, reduce nonspecific interactions and direct receptor-mediated endocytosis, all of which would then maximize a selective delivery of cargo. However, despite the many reports about cytotoxicity and biocompatibility, synthetic routes of nanoparticles intended to specifically interact with cells stay behind and largely unappreciated, and consequently, few attempts to link the cellular responses to the physicochemical properties of the engineered nanoparticles have been reported.

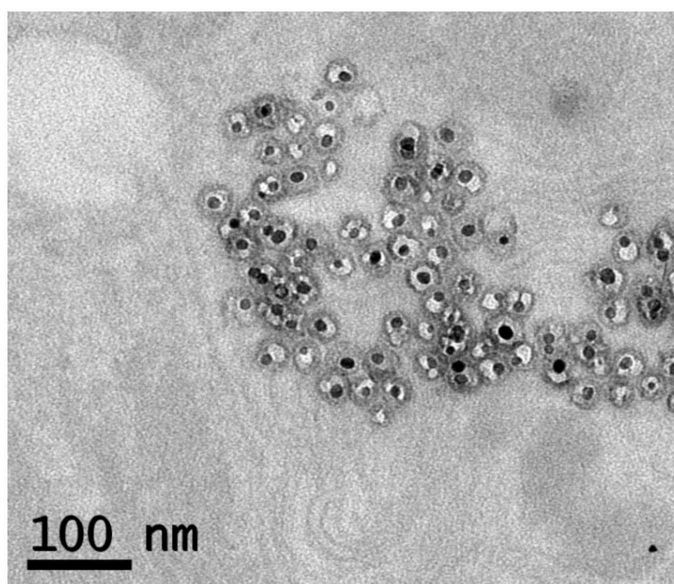


Figure 1. Partially disolved $Fe_3O_4@SiO_2$ nanoparticles after cellular uptake.

Acknowledgements: V. S. acknowledges funding from the Xunta de Galicia Regional Government (Spain) under project EM2014/035 (*Emerxentes*) and InBioMed.

References:

1. B. Dávila-Ibáñez, R. Mariño-Fernández, M. Maceira-Campos, A. García-Lorenzo, V. Martínez-Zorzano, V. Salgueiriño, *Nonhomogeneous Silica Promotes the Biologicallly Induced Delivery of Metal Ions from Silica-coated Magnetic Nanoparticles.* **J. Phys. Chemistry C** (2014), 118, 28266-28273.

2. R. Otero-Lorenzo, A. B. Dávila-Ibáñez, M. Comesaña-Hermo, M. A. Correa-Duarte, V. Salgueiriño, *Synergy effects of magnetic silica nanostructures for drug delivery applications*, **J. Materials Chemistry B** (2014), 2, 2645-2653.
3. B. Dávila-Ibáñez, N. J. Buurma, V. Salgueiriño, *Assessment of DNA Complexation onto Polyelectrolyte-coated Magnetic Silica Nanoparticles*, **Nanoscale** (2013), 5, 4797-4807.
4. B. Dávila-Ibáñez, V. Salgueirino, V. Martínez-Zorzano, R. Mariño-Fernández, A. García-Lorenzo, M. Maceira-Campos, M. Muñoz-Úbeda, E. Junquera, E. Aicart, J. Rivas, F. J. Rodríguez-Berrocal, J. L. Legido, *Magnetic Silica Nanoparticle Cellular Uptake and Cytotoxicity Regulated by Electrostatic Polyelectrolytes-DNA Loading at their Surface*, **ACS Nano** (2012), 6, 747-759.

S8-3

Amyloid fibrils: Nor only a marker of disease but also an exciting nanomaterial

Pablo Taboada[1], Silvia Barbosa[1]

[1]*Colloids and Polymers Physics Group, Faculty of Physics, Universidad de Santiago de Compostela, 15782, Campus Vida, Santiago de Compostela, Spain.*

Protein misfolding and self-assembly of certain proteins and peptides into highly ordered β-sheet-rich fibrillar assemblies known as amyloid fibrils are common features of a growing class of systemic and neurodegenerative diseases as Alzheimer´s, Parkinson´s, and Huntington´s diseases, senile systemic amyloidoses, type II diabetes and many others [1]. However, fibrillation is not exclusive of these disease-related proteins, and an important number of non-disease associated proteins have also been already found to form ordered cytotoxic aggregates and amyloid-like fibrils *in vitro* [2]. On the other hand, the exceptional physical characteristics of the amyloidal protein state as its stability, mechanical strength and resistance to degradation implies that this type of structures possess a range of potential technological applications in biotechnology and materials science [3]. Thus, we will expose some of the main aspects concerning the origin and possible mechanisms by which proteins fibrillate, with special emphasis on the factors which can both originate and influence this process for a model protein, human serum albumin. We will also perform a summary of the most remarkable physico-chemical properties of the fibrillar structures, strongly related to their 1-dimensional architecture. Finally, some potential technological applications and uses of these particular nanostructures on the biomedical, biotechnology and material science areas will be briefly discussed.

Acknowledgements: This work has been sponsored by MINECO through project MAT2013-40971-R

References:

[1] Dobson C.M. *The structural basis of protein folding and its links with human disease*. Philos. Trans. R. Soc. Lond. B 356, 133-14, 2001.
[2] Juárez J., Alatorre-Meda M., Cambón A., Topete A., Barbosa S., Taboada P., Mosquera V. *Hydration effects on the fibrillation process of a globular protein: the case of human serum albumin.* Soft Matter 8, 3608-3619, 2012.
[3] Knowles T.P.J., Buehler M.J. *Nanomechanics of functional and pathological amyloid materials.* Nat. Nanotechnol. 6, 469-479, 2011.

S8-4

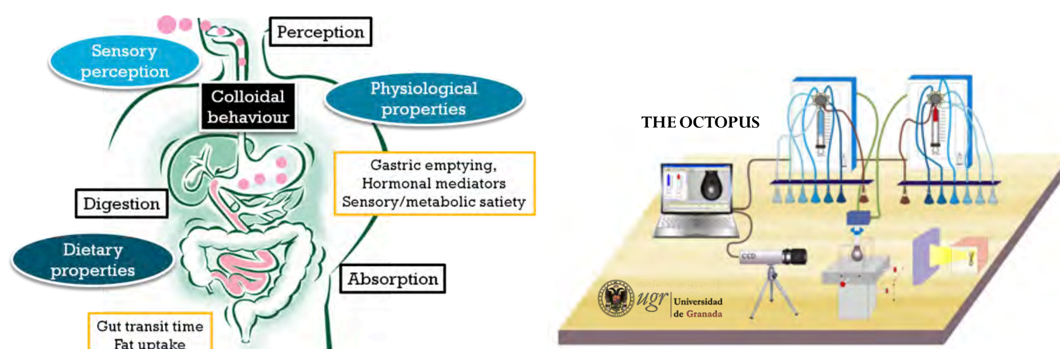
IMPACT OF INTERFACIAL STRUCTURE ON DIGESTIBILITY OF FOOD EMULSIONS

Julia Maldonado-Valderrama

University of Granada, Department of Applied Physics, Campus de Fuentenueva, s/n., 18071, Granada, Spain. *tdelcastillo@ugr.es*.

The rational design of healthier foods is generating renewed scientific interest due to the growing social and economic consequences of the obesity crisis in the developed world. Design of healthier foods to control fat uptake is a top priority in the EU: it is estimated that treating obesity and related conditions uses ~ 7% of the total healthcare budget. Improved understanding of the digestion and metabolism of lipids, and possible ways that this can be modified is of paramount importance in order to address this problem effectively. If we are to undertake the challenge of **rationally developing healthier foods with improved functionality, it is crucial that we gain an improved understanding of our consumption, digestion and absorption of lipids.**

Based on the physiological/physicochemical understanding of digestion, opportunities to affect the digestion profile, delivery and release of nutrients by food structure and composition are identified. In spite of the crucial role played by interfaces in determining the stability of emulsions and the breakdown of emulsion structure on digestion, there are still **few systematic basic studies dealing directly with the effects of digestion conditions on interfacial structures**.



In the last decade our interest has been to understand at a fundamental level, how changes in the interfacial composition and structure of processed food emulsions during digestion can influence lipolysis and digestibility of interfacial layers. To undertake this challenge we have designed **a novel methodology to study in-vitro digestion of interfacial layers: The OCTOPUS.** This equipment alows to follow *in-situ* the fate of an model interface as it passes through the gastrointestinal tract. We have have demontraded the **effect of block copolymers in reducing lipolysis** and the **impact of protein conformation in digestibility** of proteins. The latter is important owing to the link with allergenic potential which may be connected to incomplete digestion causing an inappropriate immune response in the gut.Such observations are important new generic features of the digestion process which could be potentially manipulated to control the digestion process through **functionalisation of interfaces**.

Our research work shows the value of interfacial tension as a tool to understand food digestion by **pioneering the use of *in-vitro* digestion models in a single droplet** to probe effects of digestion on interfacial structures. To systematically take into consideration the process of digestion in the

assembly line of a food product is a novel approach which allows **back engineering to reach an improved functionality** of the product.

Acknowledgements: RYC-2012-10556, COST-MPN-1106-Green Interfaces and COST-FA-1005-Infogest.

References:

Maldonado-Valderrama, J.; Wilde, P. J.; Macierzanka, A.; Mackie, A. R. "*The role of bile salts in digestion"*. Adv. Colloids Interface Sci. 165, 2011

Maldonado-Valderrama, J.; Terriza, J. A. H.; Torcello-Gomez, A.; Cabrerizo-Vilchez, M. A., "*In vitro digestion of interfacial protein structures",* Soft Matter, 9 (4), 1043-1053, 2013.

Amelia Torcello-Gómez, Miguel Wulff-Pérez, María José Gálvez-Ruiz, Antonio Martín-Rodríguez, Miguel Cabrerizo-Vílchez, Julia Maldonado-Valderrama. "*Block copolymers at interfaces: Interactions with physiological media".* Adv. Colloids Interface Sci. 206, 414-427, 2014

Maldonado-Valderrama, J. Torcello-Gómez, Del Castillo Santaella, T.;Holgado-Terriza, J. A.; Cabrerizo-Vílchez, M. A.; "*Subphase exchange experiments with the pendant drop technique".* Adv. Colloids Interface Sci. *in press* (DOI: 10.1016/J.CIS.2014.08.002).

Satelite Workshop "New & Notable in Biophysics" Lectures

W1

UNDERSTANDING HOW BIOLOGICAL MOLECULAR MOTORS WORK: IDENTIFICATION OF THE TRANSLOCATION STEP OF A REPLICATIVE DNA POLYMERASE

José A. Morin[1,5], Francisco J. Cao[2], José M. Lázaro[3], José M. Valpuesta[4], José L. Carrascosa[4], Ricardo J Arias-González[5], Margarita Salas[3] and Borja Ibarra[5]

[1] Max-Planck-Institute for the Physics of Complex Systems, Dresden, Germany
[2] Universidad Complutense de Madrid, Madrid, Spain
[3] Centro de Biología Molecular 'Severo Ochoa' (CBSO-CSIC), Madrid, Spain
[4] Centro Nacional de Biotecnología (CNB-CSIC), Madrid, Spain
[5] Instituto Madrileño de Estudios Avanzados en Nanociencia, IMDEA Nanociencia, Madrid, Spain.
borja.ibarra@imdea.org

During DNA replication replicative polymerases move in discrete mechanical steps along the DNA template. To address how the chemical cycle is coupled to mechanical motion of the enzyme, we have used optical tweezers to study the translocation mechanism of individual bacteriophage Phi29 DNA polymerases during processive DNA replication. We have determined the main kinetic parameters of the nucleotide incorporation cycle and their dependence on external load and nucleotide (dNTP) concentration. The data is inconsistent with power stroke models for translocation, instead supports a loose-coupling mechanism between chemical catalysis and mechanical translocation during DNA replication. According to this mechanism the DNA polymerase works by alternating between a dNTP/PPi-free state, which diffuses thermally between pre- and post-translocated states, and a dNTP/PPi-bound state where dNTP binding stabilizes the post-translocated state. We show that this thermal ratchet mechanism is used by the polymerase to generate work against surprisingly large opposing loads (~50 pN).

Acknowledgements: This work was supported by Spanish Ministry of Economy and Competitiveness [BFU2011-29038 to JLC, BFU2013-44202 to JMV, BFU2011-23645 to MS, FIS2010-17440, GR35/10-A-920GR35/10-A-911 to FJC, and BFU2012-31825 to BI].

W2

Super-resolution imaging of nucleosome organization

Melike Lakadamyali[1], Maria Aurelia Ricci[2], Carlo Manzo[1], Maria F. Garcia-Parajo[1,3], Maria Pia Cosma[2,3]

[1]ICFO-Institute of Photonic Sciences, Barcelona, Spain, *melike.lakadamyali@icfo.es*
[2]Center for Genomic Regulation, CRG, Barcelona, Spain
[3]Institucio Catalana de Recerca I Estudis Avancats (ICREA), Barcelona, Spain

Nucleosomes help structure chromosomes by compacting DNA into fibers. Chromatin organization likely plays an important role for regulating gene expression; however, due to the nanometer length scales involved, it has been very difficult to visualize chromatin fibers in vivo. To gain insight into how nucleosomes are arranged in vivo, we combined quantitative super-resolution nanoscopy with computer simulations to visualize and count nucleosomes along the chromatin fiber in single nuclei. Nucleosomes assembled in heterogeneous groups of varying sizes, which we named "clutches," in analogy with "egg clutches". Despite the heterogeneity in clutch size in a given nucleus, strikingly, the median number of nucleosomes and their compaction inside clutches were highly cell type specific. Ground-state pluripotent stem cells had, on average, less dense clutches containing fewer nucleosomes and clutch size strongly correlated with the pluripotency grade of induced pluripotent stem cells. RNA polymerase II preferentially associated with the smallest clutches while the large clutches were enriched in heterochromatin. Our results reveal how the chromatin fiber is formed at nanoscale level and link chromatin fiber architecture to stem cell state.

Acknowledgements: This work has been sponsored by Fundacio Cellex, Barcelona, Systems Microscopy Network (to M.L.), ERC-StG (to M.L. and M.P.C) Human Frontiers Research Program (to M.G.P)

References:

[1] Maria Aurelia Ricci, Carlo Manzo, Maria F. Garcia-Parajo, Melike Lakadamyali, Maria Pia Cosma, *"Chromatin fibers are formed by heterogenous groups of nucleosomes in vivo"*, Cell, 160, 6, 1145-1158, 2015.

W3

NANOMECHANICS OF ENERGY TRANSDUCTION IN PROTEIN-DRIVEN MEMBRANE FISSION

Vadim Frolov[1,2], Eva Rodriguez Hortelano[1], Sandra Schmid[3] and Anna Shnyrova[1]

[1]Biophysics Unit (CSIC, UPV/EHU) and Department of Biochemistry and Molecular Biology, University of the Basque Country, Leioa, Spain
[2]IKERBASQUE, Basque Foundation for Science, Bilbao, Spain
[3]UT Southwestern Medical Center, Dallas, USA

Division of cellular organelles and membranes is conducted by dedicated protein machineries. The proteins provide energy required to create extremely high membrane curvatures characteristic for lipid intermediates of membrane fission. While the overall energetic of membrane remodeling is being progressively understood, much less is known about the corresponding force fields. Here we analyze membrane fission mediated by dynamin 1, a large GTPase orchestrating scission of endocytic vesicles. We used molecular engineering to arrest dynamin machinery at different stages of fission and examine corresponding membrane structures using the lipid nanotube model. We revealed how the membrane stress fields are built up and reconfigured with the progression of the GTPase cycle of dynamin. We associated hemifission and complete fission with qualitatively different force fields and propose generic two step model of membrane fission.

Acknowledgements: this work has been sponsored by Spanish Ministry of Economy and

Competitiveness, grant BFU2012-34885

W4

Understanding the cell-extracellular matrix mechanical link: from molecular roles to emerging behaviors.

Pere Roca-Cusachs[1,2]

[1]Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain
[2]University of Barcelona, Barcelona, Spain
rocacusachs@ub.edu

Cell proliferation and differentiation, as well as key processes in development, tumorigenesis, and wound healing, are determined by mechanical stimuli transmitted between cells and their environment. However, how those stimuli are detected and regulated by cells remains largely unknown. One of the main types of structures transmitting mechanical forces to cells is that of integrin-based cell adhesions, which connect extracellular matrix proteins to the cell cytoskeleton through the transmembrane molecules integrins and different adaptor proteins. Understanding this system requires molecular biology tools to target specific proteins, biophysical tools to exert and measure forces at the subcellular level, and theoretical modelling to integrate molecular mechanical properties into cell response. We employ this approach to dissect the molecular mechanisms by which cells withstand, detect, and transmit forces, and respond to tissue rigidity. In this talk, I will explain our findings showing that different integrins and adaptor proteins are adapted to either sensing or transmitting forces. Further, I will explain how cells use the binding dynamics between integrins and the extracellular matrix to detect and respond to matrix rigidity, and the implications that this has in cancer. Finally, I will show recent findings explaining how mechanical signals are integrated by an often forgotten player – the plasma membrane.

Pere Roca-Cusachs obtained his PhD in cellular biophysics in 2007 from the Medical School at the University of Barcelona. He then worked in the lab of Prof. Michael Sheetz (Department of Biological Sciences, Columbia University) as a post-doctoral researcher until 2011. In 2011, He joined the University of Barcelona as a tenure-track lecturer. In 2012, he obtained a position as junior group leader at the Institute for bioengineering of Catalonia (IBEC). His research focuses on unraveling the physical and molecular mechanisms by which cells detect and respond to mechanical force.

W5

STRUCTURE OF THE P15^{PAF}/PCNA COMPLEX AND IMPLICATIONS FOR CLAMP SLIDING ON THE DNA DURING REPLICATION AND REPAIR

Alfredo De Biasio[1], Alain Ibáñez de Opakua[1], Gulnahar B. Mortuza[2,3], Rafael Molina[2], Tiago N. Cordeiro[4], Francisco Castillo[5], Maider Villate[1], Nekane Merino[1], Sandra Delgado[1], David Gil-Cartón[1], Irene Luque[5], Tammo Diercks[1], Pau Bernadó[4], Guillermo Montoya[2,3], Francisco J Blanco[1,6]

[1]Structural Biology Unit, CIC bioGUNE, Derio, Spain, *fblanco@cicbiogune.es*
[2]Structural Biology and Biocomputing Programme, CNIO, Madrid, Spain
[3]Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, Denmark
[4]Centre de Biochimie Structurale, Université Montpellier 1 and 2, France
[5]Department of Physical Chemistry, Universidad de Granada, Spain
[6]IKERBASQUE, Basque Foundation for Science, Bilbao, Spain

The intrinsically disordered protein p15^{PAF} is overexpressed in cancer and regulates DNA replication and repair by binding to the proliferating cell nuclear antigen (PCNA) sliding clamp [1,2,3].
We have characterized the structure of the human p15^{PAF}/PCNA complex by NMR, crystallography, and computational modeling [4]. The central PCNA interacting protein motif (PIP-box) of p15^{PAF} is tightly bound to the canonical PIP-box binding groove on the PCNA front face. In contrast to other PCNA interacting proteins, however, p15^{PAF} also contacts the inside of, and passes through, the PCNA ring. The mostly disordered p15^{PAF} chain termini thus emerge at opposite faces of the ring, but remain protected from degradation by the 20S core proteasome.
We also unveil a novel DNA binding activity of p15^{PAF}, both free and bound to PCNA, which is mainly mediated by its conserved histone-like N-terminal tail. Molecular modeling shows that a ternary complex with a duplex DNA inside the PCNA ring is energetically feasible and our electron micrographs show increased density inside the ring.
We propose that p15^{PAF} acts as a flexible drag that regulates PCNA sliding along the DNA, and may facilitate the switch from replicative to translesion synthesis polymerase binding upon DNA damage.

Acknowledgements: This work has been mainly sponsored by MINECO grant CTQ2011-28680 and Juan de la Cierva-2010 contract to Alfredo De Biasio.

References:

[1] Alfredo De Biasio, et al. p15(PAF) Is an Intrinsically Disordered Protein with Nonrandom Structural Preferences at Sites of Interaction with Other Proteins. Biophys J 106, 4, 865-74, 2014.
[2] Chanlu Xie et al. Proliferating cell unclear antigen-associated factor (PAF15): A novel oncogene. Int J Biochem Cell Biol 50, 127-31, 2014.
[3] Alfredo De Biasio and Francisco J. Blanco. Proliferating cell nuclear antigen structure and interactions: too many partners for one dancer? Adv Protein Chem Struct Biol 91, 1-36, 2013.
[4] Alfredo De Biasio et al. Structure of p15(PAF)-PCNA complex and implications for clamp sliding during DNA replication and repair. Nat Commun. 12, 6, 6439, 2015.

Awards Lectures

A1

STRUCTURAL INSIGHTS INTO BACTERIAL PATHOGENESIS

Juan A. Hermoso[1]

[1]Dept. Crystallography & Structural Biology. Instituto de Química-Física Rocasolano. Madrid, Spain. E-mail: *xjuan@iqfr.csic.es*

Antimicrobial resistance is one of the most serious health threats. The unceasing evolution of microbial species, under the selective pressure of antibiotic use, continues to present an urgent and compelling need for continued robust discovery efforts to find useful, new therapeutic, and preventative measures. A prerequisite for developing new control strategies is the detailed understanding of the interaction between pathogen and the host cells. Surface-exposed proteins of pathogenic bacteria are considered as potential virulence factors through their direct contribution to host-pathogen interactions. Besides, bacterial surface proteins are also involved in critical events such as pathogenesis, cell division, bacterial fitness and elimination of competitors among others.
Our main goal is to generate the knowledge, based on a biophysical study of some critical bacterial cell wall remodeling processes, to provide new pharmacological targets in the fight against some of the most dangerous multidrug-resistant pathogens. We focused on molecular aspects of (i) the virulence mechanisms mediated by pneumococcal surface proteins, (ii) characterization of the pneumococcal divisome, (iii) cell-wall recycling and antibiotics resistance in G(-) pathogens, (iv) Characterization of multidrug resistance mechanisms in pathogen MRSA and (v) Synthesis and regulation of cell wall mediated by non-canonical D-amino acids (NCDAA). In this sense, we have solved the three-dimensional structures of pivotal surface proteins and unraveled their implications in pneumococcal pathogenesis [1][2][3][4]; we have solved the essential protein in pneumococcal division and a mechanism of regulation was proposed [5]; we have characterized for the first time the proteins producing a broad spectrum of NCDAA [6] as well as some of the enzymatic machineries involved in cell-wall recycling [7][8]. Besides, structural evidences of an allosteric mechanism driving multi-resistance in MRSA have been provided [9][10][11]. Along the talk some of these examples will be explained, stressing the relevance of structural studies in unraveling physiological function.

Acknowledgements: This work has been sponsored by MINECO, Seventh Framework Programme - EU, NIH -USA, Autonomous Community of Madrid and CSIC.

References:

[1] Hermoso et al., "*Insights into pneumococcal pathogenesis from crystal structure of the modular teichoic acid phosphorylcholine esterase Pce*", Nature Structural & Molecular Biology, Vol.12, N 6, 533-538, (2005).
[2] Molina et al., "*Crystal structure of CbpF, a bifunctional choline-binding protein and autolysis regulator from Streptococcus pneumoniae*", EMBO reports, 10, 246-251, (2009).
[3] Pérez-Dorado et al., "*Insights into pneumococcal fratricide from crystal structure of the modular Killing Factor LytC*", Nature Structural & Molecular Biology, Vol.17, N 5; 576-581, (2010).
[4] Saleh et al, "*Molecular architecture of Streptococcus pneumoniae surface thioredoxin-fold lipoproteins crucial for extracellular oxidative stress resistance and maintenance of virulence*", EMBO Molecular Medicine, 5, 1852-1870, (2013).
[5] Bartual et al., "*Structural basis of PcsB-mediated cell separation in Streptococcus pneumoniae",* Nature Communications, 5, (2014).
[6] Espaillat et al., "*Structural bases for the broad specificity of a new family of amino acid racemases*", Acta Crystallographica, D70, 79-90, (2014).
[7] Martínez-Caballero et al. "*Reaction products and the X-ray structure of AmpDh2, a virulence determinant of Pseudomonas aeruginosa*", JACS, 135, 10318-10321, (2013).

[8] Lee et al., "*Cell-Wall Remodeling by the Zinc-Protease AmpDh3 from Pseudomonas aeruginosa*", JACS, 12605-12607, (2013).
[9] Otero et al., "*How allosteric control of Staphylococcus aureus penicillin binding protein 2a enables methicillin resistance and physiological function*", Proceedings of National Academy of Sciences USA, 110, 16808-16813, (2013).
[10] Fishovitz et al., "*Disruption of Allosteric Response as an Unprecedented Mechanism of Resistance to Antibiotics*", JACS, 136, 9814-9817, (2014).
[11] Bouley et al., "*Discovery of Antibiotic (E)-3-(3-Carboxyphenyl)-2-(4-cyanostyryl)quinazolin-4(3H)-one*", Journal of the American Chemical Society, 137, 1738−1741, (2015).

A2

Biointeractomics of Cytochrome *c*: From Transient Life to Stable Death

Irene Díaz-Moreno

[1]IBVF – cicCartuja, Universidad de Sevilla – CSIC, Sevilla, Spain. Email: idiazmoreno@us.es

Cytochrome *c* (C*c*), a small soluble hemeprotein, is highly conserved along evolution. In mammals, C*c* plays a dual role in cell life and death: under homeostatic conditions, C*c* is retained inside the mitochondria and acts as an electron shuttle in the electron transfer respiratory chain. Then, gliding mechanisms of C*c* molecules shuttle electrons between respiratory complexes III and IV within mitochondrial supercomplexes, instead of carrying electrons by random diffusion across the intermembrane bulk phase[1,2]. Upon apoptotic stimuli, however, C*c* is released into the cytoplasm so as to serve as an essential key factor by binding to Apaf-1 and further assembling the apoptosome, the machinery responsible for activation of caspases. The mitochondria-to-cytoplasm C*c* translocation has been long considered as a random event, although it is an evolutionarily conserved process among organisms. This finding, along with the fact that apoptosis remains active in Apaf-1 knockout mutants but not in C*c* knockout mutants, lead one to wonder if cytoplasmic C*c* could play other possible signaling functions.

To better understand the role of C*c* in the onset of apoptosis and to harmonize the different phenotypes of Apaf-1 and C*c* knockout mutants, we have recently proposed that C*c* interacts, in apoptotic cells, with an ample set of pro-survival and anti-apoptotic proteins, thereby interfering with cell survival signaling and unlocking programmed cell death[3-5]. Within a complex regulatory network, C*c* would avoid the spatial and temporal co-existence of pro- and anti-apoptotic signals, so as to lead living cells to dye.

Whereas respiration is governed by interactions of C*c* for electron transfer within the mitochondria that are highly transient, the nucleo-cytoplasmic adducts of C*c* that drive to apoptosis are amazingly stable[6]. Altogether, these findings suggest that C*c* indeed plays a crucial role in controlling the fragile equilibrium between cell life and death.

Acknowledgements: This work has been sponsored by the Spanish Ministry of Economy and Competiveness (Grant No. BFU2012-31670/BMC) and the Regional Government of Andalusia (BIO198 and Grant No. P11-CVI-7216).

References:

[1] Moreno-Beltrán *et al.*, *"Cytochrome c_1 exhibits two binding sites for cytochrome c in plants"*, Biochim. Biophys. Acta - Bioenergetics, 1837, 1717-1729, 2014.
[2] Moreno-Beltrán et al., "Respiratory complexes III and IV can each bind two molecules of cytochrome c at low ionic strength", FEBS Lett., 589, 476-483, 2015.
[3] Martínez-Fábregas et al., "New Arabidopsis thaliana cytochrome c partners: a look into the elusive role of cytochrome c in programmed cell death in plants", Mol. Cell. Proteomics, 12, 3666-3676, 2013.
[4] Martínez-Fábregas et al., "Structural and functional analysis of novel human cytochrome c targets in apoptosis", Mol. Cell. Proteomics, 13, 1439-1456, 2014.
[5] Martínez-Fábregas et al., "A common signalosome for programmed cell death in humans and plants", Cell Death Dis., 5, e1314, 2014.
[6] Louro & Díaz-Moreno, *"Redox Proteins in Supercomplexes and Signalosomes"* (Taylor and Francis Group, CRC Press, 2015).

A3

STRUCTURAL BIOLOGY OF MACROMOLECULAR MACHINES INVOLVED IN PEPTIDOGLYCAN RECYCLING AND THEIR IMPLICATIONS IN ANTIBIOTIC RESISTANCE.

Cecilia Artola-Recolons[1], Juan A. Hermoso[1]

[1] Instituto Química Física "Rocasolano", CSIC. Department of Crystallography and Structural Biology , xcecilia@iqfr.csic.es; xjuan@iqfr.csic.es

The Lytic transglycosylases are essential bacterial enzymes that catalyze the cleavage of peptidoglycan. The degradative activity of lytic transglycosylases MltE and MltC from *Escherichia coli* catalyzes the cell wall recycling, which is an integral event in the existence of bacteria. The crystallographic structure of theses enzymes shed light on how MltE and MltC recognize their substrates (the cell wall peptidoglycan), explaining their endolytic or exolytic activity and the way they are attached to the inner leaflet of the outer membrane.
Besides, the zinc proteases AmpDh2 and AmpDh3 are virulence determinant of *Pseudomonas aeruginosa*. The reaction products of these two enzymes with the cell wall are understood with their X-ray structures in complex with their turnover products. Both proteins are unique in this organism and they complement each other in the turnover and maturation of the cell wall.

References:

[1] Cecilia Artola-Recolons, Cesar Carrasco-López, Leticia Irene Llarrull, Malika Kumarasiri, Elena Lastochkin, Iñaki Martínez de Ilarduya, Kathrin Meindl, Isabel Usón, Shahriar Mobashery and Juan A. Hermoso, *"High-Resolution Crystal Structure of an Outer Membrane-Anchored Endolytic Peptidoglycan Lytic Transglycosylase (MltE) from Escherichia coli"*, Biochemistry, 50, 2384-2386, 2011.

[2] Siseth Martínez-Caballero, Mijoon Lee, Cecilia Artola-Recolons, César Carrasco-López, Dusan Hesek, Edward Spink, Elena Lastochkin, Weilie Zhang, Lance M. Hellman, Bill Boggess, Shahriar Mobashery and Juan A. Hermoso, *"Reaction products and the X-ray structure of AmpDh2, a virulence determinant of Pseudomonas aeruginosa"* , Journal of the American Chemical Society, 135, 10318-10321, 20013.

[3] Mijoon Lee, Cecilia Artola-Recolons, Cesar Carrasco-López, Siseth Martínez-Caballero., Dusan Hesek, Eduard Spink, Elena Lastochkin., Zhang, W, Hellman, L., Bill Boggess, Juan Hermoso and Shahriar Mobashery, *"Cell-Wall Remodeling by the Zinc-Protease AmpDh3 from Pseudomonas aeruginosa"*, Journal of the American Chemical Society, 12604-12607, 2013.

[4] Cecilia Artola-Recolons, Mijoon Lee, Noelia Bernardo-García, Blas Blázquez, Dusan Hesek, Sergio Bartual, Kiran .V. Mahasenan, Elena Lastochkin, Hualian Pi, K. Meindl, William Boggess, Isabel Uson, Jed F. Fisher, Shahriar Mobashery and Juan A. Hermoso, *Structure and Cell Wall Cleavage by Modular Lytic Transglycosylase MltC of Escherichia coli*, ACS Chemical Biology, 9, 2058-2066, 2014.

Oral Communications

O1-1, P1-1

MOLECULAR RECOGNITION OF GLYCOSAMINOGLYCAN OLIGOSACCHARIDES BY LANGERIN

Jesús Angulo,[1,2] Juan C. Muñoz-García,[1] Eric Chabrol,[3] José L. de Paz,[1] Javier Rojo, [1] Franck Fieschi,[3] *Pedro M. Nieto*[1]

[1]Glycosystems Laboratory, Instituto de Investigaciones Químicas (CSIC-US), Américo Vespucio 49, 41092, Sevilla, Spain. *e-mail: pedro.nieto@iiq.csic.es*
[2]School of Farmacy, University of East Anglia, Norwich Research Park, Norwich, NR4 7TJ, UK.
[3]Membranes & Pathogens Group, Université Joseph Fourier, CEA, CNRS, Institut de Biologie Structurale 71 avenue des Martyrs, CS 10090, 38044 Grenoble Cedex 9 Grenoble, France

Epidermal Langerhans cells (LCs) constitute a subset of dendritic cells that express langerin, a C-type lectin which is a crucial component of Birbeck granules (BGs), subdomains of the endosomal compartment specific to LCs.[1] Langerin acts as a pathogen receptor by binding to surface glycoconjugates of a number of microorganisms (fungi, mycobacteria, viruses). Notably, langerin can prevent transmission of HIV from LCs to T cells by mediating internalization into BGs and degradation of the virus. Within the C-type family of lectins, langerin seems to be a unique receptor as it has shown to have dual specificity, being able to recognize both, mannosylated and sulfated glycans, via a single C-type carbohydrate recognition domain (CRD).[2]
In this work we have applied transient NMR spectroscopic techniques (STD NMR and transfer-NOE) to study the interactions of sulfated glycosaminoglycan (GAG) ligands to the extracellular domain (ECD) of langerin in solution. Using ligands with diverse sulfation patterns, lengths and cations, we have demonstrated that Langerin interacts with GAG using two alternative binding sites depending of the sulfation pattern, the length of the chain and presence of Ca^{2+} ions.[3]

Acknowledgements: This work has been sponsored by the CSIC (Grant No. 201180E021) and the Spanish Ministry of Science and Innovation (Grant No. CTQ2009-07168).

References:

[1] M. Thépaut, J. Valladeau, A. Nurisso, R. Kahn, B. Arnou, C. Vivès, S. Saeland, C. Ebel, C. Monnier, C. Dezutter-Dambuyant, A. Imberty, and F. Fieschi, *"Structural studies of Langerin and Birbeck granule: A macromolecular organization model"*, Biochemistry *48*, 2684-2698, 2009.
[2] H. Feinberg, M. E. Taylor, N. Razi, R. McBride, Y. A. Knirel, S. A. Graham, K. Drickamer, and W. I. Weis, "Structural Basis for Langerin Recognition of Diverse Pathogen and Mammalian Glycans through a Single Binding Site", J. Mol. Biol. 405, 1027-1039, 2011.
[3] J. C. Muñoz-García, E. Chabrol, R. R. Vivès, A. Thomas, J. L. de Paz, J. Rojo, A. Imberty, F. Fieschi, P. M. Nieto, and J. Angulo, *"Langerin–Heparin Interaction: Two Binding Sites for Small and Large Ligands As Revealed by a Combination of NMR Spectroscopy and Cross-Linking Mapping Experiments"*, J. Am. Chem. Soc., 137, 4100-4110, 2015.

O1-2, P1-2

New insights into the chaperones system in *Mycoplasma genitalium*

Maria Adell-Morunol[‡], Luca Martinelli[‡], Martha Brennich[†], Ignacio Fita[‡] and Bárbara M. Calisto[†]
[‡]Instituto de Biología Molecular de Barcelona (IBMB-CSIC), Barcelona, Spain *mamcri@ibmb.csic.es*
[†]Structural Biology Group, European Synchrotron Radiation Facility, Grenoble, France

The human pathogen *Mycoplasma genitalium*, one of the smallest self-replicating microorganisms, is characterized by the presence of a unique cytoskeleton protrusion called the Terminal Organelle (TO)[1] which is known to be involved in key cellular processes such as cell division, adhesion to host cells, motility and virulence[3]. The structure of the TO is composed by three main parts: the terminal button (distal with respect to the cell body), the electrodense core and the wheel complex (proximal with respect to the cell body[2]. We have characterized the chaperone DnaK system from *M. genitalium* and investigated its possible role in the formation and functioning of the TO. Here we present the X-ray crystal structures, at about 2 Å resolution, of a construct of DnaK (from a clon kindly provided by Dr. J. Piñol) including the Nucleotide and the Substrate Binding Domains (respectively, NBD and SBD) bound to a putative substrate and to the nucleotidic co-factors ADP or AMP-PNP. Moreover, we also present the structures of NBD, at about 1.5 Å resolution, in its apo form and in complex with ADP, ATP or AMP-PNP, which helped to better understand nucleotide hydrolysis.
Surface Plasmon Resonance (SPR), and Small Angle X-ray Scattering (SAXS) coupled to HPLC (a relatively new and powerful variant of the technique) allowed, respectively, to detect the interaction between DnaK and the nucleotide exchange factor GrpE and to model DnaK in solution alone and in complex with GrpE.

This work has been sponsored by Plan Nacional 2010-2014 Ministerio Economia y Competitividad SPAIN. Ref: BFU2009-09268

References:

[1] Fraser, C. M., J. D. Gocayne, O. White, M. D. Adams, R. A. Clayton, R. D. Fleischmann, C. J. Bult, A. R. Kerlavage, G. Sutton, J. M. Kelley, R. D. Fritchman, J. F. Weidman, K. V. Small, M. Sandusky, J. Fuhrmann, D. Nguyen, T. R. Utterback, D. M. Saudek, C. A. Phillips, J. M. Merrick, J. F. Tomb, B. A. Dougherty, K. F. Bott, P. C. Hu, T. S. Lucier, S. N. Peterson, H. O. Smith, C. A. Hutchison, 3rd and J. C. Venter (1995). "*The minimal gene complement of Mycoplasma genitalium*." Science **270**(5235): 397-403.
[2] Henderson, G. P. and G. J. Jensen (2006). "*Three-dimensional structure of Mycoplasma pneumoniae's attachment organelle and a model for its role in gliding motility*." Mol Microbiol **60**(2): 376-385.
[3] Krause, D. C. and M. F. Balish (2001). "Structure, function, and assembly of the terminal organelle of Mycoplasma pneumoniae." FEMS Microbiol Lett 198(1): 1-7.

O1-3, P1-3

CHITOSAN-GOLD NANOCOMPOSITES: CONTROLLED SIZE SYNTHESIS AND STABILITY

MM Collado-González[a], MG Montalbán[b], R Trigo[b], V Fernandez Espin[a], JG Hernández Cifre[a], J García de la Torre[a], G Villora[b], and F Guillermo Díaz Baños[a].

[a]Department of Physical Chemistry; [b]Department of Chemical Engineering, School of Chemistry, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, 30071 Murcia, Spain. fgb@um.es

Key factors in a system to be considered good drug delivery system are size and stability at physiological conditions. Gold nanoparticles (AuNPs) have been proposed for several applications in biomedicine, but bare nanoparticles in solution tend to aggregate so they are coated with small charged molecules (i.e. citrate) which act as stabilizing agents via electrostatic repulsion. However, if the medium has sufficiently high ionic strength (I) or low pH, that electrostatic interaction is screened and aggregation occurs. A practical solution is to add some biocompatible polymer that is adsorbed on the nanoparticle surface. Chitosan is a linear amino-polysaccharide which can be used for this purpose after interacting with negatively charged citrate on the surface of AuNP.
AuNPs of an average size of 10 nm show in UV-Vis spectroscopy an absorption peak at 520 nm. When we add a salt to the solution above a certain level, AuNP aggregate and, as a consequence, the peak shifts to wavelengths higher than 600 nm. If moderately concentrated chitosan is added to a no salted AuNP suspension, DLS analysis suggest that AuNP are attached to CS aggregates, which form a trapping network for nanoparticles and UV-Vis analysis shows a maximum at 524 nm, which indicates that no AuNP aggregation has occurred. Interestingly, if then $NaNO_3$ is added up to I=0.25 M, DLS and UV-Vis results show that ionic strength doesn't generate AuNP aggregation. In addition, TEM images suggest that we have obtained stable nanocomposites (NCs) consisting of CS with AuNP on the surface.
We have also checked the stability of these NCs when pH changes (pH=2, pH=4 and pH=6). In all conditions the chitosan-AuNP interaction avoids aggregation of gold nanoparticles. In addition, time and temperature stability was tested keeping NCs at 4°C for two months or exposing them to different temperatures in a range between 25 °C up to 60°C during different periods of time. In all cases NCs maintained the same size.
A synthesis to reduce the size of chitosan-AuNP nanocomposites have been developed through the controlled addition of chitosan to a AuNPs suspension. DLS measurements show that the initial results are promising.

Acknowledgments: This work was performed within a "Grupo de Excelencia de la Región de Murcia" (grant 04531/GERM/06) with financial support also provided by Ministerio de Economía y Competitividad within project CTQ2012-33717 including FEDER funds. Work was also partially supported by the European Commission (FEDER/ERDF), the Spanish MICINN (CICYT Ref. CTQ2011-25613) and SÉNECA foundation (Ref. 1195/PI/09).

O1-4, P1-4

ROLE OF DECORIN CORE PROTEIN IN COLLAGEN ORGANISATION IN CONGENITAL STROMAL CORNEAL DYSTROPHY (CSCD)

Christina S. Kamma-Lorger[1,2], Christian Pinali[2], Juan Carlos Martínez[1], Jon Harris[2], Robert D. Young[2], Cecilie Bredrup[3], Eva Crosas[1], Marc Malfois[1], Eyvind Rødahl[3,4], Carlo Knupp[2], Keith M. Meek[2]

[1]NCD-BL11, ALBA Synchrotron Light Source, Cerdanyola del Vallés, 08290, Barcelona, Spain ckamma@cells.es
[2]Structural Biophysics Research Group, School of Optometry and Vision Sciences, Cardiff University, Cardiff, CF24 4HQ, UK
[3]Department of Ophthalmology, Haukeland University Hospital, 5021 Bergen, Norway
[4]Department of Clinical Medicine, University of Bergen, 5020 Bergen, Norway

The role of Decorin in organising the extracellular matrix was examined in normal human cornea and in a case of Congenital Stromal Corneal Dystrophy (CSCD) with corneal clouding caused by a truncating mutation (c967delT) on the Decorin (DCN) gene[1-4]. Normal human Decorin protein and the truncated one were reconstructed in silico using homology modelling techniques to explore structural changes in the diseased protein. Corneal CSCD specimens were also examined using 3-D electron tomography and Small Angle X-ray diffraction (SAXS), to image the collagen-proteoglycan arrangement and to quantify fibrillar diameters, respectively. Homology modelling showed that truncated Decorin had a different spatial geometry to the normal one, with the truncation removing a major part of the site that interacts with collagen, compromising its ability to bind effectively. Electron tomography showed regions of abnormal stroma, where collagen fibrils came together to form thicker fibrillar structures (Fig. 1), showing that Decorin plays a key role in the maintenance of the order in the normal corneal extracellular matrix.

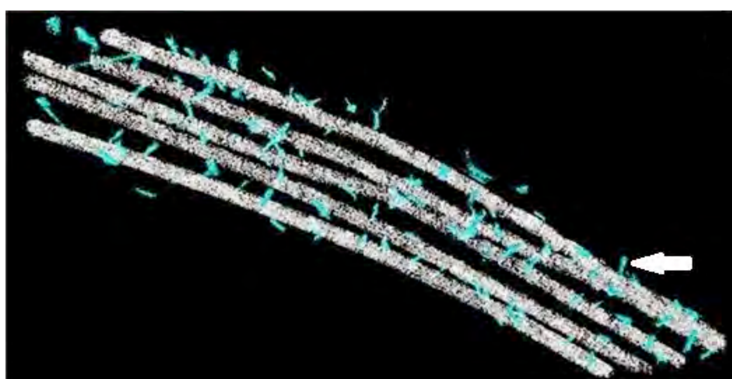


Figure 1: Longitudinal 3D segmentation showing the association of separate collagen fibrils (white arrow) in the corneal stroma, as it was observed in the CSCD cornea

Averaged diameter of individual fibrils throughout the full thickness of the tissue remained normal. Differences in fibrillar diameter were more pronounced in 100 micron sections throughout the depth of the cornea. Our findings confirm that CS/DS proteoglycans, like Decorin, form longer chains that extend among several collagen fibrils and hence help to organise collagen in the extracellular matrix in a pseudo hexagonal arrangement, which is an essential property for maintaining transparency in the cornea.

Acknowledgements: This work has been sponsored by Medical Research Council 5-year programme grants (G0600755 and 503626) and a Biotechnology and Biological Sciences Research Council research grant (BB/F022077). Prof. Keith M. Meek is a Royal Society-Wolfson Research Merit Award Holder.

References:

[1] C. Bredrup, P. M. Knappskog, J. Majewski, E. Rødahl, H. Boman, *"Congenital stromal dystrophy of the cornea caused by a mutation in the decorin gene"*, Invest Ophthalmol Vis Sci., 46, 420-426, 2005

[2] E. Rødahl, R. Van Ginderdeuren, P. M. Knappskog, C. Bredrup, and H. Boman, *"A second decorin frame shift mutation in a family with congenital stromal corneal dystrophy"*, Am J Ophthalmol., 142, 520-521, 2006

[3] J. Kim, H., J. M. Ko, I. Lee, J. Y. Kim, M. J. Kim, and H. Tchah, "A novel mutation of the decorin gene identified in a korean family with congenital hereditary stromal dystrophy", Cornea, 30, 1473-1477, 2011

[4] Y. Jing, P. R. Kumar, L. Zhu, D. P.Edward, S. Tao, LWang, R. Chuck, C. Zhang, *"Novel decorin mutation in a Chinese family with congenital stromal corneal dystrophy"*, Cornea, 33, 288-293, 2014

O2-1, P2-1

A MASS-SPECTROMETRY-BASED METHOD TO STUDY THE REDOX STATE OF CYSTEINES IN TITIN

Elías Herrero-Galán, Carmen Suay-Corredera, Cristina Sánchez-González, Elena Bonzón-Kulichenko, Jesús Vázquez and Jorge Alegre-Cebollada

Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain.
elias.herrero@cnic.es, jalegre@cnic.es

Contractility of cardiac muscle depends on the concerted action of sarcomeric proteins, among which titin is especially relevant due to its central role in muscle elasticity. This fact has been recently evidenced by the discovery that mutations in the titin gene lead to pathologies that entail aberrant changes in the elasticity of the heart. The elastic properties of titin derive from its molecular architecture, composed of random coil regions that act as entropic springs easy to extend, and tandem immunoglobulin (Ig) domains, able to unfold and refold under mechanical force. Variations in the mechanical folding/unfolding properties of these Ig domains cause changes in the elasticity of titin. In this sense, strain-induced posttranslational modifications of buried cysteines have been shown to be key regulators of the folding dynamics of titin Ig domains[1]. However, the extent and specific residues targeted by these modifications *in vivo* remain unexplored. We have optimized a method for in-gel detection of thiols and used mass spectrometry and high resolution structure modelling to study the redox state of cysteine residues and predict the presence of disulfides and other redox posttranslational modifications in the different Ig domains of titin. Furthermore, by means of single-molecule atomic force spectroscopy measurements, we aim to determine the effect of this modifications in the elasticity of the protein. This approach may lead to a better understanding of how the elasticity of the heart is modulated in health and disease.

Acknowledgements: This work has been sponsored by FP7-PEOPLE-2010-COFUND-267149 from the Marie Curie International Incoming Fellowship (J. A.-C.).

References:

[1] Jorge Alegre-Cebollada, Pallav Kosuri, David Giganti, Edward Eckels, Jaime Andrés Rivas-Pardo, Nazha Hamdani, Chad M. Warren, R. John Solaro, Wolfang A. Linke, Julio M. Fernández, *"S-Glutathionylation of Cryptic Cysteines Enhances Titin Elasticity by Blocking Protein Folding"*, Cell, 156, 6, 1235-1246, 2014.

O2-2, P2-2

MECHANICS OF THE CELL CONSTRICTION DURING DIVISION

Elena Beltrán-Heredia[1, 2†], Víctor Almendo-Vedia[1, 2], Francisco Monroy[2] and Francisco J. Cao[1]

[1]*Departamento de Física Atómica, Molecular y Nuclear, Universidad Complutense de Madrid, 28040, Madrid, Spain.*
[2]Departamento de Química Física I, Universidad Complutense de Madrid, 28040, Madrid, Spain.

[†]*elenabel@ucm.es*

Cell constriction is an important cytokinetic phase preceding division. Before splitting in two daughters, symmetrically dividing cells accommodate theirs duplicated contents into spatially separated compartments defined by a stable fission site located at midcell. Constriction is a non-spontaneous process which involves large membrane deformations at the site of fission, a division route entailing a strong breakage of symmetry in the mother cell. In this work, we investigate the mechanical route for symmetric constriction by computing the bending energy of deformed vesicles with rotational symmetry. Analytical expressions are obtained for the main magnitudes. These equations provide an easy and compact way to predict minimal requirements for successful constriction and the values of its relevant properties. Thus, they can be useful for the design of synthetic divisomes and give good predictions for magnitudes including constriction energy, length of the constriction zone, volume and area of the vesicle, and the stability coefficient for symmetric constriction.

Acknowledgements: This work was supported by Ministerio de Ciencia e Innovación (Spain) through Grants No. FIS2010-17440 (F.J.C.), No. FIS2012-35723 (F.M.), and No. CSD2007-0010 (F.M.) (the last one as part of the Consolider Ingenio en Nanociencia Molecular Grant); Ministerio de Economía y Competitividad (Spain) Grant No. FIS2009-14650-C02-01 (F.M); and Comunidad Autónoma de Madrid (Spain) Grant No. S2009MAT-1507 (F.M.). V.A-V acknowledges support from Ministerio de Educación, Cultura y Deporte (Spain) through the Becas de Colaboracion program and E. B acknowledges support from Ministerio de Educación, Cultura y Deporte (Spain) through the Becas FPU program (FPU 13/02826).

References:

[1] Víctor Almendro-Vedia, Francisco Monroy and Francisco Javier Cao, *"Mechanics of Constriction during Cell Division: A Variational Approach"*, Plos ONE, 8, 8, 13, 2013.

[2] Víctor Almendro-Vedia, Francisco Monroy and Francisco Javier Cao, *"Analytical results for cell constriction dominated by bending energy"*, Physical Review E, 91, 012713, 12, 2014.

O2-3, P2-3

The actin cytoskeleton modulates the activation of invariant NKT cells by segregating CD1d nanoclusters on antigen presenting cells

Juan A. Torreno-Pina [1], Carlo Manzo [1], Mariolina Salio [2], Michael Aichinger [2,3], Dawn Shepherd [2], Gurdyal S. Besra [4], Vincenzo Cerundolo [2,6] and Maria F. Garcia-Parajo [1,5,6]

[1]ICFO – Institut de Ciencies Fotoniques, Mediterranean Technology Park, 08860 Castelldefels (Barcelona), Spain.
[2]MRC Human Immunology Unit, Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, OX3 9DS, University of Oxford, United Kingdom.
[3]Present Address: EveliQure Biotechnologies GmbH, Campus Vienna Biocenter, Helmut-Qualtinger-Gasse 2, Staircase 1, 3rd floor, A-1030 Vienna, Austria.
[4]School of Biosciences, University of Birmingham, Edgbaston, Birmingham, B11 2TT, UK.
[5]ICREA-Institució Catalana de Recerca i Estudis Avançats, 08010 Barcelona, Spain.
[6]Co-senior author.

The ability of invariant NKT cells (iNKT cells) to recognize CD1d bound endogenous lipid antigens represents a distinct immune recognition strategy, which underscores the constitutive memory phenotype of iNKT cells. However, the mechanisms regulating activation of iNKT cells remain unclear. By using super-resolution nanoscopy and dual-color single particle tracking, we demonstrate that CD1d molecules form nanoclusters at the surface of antigen presenting cells (APCs), whose size, density and lateral mobility is constrained by interactions between CD1d cytosolic tail and the actin cytoskeleton. Formation of larger nanoclusters upon disruption of actin cytoskeleton interactions enhances iNKT cell activation. Importantly, and consistently with iNKT cell activation during inflammatory conditions, exposure of APCs to the TLR7/8 agonist R848 increases nanocluster density and iNKT cell activation. Overall, these results define a novel mechanism that modulates iNKT cell autoreactivity based on the tight control by the APC cytoskeleton of the sizes and densities of endogenous antigen loaded CD1d nanoclusters [1].

References:

[1] Juan A. Torreno-Pina et al., "The actin cytoskeleton modulates the activation of invariant NKT cells by segregating CD1d nanoclusters on antigen presenting cells", submitted.

O3-1, P3-1

NATURAL LIGANDS RESTORE THE FUNCTION OF A CANCER-ASSOCIATED POLYMORPHISM IN NQO1

Encarnación Medina Carmona[1], Rogelio Palomino Morales[2], Julian F. Fuchs[3], David J. Timson[4], Ángel Luis Pey[1].

[1]Department of Physical Chemistry, University of Granada, Granada, Spain
[2]Department of Biochemistry and Molecular Biology I, University of Granada, Granada, Spain
[3]Center for Molecular Biosciences, Leopold-Franzens University, Innsbruck, Austria.
[4] School of Biological Sciences and Institute for Global Food Security, Queen´s University Belfast, Belfast, UK.

NAD(P)H:quinone oxidoreductase 1 (NQO1) is a stress-inducible flavoprotein essential for the antioxidant defense and implicated in the molecular pathology of some cancers. In particular two polymorphic forms of human NQO1, p.P187S and p.R139W, have been associated to increased predisposition to cancer and reduce the ability of NQO1 to activate pro-drugs in cancer treatment. In this work, we explore the effects of these two polymorphisms on the NQO1 conformational stability, protein dynamics and intracellular stability and functionality to understand the molecular alterations caused by these polymorphisms.
Detailed thermal stability studies show that both polymorphisms, especially p.P187S, destabilize the NQO1 dimer while addition of FAD [1] and dicoumarol, a potent inhibitor of NQO1, restores the stability of the polymorphic forms to wild-type levels. Furthermore, activity measurements and direct titrations indicate that p.P187S has reduced activity due to a lower binding affinity for FAD. Proteolysis experiments reveal an increased flexibility of the C-terminal domain of p.P187S, which is corrected by FAD and dicoumarol. Molecular dynamic simulations show that changes in global stability, i.e. destabilization induced by polymorphisms and stabilization caused by ligands, are linked to the dynamics of the dimer interface, while the low activity and affinity for FAD in p.P187S is explained by increased fluctuations at the FAD binding site. Experiments in cell model systems support that the stability and function of p.P187S can be rescued by native state ligands.
Overall, we propose that alterations in protein dynamics are fundamental to understand loss-of-function in p.P187S and to direct the development of new pharmacophores to treat patients bearing this polymorphism.

Acknowledgements: This work has been sponsored by grants from MINECO (CSD2009-00088 and BIO2012-34937) and Junta de Andalucia (CTS11-07187). E.M.C. is supported by a predoctoral fellowship from Junta de Andalucia. A.L.P. is recipient of a Ramón y Cajal research contract from MINECO-University of Granada (RYC2009-04147).

References:

[1] Angel L. Pey, Clare F. Megarity, David J. Timson, "FAD binding overcomes defects in activity and stability displayed by cancer-associated variants of human NQO1", Biochim.Biophys.Acta (Mol.Bas.Dis). 1842,1463-1473, 2014.

O3-2, P3-2

UNDERSTANDING PROTEIN MISFOLDING AND AMYLOID FIBRIL FORMATION: SEQUENTIAL FOLDING UPON SELF-ASSEMBLY

Nunilo Cremades[1] Serene W. Chen[2] , Srdja Drakulic[3], Myriam Ouberai[4], Rocío Arranz[3], Carlos Alfonso[5], Germán Rivas[5], José María Valpuesta[3] and Christopher M. Dobson[2]

[1] Institute for Biocomputation and Physics of Complex Systems (BIFI), University of Zaragoza, Zaragoza, Spain. ncc@unizar.es
[2] Department of Chemistry, University of Cambridge, Cambridge, United Kingdom
[3] Department of Macromolecular Structure, Centro Nacional de Biotecnología (CNB-CSIC), Madrid, Spain
[4] Nanoscience Centre, University of Cambridge, Cambridge, United Kingdom
[5] Department of Cellular and Molecular Biology, Centro de Investigaciones Biológicas (CIB-CSIC), Madrid, Spain

Protein misfolding and amyloid fibril formation is the hallmark of an increasing number of human disorders such as Alzheimer's and Parkinson's diseases but there is currently very little understanding of the mechanisms by which normally monomeric proteins self-assemble in this highly organized fibrillar species with a common cross-β structure, in part due to the lack of knowledge of the nature of the intermediate species formed during this process. Importantly, oligomeric forms of amyloid aggregates have been detected in the brains and tissues of patients suffering from neurodegenerative disorders and it is widely thought that such species are key pathogenic agents in the development and spreading of disease; however their study has been proven to be extremely challenging, primarily as a result of their intrinsic transient nature and high levels of heterogeneity.

We have recently described studies of the aggregation of α-synuclein, the protein whose amyloid-like deposition is the hallmark of Parkinson's disease, by using single-molecule fluorescence techniques that revealed the presence of two distinct forms of oligomeric species during fibril formation, with different conformations and neurotoxic properties, and established the rate of conversion between them [1]. In order to characterize in more detail the nature of the toxic oligomeric forms, we have isolated them and used a wide variety of biophysical methods to define and minimize their degree of heterogeneity [2]. Our approach has allowed us to identify distinct subgroups of oligomeric structures and define their overall structural properties and molecular architectures. Indeed, we have been able to use cryo-EM image reconstruction techniques to obtain three-dimensional structural models for the different subgroups of amyloid oligomers, revealing the quaternary structural architectures of stable toxic amyloid oligomers. The results of our studies provide the basis for a more complete understanding of the nature of the misfolding and self-assembly of polypeptides into β-sheet rich amyloid aggregates, providing a unifying view of the protein misfolding process.

Acknowledgements: This work has been sponsored by the Agency for Science, Technology and Research, Singapore (SWC), the "La Caixa" foundation (SD), the BBSRC through Grants BB/H003843/1 (M.O.) and BB/E019927/1 (CMD), the Spanish Ministry of Economy and Competitiveness through Grants BFU2013-44202 (JMV) and BIO2011-28941-C03-03 (CA and GR), the Spanish, the Madrid Regional Government through Grant S2013/MIT-2807 (JMV), the Wellcome Trust, the Leverhulme Trust, the European Commission through project LSHM-CT-2006-037525 (CMD), the Engineering and Physical Sciences Research Council (CMD) and the Royal Society (Dorothy Hodgkin Fellowship - NC). NC is currently a Ramón y Cajal Researcher sponsored by the Spanish Ministry of Economy and Competitiveness (RYC-2012-12068).

References:

[1] Nunilo Cremades, Samuel I Cohen, Emma Deas, Andery Y Abramov, Allen Y Chen, Angel Orte, Massimo Sandal, Richard W Clarke, Paul Dunne, Francesco A Aprile, Carlos W Bertoncini, Nicholas W Wood, Tuomas PJ Knowles, Christopher M Dobson & David Klenerman. *Direct observation of the interconversion of normal and toxic forms of alpha-synuclein*. Cell, 149(5): 1048-59, 2012.

[2] Serene W Chen, Srdja Drakulic, Emma Deas, Myriam Ouberai, Francesco A Aprile, Rocío Arranz, Samuel Ness, Cintia Roodveldt, Tim Guilliams, Erwin De-Genst, David Klenerman, Nicholas W Wood, Tuomas PJ Knowles, Carlos Alfonso, Germán Rivas, Andrey A Abramov, José María Valpuesta, Christopher M Dobson & Nunilo Cremades. *Structural characterization of toxic oligomers that are kinetically trapped during α-synuclein fibril formation*. Proc. Natl. Acad. Sci U.S.A. Apr 8. pii: 201421204, 2015.

O3-3, P3-3

Towards an improved decomposition of time-dependent SAXS data from fibrillating proteins

Fátima Herranz-Trillo[1,2], Romà Tauler[3], Bente Vestergaard[1] & Pau Bernadó[2]

[1] Department of Drug Design and Pharmacology, University of Copenhagen, Denmark.

[2] Centre de Biochimie Structurale. CNRS UMR-5048, INSERM U-1054, Université de Montpellier I et II, France. *herranztrillo@cbs.cnrs.fr*

[3] Department of Environmental Chemistry, Institute of Chemistry and Environmental Research, CSIC, Barcelona, Spain.

In amyloid pathologies (e.g. Parkinson's or Alzheimer's diseases) there are indications that oligomeric aggregated precursors of fibrillation, and not mature fibrils, are the main cause of cytotoxicity and neuronal damage. Hence the importance of characterizing early stages in the fibrillation process.
The structural analysis of these oligomeric species is a major challenge due to their instability, low relative concentration, the difficulties for isolation, and the equilibrium between species of very different sizes, present at any time point during the fibrillation process [1]. Mechanistic studies normally monitor individual species of the fibrillation process, such as mature fibres, whereas the other species remain invisible.
In SAXS studies of fibrillation, the resulting individual scattering pattern measured at different time-points throughout the fibrillation process, is a sum of the contributions from each component of the mixture. We propose the use of an iterative multivariate curve resolution procedure based on an alternating least squares (MCR-ALS [2,3]) chemometrics algorithm to estimate the pure SAXS spectra for each of the individual components of the mixture, as well as their individual populations. High-quality experimental data, using the setup described previously by our group [4], has been collected for alpha-synuclein. MCR-ALS analysis estimates the presence of four species in solution and provides the individual scattering signal for each component. The scattering properties of these components suggest the coexistence of the native (monomeric) species with a soluble oligomer and two much larger species corresponding to two fibril types. Further structural analysis of the resulting SAXS curves will be performed to obtain the low-resolution model of the cytotoxic oligomer and the two fibril forms. We will compare data from wildtype alpha-synuclein fibrillation with already published results [5] based on very laborious non-automated data analysis. If successful, we will apply MCR-ALS analysis to fibrillation data from familial mutants, causing early onset fibrillation.

Acknowledgements:

This work has been sponsored by ANR-SPIN-HD (France), Institut national de la santé et de la recherche médicale (INSERM, France), University of Copenhagen and Neuromed (SUDOE).

References:

[1] Bente Vestergaard, Minna Groenning, Manfred Roessle, Jette S. Kastrup, Marco van de Weert, James M. Flink, Sven Frokjaer, Michael Gajhede, Dmitri I. Svergun. *"A helical structural nucleus is the primary elongating unit of insulin amyloid fibrils", PLoS Biology 5(5),* e134 (2007)
[2] Romà Tauler. *"Multivariate curve resolution applied to second order data"*, Chemom. Intell. Lab. Syst. 30, 133 (1995)
[3] Jascha Blobel, Pau Bernadó, Dmitri I. Svergun, Romà Tauler, Miquel Pons. *"Low-resolution structures of transient protein-protein complexes using small-angle X-ray scattering"* J. Am. Chem. Soc. 131, 4378 (2009)

[4] Annette E. Langkilde, Bente Vestergaard. *"Structural Characterization of Prefibrillar Intermediates and Amyloid Fibrils by Small-Angle X-Ray Scattering"* Meth. Mol. Biol. 849, 137 (2012)
[5] Lise Giehm, Dmitri I. Svergun, Daniel E. Otzen, Bente Vestergaard. *"Low-resolution structure of a vesicle disrupting alpha-synuclein oligomer that accumulates during fibrillation" Proc. Natl. Acad. Sci. U. S. A.* 108, 3246–51 (2011)

O3-4, P3-4

Mechanical Force Modulates the Unfolding Pathways of the Cold-Shock Protein B from *Thermotoga Maritima*.

Jörg Schönfelder[1,2], Raúl Pérez-Jiménez[3]and Victor Muñoz[1,2,5]

[1] IMDEA Nanociencia, Nanobiosystems, Madrid, Spain.
[2] CNB, Centro Nacional de Biotecnología, CSIC, Madrid, Spain.
[3] CIC nanoGUNE, Department of Bionanomechanics, San Sebastián, Spain.
[4] IKERBASQUE, Basque Foundation for Science, Bilbao, Spain.
[5] University of Maryland, Department of Chemistry and Biochemistry, College Park, MD, USA.
E-mail: jschoenfelder@cnb.csic.es , r.perezjimenez@nanogune.eu , vmunoz@cnb.csic.es

Single molecule force spectroscopy (SMFS) has become an essential experimental technique allowing investigating protein dynamics through the application of a mechanical force[1]. Our approach is to probe the mechanical properties of the small 66 residue containing cold-shock protein B from *Thermotoga Maritima* (Csp), which consists of 5 β-strands forming a compact barrel using the force clamp atomic force microscope (AFM) (Luigs & Neumann) [2]. The force clamp AFM allows the application of a controlled constant mechanical force on the protein sample by using a PID feedback loop.
The Csp is one of the most studied examples of a single protein domain showing a clear 2-state behavior and having a fast folding rate in the range of ms, which has been shown in kinetic ensemble measurements [3]. Moreover, as it has been recently reported the Csp shows a high mechanical stability and a clear 2-state unfolding pattern when the protein is placed under a rapid and uncontrolled high mechanical force, using the so-called constant velocity mode of the AFM [4]. In our approach, in order to conduct the SMFS experiments with the force clamp AFM we built one polyprotein construct using biomolecular techniques consisting of the Csp domain flanked by three Titin I27 domains on each side. This enabled us to detect and measure the mechanical unfolding pattern of the individual Csp domain.
First, our result confirmed the single-step 2-state mechanical unfolding behavior of the Csp[4]. However in a lower force regime during force ramp and force clamp experiments, beside the single step unfolding we were also able to detect a high fraction of traces that display a clear mechanical multiple-step unfolding behavior for the Csp. We could reveal that Csp unfolds through up to 5 unfolding steps (4 intermediates). Surprisingly, we found that the probability of Csp to unfold via different pathways changed for different constant forces and was highest between 40 and 60pN constant force. Thus we conclude that mechanical force modulates the unfolding pathway of Csp between 20 and 80pN and reveals otherwise hidden intermediate states.

References:

[1] KC. Neuman, A, Nagy,"Single-molecule force spectroscopy: optical tweezers, magnetic tweezers and atomic force microscopy." Nat Methods. 5(6):491-505A 2008.
[2] R.Perez-Jimenez, A.Alonso-Caballero,R.Berkovich,D.Franco D, MW Chen, P.Richard, CL.Badilla, JM.Fernandez, "Probing the effect of force on HIV-1 receptor CD4.", ACS Nano. 28;8(10):10313-202014 Oct.
[3] D.Perl, C.Welker, T.Schindler, K.Schröder, MA.Marahiel, R.Jaenicke, FX.Schmid, "Conservation of rapid two-state folding in mesophilic, thermophilic and hyperthermophilic cold shock proteins"Nature Struct. Biol. Volume 5, Number 3, 229-35 1998.
[4] T.Hoffmann, K.M.Tych, D.J. Brockwell, L. Dougan, "Single-molecule force spectroscopy identifies a small cold shock protein as being mechanically robust."J. Phys. Chem. B. 117, 1819−1826 2013.

O4-1, P4-1

NEW INSIGHTS INTO SYNAPTOTAGMIN-1 BINDING

Ángel Pérez-Lara[1], Anusa Thapa[2], Sarah Nyenhuis[2], Partho Halder[1], Michael Tietzel[3], Kai Tittmann[3], David S. Cafiso[2], Reinhard Jahn[1].

[1]Department of Neurobiology, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany
[2]Center for Membrane Biology and Department of Molecular Physiology and Biological Physics, University of Virginia, Charlottesville, Virginia, USA
[3]Department of Molecular Enzymology, Göttingen Center for Molecular Biosciences, Georg-August University Göttingen, Göttingen, Germany

e-mail address: *fperezl@gwdg.de*

Synaptotagmin-1 is the main Ca^{2+}-sensor during the fast synchronous neurotransmitter release in neurons. Upon Ca^{2+} influx, synaptotagmin-1 binds to the presynaptic membrane, promoting SNARE mediated fusion between the synaptic vesicle and the plasma membrane. Here, we use thermodynamic, kinetic and structural methods under well-defined conditions to characterize binding of synaptotagmin-1 to its main lipid effectors (phosphatidylserine and phosphoinositides), resulting in a refined mechanistic model for synaptotagmin-1 binding. Accordingly, in the absence of Ca^{2+} synaptotagmin-1 binds to PI(4,5)P_2 on the plasma membrane via its C2B polybasic patch and promotes priming/docking of synaptic vesicles. Upon Ca^{2+} influx, the Ca^{2+}-binding sites bind PS, allowing for the penetration of synaptotagmin-1 into the plasma membrane. Additionally, Ca^{2+} increases the affinity of the polybasic patch to PI(4,5)P_2 by screening the negative charges of the Ca^{2+} binding site located in the C2B domain. Both of these events decrease the dissociation rate of synaptotagmin-1 binding and probably constitute the main trigger of SNARE-dependent exocytosis.

Acknowledgements: This research was supported by Deustsche Forschungsgemeinschaft (SFB803) and the National Institutes of Health (P01 GM072694 to R.J. and D.S.C.).

O4-2, P4-2

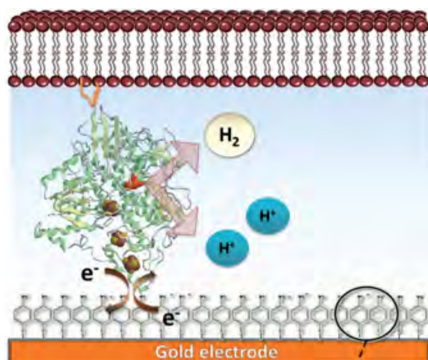
Induction of a Proton Gradient across a Gold-Supported Biomimetic Membrane by Electroenzymatic H_2 Oxidation

Marisela Vélez [1], Oscar Gutiérrez-Sanz[1], Cristina Tapia[1], Marta C. Marques[2], Sonia Zacarias[2], , Inés A. C. Pereira[2], and Antonio L. De Lacey[1]

[1]*Instituto de Catálisis y Petroleoquímica, CSIC, c/Marie Curie 2, 28049 Madrid (Spain)* marisela.velez@icp.csic.es

[2]Instituto de Tecnologia Quimica e Biologica, Universida de Nova de Lisboa, Apartado 127, 2781-901 Oeiras (Portugal)

We show that the proton concentration at an electrode/phospholipid-bilayer interface can be controlled and monitored electrochemically by immobilizing a membrane-bound hydrogenase. This artificial construct mimics an energy-transduction mechanisms widely used in living organisms. During photosynthesis and respiration, light and chemical energy are stored in the form of an electrochemical gradient created across a lipid bilayer. In our artificial construct, the energy used to generate a proton gradient across the supported biomimetic membrane is derived from the electroenzymatic oxidation of H_2 [1]. This method controlling and monitoring and the membrane potential in bioelectronic devices could be used to store energy produced from H_2 for several applications, such as artificial adenosine triphosphate (ATP) production, drug testing in a biomimetic environment, and the development of new biosensors, as well as for fundamental studies of cell metabolism. Future studies aim to couple the developed system to the activity of an ATP synthase.



Schematic representation of Dv-SeHase immobilized covalently on a Au electrode modified with a SAM of 4-ATP and oriented with its lipidic tail inserted into a phospholipid bilayer (Au/4-ATP/Dv- SeHase/PhBL), in accordance with previous studies[2].

Acknowledgements: This research was funded by the Spanish MINECO (project CTQ2012-32448) and by the Fundaçao para a Ciência e a Tecnologia (project PTDC/BBB-BEP/0934/2012). O.G.-S. thanks MINECO for an FPI grant

References:

[1] Gutiérrez-Sanz, O., Tapia, C., Marques, M., Zacarias, S., Vélez, M., Pereira, I.A.C., De Lacey, A.L. *"Induction of a Proton Gradient across a Gold-Supported BiomimeticMembrane by Electroenzymatic H2 Oxidation"*, Angew. Chem. Int. Ed. 2015, 54, 2684 –2687.

[2] Gutiérrez-Sanchez,C., Olea, D. , Marques, M. , Fernández, V. M., Pereira, I. A. C. , Vélez, M. , De Lacey, A. L. ," *Oriented Immobilization of a Membrane-Bound Hydrogenase onto an Electrode for Direct Electron Transfer"* Langmuir 2011, 27, 6449 – 6457

O4-3, P4-3

INTERACTION OF NEW FLUORESCENT CONJUGATED POLYELECTROLYTES WITH MODEL MEMBRANES: THEIR POTENTIAL USE AS FLUORESCENT MEMBRANE MARKERS

Zehra Kahveci, Maria José Martínez-Tomé, Rebeca Vázquez, Amalia Mira, Ricardo Mallavia and C. Reyes Mateo[1]

[1]Instituto de Biología Molecular y Celular, Universidad Miguel Hernández de Elche, 03202, Elche (Alicante), Spain, rmateo@umh.es

Conjugated polyelectrolytes (CPEs) are polymers with delocalized π-electron systems, which show strong absorption and high efficiencies in both photoluminescence and electroluminescence, containing ionic side groups to facilitate their water solubilization. In general, fluorescent CPEs have high photostability and low cytotoxicity and can undergo spontaneous self-assembly through reversible, electrostatic, and/or hydrophobic interactions with some other species, generally of opposite charge, resulting in supramolecular structures with interesting optical and material properties [1,2].

In the present work, we have explored the interaction of new fluorescent CPEs, previously synthesized in our laboratories, with model lipid membranes in order to evaluate their potential use as fluorescent membrane markers. To this end we have selected the cationic fluorescent polyfluorenes HTMA-PFP and HTMA-PFNT which show blue and red emission respectively. These CPEs have been studied with model membranes of *E.coli* (DOPG:DOPE:Cardiolipine) and mammalian (DOPC:Cholesterol). The study was carried out using different biophysical techniques, mainly fluorescence spectroscopy and microscopy. Results indicate that both polyelectrolytes show more affinity for *E.coli* membranes than for mammalian membranes, as well as a different mechanism of interaction and final location in the bilayer. Whilst the polyelectrolytes are embedded within the lipid bilayer in the *E.coli* model membrane, they remain close to the surface, forming aggregates that are sensitive to the physical state of the lipid bilayer, in mammalian model membranes. Additional experiments via fluorescence microscopy show that the polyelectrolytes label the lipid bilayer without altering the morphology of the vesicles and allowing their visualization in blue and in red colours.

Results confirm the appropriateness of using HTMA-PFP and HTMA-PFNT as fluorescent membrane markers and suggest that, given their behavior towards different membrane models, they could be used for selective recognition and imaging bacteria over mammalian cells.

Acknowledgements: This work has been sponsored by…

References:

[1] Y. Liu, K. Ogawa, K..S. Schanze, *"Conjugated polyelectrolytes as fluorescent sensors"*, Journal of Photochemistry and Photobiology C: Photochemistry Reviews, 10 (4), 173-190, 2009

[2] A.T. Ngo, P. Karam, G. Cosa, *"Conjugated polyelectrolyte-lipid interactions: Opportunities in biosensing"*, Pure and Applied Chemistry, 83 (1), 43-55, 2011

O4-4, P4-4

The effect of cholesterol on the long-range network of interactions established among sea anemone sticholysin II residues at the water-membrane interface

Sara García-Linares[1], Ida Alm[2], Terhi Maula[2], José G. Gavilanes[1*], J. Peter Slotte[2] and Álvaro Martínez-del-Pozo[1*]

[1]Departamento de Bioquímica y Biología Molecular I, Universidad Complutense, Madrid, Spain.
[2]Biochemistry, Department of Biosciences, Åbo Akademi University, Turku, Finland.

*Authors to whom correspondence should be addressed; E-Mail: alvaromp@quim.ucm.es (AMP) or ppgf@bbm1.ucm.es (JGF); Tel.: 34 91 394 4158; Fax: 34 91 394 4159.

Actinoporins are a group of sea anemones α-pore forming proteins with a therapeutic potential which includes different pharmacological effects, their presumable anticancer activities, and their use in the construction of specific immunotoxins [1,2,3-8]. Sticholysin II (StnII) from *Stichodactila helianthus* is one of its best characterized representatives [9,10]. In aqueous solution actinoporins remain stably folded but upon interaction with lipid bilayers they oligomerize to form a pore [9,10,11]. This event is triggered by the presence of sphingomyelin (SM), but cholesterol (Chol) eases the formation of the pores [12,13,14-18]. Membrane attachment and pore formation require a series of conformational changes involving long-distance rearrangements of many of the residues located protein-membrane interface. Thus, the influence of the presence of Chol on membrane recognition, oligomerization, and/or final pore formation has been now studied using eight different StnII variants. The purified proteins were characterized from structural and functional points of view, in terms of their ability to interact with model membranes in presence or absence of Chol. The results obtained frame Chol not only as an important partner of SM for functional membrane recognition but also as a molecule which significantly reduces the structural requirements for the mentioned conformational rearrangements to occur. In addition, it is also shown that the interaction provided by the guanidinium group of Arg51 is strictly required for membrane recognition, independently of the presence of Chol.

Acknowledgements: The work was funded by generous grants from the Sigrid Juselius Foundation (JPS), the Åbo Akademi Foundation (JPS), and BFU2012-32404 from the Spanish Ministerio de Ciencia e Innovación (JGG and AMP) and a FPU fellowship granted to S.G.-L.

References:

[1] D. Suput, *"In vivo effects of cnidarian toxins and venoms"*, Toxicon, 54, 1190-1200, 2009.

[2] M. Tejuca, G. Anderluh, P. Macek, R. Marcet, D. Torres, J. Sarracent, C. Alvarez, M.E. Lanio, M. Dalla Serra, G. Menestrina, *"Antiparasite activity of sea-anemone cytolysins on giardia duodenalis and specific targeting with anti-giardia antibodies"*, Int J Parasitol, 29, 489-498, 1999.

[3] M. Thomson, R.L. Moritz, R.J. Simpson, R.S. Norton, *"Tenebrosin-a, a new cardiostimulant protein from the australian sea anemone actinia tenebrosa"*, Biochem Int, 15, 711-718, 1987.

[4] R.S. Norton, G. Bobek, J.O. Ivanov, M. Thomson, E. Fiala-Beer, R.L. Moritz, R.J. Simpson, *"Purification and characterisation of proteins with cardiac stimulatory and haemolytic activity from the anemone actinia tenebrosa"*, Toxicon, 28, 29-41, 1990.

O5-1, P5-1

STRUCTURE OF THE HUMAN TSG101-UEV DOMAIN IN COMPLEX WITH THE PTAP MOTIF OF VIRAL L-DOMAINS

Julio Bacarizo, Montserrat Andujar-Sánchez, Emilia Ortiz-Salmerón, Marina Plaza-Garrido and Ana Cámara-Artigas

Department of Chemistry and Physics, Research Centre for Agricultural and Food Biotechnology (BITAL), University of Almería, Agrifood Campus of International Excellence (ceiA3), 04120 Almería, SPAIN; e-mail: acamara@ual.es

Some virus scape from the cell by interacting with proteins of the ESRCT (Endosomal Sorting Complexes Required for Transport) machinery through the called Late domains (L-domains). These are present in the Gag proteins of a number of retroviruses and in the matrix proteins of the rhabdoviruses and filoviruses. The L-domains contain highly conserved motifs (PTAP, PPXY, and YXXL) that are essential in the interaction with the host proteins. One of the ESCRT proteins that interact with these L-domains is the TSG101 (Tumor susceptibility Gene 101). The interaction takes places through its UEV domain (Ubiquitin E2 variant) and has been targeted as a novel antiviral strategy. To develop drugs efficient to inhibit the interaction of the late domains with the TSG101-UEV domain we need to know in deep their binding. The crystal structure of the TSG101-UEV domain in complex with several L-domains have been solved: TSG101-UEV / L-domain PTAP motif of the Ebola virus VP40 matrix protein and TSG101-UEV / L-domain PTAP motif of the p19 gag protein of the Human T-cell Leukemia type I virus. Besides, we have solved the structure of the unliganded TSG101-UEV domain in two different crystal forms. Here we present the structural analysis of these complexes and their comparison with the unliganded structure.

Acknowledgements: This research was funded by the Spanish Ministry of Science and Innovation (Spain) and Ministry of Economy and Competitiveness (Spain) and FEDER (EU) [BIO2009-13261-C02-01/02 and BIO2012-39922-C02-01/02], Andalusian Regional Government (Spain) and FEDER (EU) [P09-CVI-5063]. Data collection was supported by European Synchrotron Radiation Facility (ESRF), Grenoble (France) [BAG proposals MX-1406, MX-1541 and MX-1629]; and ALBA (Barcelona, Spain) [BAG 2012010072 and 2012100378].

O5-2, P5-2

In-vivo-like study of the excluded volume effects on the kinetics of enzymatic reactions

Cristina Balcells[1], Claudia Hernández[1], Mireia Via[1], Isabel Pastor[2], Josep Lluís Garcés[3], Sergio Madurga[1], Eudald Vilaseca[1], Marta Cascante[4] and Francesc Mas[1]

[1] Department of Physical Chemistry and Research Institute of Theoretical and Computational Chemistry (IQTCUB) of Barcelona University, Barcelona (Spain); *crisgatsu@gmail.com*
[2] Small Biosystems Lab, Department of Fundamental Physics, University of Barcelona, Barcelona (Spain) and CIBER-BBN, Carlos III Health Institute, Madrid (Spain);
[3] Department of Chemistry, and AGROTECNIO, University of Lleida (UdL), Lleida (Spain);
[4] Department of Biochemistry and Molecular Biology and Institute of Biomedicine (IBUB) of Barcelona University, Barcelona (Spain)

The cell is a heterogeneously distributed and highly crowded medium in which a wide variety of physical and chemical processes take place. Until recently, each single process had been studied as an independent and isolated event, as close to ideality as possible. Still, this is quite unrealistic, both in terms of intermolecular interactions and in the fraction of occupied volume, which can be up to 300-400 g/L. Volume exclusion is one of the most relevant entropic effects occurring inside the cell, since it gives raise to steric repulsions, depletion forces and directly impacts on diffusion, interactions, kinetics and conformational equilibriums of biopolymers [1].

In the present work, the volume exclusion problem, also known as macromolecular crowding, has been applied to the field of enzyme kinetics. It has been approached by adding neutral, relatively inert polymers, which act as crowding agents or obstacles, in the media of given enzymatic reactions. The concentration and size of these obstacles have been changed systematically while studying the kinetic behavior of four differently-sized enzymes: α-Chymotrypsin (α-Chy, 25 kDa) [2], Horseradish Peroxidase (HRP, 42 kDa) [3], Alkaline Phosphatase (ALKP, 104 kDa) [4] and Lactate Dehydrogenase (LDH, 140 kDa) [5].

Results, in both experiment [6] and simulation [7], indicate that the performance of a certain enzyme depends on the amount of excluded volume, regardless of the enzymatic system. However, only large, oligomeric proteins display an obstacle size-dependent behavior. In this regard, the enzyme-crowding agent ratio can have a significant impact on the kinetics of a given reaction. Besides, it has been shown that such crowding can hinder diffusion to the extent of being capable of altering reaction control from activation to diffusion.

References:

[1] HX. Zhou, G. Rivas, A.P. Minton, *Annu. Rev. Biophys*. **2008,** 37, 375-397; I. M. Kuznetsova, K.K. Turoverov and V.N. Uversky, *Int. J. Mol. Sci.* **2014**, 15, 23090–23140.
[2] I. Pastor, E. Vilaseca, S. Madurga, M. Cascante, F. Mas *J. Phys. Chem. B*. **2011** 115(5), 1115–21.
[3] L. Pitulice, I. Pastor, E. Vilaseca, S. Madurga, A. Isvoran, M. Cascante, F. Mas., *J. Biocatal. Biotransform*., **2013**, 2, 1-5.
[4] C. Balcells., C. Hernández, M. Via, I. Pastor, J.L. Garcés, S. Madurga, M. Cascante, F. Mas, in preparation **2015.**
[5] C. Balcells., I. Pastor, E. Vilaseca, S. Madurga, M. Cascante, F. Mas *J. Phys. Chem. B*. **2014,** 118 , 4062-4068.
[6] I. Pastor, L. Pitulice, C. Balcells, E. Vilaseca, S. Madurga, A. Isvoran, F. Mas, *Biophys. Chem.,* **2014**, 185, 8–13.
[7] L. Pitulice, E. Vilaseca, I. Pastor, S. Madurga, J.L. Garcés, A. Isvoran, F. Mas, *Math. Bios.,* **2014**, 251, 72-82.

O5-3, P5-3

Identification of compounds that inhibits bacterial diguanylate cyclases involved in biofilm formation from theraputics drugs

Helton J. Wiggers[1], Éverton E. D. Silva[1]; Juliana Cheleski; Naiara U. Torres; Ederson Crusca; Marcos V. A. S. Navarro[1]

[1]Instituto de Física de São Carlos, Universidade de São Paulo, Av. Trabalhador Sancarlense 400, 13560-970, São Carlos, Brazil *wiggers@ursa.ifsc.usp.br*

Cyclic dimeric guanosine monophosphate (c-di-GMP) is a common, bacterial second messenger that regulates cellular processes in bacteria. High concentrations of c-di-GMP usually implies in biofilms formation, which are highly resistant to treatment with antibiotics and represent the predominant phenotype in most chronic infections. The c-di-GMP is synthesized from two GTP molecules by enzymes diguanylate cyclase (DGC) belonging to GGDEF family, these enzymes are attractive anti-biofilm targets for drug design. A drug repositioning strategy was applied in order to select potential diguanylate cyclase inhibitors from FDA-approved drugs. Using consensus scoring of docking, shape and electrostatic similarities ten compounds were selected for biochemical assay resulting in the discovery of antinflamatory and antihypertensive drugs as DGC inhibitors at micromolar range. Mass spectrometry was used to confirm the compounds binding to DGC and probe the GTP site. The approved drugs identified as DGC inhibitors showed anti-biofilm activity and are excellent starting compound for DGC potency optimization.

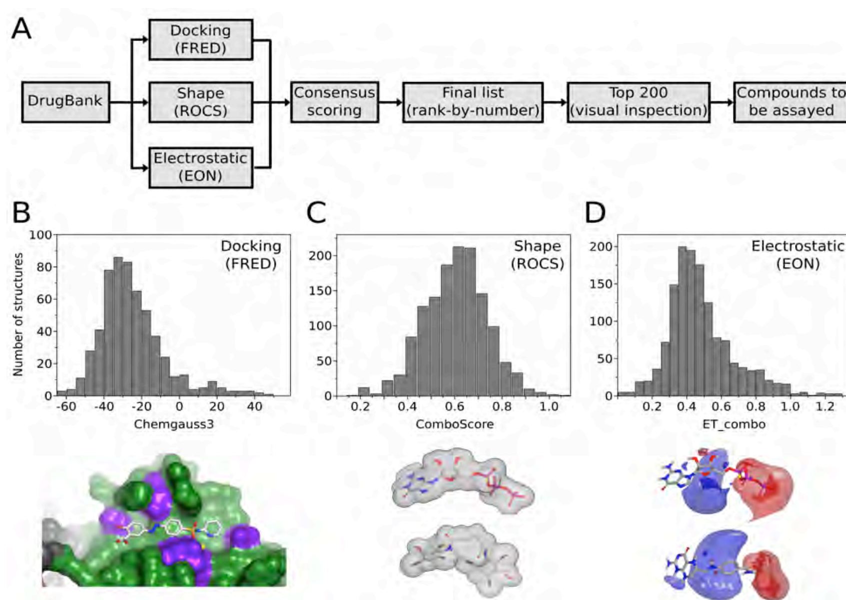


Figure 1. (A) The flowchart of virtual screening strategy employed for selecting the compounds for biochemical assay and histograms of (B) calculated binding energy by FRED program and Docking pose of sulfasalazine in the DGC PleD structure, (C) shape similarity calculated by ROCS program and shape comparison between the query GTP-α-S and iodipamide, (D) electrostatic similarity calculated by the EON program and electrostatic comparison of the query GTP- α-S and folic acid.

Acknowledgements: This work has been sponsored by FAPESP (grant 2010/ 19109-4) and Openeye Scientific Software for providing us with their program package

References

[1] De N, Navarro MVAS, Raghavan R V, Sondermann H. 2009. Determinants for the activation and autoinhibition of the diguanylate cyclase response regulator WspR. J. Mol. Biol. 393:619–33

[2]] H.J. Wiggers, J.R. Rocha, J. Cheleski, C.A. Montanari, Integration of ligand- and target-based virtual screening for the discovery of cruzain inhibitors, Molecular Informatics, 30 (2011) 565-578.

O5-4, P5-4

Revisiting the riboflavin kinase catalytic cycle of bacterial FAD Synthetase.

María Sebastián **[1,2], Ana Serrano [1,2*], Beatriz Herguedas[1,2**] Milagros Medina[1,2].**

[1] Departamento de Bioquímica y Biología Molecular y Celular, Facultad de Ciencias, and Instituto de Biocomputación y Física de Sistemas Complejos (BIFI), Universidad de Zaragoza, 50009-Zaragoza, Spain. *m.sebastian.valverde@gmail.com*
[2] Departamento de Bioquímica y Biología Molecular y Celular, Facultad de Ciencias, and Instituto de Biocomputación y Física de Sistemas Complejos (BIFI), Universidad de Zaragoza, 50009-Zaragoza, Spain.

[*±]Present address: Centro de Investigaciones Biológicas, CSIC, Ramiro de Maeztu 9, E-28040 Madrid, Spain
[**]Present address: MRC Laboratory of Molecular Biology, Francis Crick Avenue, CB2 0QH, Cambridge, UK

The FAD Synthetase (FADS) from *Corynebacterium ammoniagenes* is a bifunctional enzyme that catalyses the transformation of riboflavin (RF) into flavin adenine dinucleotide (FAD) in two steps.[1] First RF is phosphorilated to flavin mononucleotide (FMN) by a riboflavin kinase activity, being subsequent converted into FAD by an adenylyl-transferase activity. These activities are located in two different modules of the enzyme: the C-terminal domain performs the riboflavin kinase activity (RFK), while the N-terminal module is responsible from the FMN transformation into FAD. The FADS from the non pathogenic organism *C. ammoniagenes* shows structural similarities with those from other pathogens such as *M. tuberculosis* or *S. pneumonia*, being enzymes from C. ammoniagenes ususally used as models for other organisms. Due to the essentiality of the catalytic activities of FADS for cell survival and to their structural differences related to mammal enzymes involved in similar functions, FADS appears as a potential drug target.[2] In this context, a deeper characterization of its catalytic cycle is advantageous in the development of new antibacterial drugs. In this work, we use the rapid mixing technique, stopped flow, to identify and quantify the different individual steps of the RFK activity catalytic cycle. This is, to determine the ligands binding and dissociation order, and the characteristic constants that define every single process. [3]Due to the complexity of the FADS catalytic cycle, we used a truncated form of the enzyme consisting on the fully active RFK domain.

1. Frago, S., et al., *Structural analysis of FAD synthetase from Corynebacterium ammoniagenes.* BMC Microbiol, 2008. **8**: p. 160.
2. Serrano, A., et al., *The prokaryotic FAD Synthetase family: a potential drug target.* Current Pharm Design, 2013. **In press**.
3. Frago, S., A. Velázquez-Campoy, and M. Medina, *The puzzle of ligand binding to Corynebacterium ammoniagenes FAD synthetase.* J Biol Chem, 2009. **284**(11): p. 6610-9.

O6-1, P6-1

Unconventional EGF-induced ERK1/2-mediated Kv1.3 endocytosis

Ramón Martínez-Mármol[1, 2], Núria Comes[1], Mireia Pérez-Verdaguer[1], Jesusa Capera[1], Katarzyna Styrczewska[1], Lluís Pujadas[2], Eduardo Soriano[2,3], Alexander Sorkin[4], Antonio Felipe[1]

[1]Molecular Physiology Laboratory, Departament de Bioquímica i Biologia Molecular, Institut de Biomedicina (IBUB), Universitat de Barcelona, Barcelona, Spain. *afelipe@ub.edu.*
[2]Departament de Biologia Celular, Universitat de Barcelona, Barcelona, Spain.
[3]Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), ISCIII, Madrid, Spain. Vall d´Hebron Institute of Research (VHIR) and Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain.
[4]Department of Cell Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA.

The voltage-dependent potassium channel Kv1.3 plays important roles in immunity, neuronal development and sensory discrimination. Regulation of Kv1.3 by kinase signaling has been extensively studied. In this context, EGF binds to specific receptors (EGFR, ERB3) and triggers important tyrosine kinase-dependent signaling, which strongly down-regulates Kv1.3 currents. Here, we show that Kv1.3 undergoes EGF-dependent endocytosis. This EGF-mediated mechanism is highly relevant because is involved in adult neural stem cell fate determination. Using a wide repertoire of techniques, we demonstrated that changes in Kv1.3 subcellular distribution upon EGFR activation were due to massive Kv1.3 clathrin-dependent endocytosis, which was followed by targeting the Kv1.3 channels to the lysosomal degradative pathway. Interestingly, our results further revealed that relevant tyrosines and other interacting motifs, such as PDZ and SH3 domains, were apparently not involved in the EGF-dependent Kv1.3 internalization. However, a new, and yet undescribed mechanism, of ERK1/2-mediated threonine phosphorylation is crucial for the EGF-mediated Kv1.3 endocytosis. Our results demonstrate that EGF triggers the down regulation of Kv1.3 activity and its expression at the cell surface, which is important for the development and migration of adult neural progenitors.

Supported by BFU2014-54928-R, BFU2011-23268 and CSD2008-00005 (MINECO, Spain)

O6-2, P6-2

THE ROLE OF MEMBRANE HETEROGENEITY ON RECEPTOR DIFFUSION AND FUNCTION: ARE DIFFUSION CONSTANTS CONSTANT?

Carlo Manzo[1,*], Juan A. Torreno-Pina[1], Pietro Massignan[1], Gerald J. Lapeyre, Jr.[1], Maciej Lewenstein[1,2], and Maria F. Garcia-Parajo[1,2]

[1]ICFO – Institut de Ciències Fotòniques, Castelldefels (Barcelona), SPAIN
[2]ICREA – Institució Catalana de Recerca i Estudis Avançats, Barcelona, SPAIN
[*]E-mail: carlo.manzo@icfo.es

The concept of diffusion is essential to modern physics and has influenced nearly every field of science. According to Fick's law, the diffusion coefficient (or diffusivity) determines the proportionality between the molar flux due to thermal motion of molecules and the gradient of the molecular concentration. At low Reynolds numbers, the Stokes-Einstein relation defines the dependence of diffusivity on the size of diffusing objects and the temperature and viscosity of the medium, therefore the diffusivity is typically considered constant for fixed experimental conditions. In addition, the diffusion law can be equivalently determined either via a phenomenological approach (based on the description of an ensemble of particles) or the atomistic one (based on random walk of single particles), thus implying the ergodicity of the process [1].

The advent of fluorescence-based single-molecule techniques has allowed us to monitor the diffusion of single particle in living systems with unprecedented spatiotemporal resolution. The study of molecular diffusion is relevant to understand mechanisms of reaction kinetics regulating cellular function as well as to probe the structure of the diffusive environment at the molecular scale. These experiments have shown that the transport of molecules in living systems often deviates from ordinary Brownian diffusion, due to the crowding and heterogeneity of the environment. In addition, such heterogeneity might lead to nonergodic behavior, i.e. the non-equivalence of temporal and spatial averages [1].

Within this context, we have recently shown that the motion of a pathogen-recognition receptor in living cell membranes displays spatiotemporal heterogeneity characterized by random changes of diffusivity. Remarkably, this behavior produces anomalous diffusion with signatures of weak ergodicity breaking and aging [2] and can be interpreted by a novel model of ordinary diffusion in multi-scale random media [3]. In addition, a comparative analysis of mutated forms of the receptor evidences the role that its structure and interactions with other cellular components have on receptor dynamics [2]. Since the mutations differently impair receptor function, we establish a link between nonergodicity and the regulation of functional mechanisms, such as the capacity for pathogen recognition and internalization [2,4].

Acknowledgements: This work has been supported by Fundació Cellex, Generalitat de Catalunya, the European Commission, the HFSP, ERC AdG Osyris, and the Spanish Ministry of Science and Innovation.

References:

[1] C. Manzo, M. F. Garcia-Parajo, *"Single particle tracking: from methods to biophysical insights"*, Rep. Prog. Phys., (under review).
[2] C. Manzo, J. A. Torreno-Pina, P. Massignan, G. J. Lapeyre, Jr., M. Lewenstein, M. F. Garcia Parajo, *"Weak Ergodicity Breaking of Receptor Motion in Living Cells Stemming from Random Diffusivity"*, Phys. Rev. X, 5, 011021, 2015.
[3] P. Massignan, C. Manzo, J. A. Torreno-Pina, Jr., M. F. Garcia Parajo, M. Lewenstein, G. J. Lapeyre, *"Nonergodic Subdiffusion from Brownian Motion in an Inhomogeneous Medium"*, Phys. Rev. Lett., 112, 150603, 2014.

[4] J. A. Torreno-Pina, B. M. Castro, C. Manzo, S. I. Buschow, A. Cambi, M. F. Garcia Parajo, *"Enhanced receptor–clathrin interactions induced by N-glycan–mediated membrane micropatterning"*, Proc. Natl. Acad. Sci. USA, 111, 11037-11042, 2014.

O6-3, P6-3

Global characterization of the bimodular ligand binding domains of PP2249 and PP1228: two paralogous chemoreceptors from *Pseudomonas putida*

Álvaro Ortega[1], Andrés Corral-Lugo[1], Bertrand Morel[1] and Tino Krell[1]

[1]Estación Experimental del Zaidín-CSIC. Granada, Spain. *alvaro.ortega@csic.es.*

Bacteria need to constantly adapt to their environmental conditions for survival. In order to do so, they must be able to detect and react to advantageous or threatening cues through different signal recognition and transduction mechanisms. One of the most extended is that composed by the methyl-accepting chemotaxis proteins (MCP), which allows the organism to carry out an effective displacement by adapting the rotatory movement of its flagella according to the molecular signal detected by a periplasmic chemoreceptor.

Chemoreceptors are composed generally by a ligand binding domain (LBD), a HAMP region and a methyl-accepting domain that works as the signaling domain and can be modulated by other accessory proteins. Chemoreceptors can be classified by the size and fold of their LBD. Whereas the best studied bacterial model *E. coli* contains only 5 chemoreceptors with LBD's of around 150 aa (Cluster I MCP), there are organisms with many more chemoreceptors in their genomes. These may include LBD's of up to 300 aa (Cluster II MCP) that are bimodular and able to recognize two different signals at a time [1,2]. These second group of chemoreceptor are less studied and therefore worse described.

Here we present the characterization of the LBD's of two paralogous bimodular chemoreceptors of *Pseudomonas putida*, PP2249 and PP1228, that have a D-PDC-like [3] cluster II fold, albeit with a small difference in size. They present a monomeric state in solution, as shown by Analytical Ultracentrifugation (AUC) and Small Angle X-ray Scattering (SAXS), that is not modified upon recognition of their cognate ligands. A low resolution structure by SAXS of the recombinant proteins in solution is shown to be in good agreement with the rather elongated 3D homology model extracted from their sequences. Their secondary structures are conserved in the apo and ligand-bound forms, while their thermodynamic stabilities undergo important increases as extracted from Circular Dichroism (CD) and Differential Scanning Calorimetry (DSC) experiments respectively.

A.O. acknowledges a CSIC JAE-Doc contract co-funded by the European Social Fund.

References:

[1] E. Pineda-Molina, J.A. Reyes-Darias, J. Lacal, J.L. Ramos, J.M. García-Ruiz, J.A. Gavira and T. Krell "*Evidence for chemoreceptors with bimodular ligand binding regions harboring two signal-binding sites*". **Proc. Acad. Natl. Sci. USA.** 109, 18926-18931, 2012.

[2] J. Lacal, C. García-Fontana, F. Muñoz-Martínez, J.L. Ramos, T. Krell. "*Sensing of environmental signals: classification of chemoreceptors according to the size of their ligand binding regions*". **Environ. Microbiol.** 12, 2873-2884, 2010.

[3] Z. Zhang and W.A. Hendrickson. "Structural characterization of the predominant family of histidine kinase domains" J. Mol. Biol. 400, 335-353, 2010.

O7-1, P7-1

SINGLE-STRANDED RNA INTERACTION WITH LONG HUMAN TELOMERIC RNA UNVEILED AT THE SINGLE-MOLECULE LEVEL

Irene Gutiérrez[1], Miguel Garavís[2, 3], Santiago Casado[1], Carlos González[2], Alfredo Villasante[3] and J. Ricardo Arias-González[1,4]

[1]Instituto Madrileño de Estudios Avanzados en Nanociencia (IMDEA Nanociencia), C/Faraday 9, Cantoblanco, 28049 Madrid, Spain *irene.gutierrez@imdea.org*
[2]Instituto de Química Física Rocasolano, CSIC, C/Serrano 119, 28006 Madrid, Spain
[3]Centro de Biología Molecular "Severo Ochoa" CSIC-UAM, C/Nicolás Cabrera 1, 28049 Madrid, Spain
[4]CNB-CSIC-IMDEA Nanociencia Associated Unit "Unidad de Nanobiotecnología"

Telomeres are nucleoprotein structures that protect chromosome ends from being recognized as DNA breaks[1,2]. Each telomere end terminates in a G-rich single-stranded (ss) overhang able to self-fold into a four-stranded structure known as G-quadruplex. These non-canonical structures have a role in telomere end-protection and therefore in chromosome stability and in senescence as a barrier to tumorigenesis[3,4]. Furthermore G-quadruplexes are known to be present in the promoter regions of oncogenes[5]. We and others previously studied the mechanical unfolding of long human telomeric RNA (TERRA).[6,7] Here, we study the unfolding dynamics of TERRA in the presence of extra ssRNA by a combination of optical tweezers[8,9] and atomic force microscopy. We find that ssRNA interacts with TERRA and, although it does not significantly change its mechanical stability, it interferes with the unfolding dynamics of the G-quadruplex. In the cellular context, single-stranded segments often appear nearby a G-quadruplex. Our results then suggest that single-stranded tracts may compete for the binding to a G-quadruplex, which is important to understand G-quadruplex-binding drugs and the mechanical activity of the telomerase.

Acknowledgements: This work has been sponsored by Fundación IMDEA Nanociencia

References:

1. Titia de Lange. "Shelterin: the protein complex that shapes and safeguards human telomeres". Genes Dev., 19, 2100-2110, 2005.
2. Elizabeth H. Blackburn. "*Structure and function of telomeres*". Nature, 350, 569-573, 1991.
3. Stephen Neidle. "*The structure of quadruplex nucleic acids and their drug complexes*". Current Opinion in Structural Biology, 19, 239-250, 2009.
4. T. Ou, Y.Lu, J.Tan, Z. Huang, K.Wong and L.Gu. "*G-Quadruplexes: Targets in anticancer drug design*".ChemMedChem, 3, 690-713, 2008.
5. P. Rawal, V. B. R. Kummarasetti, J. Ravindran, N. Kumar, K. Halder, R. Sharma, M. Mukerji, S. K. Das and S. Chowdhury. "*Genome-wide prediction of G4 DNA as regulatory motifs: Role in Escherichia coli global regulation*". Genome Research, 16, 644-655, 2006.
6. Miguel Garavís, Rebeca Bocanegra, Elías Herrero-Galán, Carlos González, Alfredo Villasante and J. Ricardo Arias-Gonzalez. "*Mechanical unfolding of long human telomeric RNA (TERRA)*". Chem. Commun., 49, 6397-6399, 2013.
7. Philip M. Yangyuoru, Amy Y. Q. Zhang, Zhe Shi, Deepak Koirala, Shankar Balasubramanian and Hanbin Mao. "*Mechanochemical Properties of Individual Human Telomeric RNA (TERRA) G-Quadruplexes*". ChemBioChem, 14, 1931 – 1935, 2013.
8. Silvia Hormeño and J. Ricardo Arias-González. "*Exploring mechanochemical processes in the cell with optical tweezers*". Biol.Cell, 98 (12), 679-695, 2006.
9. J.Ricardo Arias-González. "*Single-molecule portrait of DNA and RNA double helices*". Integrative Biology, 6, 904-925, 2014.

O7-2, P7-2

Towards visualizing DNA repair at the single molecule level combining magnetic tweezers and TIRF microscopy

Julene Madariaga-Marcos[1], Mark S. Dillingham[2] and Fernando Moreno-Herrero[1]

[1] Department of Macromolecular Structures, Centro Nacional de Biotecnología, Consejo Superior de Investigaciones Científicas, Madrid, Spain. *E-mail: jmadariaga@cnb.csic.es*
[2] DNA:Protein Interactions Unit, School of Biochemistry, University of Bristol, Bristol, United Kingdom

Double-strand breaks (DSB) are a source of DNA damage frequently produced during the normal metabolism of cells. If not properly repaired, DSB can lead to genomic instability, developmental defects and cancer. Fortunately, cells posses robust repair mechanisms, such as homologous recombination, which relies in using the sister chromatid as a template to copy and repair the damaged DNA strand [1]. In bacteria, it has been extensively described how helicases and nucleases like AddAB/RecBCD perform the first step in this repair process, unwinding and degrading DNA, regulated by Chi recombination sequences [2-4]. We have previously characterized the unwinding activity of the AddAB helicase-nuclease using AFM [3] and a Magnetic Tweezers (MT) setup [5]. Recently, we have developed a hybrid setup incorporating total internal reflection fluorescence (TIRF) microscopy to our MT. This is a powerful approach because it will allow us to correlate biological activity with precise positioning and stoichiometries (directly observed by fluorescence) of the proteins. We 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 is quite straightforward for our MT and allows switching to 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.

Acknowledgements: J.M.M. acknowledges support of the Basque Government through a Predoc fellowship (ref PRE_2013_11_1174).

References:

[1] Claire Wyman and Roland Kanaar , *"DNA Double-Strand Break Repair: All's Well that Ends Well"*, Annu. Rev. Genet., 40, 363-83, 2006.
[2] Piero C. Bianco and Stephen C. Kowalczykowski, *"The recombination hotspot Chi is recognized by the translocating RecBCD enzyme as the single strand of DNA containing the sequence 5'-GCTGGTGG-3'"*, Proc. Natl. Acad. Sci. USA, 94, 6706–11, 1997.
[3] Joseph T.P. Yeeles et al., *"Recombination Hotspots and Single-Stranded DNA Binding Proteins Couple DNA Translocation to DNA Unwinding by the AddAB Helicase-Nuclease"*, Mol. Cell, 42, 806-16, 2011.
[4] Dale B. Wigley, *"Bacterial DNA repair: recent insights into the mechanism of RecBCD, AddAB and AdnAB"*, Nat. Rev. Microbiol., 11, 9-13, 2013.
[5] Carolina Carrasco et al., *"On the mechanism of recombination hotspot scanning during double-stranded DNA break resection"*, Proc. Natl. Acad. Sci. USA, 110(28), E2562-71, 2013.

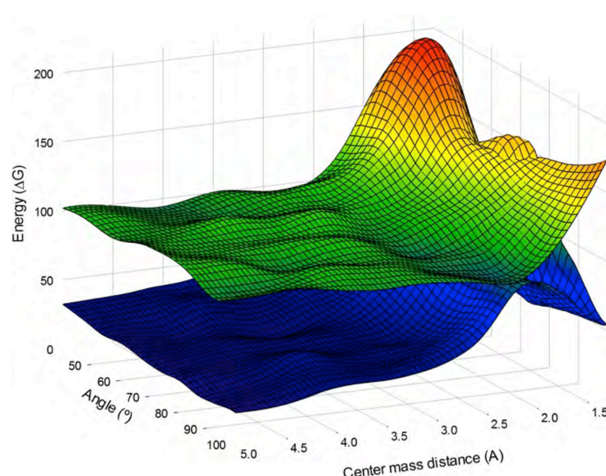
O7-3, P7-3

Ultrafast photochemical reactions in DNA: a QM/MM study

Jesús I. Mendieta-Moreno [1,2], Paulino Gómez-Puertas [2], Jesús Mendieta [2] and José Ortega [1]

[1] Departamento de Física Teórica de la Materia Condensada, Universidad Autónoma de Madrid, Madrid, Spain
[2] Molecular Modelling Group, Centro de Biología Molecular Severo Ochoa, Madrid, Spain

Ultraviolet (UV) light may induce photochemical reactions in DNA that corrupt the genetic information (photo-damage). The theoretical modeling of these processes is a great scientific challenge. Firstly, the reaction center has to be described using *first-principles* (quantum) molecular dynamics (MD) techniques. Secondly, the description of DNA has to be realistic, taking properly into account the environment of the reaction center (rest of the DNA and solvent). Moreover, the computational techniques must present an excellent balance between accuracy and computational efficiency, in order to properly explore the conformational space for the reaction. Finally, *non-adiabatic* MD simulations may be necessary to fully understand the mechanism of the reaction.
In this work we analyze the formation of a cyclobutene thymine dimer in DNA induced by UV light using a recently developed QM/MM MD technique, Fireball/Amber [1]. We explore the conformational space for the thymine dimerization reaction by means of long (~ 10^6 time steps) steered MD simulations for DNA in both the ground and excited states. This allows us to generate free energy maps and characterize the conical intersection for the reaction. Using all this information we can also determine the most likely path for this photo-induced reaction and the relationship between conformation and propensity for dimerization after UV light absorption.



References:

[1] J.I. Mendieta-Moreno, R. Walker, J.P. Lewis, P. Gomez-Puertas, J. Mendieta, J. Ortega, FIREBALL/AMBER: An efficient local-orbital DFT QM/MM method for biomolecular systems, Journal of Chemical Theory and Computation, 10, 2185-2193, 2014

O7-4, P7-4

UNDERSTANDING THE MECHANISMS OF DNA CONDENSATION BY THE BACTERIAL PROTEIN ParB/Spo0J

Cesar L. Pastrana[1], James A. Taylor [2], Gemma L. Fisher[2], Mark S. Dillingham[2] and Fernando Moreno-Herrero[1]

[1] Department of Macromolecular Structures, Centro Nacional de Biotecnología, Consejo Superior de Investigaciones Científicas, Madrid, Spain.

[2] DNA:Protein Interactions Unit, School of Biochemistry, University of Bristol, Bristol, United Kingdom.

The condensation and dynamic re-organization of the chromosome is crucial to the cell cycle of all living organisms. In several bacterial chromosomes, this process is dependent on the interactions of ParB proteins with centromere-like DNA sequences called *parS* that are located close to the origin of replication [1, 2]. We studied ParB-dependent condensation using magnetic tweezers, showing a strong reduction of the extension at forces < 2 pN that is reversible by protein unbinding or by force. The condensation process is not engaged with the formation of ordered structures, as reported by freely-orbiting magnetic tweezers experiments where a clear rotation trend was not measured. In fact, condensation is observed both in presence or absence of *parS* sites, indicating a non-specific mode of binding and a sequence-independent mechanism. These results are in agreement with previous models [3, 4] that envision looping and bridging of remote DNA regions mediated by Brownian motion. We provide deeper insight, confirming *in-trans* interactions using double-tethered beads and *in-cis* interactions in single supercoiled DNA molecules [5]. In order to determine the role of *parS* sequences, we explore different ParB mutants at the specific and non-specific domains. Our data suggest a novel DNA binding site responsible of non-specific binding. These results help us to understand the mechanism responsible of chromosome condensation and its implications for chromosme segregation.

References

[1] Gruber, S. and J. Errington, *Recruitment of condensin to replication origin regions by ParB/SpoOJ promotes chromosome segregation in B. subtilis.* Cell, 2009. **137**(4): p. 685-96.

[2] Sullivan, N.L., et al. *Recruitment of SMC by ParB-parS organizes the origin region and promotes efficient chromosome segregation.* Cell, 2009. **137**(4): p. 697-707.

[3] Graham, T.G., et al., *ParB spreading requires DNA bridging.* Genes Dev, 2014. **28(**11): p. 1228- 1238

[4] Broedersz, C.P. et al., *Condensation and localization of the partitioning protein ParB on the bacterial chromosome*. Proc Natl Acad Sci USA. 2014. **111**(24): p. 8809-8833.

[5] Taylor, J.A., et al., *Specific and non-specific interactions of ParB with DNA: implications for chromosome segregation.* Nucleic Acids Res, 2015. **43**(2): p. 719-31.

O8-1, P8-1

NATURAL EXTRACTS INHIBIT THE LIPOLYSIS USING A SINGLE DROP METHOD.

del Castillo-Santaella Teresa[1], Maldonado-Valderrama Julia[1], Rivadeneira-Ruiz Ceferino[2], Rondon-Rodriguez Deyanira[2], Cabrerizo-Vílchez Miguel Ángel[1] and Gálvez-Ruiz MªJosé[1].

[1] University of Granada, Department of Applied Physics, Campus de Fuentenueva, s/n., 18071, Granada, Spain. tdelcastillo@ugr.es
[2] Biosearch Life S.A., Department of Natural Products. Camino de Purchil, 66, 18004, Granada, Spain.

Fat digestion is an interfacial process due to the apolar nature of lipids which are the substrate of water-soluble lipases. The major part of the lipolysis of emulsified fat takes place in the duodenum where enzymes secreted by the pancreas have a high capacity for fat digestion[1–3]. Therefore the rate of lipolysis is controlled by enzyme ability to access the interface of its emulsified substrate. This in turn is controlled by the physicochemical characteristics of the oil/water interface; such as interfacial structure/composition and droplet surface area. Inhibition of lipase activity is currently one of the main approaches to reduce fat intake in the diet. There are in fact many commercial drugs available but produce side effects. In this sense, an interesting alternative is the use of natural extracts with inhibiting properties and less side-effects. At any rate, in order to obtain the desired effect it is crucial to improve the understanding of molecular mechanisms underlying lipase inhibition.

We have designed a generic study to address lipolysis from an interfacial perspective, by measuring the inhibition caused by commercial drugs with the pendant drop technique[4]. The evolution of the interfacial tension of a physiologically relevant lipase solution provides a control for the lipolysis. The interfacial tension of the same lipase mixture in the presence of Xenical, a medical lipase inhibitor, provides the control for the inhibition profile. From these data we can quantify the inhibition of new natural products.

We demonstrate that the inhibition mechanism can be correlated with the reduction of the interfacial activity of lipase under the physiological conditions in the duodenum. Likewise, based on dilatational response of lipase in the presence and absence of inhibiting products, we discuss the conformational changes induced in the molecule or blocking of adsorption sites as possible origins of the inhibiting effect. Such observations provide insight into the lipase inhibition mechanism and opportunities to affect the digestion profile, delivery and release of nutrients can be identified.

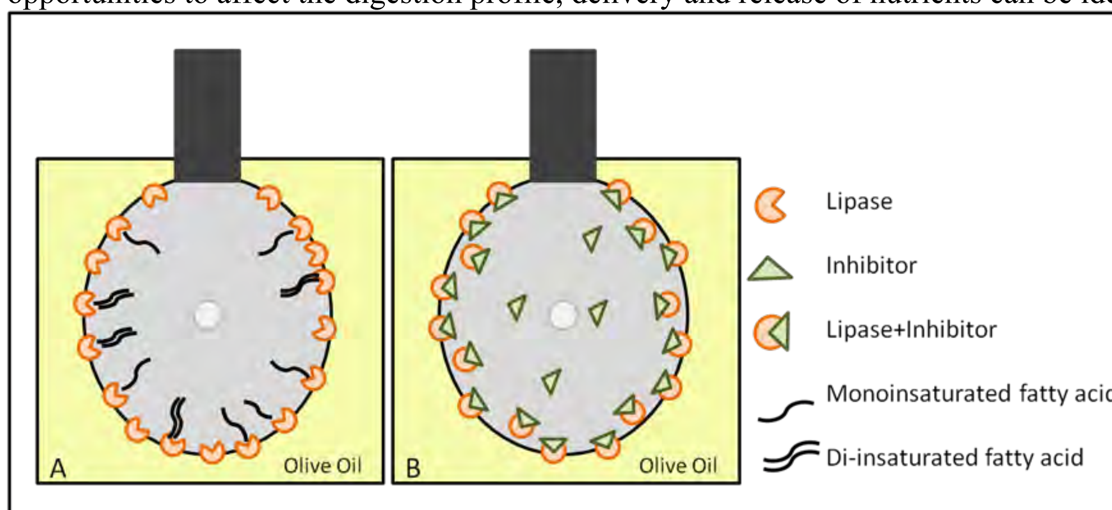


Figure 1. Simplified schematic representation of the adsorbed lipase protein layer into olive oil - water interface. A) Lipase B) Lipase and inhibitor.

Acknowledgements: This work has been sponsored by CDTI (FEDER INNTERCONECTA: ITC-20131081), RYC-2012-10556, MAT2011-23339, MAT2012-36270-C04-02. COST-MPN-1106-Green Interfaces and COST-FA-1005-Infogest.

References:

[1] Wilde, P. J.; Chu, B. S., "*Interfacial and colloidal aspects of lipid digestion*", Adv. Colloid Interface Sci., 165 (1), 14-22, 2011.

[2] Reis, P.; Holmberg, K.; Watzke, H.; Leser, M. E.; Miller, R., "*Lipases at interfaces: A review*", Advances in Colloid and Interface Science, 147–148 (0), 237-250, 2009.

[3] Golding, M.; Wooster, T. J., "*The influence of emulsion structure and stability on lipid digestion*", Curr. Opin. Colloid Interface Sci., 15 (1-2), 90-101, 2010.

[4] Maldonado-Valderrama, J.; Terriza, J. A. H.; Torcello-Gomez, A.; Cabrerizo-Vilchez, M. A., "*In vitro digestion of interfacial protein structures*", Soft Matter, 9 (4), 1043-1053, 2013.

O8-2, P8-2

Functionalized Nanoparticles in a Biological Environment: Impact of Protein Corona.

Paola Sánchez Moreno[1], Daniele Maiolo[1], Claudia Pigliacelli[1], Ilaria Tirotta[1], Pierangelo Metrangolo[1,2], Giuseppe Resnati[1], Francesca Baldelli Bombelli[1].

[1]Centro Europeo di Nanomedicina (CEN) and Dipartimento di Chimica, Materiali e Ingegneria Chimica Giulio Natta, Politecnico di Milano, Milano, Italy.
[2]VTT Technical Research Centre of Finland Ltd, Tietotie 2, Espoo FI-02044 VTT, Finland.

Nanomedicine plays an ever-increasing role in pharmaceutical research using nano-scaled materials for the detection and treatment of human diseases. This growing multidisciplinary field has promoted the development of several nanosystems able to deliver different therapeutic agents to targeted tissues.

In a biological environment, a nanoparticle is exposed to an evolving combinatorial system containing thousands of different proteins alongside lipids and sugars, which can reconfigure the nano-bio interface forming a "corona" that defines the biological identity of the particle (1). The proteins adsorbed onto the original nanoparticle surface can mask targeting ligands and furthermore interact with specific plasma membrane receptors on monocytes and various subsets of tissue macrophages, promoting rapid recognition and removal of the intravenously injected nanoparticles. Huge efforts have been done to better understand how the physicochemical properties of nano-scaled materials affect their interaction with the highly complex surrounding biological milieu and with cells (2-4)_ENREF_3_ENREF_4. This improved understanding can be used to develop intelligent strategies in the design of new therapies with controlled environmental interaction and optimized biological functionality.

In this study, different surface functionalizations of nanoparticles designed for biomedical applications are shown. We present here a complementary analysis to address the different aspects governing the formation and function of the protein corona in the bio-environment. The data highlight the importance of using complementary characterization techniques to analyse different aspects of the protein corona, thus guiding the design of successful nanomedicines.

References:

[1] Tenzer S, *et al.* (2013) Rapid formation of plasma protein corona critically affects nanoparticle pathophysiology. *Nat Nanotechnol* 8(10):772-U1000

[2] Monopoli MP, *et al.* (2011) Physical-Chemical Aspects of Protein Corona: Relevance to in Vitro and in Vivo Biological Impacts of Nanoparticles. *J Am Chem Soc* 133(8):2525-2534

[3] Treuel L, *et al.* (2014) Impact of Protein Modification on the Protein Corona on Nanoparticles and Nanoparticle-Cell Interactions. *Acs Nano* 8(1):503-513

[4] Del Pino P, *et al.* (2014) Protein corona formation around nanoparticles - from the past to the future. *Mater Horizons* 1(3):301-313.

O8-3, P8-3

Cationic Gemini Surfactants Induce Abnormal DNA Conformational Change at High Surfactant-DNA Molar Ratios

Elia Grueso[1], Edyta Kuliszewska[2], Emilio Roldan[1], Pilar Perez-Tejeda[1], Rafael Prado-Gotor[1] and Brecker Lothar[3]

[1] Department of Physical Chemistry, Faculty of Chemistry, University of Seville. C/ Profesor García González, s/n, 41012, Sevilla, Spain.
[2] Institute of Heavy Organic Synthesis- Ul. Energetykow 9, Kedzierzyn-Kozle 47-225, Poland.
[3] Institute of Organic Chemistry, University of Vienna, Währingerstrasse 38, A-1090 Wien, Austria.

The understanding of the interaction between double stranded DNA and cationic gemini surfactants will have a broad bearing on many important applications from drug delivery to the translocation of DNA across the cell for the purpose of gene therapy [1]. These applications require that compact DNA molecules arrive in the nucleus as well as accessibility to the cell enzymatic machinery. However, this final step is only possible if full or partial decompaction occurs. As a contribution to this field, the DNA conformational changes induced by different members of the N,N'-bis(dimethyldodecyl)-α-ω-alkanediammonium dibromide series (m-s-m, m = 12, s = 3 and 6) and the analogous series of hexadecyl gemini surfactants (m = 16, s = 3 and 6) were investigated in aqueous media by means of circular dichroism (CD), zeta potential, dynamic light scattering (DLS), viscometric, and atomic force microscopy (AFM) methods. The measurements were done by varying the gemini surfactant-DNA molar ratio, $R = C_{m\text{-}s\text{-}m}/C_{DNA}$. For the conditions investigated two significantly different conformational changes were observed, the second of them being worth noting. At the low molar ratios, all methods concurred by showing that gemini surfactants were able to form ordered aggregates which precedes DNA compaction. The second effect observed, at high molar ratios, corresponds to the transition from the compact state to a new more extended conformation. The degree of decompaction and the morphologies of the visualized structures are different not only depending on the surfactant tail´s length, but also on the spacer´s length. Importantly, this process is fully accomplished for dodecyl gemini surfactants and is only partial for the 16-s-16 series (see Figure 1).

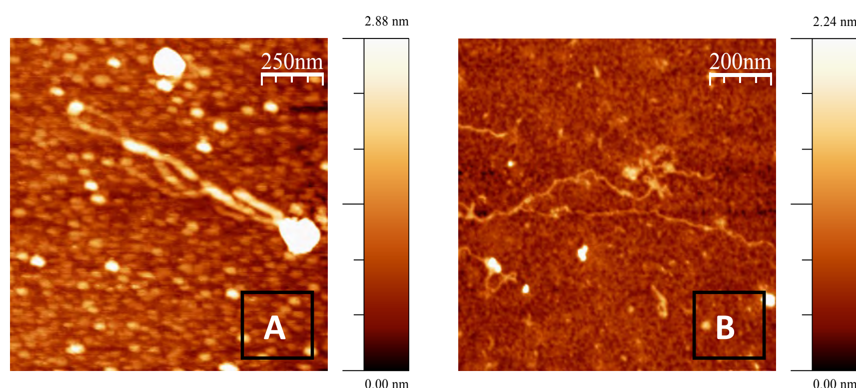


Figure 1. AFM topographic images of CT-DNA and gemini surfactants showing different degree of decompaction, $C_{DNA} = 3 \times 10^{-7}$ M (A) 12-6-12/DNA system, R = 5.0; (B) 16-6-6/DNA system, R = 5.0.

In fact, the results obtained for the 16-3-16/DNA and 16-6-16/DNA systems point out that the compaction/decompaction processes are somewhat different to those previously visualized for the

analogous monoquaternary chain surfactant CTAB [2]. These results are particularly interesting, since in spite of the ability of gemini surfactants to induce DNA compaction, such an ability was already recognized, but the DNA decompaction up to now had not been observed [3]. These findings open a new strategy for the design of non-toxic carriers, based on the gemini surfactants, for drug delivery and gene therapy applications. In a first stage, the compacted DNA-surfactant complexes would be introduced into the cell nucleus. Then, once the DNA has adapted its structure to the tine specific sites in the cell, the addition of more quantity of a gemini surfactant would make the polymer accessible to the cell enzymatic machinery by inducing DNA decompaction.

This work has been sponsored by the Consejería de Educación y Ciencia of the Junta de Andalucía (FQM-03623)

References:

[1] M. C. Garnett. "Gene-delivery systems using catonic polymers", Crit. Rev. Ther. Drug. Carrier Syst, 16, 2, 147-207, 1999.

[2] E. Grueso, C. Cerrillos, J. Hidalgo, P. Lopez-Cornejo "Compaction and Decompaction of DNA induced by the cationic surfactant CTAB " Langmuir, 28, 30, 10968-79, 2012.

[3] Elia Grueso, Edyta Kuliszewska, Emilio Roldan, Pilar Perez-Tejeda, Rafael Prado-Gotor, Brecker Lothar "DNA Conformational Changes Induced by Cationic Gemini Surfactants: The Key to Switch DNA Compact Structures into Elongated Forms " RSC Advances, 5, 29433-46, 2015.

O8-4, P8-4

LIPID-CATION-DNA COMPLEXES: INTERFACIAL CHARACTERIZATION AND MODELING

Germán Luque Caballero[1*], Teresa del Castillo Santaella[2], Julia Maldonado Valderrama[2], Manuel Quesada Pérez[3] and Alberto Martín Molina[2]

[1]University of Granada, Granada, Spain, *gluque@ugr.es*
[2]University of Granada, Granada, Spain
[3]University of Jaén, Linares, Spain

Multivalent cations are able to mediate the interaction between DNA and negatively charged membranes. As a result, spontaneous formation of lipid-cation-DNA ternary complexes has been observed both in bulk and at interfaces[1]. The former is related to the production of gene delivery vectors, known as anionic lipoplexes, and the latter is related to the multivalent-cation-mediated DNA binding to phospholipid monolayers at the air-water interface. In this study, we analyze the effect on the monolayer properties upon the DNA binding mediated by divalent cations. Namely, monolayer state and molecular packing are characterized as a function of the lateral pressure and the structures formed upon ternary complexation are visualized by AFM. Furthermore, the role of electrostatic interactions can be inferred from surface potential measurements. Indeed, Monte Carlo simulations have predicted the condensation of a polyanion onto a like-charge surface in the presence of multivalent cations, considering purely electrostatic interactions. The combination of experimental characterization and simulations allow us to improve our understanding of the different interactions involved in the formation, stability and function of anionic lipoplexes.

Acknowledgements: This work has been sponsored by the Junta de Andalucia rearch project P09-FQM-4698. [2]

References:

[1] Alberto Martín Molina, Germán Luque Caballero, Manuel Quesada Pérez, Jordi Faraudo and Julia Maldonado Valderrama, *"Adsorption of DNA onto anionic lipid surfaces"*, Advances in Colloid and Interface Science, 206, 172-185, 2014.
[2] Lipoplex project, http://wdb.ugr.es/~lipoplex/

Poster Presentations

P1-5

Functional and structural characterization of the Human mTOR Complex II

Elena Aranda Serrano[1], David Gil Cartón[2], Nuria Roldán[1], Jesús Pérez-Gil[1] and Begoña García-Alvarez[1]

[1]Departament of Biochemistry, Faculty of Biology, Complutense University of Madrid, Spain. elena.aranda.serrano@gmail.com
[2]Structural Biology Unit, CIC bioGUNE, Parque Tecnológico de Bizkaia, Edificio 800, 48160 Derio, Spain.

The mTOR("mammalian Target Of Rapamycin") signaling pathway is crucial in regulating cell growth and proliferation. mTOR serine/threonine kinase exists in two structurally and functionally distinct mTORC1 and mTORC2 complexes. Here we report functional and structural studies of the less characterized mTORC2 complex. This complex controls cell proliferation and survival through the phosphorylation and activation of Akt/PKB kinase. It has five components: mTOR, mLST8, Rictor (Raptor Independent Companion of mTOR), mSIN1 (mammalian Stress Interacting protein-activated protein kinase 1), and Protor-1 (Protein observed with Rictor-1). We purified mTORC2 from mammalian HEK-293T cells that stably express a mSIN1-Histag variant. We tested the integrity of the purified complex inside the cells. Moreover, depending of the cell type, purified mTORC2 can activate either apoptosis or autophagy. We also demonstrated that the AKT phosphorylated state in Ser-473 is altered in transfected cells previously stimulated with the receptor factors suggesting the negative feedback between mTORC1 and mTORC2. Finally, we have started the structural characterization of the mTOR complex using electron microscopy negative staining. Preliminary results have been obtained from average 2D images of individual particles of the complex.

Acknowledgements: This work has been sponsored by a B.G.A Ramon & Cajal contract and a grant from the Spanish Ministry of Economy and Competitivity (BIO2012-30733).

References:

[1] Calvin K. Yip, Kazuyoshi Murata, Thomas Walz, David M. Sabatini, and Seong A. Kang, , *"Structure of the Human mTOR Complex I and Its Implications for Rapamycin Inhibition"*, Molecular Cell, 38, 768-774, June 11, 2010.

[2] Won Jun Oh and Estela Jacinto. "*mTOR complex 2 signaling and functions"* , Cell Cycle 10:14, 2305-2316, July 15, 2011

[3] Pengda Liu, Wenjian Gan, Hiroyuki Inuzuka, Adam S. Lazorchak, Daming Gao, Omotooke Arojo, Dou Liu, Lixin Wan, Bo Zhai, Yonghao Yu, Min Yuan, Byeong Mo Kim, Shavali Shaik, Suchithra Menon, Steven P. Gygi, Tae Ho Lee, John M. Asara, Brendan D. Manning, John Blenis, Bing Su and Wenyi Wei *"Sin1 phosphorylation impairs mTORC2 complex integrity and inhibits downstream Akt signalling to suppress tumorigenesis"*, Nature Cell Biology, volume 15, 11, 2013

[4] Alessandra Adami, Begoña García-Álvarez,Ernesto Arias-Palomo, David Barford and Oscar Llorca, "*Structure of TOR and Its Complex with KOG1"*, Molecular Cell 27, 509-516, 2007.

P1-6

THE ROLE OF MOLECULAR STRUCTURE IN THE INTERACTION BETWEEN MASLINIC ACID DERIVATIVES AND ALBUMIN

F. Galisteo-González[1], J. A. Molina-Bolívar[2], C. Carnero Ruiz[2], M. Medina-O´ Donnell[3], A. Parra[3]

[1]Dept of Applied Physics, University of Granada, Granada, Spain (galisteo@ugr.es)
[2] Dept of Applied Physics II, University of Málaga, Málaga, Spain
[3]Dept of Organic Chemistry, University of Granada, Granada, Spain

Interaction of disuccinylmaslinic acid (SMA) and diacetyl maslinic acid (DMA) with bovine serum albumin (BSA) has been investigated by steady-state fluorescence under different experimental conditions. From the temperature dependence of the binding process an extensive analysis of thermodynamic parameters has been made in connection with the drug structure. SMA binds to BSA mainly through electrostatic interactions at physiological pH (7.4) and low ionic strength. An increased electrolyte concentration provoked hydrogen bonds and van der Waals forces to control the complex formation. When pH was higher than the isoelectric point of albumin (i.e.p. 4.9) the attachment of the drug was favored by both negative enthalpy and positive entropy changes. These results suggest a dominance of electrostatic forces in the association process. Conversely, at pH values lower than the i.e.p., the unfavorable negative entropy changes prompt the involvement of hydrogen bonds in the binding. In the case of DMA, hydrophobic interactions were the dominant intermolecular forces in the binding to BSA. A noteworthy enthalpy-entropy compensation phenomenon has been detected. The binding processes -controlled mainly by hydrogen bonds and van der Waals interactions (SMA-BSA) or by hydrophobic forces (DMA-BSA)- fall in the same compensation line. The observation of this entropy-enthalpy compensation suggests that water reorganization plays an important role in the binding of both drugs to BSA.

P1-7

The mechanochemistry of a structural Zinc finger

Judit Perales-Calvo, Ainhoa Lezamiz and Sergi Garcia-Manyes

Department of Physics and Randall Division of Cell and Molecular Biophysics, King's College London, Strand, WC2R 2LS, London, UK

Zinc fingers are highly ubiquitous structural motifs that provide stability to proteins, thus contributing to their correct folding. Despite the high thermodynamic stability of the $ZnCys_4$ centres, their kinetic properties display remarkable lability. Here we use a combination of protein engineering techniques with single molecule force spectroscopy AFM to uncover the surprising mechanical lability (~90 pN) of the individual Zn-S bonds that form the two equivalent zinc finger motifs embedded within the structure of the multidomain DnaJ chaperone. Rational mutations within the zinc coordinating residues enable direct identification of the chemical determinants that regulate the interplay between zinc binding –requiring the presence of all 4 cysteines– and disulfide bond formation. Finally, our observations show that peptide binding drastically increases the mechanical stability of DnaJ. Altogether, our experimental approach offers a detailed, atomistic vista on the fine chemical mechanisms that govern the nanomechanics of individual, naturally occurring zinc fingers.

P2-4

TOWARDS THE CHARACTERIZATION OF MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE BY DSC AND MALDI-TOF MS ANALYSES OF BLOOD SERUM PROTEOME

Francisca Barceló[1], Rosa M. Gomila[2], José L. Merino[3], Regina Alemany[1], Oliver Vogler[1], Ivan de Paúl[3]; Jaume Segura[3], Albert Pérez-Montaña[4], Bernardo López[4], Antonia Sampol[4], Joan Besalduch[4], Teresa Gutierrez[5] and José Portugal[6]

[1]Clinical and Translational Research group. University of the Balearic Islands. Palma de Mallorca, Spain. francisca.barcelo@uib.es
[2]Servicios Científico-técnicos, University of the Balearic Islands. Palma de Mallorca, Spain
[3]Electronic Systems Group. University of the Balearic Islands. Palma de Mallorca, Spain
[4]Servicio de Hematología y Hemoterapia HUSE; IdisPa. Hospital Universitario Son Espases. Palma de Mallorca, Spain
[5]Fundació Banc de Sang i Teixits de les Illes Balears. Gobierno Balear, Spain
[6]Instituto de Biología Molecular de Barcelona, CSIC, Parc Cientific de Barcelona. Barcelona, Spain

Monoclonal gammopathy of undetermined significance (MGUS) is a pre-malignant dyscrasia that can precede the development of multiple myeloma, a malignant neoplasia. There are no reliable biologic markers that predict which individual with MGUS will progress to MM or related condition. Therefore, improved methods and molecular biomarkers for MGUS diagnosis and its clinical evolution are needed. We are examining the suitability of DSC and MALDI-TOF MS analyses of blood sera from MGUS patients for diagnosis and clinical outcomes. DSC thermograms of serum samples distinguished healthy samples from MGUS individuals, and they showed closed connection with different peculiarities of MGUS pathology [1]. MALDI-TOF MS methodology, based on different analytical properties, is being explored to provide a complementary approach for screening MGUS disease. We have found that MGUS patients display blood serum MS profiles and peak intensities that differ from those in healthy control individuals. Our results provide novel insights into the altered protein thermogram and peptidome profile associated with MGUS. They support that DSC and MALDI-TOF techniques are promising tools for the early diagnosis and monitoring of MGUS.

Acknowledgements: This work has been sponsored by grants from the “Direcció General d’Universitats i Recerca del Govern de les Illes Balears” and the FEDER program of the European Community.

References:

[1] Francisca Barceló, Joan J. Cerdà, Antonio Gutiérrez, Teresa Jimenez-Marco, M. Antonia Durán *et al. Characterization of Monoclonal Gammopathy of Undetermined Significance by Calorimetric Analysis of Blood Serum Proteome*. Plos One 10(3):e0120316, 2015

P3-5

Structural and Functional Characterization of MeCP2, a Protein Target Associated with Rett Syndrome

Rafael Claveria-Gimeno[1,2,3], Pilar Maria Lanuza[1], Olga de la Caridad Jorge[4], Sonia Vega[1], Olga Abian[1,2,3,5], Manel Esteller[4,6], Adrian Velazquez-Campoy[1,3,7]

[1]Institute of Biocomputation and Physics of Complex Systems (BIFI), Joint Unit IQFR-CSIC-BIFI, Universidad de Zaragoza, Zaragoza, Spain (*rafacg@bifi.es*)
[2]Instituto Aragonés de Ciencias de la Salud (IACS), Zaragoza, Spain
[3]IIS Aragón, Zaragoza, Spain
[4]Cancer Epigenetics and Biology Program (PEBC), Bellvitge Biomedical Research Institute (IDIBELL), Barcelona, Spain
[5]Centro de Investigación Biomédica en Red en el Área Temática de Enfermedades Hepáticas y Digestivas (CIBERehd), Barcelona, Spain
[6]Department of Physiological Sciences II, School of Medicine, University of Barcelona, Barcelona, Spain, and Generalitat de Catalunya, Institucio Catalana de Recerca i Estudis Avançats, 08010 Barcelona, Spain
[7]Fundacion ARAID, Government of Aragon, Zaragoza, Spain, and Department of Biochemistry and Molecular and Cell Biology, Universidad de Zaragoza, Zaragoza, Spain

Methyl CpG binding protein 2 (MeCP2) is a DNA binding protein involved in gene expression regulation that preferentially interacts with methylated DNA regions, though it also interacts with multiple protein partners. Certain mutations in MeCP2 are associated with Rett syndrome, an important neurodevelopmental disorder affecting young girls. Those clinically-relevant mutations in MeCP2 may affect its ability to fold and/or to interact properly with DNA, thus, hindering its multiple functions.
MeCP2 is an intrinsically disordered protein. The majority of its polypeptide chain is considered to be unstructured under physiological conditions. Unstructured regions are important because they provide the required structural plasticity for establishing multiple interactions with different binding partners with a low entropic penalty, through processes where partial folding and binding are intimately coupled.
We have carried out a biophysical characterization of the structural stability of the methyl binding domain (MDB) and other MeCP2 variants by fluorescence and circular dichroism. The impact of methylated and unmethylated DNA interaction on that stability was also assessed. Large DNA stabilization effects suggest that the interaction with DNA is coupled to a rearrangement of the protein conformation. In addition, the interaction between MBD MeCP2 and methylated/unmethylated DNA has been studied directly by isothermal titration calorimetry (ITC). The results indicate that the interaction of MeCP2 with DNA is coupled to the structuring of the protein conformation and it is strongly modulated by external factors (e.g. pH, ionic strength).

Acknowledgements: This work has been sponsored by Spanish Ministerio de Economía y Competitividad (BFU2013-47064-P), Spanish Ministerio de Educación, Cultura y Deporte (Grant FPU13/3870), Miguel Servet Program from Instituto de Salud Carlos III (CP07/00289), Diputación General de Aragón (Protein Targets Group B89)

P3-6

The effect of N-terminal acetylation on SDS-induced α-Synuclein amyloid aggregation.

David Ruzafa[1,4], Yuriko S. Hernández-Gómez[2], Giovanni Bisello[2], Bertrand Morel[3], Francisco Conejero-Lara[1].

[1] *Departamento de Química Física e Instituto de Biotecnología, Facultad de Ciencias, Universidad de Granada,18071, Granada, Spain.*
[2] *Dipartimento di Science del Farmaco, Università degli Studi di Padova, 35020, Padova, Italy.*
[3] *Grupo de Microbiología Ambiental y Biodegradación. Estación experimental del Zaidín,Consejo Superior de Investigaciones Científicas, 18008,Granada, Spain.*
[4] ruzafa@ugr.es

The Parkinson's disease (PD) is the most common neurodegenerative motor system disorder. PD is characterized by the loss of dopaminergic neurons in the "*substantia nigra*" and the appearance of intraneuronal inclusions, known as Lewy bodies whose major components are fibrilar aggregates of a-Synuclein (a-Syn) and its oligomeric forms. a-Syn oligomerization is believed to play a key role in the progress of PD [1].

Although a-Syn has always been described as an intrinsically disordered protein, however it adopts a-helical structures and binds to negatively charged membranes [2] inducing curvature in them. This has suggested a membrane remodeling function for a-Syn. Recent evidence suggests that unfolded a-Syn monomers exists under physiological conditions in equilibrium with oligomeric forms. Moreover, this equilibrium appears to be modified by N-terminal acetylation [3]. This posttranscriptional modification increases the hydrophobicity of the N-terminal region, stabilizes its a-helical structure, enhances the affinity with anionic lipid surfaces and somehow decreases the aggregation propensity[4]. However, the underlying mechanisms of these effects remain unclear.

Sodium dodecyl sulfate (SDS) is an anionic surfactant commonly used in biophysical studies to mimic membrane environments for proteins and it has been extensively used with a-Syn[5]. Low SDS concentrations (0.5-2 mM) stabilize oligomeric and partially folded states and increase amyloid fibrillation, whereas higher SDS concentrations decrease this amyloidogenic propensity with a maximum a-helix content.

In this work we compare the formation of partially folded oligomers of N-acetylated and unacetylated a-Syn in presence of low concentrations of SDS and explain the different behavior related to their amyloidogenic propensity. We find that the SDS-associated dynamic oligomers constitute optimal species for spontaneous and efficient formation of amyloid nuclei, which further drive lag-free amyloid fibrillation. N-acetylation appears to stabilize a-Syn interaction with SDS micelles thereby reducing the population of amyloidogenic species.

Acknowledgements: This work has been sponsored by the MECD (grant BIO2009-07317), and the European Regional Development Fund of the European Union.

References:

(1)Lorenzen, N.; Nielsen, S. B.; Buell, A. K.; Kaspersen, J. D.; Arosio, P.; Vad, B. S.; Paslawski, W.; Christiansen, G.; Valnickova-Hansen, Z.; Andreasen, M.; Enghild, J. J.; Pedersen, J. S.; Dobson, C. M.; Knowles, T. P.; Otzen, D. E. *Journal of the American Chemical Society* **2014**.
(2)Trexler, A. J.; Rhoades, E. *Biochemistry* **2009**, *48*, 2304-2306.
(3) Bartels, T.; Choi, J. G.; Selkoe, D. J. *Nature* **2011**, *477*, 107-U123.
(4) Kang, L.; Moriarty, G. M.; Woods, L. A.; Ashcroft, A. E.; Radford, S. E.; Baum, J. *Protein science : a publication of the Protein Society* **2012**, *21*, 911-917.
(5) Ahmad, M. F.; Ramakrishna, T.; Raman, B.; Rao Ch, M. *Journal of molecular biology* **2006**, *364*, 1061-1072.

P3-7

SINGLE MOLECULE STUDIES OF THE FIRST AMYLOIDOGENIC STEP

Fabio Castello[1], Salvador Casares[2], Maria J. Ruedas-Rama[1] and Angel Orte[1]

[1]Department of Physical Chemistry, Faculty of Pharmacy. University of Granada. Campus Cartuja, 18071, Granada (Spain). *E-mail: angelort@ugr.es*
[2]Department of Physical Chemistry, Faculty of Sciences. University of Granada. Campus Fuentenueva, 18071, Granada (Spain)

One of the current major biomedical challenges is the structural and dynamic characterization of the on-pathway intermediates involved in the mechanism of amyloid fibril formation. Nucleation into oligomeric structures plays a central role in the neuronal toxicity of amyloids deposition. Single-molecule fluorescence (SMF) allows to study those soluble intermediate oligomers at the molecular level [1, 2], providing new insights into heterogeneous systems.
We focused on the formation of the early oligomeric aggregates of the highly amyloidogenic N47A mutant of the α-spectrin SH3 domain as a model, and employed a multiparameter, dual-color excitation SMF approach to extract the size distributions and the intra-oligomer FRET efficiency of the detected oligomers. Our experiments revealed the presence of aggregated species under aggregation conditions, but yet in the absence of incubation. These species were small in size, mostly dimers, but showing a low FRET efficiency, what suggests a loose molecular organization. The presence of these labile, small, low-FRET oligomers was clearly detected, even at the low concentration ranges employed in SMF experiments. We determined the value of the dissociation equilibrium constant of these oligomeric species using the single molecule event rate of the oligomers at different protein concentrations. The direct detection of these oligomers demonstrates the crucial role of nonspecific interactions as the first nucleation event in amyloid fibril formation.

Acknowledgements: This work has been sponsored by grant P10-FQM-6154 from the Conserjeria de Economia, Innovacion, Ciencia y Empleo (Junta de Andalucia).

References:

[1] A. Orte, N.R. Birkett, R.W. Clarke, G.L. Devlin, C.M. Dobson, D. Klenerman, “Direct characterization of amyloidogenic oligomers by single-molecule fluorescence”, Proc. Natl. Acad. Sci. U.S.A., 105, 14424-14429, 2008.
[2] P. Narayan, A. Orte, R.W. Clarke, B. Bolognesi, S. Hook, K.A. Ganzinger, S. Meehan, M.R. Wilson, C.M. Dobson, D. Klenerman, “The extracellular chaperone clusterin sequesters oligomeric forms of the amyloid-β_{1-40} peptide”, Nature Struct. Mol. Biol., 19, 79-83, 2012.

P3-8

Comparative mutational studies reveal conservation of site-specific amino acid preferences over billions of years

Fadia Manssour-Triedo[1], Valeria A. Risso[1], Alvaro Inglés-Prieto[1], Raquel Godoy-Ruiz[1], Jose A. Gavira[2], Beatriz Ibarra-Molero[1] and Jose M. Sanchez-Ruiz[1].

[1]Departamento de Química Física, Facultad de Ciencias, Universidad de Granada, 18071- Granada, Spain. *manssour@ugr.es*
[1]Departamento de Química Física, Facultad de Ciencias, Universidad de Granada, 18071- Granada, Spain
[2]Laboratorio de Estudios Cristalográficos, Instituto Andaluz de Ciencias de la Tierra (Consejo Superior de Investigaciones Científicas- Universidad de Granada), Avenida de las Palmeras 4, 18100- Armilla, Granada, Spain.

It has recently been debated whether site-specific amino acid preferences remain approximately constant during evolution or whether, due to coevolution of sites, they change strongly. To address this issue from an experimental point of view, we have carried out an extensive mutational analysis of proteins encoded by reconstructed ancestral sequences corresponding to Precambrian nodes in the evolution of thioredoxins [1, 2] and they were compared with the corresponding variants in one of its modern descendant (*E. coli* thioredoxin). In particular, a total of 25 variants were obtained involving exchanges between highly similar amino acids (E/D, I/V) [3, 4] and their thermal stability was determined by Differential Scaning Calorimetry experiments.
Our results suggest that generally, site-specific amino acid preferences appear to remain conserved throughout evolutionary history despite local sequence divergence. Furthermore, we provide experimental evidence that in some cases this conservation may involve a structural switch mechanism implying a kind of structural memory effect in proteins. This result may have potential implications in understanding molecular evolution.

Acknowledgements: This work has been supported by BIO2012-34937 and CSD2009-00088 from the Spanish Ministery of Economy and Competitiveness. F. M-T was supported by a research contract by Spanish Ministery of Economy and Competitiveness.

References:

[1]Ingles-Prieto A, Ibarra-Molero B , Delgado-Delgado A, Perez-Jimenez R, Fernandez JM, Gaucher EA, Sanchez-Ruiz JM, Gavira JA. *"Conservation of protein structure over four billion years"*. Structure 21:1690-1697, 2013.
[2]Perez-Jimenez R, Ingles-Prieto A, Zhao AM, Sanchez-Romero I, Alegre-Cebollada J, Kosuri P, Garcia.Manyes S, Kappock TJ, Tanokura M, Holmgren A, Sanchez-Ruiz JM *"Single-molecule pleoenzymology probes the chemistry of resurrected enzymes"*. Nat Struct Mol Biol 18:592-596, 2011.
[3]Godoy-Ruiz R, Perez-Jimenez R, Ibarra-Molero B and Sanchez-Ruiz JM. "*Relation between protein stability, evolution and structure, as probed by carboxylic acid mutations"*. *J Mol Biol* 336:313-318, 2004.
[4]Godoy-Ruiz R, Perez-Jimenez R, Ibarra-Molero B and Sanchez-Ruiz JM. *"A stability pattern of protein hydrophobic mutations that reflects evolutionary structural optimitzation"*. Biophys J 89:3320-3331, 2005.

P3-9

Unfolding from the N- or C- termini? Copper tells the story

Amy E.M. Beedle[1], Ainhoa Lezamiz[2], Guillaume Stirnemann[3] and *Sergi Garcia-Manyes*[1,2*]

[1]Department of Physics and [2]Randall Division of Cell and Molecular Biophysics, King's College London, WC2R 2LS, London, UK.
[3] CNRS Laboratoire de Biochimie Théorique, Institut de Biologie Physico-Chimie, Paris, 75005, France

Understanding the directionality and sequence of protein unfolding is crucial to elucidate the underlying folding free energy landscape. An extra layer of complexity is added in metalloproteins, whereby a metal cofactor participates in the correct and functional fold of the protein. However, the precise mechanisms by which such organometallic interactions are dynamically broken and reformed upon (un)folding remain largely unknown. Here we use single molecule force spectroscopy AFM combined with protein engineering techniques and MD simulations to study the individual unfolding pathways of the model blue copper proteins azurin and plastocyanin. By using the nanomechanical properties of the native copper centre as a structurally-embedded molecular reporter, we demonstrate that both proteins unfold via two independent, competing pathways. Our results provide experimental evidence of a novel kinetic partitioning scenario whereby the protein can stochastically unfold through two distinct main unfolding transition states placed at the N- and C- termini that precisely dictate the unfolding sense along the termini direction.

P3-10

SINGLE SUBSTITUTIONS IN THE β2-α2 LOOP REGION SCULPTURE THE PrP AMYLOID STATE

Javier A. Martínez[1], Rosa M. Sánchez[1], Milagros Castellanos[2], Natalia Makarava[3], Ilia V. Baskakov[3], Adriano Aguzzi[4] and María Gasset[1]

[1]Insto Química-Física "Rocasolano", Consejo Superior de Investigaciones Científicas, Madrid, Spain. jmartinez@iqfr.csic.es
[2]Centro Nacional de Biotecnología, Consejo Superior de Investigaciones Científicas, Madrid, Spain; IMDEA-Nanociencia, Madrid, Spain
[3]Center for Biomedical Engineering and Technology; University of Maryland School of Medicine; Baltimore, MD USA.
[4]Institute of Neuropathology, University Hospital of Zurich, Zurich, Switzerland.

The structure and sequence of PrP β2-α2 loop modulates conversion propensity and interspecies prion transmission. Here we have characterized the α-fold stability, fibril formation and amyloid state of rHaPrP(23-231) with NN (wt, rigid loop), SN (mouse-like flexible loop) and NT (elk-like hyper rigid loop) at 170 and 174 positions respectively. We found that these substitutions slightly impact the α-fold stability and fibrillation propensity, but largely modify the amyloid state. The formed fibrils differ notably in their spectroscopically features, shape, dimensions, ultrastructure and surface reactivity. These findings support the hypothesis that the regulatory effects ascribed to the β2-α2 loop region may arise from its effects on the disease-associated state rather than the cellular α-helical state.

Acknowledgements: This work has been sponsored by Ministerio de Economía y Competitividad (BFU2009-07971 and SAF2014-52661 to MG, BIO2011-28092 and CSD2009-00088 to MC), Fundación CIEN (MG), Raman Health (MG) and the National Institute of Health (grants NS045585 and NS074998 to IVB). JM was supported by a FPI-research contract and a FPI-short staying grant, MC by a Juan de la Cierva Postdoctoral contract.

P4-5

End-product diacylglycerol enhances activity of phosphatidylinositol phospholipase C through changes in membrane lipid domain structure.

Hasna Ahyayauch[1,2], Jesús Sot[1], M. Isabel Collado[3], Nerea Huarte[1], José Requejo-Isidro[1], Alicia Alonso[1], and Félix M. Goñi[1]

[1]Unidad de Biofísica (CSIC, UPV/EHU) and Departamento de Bioquímica, Universidad del País Vasco, P.O. Box 644, 48080 Bilbao, Spain.
[2]Institut Supérieur des Professions Infirmières et des Techniques de Santé, Av. Hassan II Km 4, Route de Casablanca, 1000 Rabat, Morocco.
[3]SGiker. Servicios Generales de Investigación UPV/EHU, Barrio Sarriena s/n. 48940
Leioa, Bizkaia, Spain.

DAG-induced activation of PI-PLC has been studied using as substrates vesicles containing PI, either pure or in mixtures with DMPC, DSPC, sphingomyelin, or galactosylceramide. At 22 ºC DAG at 33 mol% increases PI-PLC activity in all the mixtures, but not in pure PI bilayers. DAG also causes an overall decrease in DPH polarization (decreased molecular order) in all samples, and increased overall enzyme binding. Confocal fluorescence microscopy examination of GUV of all the compositions under study, with or without DAG, and quantitative evaluation of the phase behaviour using LAURDAN generalized polarization, and of enzyme binding to the various domains, indicate that DAG activates PI-PLC whenever it can generate fluid domains to which the enzyme can bind with high affinity. In the specific case of PI:DMPC bilayers at 22 ºC DAG induced increased enzyme binding and activation, but no microscopic domain separation was observed, the presence of DAG-generated nanodomains is proposed instead for this system. In PI:galactosylceramide mixtures DAG may exert its activation role through the generation of small vesicles, that PI-PLC is known to degrade at higher rates. In general our results indicate that global measurements using fluorescent probes in vesicle suspensions in cuvette are not enough to understand DAG effects that take place at the domain level. The above data reinforce the idea of DAG as an important physical agent regulating membrane or cell properties.

Acknowledgements: This work has been sponsored in part by grants BFU2012-36241 (F.M.G.) and BFU2011-28566 (A.A) from the Spanish Ministry of Economy, and grants IT849-13 (F.M.G.) and IT838-13 (A.A.) from the Basque Government

P4-6

EFFECT OF HYALURONAN PRE-TREATEMENT ON THE LATERAL STRUCTURE OF PULMONARY SURFACTANT INTERFACIAL FILMS

Raquel Arroyo[1], Mercedes Echaide[1], Elena Lopez-Rodriguez[1,2], Jesús Pérez-Gil[1]

[1]Department of Biochemistry and Molecular Biology, UCM, Madrid, Spain, raquel.arro@gmail.com
[2] Present address: Functional and Applied Anatomy, Hannover Medical School, Hannover, Germany

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. It has been described that inactivation of the surfactant complex may be produced by different agents such as serum, meconium or cholesterol, leaked into the airspaces associated with lung injury and inflammation, and that this surfactant inactivation contributes to respiratory failure.
Hyaluronan (HA), a linear polysaccharide, has shown ability to reverse the effect of inhibitory substances towards pulmonary surfactant. Pulmonary surfactant pre-exposed to HA gains significant resistance to inactivation even after removal of the polymer [1]. HA affects surfactant membranes, modifying their aggregation state and the number and size of phase-segregated lipid domains.
With the aim of understanding the structural changes and mechanisms involved in HA-promoted higher resistance of surfactant layers to inactivation, we have analyzed the effect of HA on the structure of interfacial surfactant films transferred onto solid supports, by epifluorescence and atomic force microscopy (AFM). The experiments have been carried out in a Langmuir-Wilhelmy Balance, where films made of native porcine pulmonary surfactant (NS) or of its reconstituted organic extract (OE), which is the basis for clinical surfactants, have been compared, with and without pre-exposition to HA. To analyze the lateral structure of surface films by epifluorescence microscopy, Rhodamine-DOPE labeled surfactant films have been transferred upon increasing pressure. Interfacial films for AFM have been transferred onto mica surfaces at a constant pressure. The effect of HA on the packing state of lipids has been assessed in parallel by analyzing the fluorescence of surfactant doped with LAURDAN.
These experiments reveal differences and changes in lateral organization as a consequence of exposure to HA, which correlate with the changes previously described for bilayers in GUVs made of pulmonary surfactant.

References:

[1] E. Lopez-Rodriguez, A. Cruz, R.P. Richter, H.W. Taeusch, J. Pérez-Gil, "Transient Exposure of Pulmonary Surfactant to Hyaluronan Promotes Structural and Compositional Transformations into a Higly Activate State", Journal of Biological Chemistry, 288, 41, 29872-29881, 2013.

P4-7

Interactions of Cationic Peptides Derived from *Galleria mellonella* Decropin D-like with Membrane Models and Antimicrobial Activity

José Oñate[1], Marcela Manrique-Moreno[1], Steven Trier[2], Chad Lady[2], Rodrigo Torres[3], Edwin Patiño*[1]

[1] Universidad de Antioquia, Grupo de Bioquímica Estructural de Macromoléculas, Medellín, Colombia
[2] Universidad de los Andes, Grupo de Biofísica, Bogotá, Colombia.
[3]Universidad Industrial de Santander, Grupo de Investigación en Bioquímica y Microbiología, Bucaramanga, Colombia.
e-mail: edwin.patino@udea.edu.co

Antimicrobial peptides (AMPs) are essential components of the innate immunity of organisms. Isolated from animals, plants, fungi, and bacteria, AMPs are considered promising alternatives to conventional antibiotics [1]. In this study, the neutral antimicrobial peptide Cecropin D-like *Galleria mellonella*, [2] (WT) was used as framework to study the effect of increasing charge on antimicrobial and cytotoxic activity of two novel peptides. Based on the fact that charge is closely associated with the antimicrobial activity in peptides, and it also plays an important role in the hemolytic activity, two peptides known as M1 and M2 were designed with charges of +5 and +9, respectively. The antimicrobial experiments show that these modifications reach a MIC ranged from 2 to 6 μM in Gram-negative bacteria in contrast to WT peptide, which does not have antimicrobial activity. Bacteria viability assays were also performed by measuring the ATPase activity (BacTiter-Glo kit), showing that an increase of positive charge in the sequence, decreases the viability below 39% in Gram-negative bacteria treated with the modified peptides. Cytotoxic experiments in human red blood cells showed that M1 had the highest hemolytic activity, reaching a hemolyitic concentration (HC_{50}) of 27 μM, whereas M2 peptide had the highest therapeutic index (37.6) in *E. coli* BL21.
In order to further investigate the peptide mechanism of action we measured the release of quenched Calcein from Small Unilamellar Vesicles (SUVs) composed of mixtures of palmitoyloleoyl phosphatidylglycerol (POPG), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoylphosphatidylethanolamine (POPE). The results showed that all the peptides permeabilize the model POPG membranes, whereas in POPC, POPG:POPC and POPG:POPE, only M1 and M2 induced a notable permeabilization. The interaction of peptides with phospholipids at the level of the glycerol backbone and hydrophobic domain was then studied through generalized polarization (GP) using Laurdan and fluorescence anisotropy using 1,6-diphenyl-1,3,5-hexatriene (DPH). The results suggest that peptides order the structure at the level of the glycerol backbone and on hydrophobic domain in DMPG, whereas in DMPC:DMPG SUVs, only M1 and M2 peptides increased the order of bilayers. Together, our data confirm a better interaction of cationic peptides with mixture lipids and the potential role of a rigidifying effect inside them for release of Calcein in permeabilized membranes.

REFERENCES

[1] C.D. Fjell, J.A. Hiss, R.E. Hancock, G. Schneider, Designing antimicrobial peptides: form follows function, Nat Rev Drug Discov, 11 (2012) 37-51.
[2] M. Cytryńska, P. Mak, A. Zdybicka-Barabas, P. Suder, T. Jakubowicz, Purification and characterization of eight peptides from Galleria mellonella immune hemolymph, Peptides, 28 (2007) 533-546.

P4-8

DYNAMIC BEHAVIOUR OF TACROLIMUS, AN IMMUNOSUPPRESSIVE DRUG, IN PULMONARY SURFACTANT FILMS

Alberto Hidalgo[1], Francesca Salis[2], Guillermo Orellana[2], Jesús Perez-Gil[1] and Antonio Cruz[1]

[1]Department of Biochemistry, Faculty of Biology, and [2]Department of Organic Chemistry, Faculty of Chesmitry, Complutense University, Madrid, SPAIN. *e-mail: albertohidalgo@ucm.es*

The respiratory surface is a relevant site for drug entry, not only intended for local but also for systemic treatments. Nevertheless, to consider lungs as a target for drug delivery, it is essential to take into account that 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).

PS is synthesised by type II pneumocytes and secreted in the form of multilamellar structures. It forms a film in charge of reducing the surface tension at the air-liquid interface to values below 2mN/m, to prevent pulmonary collapse during expiration and so minimizing the work during inspiration. It has also unique biophysical properties to adsorb very rapidly (in few seconds) into the air-liquid interface and, once there, to spread efficiently along it. Therefore, PS could offer novel opportunities to vehiculize different drugs efficiently, while hiding and protecting them from clearance in the lung. Nevertheless, drug impact on pulmonary surfactant needs to be considered in a case by case basis.

In the present work we have evaluated the dynamic behaviour of Tacrolimus, an immunosuppressive drug, in interfacial films (including DPPC as the simplest model of PS and the organic extract of PS, containing all the lipids plus the hydrophobic proteins SP-B and SP-C). We mainly looked for structural and functional changes associated with the impact of the drug on surfactant activity once the drug is distributed along the film and whether it is squeezed out from the interface during compression-expansion dynamic cycling. We observed that Tacrolimus affects the lateral structure of DPPC monolayers, inhibiting compression-driven domain formation associated with expanded-to-condensed lateral phase transitions. Interestingly, after five compression-expansion cycles, this effect is apparently reverted, suggesting that surfactant films can be progressively refined and depurated from the drug during interfacial dynamics.

The experiments were carried out using the Langmuir-Blodgett technique to prepare supported films to analyse the structure of drug-loaded films at different drug/lipid ratios under an epifluorescence microscope. In parallel, functional assays have been carried out in a captive bubble surfactometer that mimics breathing compression-expansion dynamics.

P4-9

STEROID MOLECULAR PROPERTIES INFLUENCE THE BIOPHYSICAL STATE OF STEROID-CONTAINING MEMBRANES

Jorge J. Wenz

Instituto de Investigaciones Bioquímicas de Bahía Blanca, CONICET-UNS. Bahía Blanca, Argentina.
E-mail: *jwenz@criba.edu.ar*

The effects of steroids on the biophysical state of membranes were investigated by analyzing the ordering, rigidifying, condensing and/or raft promoting activity on membranes of a library of 82 steroids. Based on the documented membrane activity, steroids were classified by means of a categorical variable [1] into three possible categories: disrupters, neutrals or promoters of such activity. All steroids were subjected to geometry optimization using the semi-empirical procedure AM1 and 245 molecular descriptors were next computed [2] on the low energy conformations. After the removal of the invariant descriptors, the remaining 93 were correlated with membrane activity through principal component analysis (PCA) [3] and mean contrasting.

Employing three principal components (71 % of explained variance), the PCA score plot showed two well-defined clusters of steroids reflecting similarities in their molecular properties. After the identification and counting of cluster´s members (Table) it was found that disrupter steroids represent around 94 % of the population of cluster 1. On the other hand, the 100 % of the promoter and the 95 % of the neutral steroids were located in cluster 2.

Distribution of steroids in clusters according to their activity on membranes.

Category of membrane activity	% in cluster		% in category		
	cluster 1	cluster 2	cluster 1	cluster 2	
Disrupter steroids	93.8	13.6	62.5	37.5	100
Neutral steroids	6.2	28.8	5.0	95.0	100
Promoter steroids	0	57.6	0	100	100
	100	100			

Collectively, these findings indicate that steroids having similarities in some molecular properties have similar activity on membranes, and that some of steroid molecular properties influence the biophysical state of steroid-containing membranes. By means of a thorough analysis of the PCA scores and loading it was found that the area, log P, volume, mass, refractivity, number of rotatable bonds and polarizability are the most relevant properties in determining the effect of steroids on membranes. A mean contrasting test revealed significant differences ($p<0.001$) in all of these properties among the promoter and the disrupter populations, agreeing and reinforcing the preceding conclusions.

References:

[1] J.J. Wenz, *Predicting the effect of steroids on membrane biophysical properties based on the molecular structure*, Biochim.Biophys.Acta, 1818, 896-906, (2012).
[2] Dragon, Talete SRL, Milano Chemometrics and QSAR Reseach Group, Milano, Italy, (2007).
[3] The Unscrumbler, Camo Process As, Oslo, Norway. http://www.camo.no, (2007).

P4-10

Localization of idebenone and idebenol in membranes by using solid-state NMR

Victoria Gómez-Murcia, Ana M. de Godos, Monika Schneider, Senena Corbalán-García and Juan C. Gómez-Fernández

Departamento de Bioquímica y Biología Molecular A, Universidad de Murcia, IMIB-Arrixaca, Campus of International Excellence Mare Nostrum, Murcia, Spain

Idebenone is a synthetic analogue of coenzyme Q, which is used to treat a number of pathological conditions. We have studied its interaction of both oxidized and reduced idebenone with 1-palmitoyl-2-oleoyl-*sn*- glycero-3-phosphocholine (POPC) membranes by using ^{1}H NOESY MAS-NMR based on proton-proton cross-peaks between capsaicin and 1-palmitoyl-2-oleoyl-*sn*- glycero-3-phosphocholine resonances. The location profile of this molecule was determined in a fluid membrane concluding that the benzoquinol ring in the case of idebenol or the benzoquinone ring in the case of benzoquinol is located near the lipid/water interphase of POPC membranes, but the terminal hidroxyl group at the end of the hydrophobic chain is also located at the lipid-water interface close to the glycerol backbone. This implies that the lateral chain that ends in a hydroxyl group, is twisted and therefore this disposition in the membrane is different from that of ubiquinol or ubiquinone. This difference, derived from their different liphophilic/hydrophobic balance, may explain, at least in part, the different properties of idebenone with respect to coenzyme Q and why it cannot be considered simply as an analog of ubiquinone.

P4-11

COORDINATING CURVATURE SCAFFOLDING AND MEMBRANE INSERTION DURING DYNAMIN-MEDIATED MEMBRANE FISSION

Anna V. Shnyrova[1], Eva Rodriguez Hortelano[1], Juha-Pekka Mattila[2], Sandra L. Schmid[2], Vadim A. Frolov[1,3]

[1]Biophysics Unit (CSIC, UPV/EHU) and Department of Biochemistry and Molecular Biology, University of the Basque Country, Leioa, Spain. *anna.shnyrova@ehu.eus*
[2]Department of Cell Biology, University of Texas Southwestern Medical Center, Dallas, TX, USA.
[3]IKERBASQUE, Basque Foundation for Science, Bilbao, Spain.

Dynamin-1 is a large GTPase that mediates membrane fission during endocytosis. Membrane scission can be reconstituted in a minimal system containing only purified dynamin-1 and a lipid membrane. Dynamin driven membrane fission requires polymerization of dynamin into helices and/or rings imposing high membrane curvature on a nano-cylindrical membrane template. The efficiency of fission is further controlled by the shallow membrane insertion of a hydrophobic loop in the pleckstrin homology (PH) domain of dynamin. A dynamin polymer forms a protein scaffold that is likely to stabilize the constricted membrane geometry, while membrane wedging by the PH domain perturbs and destabilizes the lipid bilayer. We report that a delicate balance between these two on-membrane activities of dynamin is required for well-timed and efficient fission. By using a mutant with altered self-assembly and insertion properties we detected that conformational stabilization of local membrane-perturbing activity of dynamin (high membrane insertion state) is sufficient to produce membrane hemifission without GTP. The hemifission configuration depends on the extent of self-assembly of the mutant: long polymers as well as small oligomers are ineffective in fission, but still produce high membrane curvature; structures comparable in size to a dynamin ring (~10 units) are the effective hemifission makers. Thus, restricted polymerization is required to localize and further coordinate membrane wedging by dynamin, resulting in membrane instabilities and fission.

P4-12

NEW "FAST AND EASY" METHOD OF MAKING GIANT UNILAMELLAR VESICLES FOR STUDYING MEMBRANE PROCESSES UNDER PHYSIOLOGICAL CONDITIONS

Ariana Velasco del Olmo[1], Vadim Frolov[1,2] and Anna Shnyrova[1]

[1]Biophysics Unit (CSIC, UPV/EHU) and Department of Biochemistry and Molecular Biology, University of the Basque Country, Leioa, Spain. [2]IKERBASQUE, Basque Foundation for Science, Bilbao, Spain.

Giant Unilamellar Vesicles (GUVs) are micron size hollow spheres that can be formed by lipids or polymers. Such membrane templates have been used for decades to study membrane organization and proteolipid interactions by direct visualization of membrane rearrangement events. Despite its usefulness, such templates have severe limitations, both in preparation (difficult and time consuming protocols, few methods are compatible with protein membrane reconstitution) and in the final product (lipid composition limited, hard to obtain GUVs at physiological ionic strength, etc.). Recently, we have developed a new method of the GUV production based upon spontaneous swelling of lipid films deposited on silica microbeads. We demonstrate fast and reproducible GUV formation from virtually any lipid composition and in physiological ionic buffers. The GUVs are easily accessible for mechanical manipulations and for reconstitution of protein activity.

P4-13

Intrinsic oligomerization capacity and pore-formation in membrane -active peptides

Edel Cunill, Orlando L. Sánchez-Muñoz and Jesús Salgado

Institute if Molecular Science (ICMol), University of Valencia, Paterna (Valencia), Spain , email: edel.cunill@uv.es

The relationship between the pore-formation activity of cationic peptides and their capacity to oligomerize in membranes has not been established yet. We have addressed this through the empirical study of 4 peptides with sequences derived from the a5 fragment of Bax and melittin. First, the oligomeric state of the peptides was determined using electrophoresis in a detergent micellar environment and fluorescence spectroscopy in vesicle membranes. The wild-type Bax-a5 fragment behaves as a dimer, but turns into monomer by a change of a single residue. On the other hand, melittin appears to be monomeric, but sequence variants of this peptide are able to oligomerize. The activity of these peptides was assayed in single vesicles by confocal fluorescence microscopy using qualitative and quantitative methods. All assayed peptides were active, and showed only small differences in the vesicle leakage kinetics, the number of pores per vesicle or the size of pores. This results suggest that a high intrinsic ability of the peptides to oligomerize is not a necessary condition for pore activity. We will discuss the consequences of this conclusion for the mechanism of pore formation as well as for the requirements in the design of better active peptides.

Acknowledgements: This work has been sponsored by MINECO, BFU2013-41648-P

P5-5

Fungal Ribotoxins as tools for the study of ribosome biogenesis in yeast.

Miriam Olombrada [1], Álvaro Martínez del Pozo[1], Vikram G. Panse[2], José G. Gavilanes Franco[1] and Lucía García-Ortega[1].

[1]Departamento de Bioquímica y Biología Molecular I, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, 28040, Madrid, Spain; molombrada@gmail.com
[2] Institute of Biochemistry, ETH Zurich, HPM F12.2, Otto-Stern-Weg 3, CH-8093 Zurich, Switzerland.

Eukaryotic ribosome biogenesis is a complex process that involves the assembly, maturation and intracellular transport of ribosomal subunits [1]. Eukaryotic ribosomes are initially assembled in the nucleolus. Pre-60S and pre-40S particles travel across the nucleoplasm towards the nuclear pore complex, reaching the cytoplasm where they still undergo several steps of maturation. Ribotoxins are a family of toxic extracellular fungal RNases that exert a highly specific ribonucleolytic activity on the rRNA within the large ribosomal subunit (60S) [2]. They inhibit protein biosynthesis by cleaving just a single phosphodiester bond of the large 25S rRNA, at a universally conserved site (the sarcin-ricin loop or SRL) with important roles in ribosome function, recycling, and biogenesis [3]. The toxic effect of ribotoxins has been related to the inability of the cleaved ribosomes to interact in optimum conditions with the elongation factors needed for a correct and efficient protein biosynthesis. However, their employment in detecting ribosome biogenesis defects is an emerging and interesting approach. Within this idea, recent results suggest that the integrity of the SRL is also essential for binding of some essential trans-acting factors as well as for the assembly of the ribosome subunits [4]. The study of ribotoxins' action on ribosomal maturation is gaining interest with the aim of using these proteins as specific tools for the study of the onset of different human ribosomopathies. The work presented deals with the influence of ribotoxins on the ribosome assembly pathway, at the molecular level, using *Saccharomyces cerevisiae* as the model organism.

References:

[1] Panse, V.G and Johnson, A.W. (2010) Maturation of eukaryotic ribosomes: acquisition of functionality. *Trends Biochem*. Sci. 35(5), 260-266.

[2] Lacadena, J., Álvarez-García, E., Carreras-Sangrà, N., Herrero-Galán, E., Alegre-Cebollada, J., García-Ortega, L., Oñaderra, M., [Gavilanes, J.G. and Martínez-del-Pozo, A. (2007) Fungal ribotoxins: molecular dissection of a family of natural killers. *FEMS Microbiol Rev*. 31, 212-237.

[3] García-Ortega, L., Alvarez-García, E., Gavilanes, J.G., Martínez-del-Pozo, A. and Joseph, S. (2010) Cleavage of the sarcin-ricin loop of 23S rRNA differentially affects EF-G and EF-Tu binding. *Nucleic Acids Res*. 38, 4108-4119.

[4] Gartmann, M., Blau, M., Armache, J.P., Mielke, T., Topf, M. and Beckmann, R. (2010) Mechanism of eIF6-mediated inhibition of ribosomal subunit joining. *J Biol Chem*. 285, 14848-14851

P5-6

A SINGLE MUTATION RESCUES CANCER-ASSOCIATED POLYMORPHIC NQO1 BY TARGETING NATIVE STATE DYNAMICS.

Encarnación Medina Carmona[1], Rogelio Palomino Morales[2], Julian F. Fuchs[3], David J. Timson[4], Angel Luis Pey[1]

[1]Department of Physical Chemistry, University of Granada, Granada, Spain
[2]Department of Biochemistry and Molecular Biology I, University of Granada, Granada, Spain
[3]Center for Molecular Biosciences, Leopold-Franzens University, Innsbruck, Austria.
[4] School of Biological Sciences and Institute for Global Food Security, Queen´s University Belfast, Belfast, UK.

NAD(P)H:quinone oxidoreductase 1 (NQO1) is a FAD-dependent antioxidant and detoxifying enzyme involved in the activation of cancer pro-drugs and stabilization of p53 and p73 oncosuppresors. A common polymorphism in NQO1 (P187S) is associated with increased cancer risk and low response to chemotherapeutics. We have recently described that P187S displays very low activity, due to a low FAD binding affinity, and reduced kinetic stability *in vitro* [1]. Interestingly, the crystal structure of P187S is virtually identical to that of the wild-type protein, suggesting that the polymorphism is affecting protein dynamics rather than the overall conformation [1,2].

Here, we have generated and characterized consensus mutations for NQO1 based on sequence alignment statistics, to determine whether they might suppress the functional and stability defects of P187S. The most remarkable mutation (H80R) increased the thermal stability of P187S to wild-type levels, and importantly, enhanced the affinity for FAD and the specific activity of P187S by many fold. Molecular dynamic simulations were used to ascertain the structural basis of the rescue of P187S by H80R, showing that the H80R overcomes dynamic alterations in the apo-state of NQO1 caused by P187S, reduces protein flexibility at the dimer interface and causes a structural switch of the Arg80 stabilizing the FAD binding site. Experiments in stably transfected HeLa cells show that H80R stabilizes the P187S polymorphism, increasing NQO1 levels by shielding the polymorphism towards proteasomal degradation. We propose that this simple approach may be useful to investigate the pathogenic mechanisms of other conformational diseases, and also to identify dynamic regions in proteins to be targeted for pharmacological intervention.

Acknowledgements: This work has been sponsored by grants from MINECO (CSD2009-00088 and BIO2012-34937) and Junta de Andalucia (CTS11-07187). A.L.P. is recipient of a Ramón y Cajal research contract from MINECO-University of Granada (RYC2009-04147). E.M.C. is supported by a predoctoral fellowship from Junta de Andalucía.

References:

[1] Angel L. Pey, Clare F. Megarity, David J. Timson, “FAD binding overcomes defects in activity and stability displayed by cancer-associated variants of human NQO1”, Biochim.Biophys.Acta (Mol.Bas.Dis). 1842,1463-1473, 2014.

[2] W.D. Lienhart, V. Gudipati, M.K. Uhl, A. Binter, S.A. Pulido, R. Saf, K. Zangger, K. Gruber, P.Macheroux. “Collapse of the native structure caused by a single amino acid exchange in human NAD(P)H:quinone oxidoreductase 1”, FEBS J., 281, 20, 4691-4704, 2014.

P5-7

REPLICATION DYNAMICS OF THE HUMAN MITOCHONDRIAL DNA POLYMERASE

Fernando Cerrón[1], Laurie S. Kaguni[2], Borja Ibarra[1].

[1] Instituto Madrileño de Estudios Avanzados, Nanoscience, Cantoblanco, 28049 Madrid, Spain.
[2]Departament of Biochemistry and Molecular Biology and Center for Mitochondrial Science and Medicine, Michigan State University, East Lansing, Michigan.

Mitochondrial DNA polymerase gamma (Pol γ) is the sole polymerase responsible for replication of the mitochondrial genome (mtDNA). It is well established that defect in mtDNA replication lead to mitochondrial dysfunction and disease. To date, approximately 150 disease mutations in Pol γ have been identified, which places Pol γ as a major locus for mitochondrial disease. To understand the molecular basis of these diseases, it is important to define the molecular mechanisms that govern the enzymatic activity of Pol γ. To this end, we are using optical tweezers to study the replicative kinetics of individual Pol γ molecules. We have described different experimental geometries to investigate the primer extension and strand displacement activity of the polymerase, and the effect of the mitochondrial Single Strand Binding (SSB) proteins on these activities.

P5-8

Characterization of a new family of Broad-spectrum racemases involved in production of noncanonical D-amino acids and cell-wall regulation

Noelia Bernardo-García[1], Cesar Carrasco-López[1], Akbar Espaillat[2,4], Natalia Pietrosemoli[3], Lisandro H. Otero[1], Laura Álvarez[2,4], Miguel A. de Pedro[4], Florencio Pazos[3], Brigid M. Davis[5], Mathew K. Waldor[5], Felipe Cava[2,4] and Juan A. Hermoso[1]

[1]Department of Crystallography and Structural Biology, Instituto de Química-Física 'Rocasolano'–CSIC, 28006 Madrid, Spain. Email: xnoelia@iqfr.csic.
[2]Department of Molecular Biology and Laboratory for Molecular Infection Medicine Sweden, Umeå Centre for Microbial Research, Umeå, University, Umeå, Sweden.
[3]Centro Nacional de Biotecnología–CSIC, 28049 Madrid, Spain.
[4]Centro de Biología Molecular 'Severo Ochoa', Universidad Autónoma de Madrid–Consejo Superior de Investigaciones Científicas (CSIC), 28049 Madrid, Spain,
[5]Division of Infectious Diseases, Brigham and Women's Hospital and Department of Microbiology and Immunobiology, Harvard Medical School and HHMI, Boston, MA 02115, USA, and

Broad-spectrum amino acid racemases (Bsrs) enable bacteria to generate noncanonical D-amino acids (NCDAA), whose roles in microbial physiology, including modulation of cell wall structure and dissolution of biofilms, are just beginning to be appreciated. We have recently described the structural and molecular features of the Bsr family [1]. We used crystallographic, mutational, biochemical and molecular simulation studies to define the molecular features of the racemases BsrV from *Vibrio cholera.* We identified conserved residues that distinguish BsrV and a newly defined family of broad-spectrum racemases from the classical alanine racemases, and found that these residues are key mediators of BsrV's multispecificity. This new family presents the enzymes located in the periplasm and not in the cytoplasm as the Ala-racemases. Other enzymes such as BsrAb from *Acinetobacter baumanii* and BsrKO from *Kingella oralis* are also able to accommodate more diverse substrates than related PLP-dependent alanine racemases. Surprisingly, NCDAA-modified cell wall peptides were found to exert a strong inhibitory effect on Bsrs activity. We propose that such modified muropeptides underlie a negative feedback loop that prevents excessive NCDAA production and controls the cell wall biosynthesis. Recent structural insights as well as a new model to explain implication of NCDAA in this process will be presented.

Reference:

[1] Espaillat, A., Carrasco-López, C., Bernardo-García, N., Pietrosemoli, N., Otero, L. H., Álvarez, L., de Pedro, M. A., Pazos, F., Davis, B.M., Waldor, M.K., Hermoso, J.A., and Cava, F. *"Structural basis for the broad specificity of a new family of amino-acid racemases"* ***Acta Crystallographica Section D Biological Crystallography***, 70, 79–90, 2014.

P5-9

EFFECT OF A MUTATION LINKED TO CHRONIC LYMPHOCYTIC LEUKEMIA ON THE SUBSTRATE SPECIFICITY OF THE EXPORT RECEPTOR CRM1

Igor Arregi[1], Marián Alonso-Mariño[1], Iraia García-Santisteban[2], Juan Jesús García-Vallejo[3], Yvette van Kooyk[3], María Ángeles Urbaneja[1], José Antonio Rodríguez[2], Sonia Bañuelos[1]

[1]Unidad de Biofísica (CSIC/UPV-EHU) and Department of Biochemistry and Molecular Biology, University of the Basque Country (UPV/EHU), Bilbao, Spain, sonia.banuelos@ehu.es
[2]Department of Genetics, Physical Anthropology and Animal Physiology, University of the Basque Country (UPV/EHU), Bilbao, Spain
[3]Department of Molecular Cell Biology and Immunology, VU University Medical Center, Amsterdam, The Netherlands

CRM1 is the main receptor mediating the nuclear export of proteins bearing a nuclear export signal (NES). A recurrent mutation in CRM1 (E571K) has been detected in chronic lymphocytic leukemia (CLL) patient samples [1], probably constituting a “driver”, oncogenic lesion. The affected residue, Glu571, locates within the NES binding groove of CRM1, and its mutation might alter the substrate recognition by CRM1. To gain insight into the molecular basis underlying CLL, we have explored the consequences of the E571K mutation on the conformational and functional properties of CRM1. Our results indicate that the E571K mutation does not significantly alter the structure neither the stability of CRM1. Furthermore, the mutant displays *in vitro* NES binding ability and export activity in cells similar to those of wild type CRM1. Nevertheless, inversion of electric charge in residue 571 might favor the binding to NES sequences relatively more electronegative. To test this hypothesis, we have compared the relative binding affinities of mutant and wild type CRM1 for NES sequences differing in the number and position of electric charges, either from natural CRM1 substrates or custom designed. We have found that the E571K mutation indeed enhances CRM1 affinity for negatively charged NESs, and by means of a quantitative cellular analysis, we show that the relative export efficiency of mutant vs. wild type CRM1 correlates with the electrostatic properties of the NESs. Altogether, our data suggest that either an excessive export of particular CRM1 substrates and/or deficient export of others, might alter the cellular homeostasis and contribute to CLL development.

References:

[1] Xose S. Puente et al. “Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia”, Nature 475, 101-105, 2011.

P5-10

Structural and Thermodynamic basis for cellulosome high-affinity protein-protein interaction

*Juliana Cheleski**, Naiara Torres, Helton J. Wiggers, Richard C. Garratt and Marcos V. A. S. Navarro

Physical Institute of Sao Carlos, University of Sao Paulo (IFSC-USP), Brazil
**cheleski@ursa.ifsc.usp.br*

One efficient strategy for degradation of plant cell wall was evolved for anaerobic bacteria and fungi, with the production of extracellular multi-enzyme complexes, known as cellulosome.[1] The structural organization of the cellulosome depends on the interaction pairs cohesin (Coh): dockerin (Doc). The conversion of cellulosic biomass into sugars by cellulosomes could result in the production of high-value products such as ethanol or organic acids from inexpensive renewable resources. The cellulosome of *Ruminococcus flavefaciens* is described as the most elaborate system identified so far.[2] The objective of this study was the structural and thermodynamic characterization of the Coh and Doc complex from *R. flavefaciens* scaffolding. The Coh from scaffolding B and Doc proteins were identified from EMBL/GenBank databases and cloned into pET-SUMO vector. High levels of expression were obtained using *E. coli*, and the proteins were purified by affinity (Talon® metal affinity resin) and size exclusion chromatography. After obtaining high purity proteins, crystallization and calorimetric assays were carried out. Two structure of Coh were determined by X-ray crystallography at 1.46 Å and 2.30 Å of resolution. The structure revealed a very flexible loop located at the putative Coh binding interface. In order to understand the impact of this loop on protein interaction, a series of structure-based site-directed mutants were constructed and their thermodynamic binding signatures were determined by Isothermal Titration Calorimetry (ITC) and Differential Scanning Calorimetry methods (DSC). ITC showed a binding stoichiometry of 1:1, enthalpy driven, with high affinity ($K_b > 10^9$ M^{-1}). Due to the tight binding affinity observed in the ITC experiments, DSC was used for accurate measurement of ΔG of interaction. Mutation at the position G94A, in the beginning of the loop, increase the binding affinity by two-fold. This is an opened conformation of the loop that has a bigger exposed surface area for solvent access and for protein pairwise binding, a competent conformation for interaction.

Acknowledgements: FAPESP (2010/19240-3)

References:

[1] Doi, R. H., and Kosugi, A. *Cellulosomes: plant-cell-wall-degrading enzyme complexes.* Nature Reviews Microbiology 2, 541-551, 2004.

[2] Rincon, M. T., Ding, S. Y., McCrae, S. I., Martin, J. C., Aurilia, V., Lamed, R., Shoham, Y., Bayer, E. A., and Flint, H. J. *Novel organization and divergent dockerin specificities in the cellulosome system of Ruminococcus flavefaciens.* Journal of Bacteriology 185, 703-713, 2013

P5-11

STRUCTURE AND BIOPHYSICAL CHARACTERIZATION OF a MATING PHEROMONE FROM THE FUNGUS *Fusarium oxysporum*

Soraya Serrano[1], Stefania Vitale[2], David Turrà[2], Álvaro Martinez-del-Pozo[3], Antonio Di Pietro[2] and Marta Bruix[1]

[1]Institute of Physical Chemistry Rocasolano, CSIC, Madrid Spain
[2]University of Cordoba, Cordoba, Spain
[3]Complutense University, Madrid, Spain

Sexual development in ascomycetous fungi is initiated by the perception of diffusible peptide pheromones *via* G protein-coupled plasma membrane receptors (GPCRs). Binding of pheromone to the cognate GPCR elicits a range of cellular responses including transcriptional reprogramming, cell cycle arrest, shmoo formation and chemotropic growth. High-resolution NMR studies of the *S. cerevisiae* α-pheromone tridecapeptide (WHWLQLKPGQPMY) in solution identified a transient type II beta-turn spanning residues 7-10, which are required for activation of the cognate receptor. Here we have characterized the α-pheromone of *Fusarium oxysporum*, a fungal pathogen of plants and humans. The chemically synthesized decapeptide WCTWRGQPCW was shown to elicit a chemotropic response in *F. oxysporum* germ tubes which is dependent on the cognate GPCR Ste2. Substitution of the conserved G_6 and Q_7 residues by alanines abolished the biological activity of the peptide. In order to understand the structural bases of its biological properties the wild type (wt) peptide and a scrambled sequence (WRWPCCWGQT) were analysed by biophysical methods including NMR spectroscopy. By HPLC, the two peptides behave as single molecular species, with no disulfide bridges. Far-UV CD was consistent with the presence of a b-turn in the wt pheromone. ^1H and ^{13}C NMR spectra were recorded and assigned in H_2O and H_2O/TFE (70/30 v/v) mixtures. ^{13}C chemical shifts confirm the oxidised state of the Cys residues in both peptides. In agreement with the CD data, the a-pheromone adopts a b-turn in H_2O involving the central $_4WRGQ_7$ sequence. By contrast, NMR data of the scrambled peptide do not provide evidence for a regular secondary structure in H_2O solution. In the presence of TFE, both peptides showed stabilised secondary structure. On the basis of the chemical shifts and assigned NOEs the 3D structures were calculated. The structure of the a-pheromone is a b-hairpin, containing the central $_4WRGQ_7$ b-turn, stabilised by strong W_4-W_{10} p/p interactions. In TFE, the scrambled sequence mainly adopts a b-turn conformation spanning the central $_4PCCW_7$ residues. Collectively, these data indicate that the hairpin adopted by the wt pheromone could be relevant for its biological activity and for the establishment of interactions with the cognate receptor or other biological partners.

Acknowledgements: Financial support from the Spanish MINECO (projects CTQ2011-22514, BIO2013- 47870-R and BFU2012-32404) is acknowledged.

P5-12

Allostery in the tau-Hsp70 complex

Javier Oroz[1,2], B. A. Nordhues[3], J. Biernat[4], E. Mandelkow[4], C. A. Dickey[3] and M. Zweckstetter[1,2]

[1]Department for NMR-Based Structural Biology, Max Planck Institute for Biophysical Chemistry, Göttingen, Germany and [2]German Center for Neurodegenerative Diseases (DZNE), Göttingen. jaor@nmr.mpibpc.mpg.de
[3]Department of Molecular Medicine, University of South Florida Health Byrd Alzheimer's Institute, University of South Florida; Tampa, Florida, USA.
[4]DZNE, Center of Advanced European Studies and Research (CAESAR), Bonn, Germany.

Under certain neuronal stress conditions, the disordered protein tau becomes defective and eventually aggregates leading to the well-characterized neuronal paired helical filaments[1]. This aggregation process is assumed to be critical in the pathological cascade of Alzheimer's diseases and several dementias, especially during the first steps of aggregation where tau can form soluble toxic oligomers[2]. ATP-driven molecular chaperones of the heat-shock protein (Hsp) family have been postulated to play a critical role inhibiting tau's gain-of-toxic function[3,4]. However, little is known about the binding and activation mechanism of neurotoxic proteins, and of disordered proteins in general, induced by Hsps[5-7]. Hsp70 undergoes dramatic allosteric changes upon repetitive cycles of ATP binding and hydrolysis[8-10] inducing conformational changes on the bound misfolded substrate towards its correct folding or promoting its proteasomal degradation[5]. Here, by means of NMR and SAXS we have characterised the regions of tau that bind to the different allosteric conformations of the human inducible Hsp72. We observe differential binding of different fragments of tau to labelled full-length Hsp72, which enable us to preliminarily determine which regions of the chaperone are involved in binding to tau. The detailed characterisation of the different tau-Hsp72 complexes will help to better understand the critical aggregation process of tau, central to Alzheimer's pathogenesis.

Acknowledgements: This work has been sponsored by a Marie Curie Postdoctoral fellowship (IEF 2013). Funding from ERC and German Research Foundation are acknowledged.

References:

[1] Barghorn S, Davies P, Mandelkow E. "Tau paired helical filaments from Alzheimer's disease brain and assembled in vitro are based on beta-structure in the core domain". Biochemistry, 43 (6), 1694-703, 2004.
[2] Ross CA, Poirier MA. "Opinion: What is the role of protein aggregation in neurodegeneration?". Nat Rev Mol Cell 6 (11), 891-8, 2005.
[3] Dou F, Netzer WJ, et al. "Chaperones increase association of tau protein with microtubules". Proc Natl Acad Sci USA 100 (2), 721-726, 2003.
[4] Luo W, Dou F, et al. "Roles of heat-shock protein 90 in maintaining and facilitating the neurodegenerative phenotype in tauopathies". Proc Natl Acad Sci USA 104 (22), 9511-6, 2007.
[5] Jinwal UK, Akoury E, et al. "Imbalance of Hsp70 family variants fosters tau accumulation". FASEB J 27 (4), 1450-9, 2013.
[6] Karagöz GE, Duarte AM, et al. "Hsp90-Tau complex reveals molecular basis for specificity in chaperone action". Cell 156 (5), 963-74, 2014.
[7] Saio T, Guan X, Rossi P, Economou A, Kalodimos CG. "Structural basis for protein antiaggregation activity of the trigger factor chaperone". Science 344 (6184), 2014.
[8] Bertelsen EB, Chang L, Gestwicki JE, Zuiderweg ER. *"Solution conformation of wild-type E. coli Hsp70 (DnaK) chaperone complexed with ADP and substrate"*. Proc Natl Acad Sci USA 106 (21), 8471-6, 2009.
[9] Zhuravleva A, Clerico EM, Gierasch LM. "An interdomain energetic tug-of-war creates the allosterically active state in Hsp70 molecular chaperones". Cell 151 (6), 1296-307, 2012.
[10] Qi R, Sarbeng EB, et al. "Allosteric opening of the polypeptide-binding site when an Hsp70 binds ATP". Nat Struct Mol Biol 20 (7), 900-7, 2013.

P5-13

Single-molecule characterization of the interaction between human Rad54 protein and double-stranded DNA

Kateryna Mykolayivna Lemishko[1], Humberto Sánchez[2] and Borja Ibarra[1]

[1] IMDEA-Nanoscience Institute, Madrid, Spain, *kateryna.lemishko@imdea.org*
[2]Department of Genetics, Erasmus MC University Medical Center, Rotterdam, Netherlands

Human Rad54 protein, a homolog of bacterial recombination protein RecA, is postulated to be essential for homologous recombination, a cellular process in which genetic information is exchanged between homologous or near homologous DNA molecules. Homologous recombination is crucial for complex DNA damage repair and collapsed replication forks recovery. It was shown that, in recombination, Rad54 cooperates with other Rad52 group proteins, such as Rad51 and Rad52 itself [1-3]. Although, the role of Rad54 in homologous recombination is not well understood, it seems that its binding properties and potent ATPase activity induce topological changes in DNA molecules [4], which can be relevant for the recombination process.
In this work, in order to develop a basis for better understanding of the role of Rad54 in homologous recombination, we aimed to characterize at the single-molecule level the interaction between human Rad54 protein and dsDNA in presence and absence of ATP, employing optical tweezers approach.

References:

[1] Paques, F., Haber, J.E., "Multiple pathways of recombination induced by double strand breaks in Saccharomyces cerevisiae", Microbiol. Mol. Biol.Rev., 63, 349-404, 1999.
[2] Sung, P., Trujillo, K.M., Van Komen, S. "*Recombination factors of Saccharomyces cerevisiae*", Mutat. Res., 451, 257-275,2000.
[3] Symington, L.S., "Role of RAD52 epistasis group genes in homologous recombination and double-strand break repair. ", Microbiol. Mol. Biol.Rev., 66, 630-670, 2002..
[4] Swagenmakers, S.M., Essers, J., de Wit, J., Hoeijmakers, J.H., Kanaar, R. "*The human RAD54 recombinational DNA repair protein is a double-stranded DNA- dependent ATPase* ", J. Biol. Chem, 273, 28292-28297, 1998.

P5-14

STUDIES OF INTERACTIONS BETWEEN DRUG AND MACROMOLECULE BY DIFFERENT SPECTROSCOPIC TECHNIQUES

Andrés Garzón[1], Iván Bravo[1], Pedro J. Pacheco[1], Carlos Alonso[2], and José Albaladejo[3]

[1]Departamento de Química Física, Facultad de Farmacia, Universidad de Castilla-La Mancha, Paseo de los estudiantes, s/n, 02071, Albacete, Spain. e-mail: andres.garzon@uclm.es
[2]Departamento de Química Orgánica, Inorgánica y Bioquímica, Facultad de Farmacia, Universidad de Castilla-La Mancha, Paseo de los estudiantes, s/n, 02071, Albacete, Spain
[3]Departamento de Química Física, Facultad de Ciencias Químicas, Universidad de Castilla-La Mancha, Avenida Camilo José Cela, 10, 13071, Ciudad Real, Spain

Studies with proteins. Currently, there is a great focus on phenolic antioxidants due to their radical scavenging activity and diverse medical applications [1]. The interactions between antioxidants as gentisic acid (GA) and bovine serum albumin (BSA) have been studied in our laboratory by Steady State Fluorescence Spectroscopy (SSFS) and Time Resolved Fluorescence Spectroscopy (TRFS) using the native fluorescence of the protein. Stern-Volmer (K_{SV}) and binding (Ka) constants, as well as the percentage of static quenching, were obtained from those studies. Thermodynamic parameters of the protein - ligand interaction (ΔH, ΔS y ΔG) were determined by varying the working temperature. The changes in the secondary structure of the protein were analyzed by UV-Vis absorption spectroscopy and FTIR.

Studies with DNA. Cisplatin is a well-known chemotherapy drug for cancer treatment which covalently binds to DNA inducing apoptosis [2]. The interactions between some no commercial cisplatin derivatives and DNA have been studied in our laboratory by means of SSFS and UV-Vis absorption spectroscopy. The Ka values determined for the set of studied compounds were compared to the values obtained for the commercially available cisplatin and related with their antitumor activities. Some guanidine compounds have also shown antitumor activity which can interact both DNA and certain target proteins [3]. The DNA affinity and interaction mechanism of a set of guanidines were analyzed by SSFS, TRFS y UV-Vis absorption spectroscopy.

Acknowledgements: The authors would like to thank the Consejería de Educación y Ciencia de la Junta de Comunidades de Castilla-La Mancha (Project PEII11-0279-8538) for supporting the research described in this article.

References:

[1] K. Ashidate, M. Kawamura, D. Mimura, et al., Eur. J. Pharmacology, 513, 173−179, 2005.
[2] M. Frezza, Q. Ping Dou, Y. Xiao, H. Samouei, et al. J. Med. Chem., 54, 6166–6176, 2011.
[3] K. Ohara, M. Smietana, A. Restouin, et al., J. Med. Chem., 50, 6465–6475, 2007.

P5-15

OPTIMIZATION OF PHENYILALANANINE HYDROXILASE STABILIZERS BY A TESTED 'ALCHEMICHAL' FREE-ENERGY APPROACH

Galano-Frutos, J.J.[1,2], Conde-Giménez, M.[1,2], Galiana, M.[2,3], Victor, B. L.[4], Brito, R. M.[4,5] and Sancho, J.[1,2]
juanjogf@gmail.com

[1]Departamento de Bioquímica y Biología Molecular y Celular, Facultad de Ciencias, Universidad de Zaragoza, Zaragoza, Spain.
[2]Instituto de Biocomputación y Física de Sistemas Complejos-BIFI (Unidad Asociada BIFI-IQFR-CSIC), Universidad de Zaragoza, Zaragoza, Spain.
[3]Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Zaragoza, Zaragoza, Spain.
[4]Departamento de Qumica, Faculdade de Ciências e Tecnologia da Universidade de Coimbra, Coimbra, Portugal.
[5]Centro de Neurociências de Coimbra, Universidade de Coimbra, Coimbra, Portugal.

Protein-ligand binding is a central event associated with both pharmaceutical activity and modulation of biological function. Designing tight-binding ligands is a key point in the challenging road to discover new small-molecule drugs. Free-energy approaches have become increasingly popular as tools to determine the binding affinity in lead optimization. This has been possible thanks to sustained improvement of force fields, sampling methods and increasing computational power. Alchemical free-energy calculation (AFEC), performs a series of unphysical 'alchemical' transformations between two structurally similar compounds[1]. Such a method allows estimating the relative difference in binding energy of two ligands of interest to a protein target with a significant reduction in computational effort. In this work we have implemented an accurate AFEC protocol for evaluating the relative binding free-energy of small ligands complexed with the enzyme phenylalanine hydroxilase (PAH). PAH is the key protein associated with Phenylketonuria (PKU)[2], an inborn, rare disease, whose mean incidence varies depending on the geographical region and the human population (the highest rate: Turkey, 1 in ~2600 births; lower rates: Japan, Finland, Philippines, more than 1 in ~100000 births; Spain, 1 in ~12 700 births)[3,4]. In previous works in our lab, two lead compounds that enhanced the thermal stability, activity and steady-state levels of PAH both *in vitro* and *in vivo* were identified[5]. The X-ray crystal structure of the complex formed by the more promising of the two was solved[6]. The presence of a coordinating Fe^{3+} ion in the ligand binding site of PAH has required rigorous parameterization. A 'bonded' and an 'unbonded' approach were followed, and in the end, the relative binding free energy results were compared with available experimental data. The one showing more accurate results was chosen in subsequent alchemical transformations from the identified candidate. The possibility of starting, in parallel, a synthesis project to obtain and test new derivatives of the selected compound, allowed us to acquire additional experimental affinity data, and therefore to continuously validate our AFEC implementation. Subsequently, the results of this work will allow us to design new promising candidates with improved binding affinity and consequently with a more potent activity.

Acknowledgements: This work has been sponsored by the European Union, specifically by the SUDOE Territorial Cooperation Programme (Interreg IV B) as part of the "Neuromed" project. Likewise, J. J. Galano is a recipient of a Ph.D. studies fellowship awarded by the Santander S.A. jointly with the University of Zaragoza.

References:

[1] A. de Ruiter and C. Oostenbrink, *"Free energy calculations of protein-ligand interactions"*, Curr. Opin. Che. Biol., 15(4), 547-52, **2011**.

[2] M. I. Flydal and A. Martínez, *"Phenylalanine hydroxylase: function, structure, and regulation",* IUBMB Life, 65(4), 341-9, **2013**.

[3] C. R. Scriver and S. Kaufman, *"Hyperphenylalaninemia: phenylalanine hydroxylase deficiency"*, In: C.R. Scriver, A. L. Beaudet, W. S. Sly, D. Valle, B. Childs, K. Kinzler, B. Vogelstein, editors, *"The Metabolic and Molecular Bases of Inherited Disease"*, 8, New York: McGraw-Hill, pp. 1667–724, **2001**.

[4] Web page of the Galician Phenylketonuric Association (ASFEGA), www.asfega.es/es/listado-enfermedades/fenilcetonuria-pku/

[5] R. Torreblanca, E. Lira-Navarrete, J. Sancho and R. Hurtado-Guerrero, *"Structural and mechanistic basis of the interaction between a pharmacological chaperone and human phenylalanine hydroxylase",* Chembiochem, 13(9), 1266-9, **2012.**

[6] A. L. Pey, M. Ying, N. Cremades, A. Velázquez-Campoy, T. Scherer, B. Thöny, J. Sancho and A. Martínez, "*Identification of pharmacological chaperones as potential therapeutic agents to treat phenylketonuria"*, J. Clin. Inv., 118(8), 2858-67, **2008**.

P5-16

BROWNIAN MOTION SIMULATIONS OF REACTION-DIFFUSION PROCESSES OF PROTEINS IN INTRACELLULAR MEDIA

Mireia Via[1], Pablo M. Blanco[1], Sergio Madurga[1], Josep Lluís Garcés[2], Eudald Vilaseca[1] and Francesc Mas[1]

[1]Department of Physical Chemistry and Research Institute of Theoretical and Computational Chemistry (IQTCUB), University of Barcelona (UB), Spain. mireiavia92@gmail.com
[2]Department of Chemistry and AGROTECNIO, University of Lleida (UdL), Spain.

The cellular cytosol is a very dense medium, with huge concentrations of biological macromolecules that, by means of non-specific interactions, have a considerable effect in processes like diffusion and reactivity. Therefore, theoretical models that describe these processes in homogeneous media are no longer valid in crowded conditions. Due to that, the mechanisms of the diffusion and reaction processes ought to be studied in these conditions in order to obtain more reliable and realistic results. In that way, new theoretical models that consider the presence of macromolecules can be proposed.

Computer simulations are a powerful tool to study reaction and diffusion processes. In this work, enzymatic reactions are simulated by means of the ReaDDy software package[1]. The stochastic movement of particles is generated by a Brownian motion algorithm, whereas the reactivity is studied with Monte Carlo simulations[2],[3]. The space-time evolution analysis of the system allows the calculation of the diffusion coefficient of the enzyme and the study of the Michaelis-Menten behaviour of its reaction in different kinetic and excluded volume conditions.

References:

[1] Johannes Schöneberg, Frank Noé, *"ReaDDy - A Software for Particle-Based Reaction-Diffusion Dynamics in Crowded Cellular Environments"*, PLoS ONE, 8(9), e74261, 2013.

[2] Eudald Vilaseca, Adriana Isvoran, Sergio Madurga, Isabel Pastor, Josep Lluís Garcés, Francesc Mas, *"New insights into diffusion in 3D crowded media by Monte Carlo simulations: effect of size, mobility and spatial distribution of obstacles"*, Physical Chemistry Chemical Physics, 13(16), 7396- 7407, 2011

[3] Laura Pitulice, Eudald Vilaseca, Isabel Pastor, Sergio Madurga, Josep Lluís Garcés, Adriana Isvoran, Francesc Mas, *"Monte Carlo simulations of enzymatic reactions in crowded media. Effect of the enzyme-obstacle relative size"*, Mathematical Biosciences, 251, 72-82, 2014

P5-17

CROWDING EFFECTS ON OLIGOMERIC ENZYMES: KINETIC ANALYSIS OF THE ALKP-CATALYZED HYDROLYSIS

Claudia Hernández[1,*], Cristina Balcells[1], Mireia Via[1], Isabel Pastor[2], Josep Lluís Garcés[3], Sergio Madurga[1], Marta Cascante[4] and Francesc Mas[1]

*[1] Department of Physical Chemistry and Research Institute of Theoretical and Computational Chemistry (IQTCUB), University of Barcelona, Spain; *chernandezcarro@gmail.com*
[2] Small Biosystems Lab, Department of Fundamental Physics, University of Barcelona, and CIBER-BBN, Carlos III Health Institute, Spain
[3] Department of Chemistry and AGROTECNIO, University of Lleida (UdL), Spain
[4] Department of Biochemistry and Molecular Biology and Institute of Biomedicine (IBUB), University of Barcelona, Spain

Studying enzymatic reactions in a medium that models the excluded volume inside the cell using synthetic polymers, provides us an insight on how metabolism is altered by the high concentrations of neighboring macromolecules surrounding any reaction. This issue is commonly referred as macromolecular crowding [1].

Kinetic behavior of Alkaline Phosphatase (ALKP) [2] and cooperative phenomena arising from it have been studied in dextran crowded media. A simple model to explain cooperativity, based on the Michaelis-Menten formalism, has been proposed. It allows us to discern how macromolecular crowding affects the cooperative behavior of the homo-dimer of ALKP. Results suggest that the effect of macromolecular crowding on this enzyme is both excluded volume and size-dependent, in accordance to what has been reported for other oligomeric enzymes such as Lactate Dehydrogenase (LDH) [3] or Malate Dehydrogenase (MDH) [4].

In particular, it has been found that v_{max} in crowded media is always lower than in dilute solution, regardless of the concentration (from 50 to 100 g/L) and size of the crowding agent (from 60 to 500 kDa). The maximum enzyme function decrease, and thus the maximum effect of excluded volume, is found for crowding agents of a size similar to the enzyme.

References:

[1] Zhou, H.X., Rivas, G., Minton, A.P., Annu. Rev. Biophys., 37, 375-397, 2008,; Kuznetsova, I. M., Turoverov, K.K., Uversky, V.N., Int. J. Mol. Sci., 15, 23090–23140, 2014; Pastor, I.; Pitulice, L. Balcells, C. Vilaseca, E. Madurga, S. Isvoran, A., Mas, F., Biophys. Chem., 185, 8–13, 2014.
[2] Balcells, C., Hernández, C., Via, M.; Pastor, I., Garcés, J.L., Madurga S., Cascante, M., Mas, F., in preparation 2015.
[3] Balcells, C., Pastor, I., Vilaseca, E., Madurga, S., Cascante, M., Mas, F., J. Phys. Chem. B., 118, 4062-4068, 2014.
[4] Poggi, C.G.; Slade, K.M., Biochemistry, 54, 260–267, 2014.

P5-18

Host Oriented Inhibitors of Late Domain Interactions as Broad-Spectrum Antivirals

Pedro Buzón[1], Francisco Castillo[1], Manuel Iglesias-Bexiga[1], Andrés Palencia[1], Bastien Cautain[2], Francisca Vicente[2], Javier Ruiz-Sanz[1], Jose C. Martínez[1], Ana Camara-Artigas[3] and Irene Luque[1].

[1]Dpto. Química-Física e Instituto de Biotecnología, Facultad de Ciencias, Universidad de Granada, 18071 Granada, Spain.
[2]Fundación MEDINA, Avda. del Conocimiento, 34,18016 Armilla, Granada, Spain.
[3]Dpto. Química-Física, Bioquímica y Química Inorgánica, Universidad de Almería, Carretera Sacramento, 04120 Almería, Spain.

Many enveloped viruses encode Late budding domains (L-domains) in their sequence. These L-domains usually contain highly conserved motifs known to mediate cellular protein-protein interactions such as PPxY and PTAP. These motifs are essential for the egress of the virions from the infected cell, which takes place through the recruitment of the ESCRT cellular machinery for sorting and scission. Because viral budding mechanisms are shared by many viruses, blocking Late domain interactions has been proposed as a promising strategy for the development of broad spectrum antivirals effective against viruses such as HIV, Ebola, Marburg, HTLV or Rabies. We present here a structural and thermodynamic characterization of L-domain binding to their cellular targets, the UEV domain of hTSG101 and the WW domains of hNEDD4, which has revealed important features of these interactions, both in terms of binding affinity and specificity, as well as cooperativity between domains. Using phage display techniques we have identified peptide sequences binding to these proteins with nanomolar dissociation constants that efficiently block viral egress in VLP systems. The thermodynamic and structural analysis of these complexes reveals additional interactions outside the canonical binding pocket, of interest for the further optimization of binding affinity and specificity. In collaboration with Fundación MEDINA, which possesses one of the largest collections of microbial extracts worldwide, we have set up cellular-based assays for the high-throughput screening of large libraries of extracts and pure compounds that, in combination with a detailed biophysical and structural analysis will allow for the identification and optimization of high affinity and specificity inhibitors of Late domain interactions as potential broad spectrum antivirals.

Supported by BIO2012-39922-C02 from the Spanish Ministry of Education and Science.

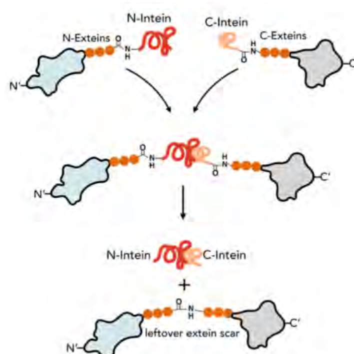
P5-19

BIOPHYSICAL CHARACTERIZATION OF THE ASSOCIATION BETWEEN In AND IC FROM *N. EQUITANS*

Verónica Gordo[1], Javier Murciano[2], Antoni Benito[1], Maria Vilanova[1], Jose C. Martínez[2] and Marc Ribó[1]

[1]Department of Biology, University of Girona, Girona, Spain. [2]Department of Chemical Physic and Biotecnology Institute, University of Granada, Granada, Spain

Inteins are protein insertion sequences that are embedded in-frame within precursor protein sequences and must be removed during the splicing process of the precursor proteins. Protein splicing is a post-translational event in which the intein is self-excised from a precursor protein and the flanking sequences, named exteins, are ligated through a new peptide bond [1]. We work with the split intein, NEQ DNA polymerase B, which hails from the hyperthermophile *Nanoarcheaum equitans*. Split inteins possess the splicing domain split in two parts, the N- and C-intein (NEQn and NEQc, respectively). Each one of them, together with its corresponding extein, is codified by different genes. This type of inteins catalyzes trans-splicing reaction where the first step is the association of the two parts (Fig. 1). We aimed the biophysical characterization of this process. We have observed a low kinetic association between NEQn and NEQc, especially at temperatures under 50°C, as expected for highly stable proteins. In addition, the thermodynamic studies revealed a strong interaction, with a Kd value in the nanomolar range. Finally, preliminary structure analysis suggested that NEQn presents more secondary and tertiary structure than other split inteins [2]. Moreover the interaction with its partner, NEQc, does not confer extra structural insights.



Representation of trans-splicing reaction

[1] Jeong Jin Choi, Ki Hoon Nam, Bokkee Min, Sang-Jin Kim, Dieter Soll and Suk-Tae Kwon. Protein Trans-splicing and Characterization of a Split Family B-type DNA Polymerase from the Hyperthermophilic Archaeal Parasite Nanoarchaeum equitans. J. Mol. Biol. (2006) 356, 1093–1106

[2] Neel H. Shah, Ertan Eryilmaz, David Cowburn, and Tom W. Muir (2014). Naturally Split Inteins Assemble through a "Capture and Collapse" Mechanism. J. Am. Chem. Soc. 2013, 135, 18673−18681

P6-4

The Kv1.3 carboxy terminal domain is involved in the KCNE4 interaction

Sara R. Roig [1], Laura Solé [1], Albert Vallejo-Gracia[1], Ramón Martínez-Mármol[1], Antonio Serrano-Albarrás[1], Anna Oliveras[1], Antonio Ferrer-Montiel[2], Gregorio Fernández-Ballester[2], Michael M. Tamkum [3], Antonio Felipe.[1]

[1]Molecular Physiology Laboratory, Departament de Bioquímica i Biologia Molecular, Institut de Biomedicina (IBUB), Universitat de Barcelona, Barcelona, Spain. *sararoig88@gmail.com.*
[2] Instituto de Biología Molecular y Celular, Universidad Miguel Hernández, Elche, Spain.
[3]Department of Biomedical Sciences and Department of Biochemistry and Molecular Biology, Colorado State University, Fort Collins, Colorado.

The voltage-dependent potassium channel Kv1.3 plays an important role in leukocytes. We demonstrated previously 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 involved in such association are unknown. Both proteins are present in leukocytes but the level of expression points to professional antigen presenting cells, such as macrophages and dendritic cells, as potential targets for this interaction. Despite of Kv1.3 and Kv1.5 can heteromerize in macrophages, and dendritic cells express Kv1.3 and Kv1.5 channels, only Kv1.3 elements are able to interact with KCNE4. Our results demonstrated that although the tertiary structure of Kv1.3 C-terminus is essential and sufficient for such interaction, this domain is apparently not involved in Kv1.3 gating modulation. We suggest that the intracellular phenotype of the Kv1.3/KCNE4 channelosome is caused by a fine balance between anterograde forward trafficking (FT) elements at the C-terminal domain of the channel and the existence of putative endoplasmic reticulum retention (ERR) motifs in KCNE4. KCNE4 association would introduce to the channelosome strong ERR elements impairing the anterograde FT mechanisms. These mechanisms would involve the COPII recognition of the YMVIEE FT signature of Kv1.3, which is crucial for the channel surface targeting. Based in our structure-function studies, the importance of the YMVIEE motif at the C-terminal domain of Kv1.3, the ERR signature of KCNE4 and the crystal structure of Kv1.2 and related KCNE isoforms; we have also proposed a docking model of the Kv1.3-KCNE4 complex. Our results pave the way for the delimitation of specific Kv1.3 molecular determinants which play a crucial role in the channelosome formation in leukocytes.

Supported by BFU2014-54928-R, BFU2011-23268 and CSD2008-00005 (MINECO, Spain)

P6-5

CHARACTERISTIC TRANSPORT MECHANISMS OF A PROTEIN ION CHANNEL INVESTIGATED USING CURRENT FLUCTUATIONS ANALYSIS

María Queralt-Martín[1], Lidón M. López[2] and Antonio Alcaraz[2]

[1]*Laboratory of Molecular Biophysics, Department of Physics, Universitat Jaume I, Castellón, Spain, mqueralt@uji.es*
[2]Laboratory of Molecular Biophysics, Department of Physics, Universitat Jaume I, Castellón, Spain

Current fluctuation analysis has been used over the years to determine successfully the physical properties of different systems. Here, we perform single-channel time-resolved current experiments in a protein channel to evaluate the different transport mechanisms governing the channel function. Using different salts of monovalent and divalent cations in a wide range of concentration and applied potentials, we analyze current fluctuations paying attention to the voltage dependence of the additional white noise that appears in the low frequency range of the spectra. We demonstrate that the channel displays two characteristic transport regimes: at low salt concentrations (10 mM to 1 M) ion permeation is controlled by the protein fixed charges that induce accumulation / exclusion of ions to preserve local electroneutrality. At high salt concentrations (> 1 M) adsorption processes associated to the binding of cations to the channel charges regulate the transport properties.

Acknowledgements: This work has been sponsored by the Spanish Ministry of Economy and Competitiveness (MINECO Project FIS2013-40473-P), the Fundació Caixa Castelló-Bancaixa (Project no. P1-1B2012-03), and Universitat Jaume I (PhD fellowship FPI-UJI 2011)

P7-5

MODELIZATION OF BINDING OF SSB PROTEINS TO ssDNA

Javier Jarillo[1†], José A. Morin[2], Elena Beltrán-Heredia[1,3], Juan P. G. Villaluenga[4], Borja Ibarra[5] and Francisco J. Cao[1]

[1]*Departamento de Física Atómica, Molecular y Nuclear, Universidad Complutense de Madrid, 28040 Madrid, Spain*
[2]Max-Planck-Institut für Physik komplexer Systeme, Nöthnitzer Straße 38, 01187 Dresden, Germany.
[3]Departamento de Química Física I, Universidad Complutense de Madrid, 28040 Madrid, Spain [4]Departamento de Física Aplicada I, Universidad Complutense de Madrid - 28040 Madrid, Spain [5]Instituto Madrileño de Estudios Avanzados, Nanoscience, Cantoblanco, 28049 Madrid, Spain

[†]*jjarillo@ucm.es*

The human mitochondrial single-stranded DNA binding protein (mtSSB) is essential for the in vivo replication of the mitochondrial genome. It binds selectively and with high affinity to ssDNA, protecting the molecule and coordinates the functional interactions of the other components of the mtDNA replisome (mainly, DNA polymeraseG and the mitochondrial helicase). Characterization of the elastic/mechanical proteins of long SSB-DNA nucleoprotein filaments is essential to understand the functions of the SSB protein. Binding of these SSB proteins to the naked ssDNA might occur in different modes, which may depend on the buffer ionic conditions and protein concentration [1]. We have used optical tweezers to characterize the elastic/mechanical properties on individual SSB-DNA nucleoprotein filaments under several ionic and protein concentration conditions. Here we propose a model to explain the binding of the SSB to individual ssDNA molecules, which is based on the decomposition of the total complex in partial chains. We study the elastic properties of the model, and compare the result with the elastic properties of the real chain.

Acknowledgements: The authors thank L. S. Kaguni for providing the mtSSB used in the study. This work has been supported by grants FPU-13/02934 (to JJ), FPU13/02826 (to EBH), BFU2012- 31825 (to BI), and FIS2010-17440 and GR35/10-A-920GR35/10-A-911 (to FJC).

References:

[1] Timothy M. Lohman, and Marilyn E. Ferrary, *"Escherichia coli single-stranded DNA-binding protein: multiple DNA-binding modes and cooperativities"*, Annual review of biochemistry, **63**, 1, 527-570, 1994.

P7-6

FORMATION OF INTERSTRAND CROSS-LINK (ICLs) IN DNA BY NITROUS ACID

J. Ortega-Castro[1], N. Hernández-Haro[2] , J. Frau1, F.Muñoz[1], J. Donoso[1] and A. Grand[2]

[1]Departament de Química, Institut Universitari d'Investigació en Ciències de la Salut (IUNICS), Universitat de les Illes Balears, Palma de Mallorca 07122, Spain.
[2]INAC, SCIB, Laboratorie "Lésions des Acides Nucleiques", UMR CEA-UJF E3, CEA-Grenoble, 17 Rue. Des Martyrs, 38054 Grenoble cedex 9, France.

World Health Organization (WHO, OMS) through the International Agency for Research on Cancer (IARC) considers proven that air pollution causes cancer. WHO sorted the air pollution as carcinogenic agent (group 1) [1]. The exposure to air pollution in cities or industrial areas is associated with changes in expression of genes, and DNA damage and repair, inflammation and oxidative stress just like shortening the length of the telomere DNA [2]. The ICL formation process inside the double-helix structure of DNA is a complex process, which starts with the formation of one lesion in the DNA. Oxygen Reactive Substances (ROS), Nitrogen Reactive Substances (RNS) and Alquilants agent (RNOx) are typical in the air pollution outdoor. These substances are being able to change the nitrogen bases and/or oxidized abasic sites formation; both ICL precursors.
ICLs formed by nitrogen rich species [3] have been studied by molecular dynamics simulations in a double helix oligonucleotide model with no-damage and damaged DNAs for obtaining information from its dynamic properties and conformational stability. The molecular dynamics show two possible symmetrical arrangements of the cross-linked guanines: the 'head-to-head conformation and the 'side-by-side' conformation, as other experimental studies suggest [4].
We have used amber tools 12.0 to create all input files to run the molecular dynamics simulations in Gromacs software package. Molecular dynamics were performed using the amber03 field to describe all parameters of the dodecamer. The systems were neutralized and were completed with a tetrahedral box of TIP3P waters. A NVT and NPT equilibration process was carried out. After, we run 500 ns molecular dynamics, enough to obtain well equilibrated MD trajectories of each system.

Acknowledgements: This work has been sponsored by Govern de les Illes Balears (grant no. AAEE27/2014 and AAEE044/2012).

References:
[1] Agency for Research on Cancer, "IARC monographs on the evaluation of carcinogenic risks to humans. Outdoor air pollution." Lyon: International., 109, 2013.
[2] D. M. DeMartini, "Genotoxicity biomarkers associated with exposure to traffic and near-road atmospheres: a review", Mutagenesis, 28, 286-505, 2013.
[3] J.L. Caulfield, J.S. Wishnok, S.R. Tannenbaum. "Nitric oxide-induced interstrand cross-links in DNA." Chemical Research in Toxicology, 16, 571 – 574, 2003.
[4] N.B. Fredrik Edfeldt, E.A. Harwood, S.T. Sigurdsson, P.B. Hopkins, B.R. Reid "Solution structure of a nitrous acid induced DNA interstrand cross-link" Nucleic Acid Research, 32, 2785-2794, 2004.

P7-7

REACTION INITIATION OF INDIVIDUAL MOLECULAR MOTORS IN SINGLE-STREAM LAMINAR FLOW CELLS

Benjamin Gollnick[1], Carolina Carrasco[1], Alberto Marín[1], Neville S. Gilhooly[2], Mark S. Dillingham[2] and Fernando Moreno-Herrero[1]

[1]Department of Macromolecular Structures, Centro Nacional de Biotecnología, CSIC, c/ Darwin 3, Campus de Cantoblanco, 28049 Madrid, Spain, *bgollnick@cnb.csic.es*.
[2]DNA:protein Interactions Unit, School of Biochemistry, Medical Sciences Building, University of Bristol, University Walk, Bristol BS8 1TD, UK.

During the last decades, single-molecule studies have revolutionised the biological sciences by providing unprecedented insights that are inaccessible to ensemble methods. In particular, surface-coupled techniques like magnetic tweezers (MT) or total internal reflection fluorescence (TIRF) microscopy have contributed seminal results on the function of individual motor proteins interacting with nucleic acid substrates. When combining the mentioned *in-singulo* approaches with one of the most widespread microfluidic flow cell designs containing only one stream of buffer, the very slow fluid velocities close to the cell borders constitute a critical issue that directly affects the behaviour of the studied molecules – especially if a precise amount of biological fuel such as adenosine triphosphate (ATP) is required for consistent measurements.

Here, we provide a continuum model for the reaction initiation kinetics of individual copies of the bacterial helicase–nuclease complex AddAB, an ATP-dependent motor protein that moves along and unwinds double-stranded DNA. By means of simulations based on finite element analysis, we elaborate a theoretical description that reproduces the typically observed translocation characteristics of the protein when investigated with permanent-magnet-based MT [1]. Relying on a Michaelis-Menten-like dependence of the mean velocity of AddAB as a function of the overall amount of biological fuel, we are able to employ this molecular motor as a sensor for the *local* (initially much smaller) ATP concentration during all stages of an experiment – at less than three micrometres above the fluid chamber surface.

Our results have important implications for different types of measurements in laminar flow cells that depend on an exact amount of a certain reactant: when flushing the respective compound in a single stream and at a rather slow volume rate, not taking into account sub-saturating effects close to any cell border may considerably bias the experimental outcome. This statement does not only apply to processes launched by the presence of nucleoside thriphosphates, but also to studies of substrate condensation, triggered e.g. by the presence of potential anticancer drugs.

[1] Benjamin Gollnick, Carolina Carrasco, Francesca Zuttion, Neville S. Gilhooly, Mark S. Dillingham and Fernando Moreno-Herrero, *"Probing DNA Helicase Kinetics with Temperature-Controlled Magnetic Tweezers"*, Small, Volume 11, Number 11, pages 1273–1284, 2015.

P8-5

INTERACTIONS OF ALLERGENIC PROTEINS AND BIG HYDROPHOBIC IONS

Leonor Pérez-Fuentes[1], Carlos Drummond[2], Jordi Faraudo[3] and Delfi Bastos-González[1]

[1]Biocolloid and Fluid Physics Group, Department of Applied Physics, University of Granada, Spain, _lpfuentes@ugr.es_
[2]CNRS, Centre de Recherche Paul Pascal (CRPP), Pessac, France
[3]Institut de Ciència de Materials de Barcelona (ICMAB-CSIC), Campus de la UAB, Barcelona, Spain

Nowadays, food allergies are a serious problem that affects a great percentage of the population. One of the most common allergies, mostly for children, comes from milk proteins, as β-casein and β-lactoglobulin. For this reason, there is a growing interest on developing techniques to extract proteins from food and systems capable of detecting the proteins, as are biosensors. For making progress in these areas, is crucial to know the properties of the proteins in solution and their interactions with other surfaces. Taking this into account, the goal of this work has been to study the physical properties of β-casein, β-lactoglobulin and BSA, as a reference protein. For the experimental work, we have covered latex nanoparticles with the different proteins and we have characterized them by electrophoresis and stability measurements. In order to compare the hydrophobicity degree of these proteins, we have used two big hydrophobic ions, which strongly interact with the proteins. These ions are the tetraphenylborate anion (Ph_4B) and the tetraphenylarsonium cation (Ph_4As), which have a very similar size, chemical structure and the same net electrical charge, being the sign of their charge the only significant difference. However, it is well known that the anions cause stronger effects over nanosystems than the cations [1, 2] because of their different hydration capabilities, so the anion (Ph_4B) is more hydrophobic than the cation (Ph_4As) [3]. These monovalent ions present a high affinity to hydrophobic interfaces, and we have observed in previous studies that they are able to produce great charge inversions over colloidal systems at very small concentrations, specially the anion [4]. This effect is more pronounced when it deals with soft-matter systems, like proteins or polymers [5]. In our study, we have found that BSA and β-lactoglobulin, both globular proteins, have a similar behavior, whereas β-casein, which is a disordered protein presents a more hydrophobic character. This is reflected by the fact that the tetraphenyl ions feel more affinity toward this protein. In addition, we can conclude that the use of tetraphenyl ions is a very suitable method in order to determine the hydrophilic/hydrophobic character of colloidal systems.

Acknowledgements: This work has been sponsored by the projects CTS-6270 (Junta de Andalucía, Spain) and MAT2012-36270-C04-02 (Ministerio de Economía y Competitividad, Spain).

References:

[1] Werner Kunz, *"Specific ion effects in colloidal and biological systems"*, Curr. Opin. Colloid Interface. Sci, 15, 34-39, 2010.

[2] Teresa López-León, Manuel J. Santander-Ortega, Juan L. Ortega-Vinuesa, Delfi Bastos-González, *"Hofmeister Effects in Colloidal Systems: Influence of the Surface Nature"*, J. Phys. Chem. C., 112, 16060-16069, 2008.

[3] Rachel Schurhammer, Georges Wipff, "Are the Hydrophobic $AsPh_4^+$ and BPh_4^- Ions Equally Solvated? A Theoretical Investigation in Aqueous and Nonaqueous Solutions Using Different Charge Distributions", J. Phys. Chem. A., 104, 11159-11168, 2000.

[4] Carles Calero, Jordi Faraudo, Delfi Bastos-González, "Interaction of Monovalent Ions with Hydrophobic and Hydrophilic Colloids: Charge Inversion and Ionic Specificity", J. Am. Chem. Soc., 133, 15025-15035, 2011.

[5] Leonor Pérez-Fuentes, Carlos Drummond, Jordi Faraudo, Delfi Bastos-González, "Anions make the difference: insights from the interaction of big cations and anions with poly(N-isopropylacrylamide) chains and microgels", (submitted).

P8-6

CELLULAR UPTAKE MECHANIMS OF LIPID NANOCAPSULES

P. Sánchez-Moreno[1], A. Salvati[2], H. Boulaiz[3], J.A. Marchal[3], J.L. Ortega-Vinuesa[1] and J.M. Peula-García[1,4]

[1]*Biocolloid and Fluid Physics Group, Department of Applied Physics, University of Granada, 18071 Granada, Spain.*
[2]*Faculty of Mathematics and Natural Sciences, Pharmacokinetics, Toxicology and Targeting, Groningen Research Institute of Pharmacy. Antonius Deusinglaan 1, 9713 AV Groningen. The Netherlands.*
[3]*Human Anatomy and Embryology Department, Regenerative Biomedicine Institute (IBIMER), University of Granada, Campus de la Salud, 18071 Granada, Spain.*
[4]*Department of Applied Physics II, University of Málaga, 29071 Málaga, Spain.*

Structured nanocapsules are generally described as colloidal systems with a core-shell structure, where the core acts as a liquid reservoir for several molecules or drugs, and the shell as a protective membrane. Their useful properties include biocompatibility and biodegradability, low toxicity, controlled release of drugs and the ability to target specific tissues. In particular, lipid nanocapsules (LNCs), consisting of an oil-filled core with a surrounding polymer shell, have special use for encapsulating and delivering hydrophobic drugs. In order to optimize the efficacy of LNCs delivery to cells, firstly, it is necessary to make a physico-chemical characterization of these since properties such as particle size, surface charge, and composition play a key role in the cellular uptake of polymeric nanoparticles [1], and then, to elucidate the underlying interactions between cells and nanomaterials and the mechanisms by which those are internalized by cells, as this will likely determine their ultimate sub-cellular fate and localization.
In this way, two different LNC systems, in which the core was constituted by olive oil and the shell by lecithin and other different biocompatible molecules such as Pluronic® F68 or chitosan, were used to investigate the uptake mechanisms in a human lung adenocarcinoma epithelial cell line (A549) analyzing the uptake and export kinetics by flow cytometry and confocal microscopy experiments.
All the obtained results point to the conclusion that the uptake mechanisms of lipid nanocapsules is the fusion with the cell membrane and then the release of the dye inside the cell. The free hydrophobic dye, once inside the cell, stains the vesicular bodies. This hypothesis could also explain the rapid decay of the fluorescence in terms of export kinetics and the non-energy dependence of the uptake rate [2].

Acknowledgements: This work has been sponsored by projects MAT2013-43922-R (European FEDER support included, MICINN, Spain), PI10/02295 (Instituto de Salud Carlos III, Fondo de Investigación Sanitaria, FEDER funds) and P07-FQM2496, P10-CTS-6270 and P07-FQM3099 (Junta de Andalucía, Spain).

References:

[1] L.A. Lane, X. Qian, A.M. Smith, S. Nie, "Physical chemistry of nanomedicine: understanding the complex behaviors of nanoparticles in vivo", Annu. Rev. Phys. Chem., 66, 521-47, 2015.
[2] A. Salvatti, C. Aberg, T. dos Santos, J. Varela, P. Pinto, I. Lynch, K. Dawson, "Experimental and theoretical comparison of intracellular import of polymeric nanoparticles and small molecules: toward models of uptake kinetics", Nanomedicine: NBM, 7, 818–826, 2011.

P8-7

Influence of the hydrophobic moieties of Poly(amidoamine)s on the condensation process and on the morphology of polymer/plasmid DNA complexes

Azahara Rata-Aguilar[1], Julia Maldonado-Valderrama[1], Juan Luis Ortega-Vinuesa[1], Antonio Martín-Rodríguez[1], Ernst Wagner[2] and *Ana Belén Jódar-Reyes[1]*

[1] Biocolloid and Fluid Physics Group, Department of Applied Physics, University of Granada, Granada, Spain, ajodar@ugr.es
[2]Department Pharmazie, Ludwig-Maximilians-Universität, München, Germany

The condensed structure of DNA in living cells has motivated extensive experimental and theoretical studies. Over the last twenty years, the number of publications devoted to DNA condensation has grown exponentially, especially due to its importance for the development of safer alternatives to viral gene delivery. Cationic polymers spontaneously form interpolyelectrolyte complexes with DNA (polyplexes) mainly due to the strong electrostatic interaction between them. The main drawback of polymeric materials for the systematic investigation of structure-activity relationships is the polydispersity of their molecular weights which contributes to the heterogeneity of the complexes formed with DNA[1]. Polymeric structures "*à la carte*" can be obtained with the help of modern polymer chemistry, allowing the rational design of condensing agents with different functional domains. In addition, it is striking, the scarce attention paid to the colloidal properties and behavior of polyplexes in spite of the key role of size, stability, electrical and interfacial properties, or morphology, in the success of the system.

In this study we evaluated the influence of different hydrophobic chains in the polymer on the condensation process of a plasmid DNA, and on the morphology of the formed complexes. We also analyzed the interaction of the polymers with a cell membrane model by means of the pendant drop technique, and correlated the colloidal properties of the complexes and its morphology with the transfection *in vitro*.

Three poly(amidoamine)s with identical cationic building blocks, but bearing different hydrophobic tails[2] were used. Poly(amidoamine)s are versatile in structure and functionality, and their unique properties make them suitable for many biomedical applications[3]. Their condensation ability was demonstrated by gel retardation and DLS at different N/P ratios, obtaining complexes of approximately 100 nm in diameter above N/P=1 regardless of the polymer. The hydrophobic contribution to the condensation of DNA was investigated by screening the electrostatic interaction with sodium chloride. The size, polydispersity index and morphologies (by TEM) of the complexes at N/P=2 were analyzed. We concluded that the hydrophobic interactions play an important role in the DNA condensation process, modulating the compaction ability, the stabilization mechanisms and the morphology of the complexes formed. Besides, the long hydrophobic moieties can improve the interaction with the cell membranes, which can promote the uptake.

Acknowledgements: The authors wish to express their appreciation for the financial support granted by the following research projects: MAT2013-43922-R - European FEDER support included - (MICINN, Spain) and P10-CTS-6270 (Junta de Andalucía, Spain). Azahara Rata-Aguilar thanks the Government of Spain (MECD) for her FPU fellowship, and the "Programa de Fortalecimiento de I+D+I", University of Granada.

References:

[1] R. Duncan, "*The dawning era of polymer therapeutics*", Nat. Rev. Drug Discov., 2(5):347–360, 2003.
[2] D. Schaffert, C. Troiber, and E. Wagner, *"New sequence-defined polyaminoamides with tailored endosomolytic properties for plasmid DNA delivery*", Bioconjugate Chem., *23* (6), 1157–1165, 2012.
[3] P. Ferruti, M.A. Marchisio, and R. Duncan, *"Poly(amido-amine)s: Biomedical Applications"*, Macromol. Rapid Commun., 23(5-6):332–355, 2002.

P8-8

FLUORESCENCE LIFETIME DENDRIMERIC SENSORS BASED ON TRIS(PHENYLENEVINYLENE)BENZENE WITH POLYAMINE AND POLYAMIDOAMINE BRANCHES

Pedro J. Pacheco[1], Iván Bravo[1], Andrés Garzón[1], Julian Rodriguez López[2], Juan Tolosa[3], Joaquín C. García[3], Jesús Canales-Vázquez[4], and José Albaladejo[5]

[1]Departamento de Química Física, Facultad de Farmacia, Universidad de Castilla-La Mancha, C/ Cronista Fco. Ballesteros Gómez, 1, 02071, Albacete, Spain. e-mail: Pedro.Pacheco@uclm.es
[2]Departamento de Química Orgánica, Inorgánica y Bioquímica, Facultad de Ciencias Químicas, Universidad de Castilla-La Mancha, Avenida Camilo José Cela, 10, 13071, Ciudad Real, Spain
[3]Departamento de Química Orgánica, Inorgánica y Bioquímica, Facultad de Farmacia, Universidad de Castilla-La Mancha, C/ Cronista Fco. Ballesteros Gómez, 1, 02071, Albacete, Spain
[4]Instituto de Energías Renovables. Universidad de Castilla-La Mancha. Campus Universitario, 02071, Albacete, Spain
[5]Departamento de Química Física, Facultad de Ciencias Químicas, Universidad de Castilla-La Mancha, Avenida Camilo José Cela, 10, 13071, Ciudad Real, Spain

The intracellular environment can be altered by different diseases and the presence of distinct drugs. Abnormal pH values are associated with inappropriate cell function, growth and division, and are observed in some common disease types such as cancer and Alzheimer. In this sense, different kinds of fluorescent probes are been extensively investigated as indicators for intracellular pH [1]. Fluorescence lifetime sensors are especially promising for biological imaging techniques since lifetime does not depend on the fluorophore concentration, fluorescence intensity, excitation wavelength and duration of light exposure [2].

A set of hybrid dendrimers bearing a polyphenylenevinylene (PPV) core with flexible polyamine and polyamidoamine (PAMAM) branches [3] were investigated as candidates to fluorescence lifetime sensors in our laboratory. Linear dependence of the fluorescence lifetime was observed in different ranges of pH. The quenching of the fluorescence lifetime at different pHs was related to the protonation state of the amines groups of the side chains. Thus, the deprotonation of the amines seems to could induce quenching by photoinduced electron transfer reactions and aggregation processes. The self-assembly processes of these set of dendrimers were also analyzed by transmission electron microscopy (TEM), dynamic Light Scattering (DLS) spectroscopy and, UV-Vis absorption and emission spectroscopy. Currently, we are studding the interactions with DNA and their applications as transfection agents.

Acknowledgements: The authors would like to thank the Consejería de Educación y Ciencia de la Junta de Comunidades de Castilla-La Mancha (Projects: PEII11-0279-8538 and PEII2014-005-A), the University of Castilla-La Mancha (Project: GI20152964) for supporting the research described in this article.

References:

[1] J. Han and K. Burgess, Chem. Rev., 110, 2709–2728, 2010.
[2] M. Y. Berezin and S. Achilefu, Chem. Rev., 110, 2641–2684, 2010.
[3] Rodrigo, A. C.; Rivilla, I; Perez-Martínez, F. C. Monteagudo, S.; Ocaña,V.; Guerra, J.; García-Martínez, J. C.; Merino,S.; Sanchez-Verdu, P.; Cena, V.; Rodríguez-Lopez, J. Biomacromolecules **2011**, 12, 1205–1213

P8-9

In-vitro digestibility of bacteriocin AS-48

del Castillo-Santaella Teresa[1], Cebrián Rubén[2], Eva Valdivia[2], Manuel Martínez-Bueno[2], Gálvez-Ruiz MªJosé[1], Maqueda Abreu Mercedes[2], *Maldonado-Valderrama Julia[1]*.

[1] University of Granada, Department of Applied Physics, Campus de Fuentenueva, s/n., 18071, Granada, Spain. julia@ugr.es
[2] University of Granada, Department of Microbiology, Campus de Fuentenueva, s/n., 18071, Granada, Spain.

AS-48 is the archetype of the growing family of circular bacteriocins (ribosomal antibacterial proteins), which are exclusively produced by Gram-positive bacteria. AS- 48 shows a broad spectrum of antibacterial activity and a remarkable stability to pH and heat, which makes it an ideal candidate for application as food biopreservative. In order to a rational utilization of AS-48 in foods it is important to address their digestibility. In this work we present the profile of AS-48 following in-vitro digestion. Owing to the mixture with biosurfactantes and free fatty acids, and the transit through the gut, the substrate for digestion is an emulsion. Also, it has been discovered recently that emulsification alters the digestion profile of proteins. Accordingly, we have studied the digestion profile of AS-48 both in bulk and at the air-water interface. Studies at the air– water interface are experimentally simpler to perform than at the oil–water interface and provide useful generic information. The digestion profile obtained demonstrates that AS-48 is less digestible at the air-water interface. Hence, emulsification might protect the AS-48 digestion. This is an important result to an optimal biotechnological application of AS-48 in the food industry.

Acknowledgements: This work has been sponsored by CDTI (FEDER INNTERCONECTA: ITC- 20131081), RYC-2012-10556, MAT2012-36270-C04-02. COST-MPN-1106-Green Interfaces and COST-FA-1005-Infogest.

References:

Maldonado-Valderrama, J.; Terriza, J. A. H.; Torcello-Gomez, A.; Cabrerizo-Vilchez, M. A., "*In vitro digestion of interfacial protein structures*", Soft Matter, 9, 1043-1053, 2013.

Amelia Torcello-Gómez, Miguel Wulff-Pérez, María José Gálvez-Ruiz, Antonio Martín-Rodríguez, Miguel Cabrerizo-Vílchez, Julia Maldonado-Valderrama. "*Block copolymers at interfaces: Interactions with physiological media*". Adv. Colloids Interface Sci. 206, 414-427, 2014.

Author Index

Author Index
(Presenting authors in bold)

Abian, Olga	P3-5
Adell-Morunol, Maria	**O1-2, P1-2**
Aguado, Alejandra	S3-4
Aguzzi, Adriano	P3-10
Ahyayauch, Hasna	**P4-5**
Aicart, Emilio	S8-1
Aichinger, Michael	O2-3, P2-3
Albaladejo, José	P5-14, P8-8
Alcaraz, Antonio	P6-5
Alegre-Cebollada, Jorge	O2-1, P2-1
Alemany, A.	S7-1
Alemany, Regina	P2-4
Alfonso, Carlos	S3-3, O3-2, P3-2
Alm, Ida	O4-4, P4-4
Almendo-Vedia, Víctor	O2-2, P2-2
Alonso, Alicia	P4-5
Alonso, Carlos	P5-14
Alonso, María Teresa	S6-4
Alonso-Mariño, Marián	P5-9
Álvarez, Laura	P5-8
Alvira, Sara	S3-3
Amorín, Manuel	S4-2
Andujar-Sánchez, Montserrat	O5-1, P5-1
Angulo, Jesús	O1-1, P1-1
Aranda Serrano, Elena	**P1-5**
Arias-González, Ricardo	W1, O7-1, P7-1
Arranz, Rocío	O3-2, P3-2
Arregi, Igor	P5-9
Arroyo, Raquel	**P4-6**
Artola-Recolons, Cecilia	**A3**
Ávila, Jesús	S3-2
Ayuso, José M.	S3-2
Bacarizo, Julio	**O5-1, P5-1**
Balcells, Cristina	**O5-2, P5-2,** P5-17
Baldelli Bombelli, Francesca	O8-2, P8-2
Bañuelos, Sonia	**P5-9**
Barbosa, Silvia	S8-3
Barceló, Francisca	**P2-4**
Barrán Berdón, Ana L.	S8-1
Barrantes, Alejandro	S3-2
Barros, Francisco	S6-3
Baskakov, Ilia V.	P3-10
Bastos-González, Delfi	P8-5
Beedle, Amy E.M.	P3-9
Bell, N. A. W.	S7-2
Beltrán-Heredia, Elena	**O2-2, P2-2,** P7-5
Benítez, María J.	S3-2
Benito, Antoni	P5-19
Bernadó, Pau	W5, O3-3, P3-3
Bernardo-García, Noelia	**P5-8**

Besalduch, Joan	P2-4
Besra, Gurdyal S.	O2-3, P2-3
Biernat, J.	P5-12
Bisello, Giovanni	P3-6
Blanco, Francisco J.	**W5**
Blanco, Pablo M.	P5-16
Bonzón-Kulichenko, Elena	O2-1, P2-1
Boulaiz, H.	P8-6
Bravo, Iván	P5-14, P8-8
Bravo, Jerónimo	**S5-1**
Bredrup, Cecilie	O1-4, P1-4
Brennich, Martha	O1-2, P1-2
Brito, R. M.	P5-15
Bruix, Marta	**P5-11**
Buzón, Pedro	**P5-18**
Cabrera, Yovana	S3-4
Cabrerizo-Vílchez, Miguel Ángel	O8-1, P8-1
Cafiso, David S.	O4-1, P4-1
Calisto, Bárbara M.	O1-2, P1-2
Camara-Artigas, Ana	O5-1, P5-1, P5-18
Camero, Sergio	S3-2
Campelo, Fèlix	**S4-3**
Campos Prieto, Luis A.	**S3-3**
Camunas-Soler, J.	S7-1
Canales-Vázquez, Jesús	P8-8
Cao, Francisco J.	W1, O2-2, P2-2, P7-5
Capera, Jesusa	O6-1, P6-1
Carnero, Ruiz C.	P1-6
Carrasco, Carolina	P7-7
Carrasco-López, Cesar	P5-8
Carrascosa, José L.	W1
Casado, Santiago	O7-1, P7-1
Casares, Salvador	P3-7
Cascante, Marta	O5-2, P5-2, P5-17
Castellanos, Milagros	P3-10
Castello, Fabio	P3-7
Castillo, Francisco	W5, P5-18
Castón, José R	**S1-3**
Cautain, Bastien	P5-18
Cava, Felipe	P5-8
Cebrián, Rubén	P8-9
Cerrón, Fernando	**P5-7**
Cerundolo, Vincenzo	O2-3, P2-3
Chabrol, Eric	O1-1, P1-1
Cheleski, Juliana	O5-3, P5-3, **P5-10**
Chen, Serene W.	O3-2, P3-2
Claveria-Gimeno, Rafael	**P3-5**
Collado, M. Isabel	P4-5
Collado-González, María del Mar	**O1-3, P1-3**
Comes, Núria	O6-1 P6-1
Conde-Giménez, M	P5-15
Conejero-Lara, Francisco	P3-6
Connell, Sean	S5-2
Corbalán-García, Senena	P4-10

Cordeiro, Tiago N.	W5
Corral-Lugo, Andrés	O6-3, P6-3
Cosma, Maria Pia	W2
Cremades, Nunilo	**O3-2, P3-2**
Crosas, Eva	O1-4, P1-4
Crusca, Ederson	O5-3, P5-3
Cruz, Antonio	P4-8
Cruz-García, Andrés	S7-3
Cuadros, Raquel	S3-2
Cunill, Edel	**P4-13**
Daura, Xavier	**S5-4**
Davis, Brigid M.	P5-8
De Biasio, Alfredo	W5
de Godos, Ana M.	P4-10
de la Peña, Pilar	**S6-3**
De Lacey, Antonio L.	O4-2 P4-2
de Paúl, Ivan	P2-4
de Paz, José L.	O1-1 P1-1
de Pedro, Miguel A.	P5-8
del Castillo Santaella, Teresa	**O8-1, P8-1**, O8-4, P8-4, P8-9
Delgado, Sandra	W5
Dhimole, Neha	S5-2
Di Pietro, Antonio	P5-11
Díaz Baños, F Guillermo	O1-3 P1-3
Díaz Moreno, Irene	**A2**
Dickey, C. A.	P5-12
Diercks, Tammo	S5-2, W5
Dillingham, Mark S.	O7-2, P7-2, O7-4, P7-4, P7-7
Dobson, Christopher M.	O3-2, P3-2
Donoso, J.	P7-6
Drakulic, Srdja	O3-2, P3-2
Drummond, Carlos	P8-5
Echaide, Mercedes	P4-6
Egea, Gustavo	S2-2
Espaillat, Akbar	P5-8
Esteller, Manel	P3-5
Faraudo, Jordi	P8-5
Felipe, Antonio	**O6-1, P6-1,** P6-4
Fernández, Cristina	S1-1
Fernández, Victoria	**S2-1**
Fernández-Ballester, Gregorio	P6-4
Fernandez Espin, V	O1-3, P1-3
Fernández-Higuero, José Angel	S3-4
Ferrer-Montiel, Antonio	P6-4
Fidelio, Gerardo D.	**L4**
Fieschi, Franck	O1-1, P1-1
Fisher Gemma, L.	O7-4, P7-4
Fita, Ignacio	O1-2, P1-2
Frau, J.	P7-6
Freire, Ernesto	**L1**
Frolov, Vadim	**W3,** P4-11, P4-12
Fuchs, Julian F.	O3-1, P3-1, P5-6
Fucini, Paola	**S5-2**
Fuertes, Alberto	S4-2

Fumagalli, Laura	**L2**
Galano-Frutos, J.J.	**P5-15**
Galiana, M.	P5-15
Galisteo-González, F.	**P1-6**
Gálvez-Ruiz, MªJosé	O8-1, P8-1, P8-9
Garavís, Miguel	O7-1 P7-1
Garcés, Josep Lluís	O5-2, P5-2, P5-16, P5-17
García, Joaquín C.	P8-8
García-Alvarez, Begoña	P1-5
García de la Torre, J.	O1-3, P1-3
García-Linares, Sara	**O4-4, P4-4**
García-Lucas, Ángel	S3-2
Garcia-Manyes, Sergi	P1-7, **P3-9**
García Martín, María Luisa	**S2-4**
García-Ortega, Lucía	P5-5
García-Parajo, María	**S2-3,** W2, O2-3, P2-3, O6-2, P6-2
García-Sancho, Javier	**S6-4**
García-Santisteban, Iraia	P5-9
García-Vallejo, Juan Jesús	P5-9
Garratt, Richard C.	P5-10
Garzón, Andrés	**P5-14**, P8-8
Gasset, María	P3-10
Gavilanes Franco, José G.	O4-4, P4-4, P5-5
Gavira, Jose A.	P3-8
Gil Cartón, David	W5, P1-5
Gilhooly, Neville S.	P7-7
Giraldo, R	**S1-1**
Godoy-Ruiz, Raquel	P3-8
Gollnick, Benjamin	**P7-7**
Gómez-Fernández, Juan C.	**P4-10**
Gómez-Murcia, Victoria	P4-10
Gómez-Puertas, Paulino	O7-3, P7-3
Gomila, Gabriel	L2, **S4-4**
Gomila, Rosa M.	P2-4
González, Carlos	O7-1, P7-1
González de Prado-Salas, Pablo	L3
González-Freire, Eva	S4-2
Goñi, Félix M.	P4-5
Göpfrich, K.	S7-2
Gordo, Verónica	**P5-19**
Gov, Nir S.	S2-2
Grand, A.	P7-6
Granja, Juan R.	**S4-2**
Grueso, Elia	**O8-3, P8-3**
Gutiérrez, Irene	**O7-1, P7-1**
Gutierrez, Teresa	P2-4
Gutiérrez-Sanz, Oscar	O4-2 P4-2
Halder, Partho	O4-1, P4-1
Harris, Jon	O1-4, P1-4
Hemmig, E.	S7-2
Heredia, Antonio	S2-1
Herguedas, Beatriz	O5-4, P5-4
Hermoso, Juan A.	**A1**, A3, P5-8
Hernández, Claudia	O5-2, P5-2, **P5-17**

Hernández, Félix S3-2
Hernández-Ainsa, Silvia **S7-2**
Hernández Cifre, JG O1-3 P1-3
Hernández-Gómez, Yuriko S. P3-6
Hernández-Haro, N. P7-6
Herranz-Trillo, Fátima **O3-3, P3-3**
Herrero-Galán, Elías **O2-1, P2-1**
Hidalgo, Alberto **P4-8**
Hierro, Aitor **S1-4**
Huarte, Nerea P4-5
Huertas, Pablo **S7-3**
Ibáñez de Opakua, Alain W5
Ibarra, Borja **W1,** P5-7, P5-13, P7-5
Ibarra-Molero, Beatriz S3-3, P3-8
Iglesias-Bexiga, Manuel P5-18
Inglés-Prieto, Alvaro P3-8
Jahn, Reinhard **L5**, O4-1, P4-1
Jarillo, Javier **P7-5**
Jiménez, Juan Salvador **S3-2**
Jódar-Reyes, Ana Belén **P8-7**
Jorge, Olga de la Caridad P3-5
Junquera, Elena **S8-1**
Kaguni, Laurie S. P5-7
Kahveci, Zehra **O4-3, P4-3**
Kaminishi, Tatsuya S5-2
Kamma-Lorger, Christina S. **O1-4, P1-4**
Keyser, U. F. S7-2
Khayet, Mohamed S2-1
Knupp, Carlo O1-4, P1-4
Krell, Tino O6-3, P6-3
Kuliszewska, Edyta O8-3, P8-3
Lady, Chad P4-7
Lakadamyali, Melike **W2**
Lanuza, Pilar Maria P3-5
Lapeyre, Jr., Gerald J. O6-2, P6-2
Lázaro, José M. W1
Lewenstein, Maciej O6-2 P6-2
Lezamiz, Ainhoa P1-7, P3-9
López, Bernardo P2-4
López, Lidón M. P6-5
López-Montero, Iván **S2-2**
Lopez-Rodriguez, Elena P4-6
Lórenz-Fonfría, Victor **S4-1**
Lothar, Brecker O8-3, P8-3
Luque, Irene **S5-3,** W5, P5-18
Luque Caballero, Germán **O8-4, P8-4**
Macias, Maria J **S1-2**
Madariaga-Marcos, Julene **O7-2, P7-2**
Madurga, Sergio O5-2, P5-2, P5-16, P5-17
Maiolo, Daniele O8-2, P8-2
Makarava, Natalia P3-10
Maldonado-Valderrama, Julia **S8-4**, O8-4, P8-4, O8-1, P8-1, P8-7, **P8-9**
Malfois, Marc O1-4, P1-4
Malhotra, Vivek S4-3

Mallavia, Ricardo	O4-3, P4-3
Mandelkow, E.	P5-12
Manrique-Moreno, Marcela	P4-7
Manssour-Triedo, Fadia	**P3-8**
Manzo, Carlo	S2-3, W2, O2-3, P2-3, **O6-2, P6-2**
Maqueda Abreu, Mercedes	P8-9
Marchal, J.A.	P8-6
Marín, Alberto	P7-7
Marques, Marta C.	O4-2, P4-2
Martín Molina, Alberto	O8-4, P8-4
Martín-Rodríguez, Antonio	P8-7
Martinelli, Luca	O1-2, P1-2
Martínez, Javier A.	**P3-10**
Martínez, Jose C.	P5-18, P5-19
Martínez, Juan Carlos	O1-4, P1-4
Martínez del Pozo, Álvaro	O4-4 P4-4, P5-5, P5-11
Martínez-Bueno, Manuel	P8-9
Martínez-Mármol, Ramón	O6-1, P6-1, P6-4
Martínez-Tomé, Maria José	O4-3, P4-3
Mas, Francesc	O5-2, P5-2, P5-16, P5-17
Massignan, Pietro	O6-2, P6-2
Mateo, C. Reyes	O4-3, P4-3
Mattila, Juha-Pekka	P4-11
Maula, Terhi	O4-4, P4-4
Medina, Milagros	O5-4, P5-4
Medina Carmona, Encarnación	**O3-1, P3-1,** P5-6
Medina-O´ Donnell, M.	P1-6
Meek, Keith M.	O1-4, P1-4
Mejías-Navarro, Fernando	S7-3
Mell, Michael	S2-2
Mellado, Mario	**S6-2**
Mendieta Jesús	O7-3, P7-3
Mendieta-Moreno, Jesús I.	**O7-3, P7-3**
Merino, José L.	P2-4
Merino, Nekane	W5
Metrangolo, Pierangelo	O8-2, P8-2
Mira, Amalia	O4-3, P4-3
Misiunas, K.	S7-2
Mivelle, Mathieu	S2-3
Molina, Rafael	W5
Molina-Bolívar, J.A.	P1-6
Molina-García, Laura	S1-1
Monroy, Francisco	S2-2, O2-2, P2-2
Montalbán, MG	O1-3 P1-3
Montenegro, Javier	S4-2
Montoya, Guillermo	W5
Morel, Bertrand	P3-6, O6-3 P6-3
Moreno-del Álamo, María	S1-1
Moreno-Herrero, Fernando	O7-2, P7-2, O7-4, P7-4, P7-7
Morin, José A.	W1, P7-5
Moro, Fernando	**S3-4**
Mortuza, Gulnahar B.	W5
Muga, Arturo	S3-4
Muñoz, F.	P7-6

Muñoz, Victor	S3-3, O3-4, P3-4
Muñoz-García, Juan C.	O1-1, P1-1
Murciano, Javier	P5-19
Mykolayivna Lemishko, Kateryna	**P5-13**
Navarro, Marcos V.A.S.	O5-3, P5-3, P5-10
Navarro, Pedro	S3-2
Navas, Paloma	S6-4
Neira, Jose Luis	S5-1
Nieto, Pedro M.	**O1-1, P1-1**
Nordhues, B. A.	P5-12
Nyenhuis, Sarah	O4-1, P4-1
Ochoa-Lizarralde, Borja	S5-2
Oliveras, Anna	P6-4
Olombrada, Miriam	**P5-5**
Oñate, José	**P4-7**
Orellana, Guillermo	P4-8
Oroz, Javier	**P5-12**
Orte, Angel	**P3-7**
Ortega, Álvaro	**O6-3, P6-3**
Ortega, José	O7-3, P7-3
Ortega-Castro, J.	**P7-6**
Ortega-Vinuesa, Juan Luis	P8-6, P8-7
Ortiz-Salmerón, Emilia	O5-1, P5-1
Otero, Lisandro H.	P5-8
Ouberai, Myriam	O3-2, P3-2
Pacheco, Pedro J.	P5-14, **P8-8**
Palencia, Andrés	P5-18
Palomino Morales, Rogelio	O3-1, P3-1, P5-6
Panjkovich, Alejandro	S5-4
Panse, Vikram G.	P5-5
Pardo, Luis A.	S6-3
Parra, A.	P1-6
Pastor, Isabel	O5-2 P5-2, P5-17
Pastrana, Cesar L.	**O7-4, P7-4**
Patiño, Edwin	P4-7
Pazos, Florencio	P5-8
Perales-Calvo, Judit	**P1-7**
Pereira, Inés A. C.	O4-2, P4-2
Pérez-Fuentes, Leonor	**P8-5**
Perez-Gil, Jesús	P1-5, P4-6, P4-8
Pérez-Jiménez, Raúl	O3-4 ,P3-4
Pérez-Lara, Ángel	**O4-1, P4-1**
Pérez-Montaña, Albert	P2-4
Perez-Tejeda, Pilar	O8-3, P8-3
Pérez-Verdaguer, Mireia	O6-1, P6-1
Peula-García, J.M.	**P8-6**
Pey, Angel Luis	O3-1, P3-1, **P5-6**
Pietrosemoli, Natalia	P5-8
Pigliacelli, Claudia	O8-2, P8-2
Pinali, Christian	O1-4, P1-4
Plaza-Garrido, Marina	O5-1, P5-1
Portugal, José	P2-4
Prado-Gotor, Rafael	O8-3, P8-3
Priegue, Juan M.	S4-2

Pujadas, Lluís	O6-1, P6-1
Queralt-Martín, María	**P6-5**
Quesada Pérez, Manuel	O8-4, P8-4
Rata-Aguilar, Azahara	P8-7
Requejo-Isidro, José	P4-5
Resnati, Giuseppe	O8-2, P8-2
Ribó, Marc	P5-19
Ricci, Maria Aurelia	W2
Risso, Valeria A.	P3-8
Ritort, Felix	**S7-1**
Rivadeneira-Ruiz, Ceferino	O8-1, P8-1
Rivas, Germán	S3-3, O3-2, P3-2
Roca-Cusachs, Pere	**W4**
Rødahl, Eyvind	O1-4, P1-4
Rodríguez, José Antonio	P5-9
Rodríguez-Garcí, Ruddi	S2-2
Rodríguez Hortelano, Eva	W3, P4-11
Rodríguez López, Julian	P8-8
Rodríguez-Vázquez, Nuria	S4-2
Roig, Sara R.	**P6-4**
Rojo, Javier	O1-1, P1-1
Rojo, Jonathan	S6-4
Roldan, Emilio	O8-3, P8-3
Roldán, Nuria	P1-5
Romero, Antonio	S3-3
Rondon-Rodriguez, Deyanira	O8-1, P8-1
Ruedas-Rama, Maria J.	P3-7
Ruiz, Federico M.	S3-3
Ruiz-Sanz, Javier	P5-18
Ruzafa, David	**P3-6**
Sadqi, Mourad	S3-3
Salas, Margarita	W1
Salgado, Jesús	P4-13
Salgueiriño, Verónica	**S8-2**
Salio, Mariolina	O2-3, P2-3
Salis, Francesca	P4-8
Salvati, A.	P8-6
Sampol, Antonia	P2-4
Sánchez, Humberto	P5-13
Sánchez, Rosa M.	P3-10
Sánchez-González, Cristina	O2-1, P2-1
Sánchez Moreno, Paola	**O8-2, P8-2,** P8-6
Sánchez-Muñoz, Orlando L.	P4-13
Sánchez-Ruiz, José Manuel	**S3-1,** P3-8, S3-3
Sancho, J.	P5-15
Schedlbauer, Andreas	S5-2
Schmid, Sandra L.	W3, P4-11
Schneider, Monika	P4-10
Schönfelder, Jörg	S3-3, **O3-4 P3-4**
Sebastián, María	**O5-4 P5-4**
Segura, Jaume	P2-4
Serrano, Ana	O5-4, P5-4
Serrano, Soraya	P5-11
Serrano-Albarrás, Antonio	P6-4

Author	Contributions
Sharma, Rajendra	S3-3
Shepherd, Dawn	O2-3 P2-3
Shnyrova, Anna V.	W3, **P4-11,** P4-12
Silva, Éverton E. D.	O5-3 P5-3
Slotte, J. Peter	O4-4 P4-4
Solé, Laura	P6-4
Somoza, Álvaro	**S7-4**
Soriano, Eduardo	O6-1, P6-1
Sorkin, Alexander	O6-1, P6-1
Sot, Jesús	P4-5
Stirnemann, Guillaume	P3-9
Styrczewska, Katarzyna	O6-1, P6-1
Suay-Corredera, Carmen	O2-1, P2-1
Taboada, Pablo	**S8-3**
Tamkum, Michael M.	P6-4
Tapia, Cristina	O4-2, P4-2
Tarazona, Pedro	**L3**
Tauler, Romà	O3-3, P3-3
Taylor, James A.	O7-4, P7-4
Thacker, V.V.	S7-2
Thapa, Anusa	O4-1, P4-1
Tietzel, Michael	O4-1, P4-1
Timson, David J.	O3-1, P3-1, P5-6
Tirotta, Ilaria	O8-2 P8-2
Tittmann, Kai	O4-1 P4-1
Tolosa, Juan	P8-8
Torreno-Pina, Juan A.	**O2-3, P2-3,** O6-2, P6-2
Torres, Naiara U.	O5-3, P5-3, P5-10
Torres, Rodrigo	P4-7
Trier, Steven	P4-7
Trigo, R	O1-3 P1-3
Turrà, David	P5-11
Urbaneja María, Ángeles	P5-9
Valdivia, Eva	P8-9
Vallejo-Gracia, Albert	P6-4
Valpuesta, José María	S3-3,W1, O3-2, P3-2
van Kooyk, Yvette	P5-9
van Zanten, Thomas S.	S2-3
Vázquez, Jesús	O2-1, P2-1
Vázquez, Rebeca	O4-3, P4-3
Vega, Sonia	P3-5
Velasco del Olmo, Ariana	**P4-12**
Velazquez-Campoy, Adrian	P3-5
Vélez, Marisela	L3, **O4-2, P4-2**
Vestergaard, Bente	O3-3, P3-3
Via, Mireia	O5-2, P5-2, **P5-16,** P5-17
Viana, Félix	**S6-1**
Vicente, Francisca	P5-18
Victor, B.L.	P5-15
Vilanova, Maria	P5-19
Vilaseca, Eudald	O5-2, P5-2, P5-16
Villaluenga, Juan P. G.	P7-5
Villasante, Alfredo	O7-1, P7-1
Villate, Maider	W5

Villora, G	O1-3, P1-3
Vitale, Stefania	P5-11
Vogler, Oliver	P2-4
Wagner, Ernst	P8-7
Waldor, Mathew K.	P5-8
Wegrecki, Marcin	S5-1
Wenz, Jorge J.	**P4-9**
Wiggers, Helton J.	**O5-3, P5-3,** P5-10
Young, Robert D.	O1-4, P1-4
Zacarias, Sonia	O4-2, P4-2
Zettl, T.	S7-2
Zweckstetter, M.	P5-12

Sponsors and collaborators

ugr Universidad de Granada

Facultad de Ciencias

Bruker Corporation

BNC peptides

TA Instruments

PRIMA DERM

ie smat

GRONTAL

Patronato de la Alhambra y Generalife
CONSEJERÍA DE EDUCACIÓN, CULTURA Y DEPORTE

CSIC

Red Temática de Estructura y Función de Proteínas

Sociedad de Biofísica de España