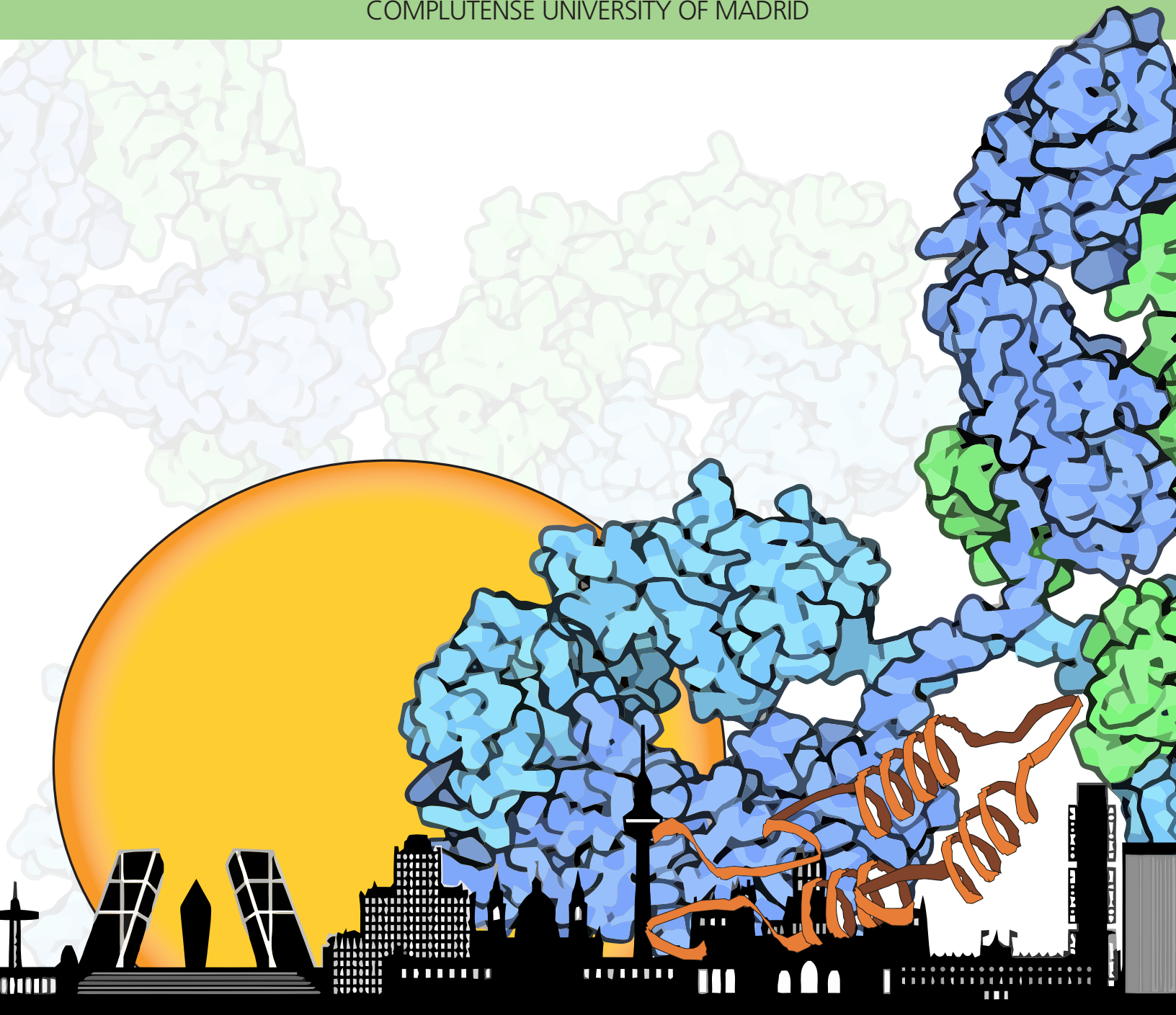


Biophysics of Proteins at Surfaces: Assembly, Activation, Signaling

OCTOBER 13–15, 2015 | MADRID, SPAIN
COMPLUTENSE UNIVERSITY OF MADRID



Organizing Committee

Félix Goñi, Universidad del Pais Vasco, Spain

Marjorie Longo, University of California, Davis, USA

Jesus Pérez-Gil, Complutense University of Madrid, Spain

Nancy Thompson, University of North Carolina, Chapel Hill, USA

Marisela Vélez, ICP CSIC, Spain

October 2015

Dear Colleagues,

We would like to welcome you to the Biophysical Society Thematic Meeting on *Biophysics of Proteins at Surfaces: Assembly, Activation, Signaling*. We have assembled a stimulating program, with lectures focusing on different aspects related to biophysics that defines fine-tuning of protein function through their assembly into biological or engineered surfaces. Particular aspects that will be covered in the Meeting will include i) the effect of the interaction with surfaces on the molecular structure of proteins and protein assemblies, with special interest in the modulation by surface-promoted orientation and two-dimensional accumulation of lipid-protein and protein-protein interactions; ii) the effect of two-dimensional organization and entropy loss on the modulation of protein function; and iii) the potential of introducing properly engineered surfaces to generate new or improved protein-based applications.

We strongly hope that the meeting will not only provide a venue for sharing recent and exciting progress, but also promote fruitful discussions and foster future collaborations in the search of general principles of surface biophysics defining and exploiting protein structure and function. The conference offers a full program with almost 50 lectures and 40 posters, bringing together around 100 well recognized scientists from different fields and countries, promising a truly international and multidisciplinary inspiring environment. We also encourage you to take part in social and cultural activities, enjoying the multicultural and cosmopolitan spirit of Madrid.

Thank you all for joining our Thematic Meeting, and we look forward to enjoying biophysics with all of you in Madrid!

Sincerely yours,

The Organizing Committee

Félix Goñi Margie Longo Jesús Pérez-Gil Nancy Thompson Marisela Vélez

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GENERAL INFORMATION

Registration Hours

The registration desk is located outside the Main Lecture Hall at the Complutense University of Madrid. Registration hours are as follows:

Tuesday, October 13	8:30 – 17:00
Wednesday, October 14	9:00 – 17:00

Instructions for Presentations

(1) Presentation Facilities:

A data projector will be available in the Main Lecture Hall at the Biology Faculty. Speakers are required to bring their laptops. Speakers are advised to preview their final presentations before the start of each session.

(2) Poster Session:

- 1) All poster sessions will be held in the Hall of the main building of the Biology Faculty.
- 2) A display board measuring 2m high and 1m width will be provided for each poster. Poster boards are numbered according to the same numbering scheme as in the abstract book.
- 3) There will be formal poster presentations on Tuesday, Wednesday, and Thursday, but all posters will be available for viewing during all three poster sessions.
- 4) During the poster session, presenters are requested to remain in front of their poster boards to meet with attendees.
- 5) All posters left uncollected at the end of the meeting will be disposed of.

Internet

Wi-Fi access is available throughout the Complutense University of Madrid.

Smoking

Please be advised that smoking is not permitted inside the Complutense University of Madrid.

Meals and Coffee Breaks

Coffee breaks and luncheons (Tuesday, Wednesday, Thursday) and a Gala Dinner (Wednesday) are included in the registration fee.

Name Badges

Name badges are required to enter all scientific sessions and poster sessions. Please wear your badge throughout the conference.

Contact

If you have any further requirements during the meeting, please contact the meeting staff at the registration desk from October 13 – 15 during registration hours.

In case of an emergency, you may contact the following organizers/staff:

Jesus Perez-Gil
Cell: +34 625805826

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Biophysics of Proteins at Surfaces: Assembly, Activation, Signaling

Madrid, Spain

October 13 – 15, 2015

PROGRAM

Scientific sessions will be held in the Main Lecture Hall at the Biology Faculty, and the poster sessions in the Hall of the main building of the Biology Faculty in the Complutense University of Madrid unless otherwise noted.

Tuesday, October 13, 2015

8:30 – 17:00	Registration/Information	Outside Main Lecture Hall
9:00 – 10:00	Opening Session	
Session I:	Oriented Protein Assembly at Biological Surfaces	
	Chair: Marisela Vélez, ICPQ CSIC, Madrid	
10:00 – 10:25	Gregor Anderluh, National Institute of Chemistry, Slovenia <i>Affecting Membrane Assembly and Insertion of a Cholesterol-dependent Cytolysin Listeriolysin O</i>	
10:25 – 10:50	Adam W. Smith, University of Akron, USA <i>Resolving Membrane Protein-Protein Interactions in Live Cells with PIE-FCCS</i>	
10:50 – 11:20	Coffee Break	
11:20 – 11:35	Kabir Biswas, National University of Singapore* <i>E-cadherin Junction Formation Involves an Active Kinetic Nucleation Process</i>	
11:35 – 11:50	Nadja Hellman, University of Mainz, Germany* <i>Surface Induced Oligomerisation—The Case of the Alpha-Toxin from S. aureus</i>	
11:50 – 12:05	Bárbara Olmeda, Complutense University of Madrid, Spain* <i>Supramolecular Assembly of Pulmonary Surfactant Protein SP-B Ensures Proper Dynamics and Structural Stability of Multilayered Films at the Respiratory Air-Liquid Interface</i>	
12:05 – 12:20	Manuel Prieto, Instituto Superior Tecnico, Universidade de Lisboa, Portugal* <i>Quantifying the Membrane Assembly of Amphitropic Proteins by homo-FRET Analysis</i>	
12:20 – 12:35	Dimitrios Morikis, University California, Riverside, USA* <i>Ligand-specific Conformational Changes in CCR7 Coupled to Selecting Different Signaling Pathways upon CCL19 and CCL21 Ligand Binding</i>	
12:35 – 13:30	Lunch	
13:30 – 15:00	Poster Session I	Hall of Biology Faculty

*Short talks selected from among submitted abstracts

Session II:	Interfacial Catalysis
	Chair: Félix Goñi, Universidad del Pais Vasco, Spain
15:00 – 15:25	Juan Carmelo Gomez-Fernandez, University of Murcia, Spain <i>Membrane Surface Activation of Protein Kinases C</i>
15:25 – 15:50	Banafshe Larijani, Ikerbasque Basque Foundation for Science and Universidad del País, Vasco, Spain <i>Protein Kinase B (PKB) and Phosphoinositide Dependent Kinase 1 (PDK1) Regulation at Membranous Compartments</i>
15:50 – 16:20	Coffee Break
16:20 – 16:35	Mary Gertrude Gutierrez, University of Southern California, USA* <i>Elucidating GPCR Functional Dependence on Plasma Membrane Composition Using Giant Unilamellar Protein-Vesicles</i>
16:35 – 16:50	Daniel G. Capelluto, Virginia Tech, USA* <i>Tom1 Modulates Binding of Tollip to Phosphatidylinositol 3-Phosphate via a Coupled Folding and Binding Mechanism</i>
16:50 – 17:05	Nathalie Reuter, University of Bergen, Norway* <i>Interplay between Weak Nonspecific Electrostatics and Cation-π Interactions Governs the Peripheral Membrane Binding of a Bacterial Phospholipase</i>
17:05 – 17:20	Sarah Perrett, Institute of Biophysics, Chinese Academy of Sciences, China* <i>Self-Assembly of Protein Nanofibrils that Display Active Enzymes</i>
17:20 – 17:35	Patrick C. Van der Wel, University of Pittsburgh School of Medicine, USA* <i>Interfacial Interactions with Cardiolipin-containing Membranes Cause Cytochrome C's Peroxidase Activity Required for Mitochondrial Apoptosis without Unfolding the Protein</i>
17:35 – 18:35	Keynote Lecture Introduced by Félix Goñi, Universidad del Pais Vasco, Spain Joshua Zimmerberg, NIH, NICHD, USA <i>Liquid/Protein Interactions in Membrane Fusion and Fission</i>

Wednesday, October 14, 2015

9:00 – 17:00	Registration/Information	Outside Main Lecture Hall
9:00 – 10:00	Plenary Lecture Introduced by Jesus Pérez-Gil, Complutense University of Madrid, Spain Allen Minton, NIDDK, NIH, USA <i>Effects of Molecular Crowding and Reversible Adsorption on Macromolecular Self-Assembly: A Mesoscopic Analysis</i>	
Session III:	Shifting from a 3D to a 2D World for Regulation and Signaling	
	Chair: Jesus Pérez-Gil, Complutense University of Madrid, Spain	
10:00 – 10:25	Marek Cieplak, Polish Academy of Sciences, Poland <i>Aqueous Amino Acids and Proteins near Solid Surfaces and Air-Water Interfaces</i>	

*Short talks selected from among submitted abstracts

10:25 – 10:50	Alicia Alonso, Unidad de Biofísica CSIC, UPV/EHU, Spain <i>The Interaction of Autophagy Proteins with Lipid Surfaces</i>	
10:50 – 11:15	Marisela Vélez, ICP CSIC, Spain <i>A Surface to Twist the Filament: A Good Strategy to Generate Force</i>	
11:15 – 11:45	Coffee Break	
11:45 – 12:00	Aurelia Honerkamp-Smith, University of Cambridge, United Kingdom* <i>Fluid Flow as a Biophysical Method for Sorting and Localization of Membrane Proteins</i>	
12:00 – 12:15	Fabio Fernandes, Instituto Superior Técnico, Portugal* <i>FRET Analysis of the Nanoscale Organization of PI(4,5)P2 in Living Cells</i>	
12:15 – 12:30	Jannik B. Larsen, University of Copenhagen, Denmark* <i>Membrane Curvature Enables N-Ras Lipid Anchor Sorting to Liquid-ordered Membrane Phases</i>	
12:30 – 12:45	Anabel-Lise Le Roux, University of Barcelona, Spain* <i>Biophysical Studies of Myristoylated Unique and SH3 Domains of Src Kinase and Their Interaction with Lipid Membranes</i>	
12:45 – 13:00	Jennie Ringberg, Biolin, Sweden* <i>QCM-D: A Powerful Surface Analysis Tool for Protein Studies</i>	
13:00 – 13:45	Lunch	
13:45 – 15:30	Poster Session II	Hall of Biology Faculty
Session IV:	Integrating Proteins and Surfaces in Biomaterials	
	Chair: Marjorie Longo, University of California, Davis, USA	
15:30 – 15:55	Ana García-Saéz, University of Tübingen, Germany <i>Oligomerization of Pore Forming Proteins in Membranes at the Single Molecule Level</i>	
15:55 – 16:20	Marjorie Longo, University of California, Davis, USA <i>Nanolipoprotein Particles Confined within Nanoporous Sol-Gel or Bound to GUVs</i>	
16:20 – 16:45	Alexey Ladokhin, KUMC, USA <i>PH-Triggered Conformational Signaling on Membrane Interfaces</i>	
16:45 – 17:15	Coffee Break	
17:15 – 17:30	Ivan Lopez-Montero, Complutense University of Madrid, Spain* <i>Structural and Nanomechanical Features of Reconstructed Spectrin—Actin Membrane Cytoskeletons for Artificial Cell Realizations: An Atomic Force Microscopy Characterization</i>	

*Short talks selected from among submitted abstracts

17:30 – 17:45	Guilherme Vilhena, Instituto de Ciencia de Materiales de Madrid, CSIC, Spain* <i>Solvent Models in Protein Adsorption Simulations: Explicit, Implicit vs Experiments</i>	
17:45 – 18:00	Karim Chouchane, LMGP, France* <i>Peptides Forming Beta-Sheets on Hydrophobic Surfaces Cooperatively Promote Insulin Amyloid Aggregation</i>	
18:00 – 18:15	Paolo Facci, National Research Council, Italy* <i>Harnessing Antibodies at an Electrode Surface: Electrical Control of IgG Conformation and Functional Activity</i>	
18:15 – 18:30	Erik G. Brandt, Stockholm University, Sweden* <i>Ubiquitin Adsorbs on the TiO₂(100) Surface by an Anchor-Lock Mechanism</i>	
21:00	Gala Dinner	Cibeles Palace

Thursday, October 15, 2015

9:00 – 10:00	Plenary Lecture Introduced by Félix Goñi, Universidad del Pais Vasco, Spain Brunno Antony, CNRS and Université de Nice Sophia Antipolis, France <i>Control of Protein Adsorption and Protein-induced Membrane Deformation by Phospholipid Unsaturation</i>	
Session V: Surface-mediated Protein Dynamics		
Chair: Félix Goñi, Universidad del Pais Vasco, Spain		
10:00 – 10:25	Patricia Bassereau, Curie Institute, France <i>Membrane Curvature Induces Scaffolding by BAR-Domain Proteins</i>	
10:25 – 10:50	Ralf Richter, CIC biomaGUNE, Spain <i>Many Weak Interactions Make a Difference—From the Self Assembly of Intrinsically Disordered Proteins to Superselective Targeting</i>	
10:50 – 11:15	Petra Schwill, Max Planck Institute of Biochemistry, Germany <i>The Role of the Membrane Structure on Protein Pattern Formation</i>	
11:15 – 11:45	Coffee Break	
11:45 – 12:00	Mariana Amaro, J. Heyrovsky Institute of Physical Chemistry of the ASCR, Czech Republic* <i>GMI Nanodomains Inhibit the Oligomerization of Membrane Bound β-Amyloid Monomers</i>	
12:00 – 12:15	Michael E. Fealey, University of Minnesota, USA* <i>Synaptic Vesicle Lipids Reveal Structured State and Antagonistic Allosteric Mechanisms of Intrinsic Disorder within Synaptotagmin I</i>	
12:15 – 12:30	Jose M. Delfino, University of Buenos Aires, Argentina* <i>Scanning Protein Surface with a Solvent Mimetic Probe: An NMR Approach</i>	

*Short talks selected from among submitted abstracts

12:30 – 12:45	German Rivas, CSIC, Spain* <i>Dynamic Interactions of Protein Elements of the Bacterial Division Machinery Evidenced in Phospholipid Bilayer Nanodiscs</i>	
12:45 – 13:00	Philip L. Yeagle, University of Connecticut, USA* <i>What Cholesterol Is Doing in the Plasma Membrane</i>	
13:00 – 13:45	Lunch	
13:45 – 15:30	Poster Session III	Hall of Biology Faculty
Session VI:	Methodological Advances	
	Chair: Marisela Vélez, ICP CSIC, Spain	
15:30 – 15:55	María García-Parajo, The Institute of Photonic Sciences, Spain <i>Spatiotemporal Organization of Receptors in Living Cell Membrane Surfaces</i>	
15:55 – 16:20	Joachim Heberle, University of Berlin, Germany <i>Protein Structural Dynamics at Surfaces as Studied by Infrared Nanospectroscopy</i>	
16:20 – 16:45	Simon Scheuring, INSERM / Aix-Marseille University, France <i>High-Speed Atomic Force Microscopy: The Dynamics and Interaction of Protein and Membrane Surfaces</i>	
16:45 – 17:15	Coffee Break	
17:15 – 17:30	Adam Cohen Simonsen, University of Southern Denmark* <i>Spatial Organization of Na⁺/K⁺-ATPase and Lipid Domains in Free-standing Membranes Captured on a Solid Support</i>	
17:30 – 17:45	Laura C. Zanetti Domingues, STFC, United Kingdom* <i>Single-Molecule Visualisation of the Mechanisms of Autoinhibition and Dysregulation of the EGFR on Living Cell Membranes</i>	
17:45 – 18:00	Chiara Rotella, University College Dublin, Ireland* <i>High Resolution Imaging Atomic Force Microscope Study of Interactions at the Membrane-Fluid Interface</i>	
18:00 – 18:15	Jonas Schartner, Biology & Biotechnology, Germany* <i>Germanium Catches Proteins in Action</i>	
18:30	Closing Remarks and Biophysical Journal Poster Awards Chair: Jesus Pérez-Gil, Complutense University of Madrid, Spain	

*Short talks selected from among submitted abstracts

SPEAKER ABSTRACTS

Affecting Membrane Assembly and Insertion of a Cholesterol-dependent Cytolysin Listeriolysin O

Gregor Anderluh^{1,2}, Matic Kisovec¹, Saša Rezelj¹, Polona Bedina Zavec¹, Marjetka Podobnik¹.
¹National Institute of Chemistry, Ljubljana, Slovenia, ²University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia.

Pore-forming toxins assemble on the surface of cellular lipid membranes in order to generate transmembrane pores. This occurs through several well-defined steps such as recognition and binding to the particular membrane receptor, oligomerisation at the plane of the membrane and insertion of part of the polypeptide chain across the membrane with consequent formation of a pore. Properties of lipid membranes and structural features of pore-forming toxin can affect each of these steps. We will present how lipid membranes composition and single-point mutation affect pore formation of listeriolysin O (LLO). LLO is the most important protein for pathogenicity of a food-borne pathogen *Listeria monocytogenes*. It belongs to the family of cholesterol-dependent cytolysins which has representatives in many different pathogenic Gram positive bacteria. LLO is unique amongst non *Listeria*-derived cholesterol-dependent cytolysins in being stable and working optimally at low pH values. LLO allows phagosome-entrapped bacteria to escape to the cytoplasm by creating huge β -barrel transmembrane pores in lipid membranes. Structural features of these pores are not yet completely understood, neither the dependence on lipid composition or pH. Here we will show that pore formation depends on lipid membrane composition, pH and single-point mutations that can have multiple effects on protein stability, rate of pore formation and properties of final pores. The results altogether indicate that the assembly of β -barrel transmembrane LLO pores exhibits significant plasticity, which is an important feature to be included in design and development of medical or nanobiotechnological applications involving cholesterol-dependent cytolysins.

Resolving Membrane Protein-Protein Interactions in Live Cells with PIE-FCCS

Adam Smith.

University of Akron, Akron, USA.

Lateral interactions in biological membranes directly influence the activity of cell surface receptors. Resolving the structure and stability of these interactions in situ is difficult because of complexity of the plasma membrane. There are few methods that can resolve the fast dynamics and short length scales that are relevant to membrane organization. I will report on two of my group's efforts to study membrane structure and organization with pulsed interleaved excitation fluorescence cross-correlation spectroscopy (PIE-FCCS) and related methods. PIE-FCCS reports on molecular associations by observing correlated diffusion in and out of a confocal detection area. In this way we can resolve protein mobility and dimerization in live cell membranes with single molecule sensitivity. I will summarize our recent efforts to resolve the clustering and activation mechanism of receptor tyrosine kinases, plexins and G protein-coupled receptors. I will also report on our efforts to study the chemical details of lipid-protein interactions. Together these projects aim to build a more complete picture of the chemical landscape that governs cell communication.

E-cadherin Junction Formation Involves an Active Kinetic Nucleation Process

Kabir Biswas¹, Kevin L. Hartman^{1,2}, Cheng-Han Yu¹, Oliver J. Harrison^{3,4,9}, Hang Song^{3,4,9}, Adam W. Smith⁵, William Huang², Wan-Chen Lin⁵, Zhenhuan Guo¹, Anup Padmanabhan¹, Sergey M. Troyanovsky⁷, Michael L. Dustin⁸, Lawrence Shapiro^{3,9}, Barry Honig^{3,4,9}, Ronen Zaidel-Bar^{1,10}, Jay T. Groves^{1,2,5}.

¹National University of Singapore, Singapore, Singapore, ²University of California, Berkeley, Berkeley, CA, USA, ³Columbia University, New York, NY, USA, ⁴Columbia University, New York, NY, USA, ⁵University of California, Berkeley, Berkeley, CA, USA, ⁶Lawrence Berkeley National Laboratory, University of California, Berkeley, CA, USA, ⁷Feinberg School of Medicine, Northwestern University, Chicago, IL, USA, ⁸University of Oxford, Headington, United Kingdom, ⁹Columbia University, New York, NY, USA, ¹⁰National University of Singapore, Singapore, Singapore.

E-cadherin-mediated cell-cell junctions play important roles in the development and maintenance of tissue structure in multi-cellular organisms. E-cadherin adhesion is thus a key element of the cellular microenvironment that provides both mechanical and biochemical signaling inputs. Here, we report in vitro reconstitution of junction-like structures between native E-cadherin in living cells and the extracellular domain of E-cadherin (E-cad-ECD) in a supported membrane. Junction formation in this hybrid live cell-supported membrane configuration requires both active processes within the living cell and a supported membrane with low E-cad-ECD mobility. The hybrid junctions recruit α -catenin, and exhibit remodeled cortical actin. Observations suggest that the initial stages of junction formation in this hybrid system depend on the trans but not the cis-interactions between E-cadherin molecules, and proceed via a nucleation process in which protrusion and retraction of filopodia play a key role.

Surface Induced Oligomerisation--the Case of the Alpha-Toxin from S.aureus

Nadja Hellmann, Markus Schwiering.
University of Mainz, Mainz, Germany.

Pore forming toxins can be found in many organisms from different taxa. Frequently the toxin is a protein, which is secreted as a monomer, binds to the membrane and undergoes a conformational change towards a membrane-spanning pore. The alpha-toxin from *S.aureus* (Hla) is able to lyse pure lipid membranes. We investigated the influence of the lipid membrane composition on the oligomerisation level by employing fluorescence spectroscopy of pyren-labelled toxin, SDS-gel electrophoresis and fluorescence microscopy in order to find out whether the toxin has a preference for regions with particular lipid composition. Increasing concentration of cholesterol and sphingomyelin (compared to phosphatidylcholine) facilitate oligomer formation and lysis in phase separating lipid mixtures. On the first glance this seems to indicate, that a preference for raft-structures exists. However, substitution of saturated SM by unsaturated SM (OSM=oleoyl SM) showed that the fluid disordered phase in particular enhances oligomerisation [1]. Taken together, these results indicate that this toxin tends to interact with or accumulate in the liquid disordered phase rather than the liquid ordered phase. This is supported by fluorescence microscopy. Simulations in comparison with experimental data indicate that oligomerisation probability rather than monomer binding affinity is enhanced by presence of SM. Thus, in contrast to some other pore forming toxins the preference for SM-containing lipid membranes seems to be not a consequence of specific interaction of the lipid binding pocket of the toxin. Possibly binding to SM keeps the toxin monomers in an orientation more suitable for oligomerisation. This work was supported by the DFG (SFB 490). [1] Schwiering, M., A. Brack, et al. (2013). "Lipid and phase specificity of alpha-toxin from *S. aureus*." *Biochim Biophys Acta* 1828(8): 1962-72.

Supramolecular Assembly of Pulmonary Surfactant Protein SP-B Ensures Proper Dynamics and Structural Stability of Multilayered Films at the Respiratory Air-Liquid Interface

Bárbara Olmeda¹, Begoña García-Álvarez¹, Manuel J. Gómez², Marta Martínez-Calle¹, Antonio Cruz¹, Jesús Pérez-Gil¹.

¹Universidad Complutense de Madrid, Madrid, Spain, ²Centro de Astrobiología (INTA-CSIC), Torrejón de Ardoz, Madrid, Spain.

Surfactant protein SP-B is essential to facilitate the formation and proper performance of surface active pulmonary surfactant films at the air-liquid interface of mammalian lungs, allowing both dynamics and mechanical stability of the film. Despite its importance, neither a structural model nor a molecular mechanism of SP-B is available.

In the present work we have purified and characterized native SP-B supramolecular assemblies to elaborate a model that supports structure-function features described for SP-B. Purification of porcine SP-B using detergent-solubilized surfactant reveals the presence of 10 nm ring-shaped particles. These rings, observed by atomic force and electron microscopy, would be assembled by oligomerization of SP-B as a multimer of dimers forming a hydrophobically coated ring at the surface of phospholipid membranes or monolayers.

Docking of rings from neighboring membranes would lead to formation of SP-B-based hydrophobic tubes, competent to facilitate the rapid flow of surface active lipids both between membranes and between surfactant membranes and the interface. The existence of these SP-B complexes not only sustain the dynamic behavior required by breathing conditions, but also explain how the protein facilitates cohesivity and mechanical stabilization of the multilayered three-dimensional structure of surfactant films at the surface of the alveolar epithelium.

Quantifying the Membrane Assembly of Amphitropic Proteins by homo-FRET Analysis

Ana M. Melo¹, Aleksander Fedorov¹, **Manuel Prieto**¹, Ana Coutinho^{1,2}.

¹Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal, ²Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal.

Transient membrane recruitment of amphitropic proteins by anionic phospholipids is a common cellular mechanism involved in the regulation of membrane signal transduction. Here, we present a homo-FRET based method for the quantitative characterization of the oligomerization state of membrane-bound proteins involved in a three-state cooperative partition/oligomerization equilibria. We assume that monomeric proteins partition into the bilayer surface and reversibly assemble into homo k -mers. Using of a combination of steady-state and time-resolved fluorescence intensity and anisotropy measurements, this method was shown to be very robust in describing the electrostatic interaction of a model fluorescently-labelled amphitropic protein (Lz-A488) with anionic lipid membranes [1,2]. The pronounced decrease detected in the fluorescence anisotropy of membrane-bound Lz-A488, and therefore the extent of homo-FRET, always correlated with the system reaching a high surface coverage by the fluorescently-labeled protein at a low lipid-to-protein (L/P) molar ratio. Anisotropy decays of Lz-A488 samples prepared with variable fractional labeling (dye-to-protein molar ratios) further confirmed the occurrence of intra-oligomeric energy transfer-induced fluorescence depolarization. A global analysis of the steady-state anisotropy data obtained under a wide range of experimental conditions (variable anionic lipid content of the liposomes, L/P molar ratio and protein fractional labeling) yielded that membrane-bound Lz-A488 self-assembled into oligomers with a stoichiometry of $k = 6 \pm 1$.

[1] Melo et al. 2013 J. Phys. Chem. 117: 2906–2917 (DOI: [dx.doi.org/10.1021/jp310396v](https://doi.org/10.1021/jp310396v))

[2] Melo et al. 2014 Phys.Chem.Chem.Phys. 16: 18105-18117 (DOI: [10.1039/c4cp00060a](https://doi.org/10.1039/c4cp00060a))

This work was supported by FCT/Portugal (PTDC/BBB-BQB/2661/2012 and RECI/CTM-POL/0342/2012). A.M. Melo current address is Dept Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut, USA.

Ligand-specific Conformational Changes in CCR7 Coupled to Selecting Different Signaling Pathways upon CCL19 and CCL21 Ligand Binding

Dimitrios Morikis, Zied Gaieb, David Lo.
University of California, Riverside, CA, USA.

Chemokine receptor type 7 (CCR7) is a G protein-coupled receptor (GPCR) that is activated by ligands CCL19 and CCL21. The ligands are expressed in different parts of the body as gradients to aid in homing of T cells and antigen-presenting dendritic cells to the lymph nodes. Although both ligands have similar structures and activate the same receptor (CCR7), they induce distinct signaling pathways. While both ligands mediate their signaling through G-protein and GRK6, only CCL19 induces CCR7 desensitization and internalization through phosphorylation by GRK3 and recruitment of β -arrestin. The functional diversity of receptor-ligand binding is related to decoupled or partially decoupled conformational changes. We will present the results of molecular dynamics (MD) simulations of free CCR7 and the complexes CCR7-CCL19 and CCR7-CCL21. We will discuss long-range conformational changes associated with receptor activation pathways upon ligand binding. Differences in the conformational changes of the three systems are quantitatively assessed with a range of MD analysis methods, including principal component analysis (PCA) and dynamic cross-correlation analysis (DCC). We observe that the presence of the ligands introduces collective motions within larger CCR7 domains, including trans-membrane helices and intra-cellular loops. Such motions may be necessary to induce large openings between intra-cellular regions that are necessary to accommodate G protein binding and to initiate intra-cellular signaling pathways. This work aims to delineate the structural elements that are responsible for biased receptor activation, and the conformational changes and long-range motions that link extra-cellular ligand binding with intra-cellular protein binding and selection of signaling pathways.

Membrane Surface Activation of Protein Kinases C

Juan Gomez-Fernandez, Senena Corbalan-Garcia.
University of Murcia, Murcia, Spain.

Classical protein kinases C are known to be important in cell physiology both in terms of health and disease. They are activated by triggering signals that induce their translocation to membranes. The consensus view is that several secondary messengers are involved in this activation, such as cytosolic Ca^{2+} and diacylglycerol. The former bridges the C2 domain to anionic phospholipids as phosphatidylserine in the membrane and diacylglycerol binds to the C1 domain. Both diacylglycerol and the increase in Ca^{2+} concentration are assumed to arise from the extracellular signal that triggers the hydrolysis of phosphatidylinositol-4,5-bisphosphate, however results obtained during the last decade indicate that this phosphoinositide itself is also responsible for modulating classical PKC activity and its localization in the plasma membrane. Novel protein kinases C, on the other hand, are known to be activated in a Ca^{2+} -independent way, with diacylglycerol/phorbol esters, playing a very important role. Recent results indicate that the C2 domain may also play an important role in this activation and, furthermore, negatively charged phospholipids are also very important in the binding of C1B domains to the membrane, participating in the activation of these isoenzymes. A picture emerges in which there is a concerted interplay of activators modulating the translocation of PKCs to the membrane, triggering conformational changes that give place to a strictly regulated activation of these enzymes.

Protein Kinase B (PKB) and Phosphoinositide dependent Kinase 1 (PDK1) regulation at membranous compartments.

Banafshe LARIJANI^{1,2,1}, Gloria De Las Heras-Martinez², Jose Requejo-Isidro², Veronique Calleja³.

¹ Ikerbasque Basque Foundation for Science and Universidad del País Vasco, Leioa, Spain, ²Biophysics Unit - CSIC, Leioa, Spain, ³CRICK INSTITUTE, LONDON, United Kingdom.

Protein Kinase B (PKB)/Akt and Phosphoinositide dependent Kinase 1 (PDK1) are members of the AGC kinase superfamily and are activated downstream of many growth factor and hormone receptors as a result of phosphoinositide 3-kinase activation. PKB and PDK1 phosphorylate a diverse set of substrates involved in many fundamental aspects of cell biology, including growth, survival, proliferation, angiogenesis, migration, and metabolism.

The most prominent site of PKB recruitment and activation is at the plasma membrane; however, this may not be the only site. Therefore we have investigated the potential for intracellular PKB activation in response to growth factor stimulation using the rapalogue dimerisation tool to inducibly and acutely recruit Akt to the endosomes or to the nuclear envelope.

We have also investigated, in cells, the mechanism of regulation of PDK1 in response to the level of negatively charged phospholipids at the plasma membrane using time resolved Forster resonance energy transfer.

Our cross-disciplinary approach has resulted in determining a refined model for the in situ, regulation of both these master regulators.

Elucidating GPCR Functional Dependence on Plasma Membrane Composition Using Giant Unilamellar Protein-Vesicles

Mary Gertrude Gutierrez, Kylee Mansfield, Noah Malmstadt.
University of Southern California, Los Angeles, USA.

Using an agarose hydration technique for protein incorporation into vesicular bilayers, we elucidate the effects of membrane composition and ordering on G Protein Coupled Receptors (GPCRs). We successfully incorporate GPCRs into model membranes in the form of giant unilamellar protein-vesicles (GUPs). Using this completely *in vitro* platform we observe that the functional rate of the human serotonin receptor, GPCR 5-HT_{1A}, and the A2A Adenosine GPCR is dependent on membrane composition and ordering. We use BODIPY-GTP γ S as our fluorescent marker to track the irreversible exchange between GDP and GTP on G proteins over time in GUPs composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), brain sphingomyelin (BSM), and cholesterol (Chol), as well as synthetic lamellar phase diblock copolymer. Furthermore, using this approach we demonstrate that the incorporated receptors display a biased orientation with the N-terminus located on the exterior (extracellular) and the C-terminus on the interior (cytosolic).

Tom1 Modulates Binding of Tollip to Phosphatidylinositol 3-Phosphate via a Coupled Folding and Binding Mechanism

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Early endosomes represent the first sorting station for vesicular ubiquitylated cargo. Tollip, through its C2 domain, associates with endosomal phosphatidylinositol 3-phosphate (PtdIns(3)P) and binds ubiquitylated cargo in these compartments via its C2 and CUE domains. Tom1, through its GAT domain, is recruited to endosomes by binding to the Tollip Tom1-binding domain (TBD) through an unknown mechanism. NMR data revealed that Tollip TBD is a natively unfolded domain that partially folds at its N-terminus when bound to Tom1 GAT through high affinity hydrophobic contacts. Furthermore, this association abrogates binding of Tollip to PtdIns(3)P by additionally targeting its C2 domain. Tom1 GAT is also able to bind ubiquitin and PtdIns(3)P at overlapping sites, albeit with modest affinity. We propose that association with Tom1 favors Tollip's release from endosomal membranes, allowing Tollip to commit to cargo trafficking.

Interplay between Weak Nonspecific Electrostatics and Cation- π Interactions Governs the Peripheral Membrane Binding of a Bacterial Phospholipase

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Bacillus thuringiensis phosphatidylinositol-specific phospholipase C (BtPI-PLC) is an amphitropic enzyme cleaving GPI-anchored proteins off the outer surface of eukaryotic plasma membranes. Amphitropic proteins bind specifically and transiently to the surface of cell membranes, and their functions are regulated upon binding.

It is commonly acknowledged that non-specific electrostatic forces are responsible for their long-range interactions with membranes. Using continuum electrostatics calculations we show how, despite having an overall negative charge (-7e), the charge distribution of BtPI-PLC leads to favorable though quite low electrostatic binding free energy with anionic membranes. The *in silico* mutation of a single, key basic residue to alanine diminishes this long-range electrostatic contribution explaining the significant decrease in the experimentally measured K_d . Multiple 500 ns-long all-atom molecular dynamics simulations of BtPI-PLC docked to mixed bilayers with varying ratio of zwitterionic lipids show that, once close to the membrane surface, short range non-specific hydrophobic interactions and specific cation- π interactions with the N(Me)₃ groups of phosphatidylcholine (PC) lipids of the membrane come into play for BtPI-PLC binding to the membrane surface[1].

Comparing our simulation results with fluorescence correlation spectroscopy measurements of the membrane affinity of the wild-type enzymes and of various mutants, we conclude that the interplay between long-range electrostatics and short range, PC specific cation- π interactions governs the specificity of BtPI-PLC for PC-rich membranes. Finally, we suggest that overlooked cation- π interactions between membranes and aromatic amino acids of amphitropic proteins may play an important role not only in membrane binding but also in lipid specificity.

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Self-Assembly of Protein Nanofibrils that Display Active Enzymes

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The ability of proteins to self-assemble into beta-sheet-rich aggregates called amyloid fibrils is considered to be universal, although certain polypeptide sequences have a particularly high propensity to adopt these conformations. In many cases the formation of amyloid fibrils is deleterious and associated with the progression of disease, but there are also examples of proteins for which the cross-beta structure represents the functional conformation. Ure2 is the protein determinant of the yeast prion [URE3]. Ure2 consists of an N-terminal prion-inducing domain that is disordered in the native state, whereas the C-terminal functional domain has a globular fold with structural similarity to glutathione transferase enzymes. The C-terminal domain shows enzymatic activity in both the soluble and fibrillar forms of Ure2. We have used a variety of biophysical approaches to investigate the structure of Ure2 fibrils and their mechanism of assembly. We have also created chimeric constructs where the prion domain is genetically fused to other enzymes of different sizes and architectures. These chimeric polypeptide constructs spontaneously self-assemble into nanofibrils with fused active enzyme subunits displayed on the amyloid fibril surface. We can measure steady-state kinetic parameters for the appended enzymes in situ within fibrils, and compare these for the identical protein constructs in solution. We have also applied microfluidic techniques to form enzymatically-active microgel particles from the chimeric self-assembling protein nanofibrils. The use of scaffolds formed from biomaterials that self-assemble under mild conditions enables the formation of catalytic microgels whilst maintaining the integrity of the encapsulated enzyme. In combination with microfluidic trapping techniques, these approaches illustrate the potential of self-assembling materials for enzyme immobilization and recycling, and for biological flow-chemistry. The design principles can be adopted to create countless other bioactive amyloid-based materials with diverse functions.

Interfacial Interactions with Cardiolipin-containing Membranes cause Cytochrome c's Peroxidase Activity Required for Mitochondrial Apoptosis without Unfolding the Protein.

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Background: Disease toxicity in Huntington's Disease and many other neurodegenerative diseases is caused at least in part by mitochondrial dysfunction, increased reactive oxygen species, and increases in mitochondrial apoptosis. These lethal processes are connected by a proapoptotic gain-of-function in mitochondrial cytochrome c, which is induced by its binding to cardiolipin in the mitochondrial inner membrane. Formation of a cardiolipin-cytochrome-c complex turns the protein into a lipid peroxidase that generates cardiolipin-derived signaling molecules required for apoptosis to occur.

Objective: Elucidate the structural changes that underlie the lethal peroxidase activity of cytochrome c induced by its binding to cardiolipin-containing membranes.

Methods: We performed structural and functional studies of the peroxidase-active state of cytochrome c as it is bound to unilamellar cardiolipin-containing lipid vesicles. Fluorescence and other optical spectroscopies were combined with unprecedented 2D and 3D magic-angle-spinning solid-state NMR to probe the conformation and dynamics of the membrane-bound protein.

Results: The vesicle-bound protein gains peroxidase activity in a cardiolipin-dependent fashion. Our structural measurements via magic-angle-spinning NMR and other techniques reveal that the membrane-bound protein retains the secondary structure and tertiary fold of its unbound native state. We also find that the protein interacts primarily with the surface of the lipid bilayers, without significantly disrupting the integrity of the lipid bilayer.

Conclusions: Large-scale unfolding and penetration of the lipid bilayer are not required for cytochrome c's membrane-induced peroxidase activity. Instead our data show that the protein gains peroxidase activity while bound to interface region of cardiolipin-containing vesicles as a peripherally bound protein that retain most of its native fold. Thus, the peroxidase activity appears to be more controlled and regulated than previously assumed, which may render it a viable and important target for disease modulation.

Liquid/Protein Interactions in Membrane Fusion and Fission

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No Abstract

Effects of Molecular Crowding and Reversible Adsorption on Macromolecular Self-assembly: a Mesoscopic Analysis**Allen Minton.**

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Previously published simplified statistical-thermodynamic models for the effect of volume exclusion ('crowding') and for the effect of surface adsorption upon the self-association of a dilute tracer protein are reviewed. A recently developed model for the cumulative effect of both crowding and adsorption on protein fibrillation is presented. This model predicts that when the volume fraction of "inert" crowder exceeds a critical value, or the enthalpy of tracer adsorption becomes more negative than a critical value, the slightly fibrillated and highly soluble tracer protein will condense onto the surface and simultaneously achieve a very high degree of fibrillation.

Aqueous Amino Acids and Proteins Near Solid Surfaces and Air-Water Interfaces**Marek Cieplak.**

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A systematic comparison of the adsorptive properties of various surfaces can be accomplished by considering a set of reference biomolecules. We have initiated such a program by selecting the twenty natural amino acids, some dipeptides, and a small protein - tryptophan cage as the reference systems for all-atom simulational studies. The surfaces compared are: ZnS, gold, cellulose I β , mica, and four faces of ZnO. The specificities, as determined through the potential of the mean force for the amino acids, are found to depend on the solid, its face and, for gold, on the choice of the force field (hydrophobic, hydrophilic, or incorporating the polarizability of the metal). We demonstrate that binding energies of dipeptides and tripeptides are smaller than the combined binding energies of their amino acidic components. The water density and polarization profiles are also surface-specific. The first water layer that forms near the strongly hydrophilic ZnO corresponds to packing at such a density that even single residues cannot reach the solid. ZnS is more hydrophobic and yields only minor articulation of water into layers. In the case of ZnS, not all amino acids can attach to the surface and when they do, the binding energies are comparable to those found for the surfaces of ZnO (and to hydrogen bonds in proteins). For the hydrophobic Au, adsorption events of tryptophan cage are driven by attraction to the strongest binding amino acids. This is not so for ZnO, ZnS and for the hydrophilic models of gold. Studies of several proteins near mica, with a net charge on its surface, indicate existence of two types of states: deformed and unfolded. Using a coarse-grained model, we also study the glassy behavior of protein layers at air-water interfaces.

The Interaction of Autophagy Proteins with Lipid Surfaces

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Autophagy, an important catabolic pathway involved in a broad spectrum of human diseases, implies the formation of double membrane-bound structures called autophagosomes (AP) which engulf material to be degraded in lytic compartments. How AP form, especially how the membrane expands and eventually closes upon itself is an area of intense research. Ubiquitin-like ATG8 has been related to both membrane expansion and membrane fusion, but the underlying molecular mechanisms are poorly understood. Here we used two minimal reconstituted systems (enzymatic and chemical conjugation) to investigate the ability of human ATG8 homologues (LC3, GABARAP and GATE-16) to mediate membrane fusion. We found that both enzymatically- and chemically-lipidated forms of GATE-16 and GABARAP proteins promote extensive membrane tethering and fusion, whereas lipidated LC3 does so to a much lesser extent. Moreover, we characterize the GATE-16/GABARAP-mediated membrane fusion as a phenomenon of full membrane fusion, independently demonstrating vesicle aggregation, intervesicular lipid mixing and intervesicular mixing of aqueous content, in the absence of vesicular content leakage. Multiple fusion events give rise to large vesicles, as seen by cryo-EM observations. We also show that both vesicle diameter and selected curvature-inducing lipids (cardiolipin, diacylglycerol and lysophosphatidyl-choline) can modulate the fusion process, smaller vesicle diameters and negative intrinsic curvature lipids (cardiolipin, diacylglycerol) facilitating fusion. These results strongly support the hypothesis of a highly bent structural fusion intermediate ("stalk") during AP biogenesis and add to the growing body of evidence that identifies lipids as important regulators of autophagy.

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A Surface to Twist the Filament: A Good Strategy to Generate Force

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We study the self-assembling behavior of FtsZ in vitro on supported lipid membranes using Atomic Force Microscopy and theoretical models that describe the polymerization in terms of a simple set of monomer-monomer interactions. FtsZ is a bacterial cytoskeletal protein that polymerizes on the inner surface of the bacterial membrane and contributes to generate the force needed for cell division. In the presence of GTP the individual protein monomers interact longitudinally to form filaments that can then aggregate to form higher order structures on the membrane surface. These filament aggregates are dynamic and exchange monomers from the solution. The final outcome of this dynamic rearrangement on the surface is the generation of force that bends the cell membrane inward. Reversible GTP-induced polymerization in vitro showed that the type of attachment to the surface and the type of lipid present on the membrane determine the shape of the filament aggregates observed. Experimental results controlling the orientation of the monomers on the surface, together with molecular dynamics simulations and theoretical models revealed that filament curvature, twist, orientation and the strength of the surface attachment are all important for determining the amount of force that the filaments can exert on the surface.

Fluid Flow as a Biophysical Method for Sorting and Localization of Membrane Proteins

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Many cells, such as leukocytes, endothelial cells, and osteoblasts, exhibit dramatic biochemical and biophysical responses to shear flow. However, the molecular-scale mechanisms of flow mechanotransduction are complex and details remain obscure [1]. It has been observed that large GPI-anchored proteins are reorganized following application of shear flow to living cells [2], but whether this is the result of advection or of active intracellular transport has not yet been determined. Here we investigate whether physiological levels of fluid flow applied to living cells can sort cell surface proteins. We use fluorescence microscopy, microfluidic manipulation, and image analysis to quantify the spatial organization of cell surface components under applied shear flow. We also investigate the contributions of the cytoskeleton and plasma membrane lipid composition to protein mobility.

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FRET Analysis of the Nanoscale Organization of PI(4,5)P₂ in Living Cells

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Phosphatidylinositol-4,5-bisphosphate (PI(4,5)P₂) is a phospholipid concentrated in the inner leaflet of the plasma membrane, to which it recruits proteins involved in several cellular functions, many of which are abrogated in the absence of PI(4,5)P₂, illustrating the importance of this lipid. Protein regulation by PI(4,5)P₂ occurs as a result of spatially and temporally localized fluctuations of its concentration in the plasma membrane. In fact, the distribution of this lipid in the plasma membrane has been proposed to be heterogeneous, and PI(4,5)P₂ clustering is detected on model membranes under specific conditions. Domains highly enriched in PI(4,5)P₂ were also reported at the plasma membrane of specific cell types. However, for most cellular models, scarce evidence has been found for PI(4,5)P₂ segregation/clustering in the plasma membrane.

Here, we aimed to characterize the distribution of PI(4,5)P₂ in the plasma membrane of cells where no heterogeneity in PI(4,5)P₂ lateral distribution had been previously detected. To this end, FRET microscopy measurements with pleckstrin homology (PH) domains tagged with different fluorescent proteins were carried out. FRET microscopy data is evaluated through comparison with the theoretical expectation for FRET in the case of a homogeneous distribution of PH domains, and evidence for the formation of PI(4,5)P₂ enriched nanodomains is obtained. Results confirm that distinct PI(4,5)P₂ local densities are found in different cellular models, suggesting that PI(4,5)P₂ organization varies significantly between eukaryotic cells. In HeLa cells, disruption of the cytoskeleton decreased the compartmentalization of PI(4,5)P₂, proving that the organization of at least a pool of PI(4,5)P₂ molecules depends on the presence of membrane-cytoskeleton interactions.

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Membrane Curvature Enables N-Ras Lipid Anchor Sorting to Liquid-ordered Membrane Phases

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In vivo observations have suggested that the trafficking and sorting of membrane-anchored Ras GTPases are regulated by partitioning between distinct sphingolipid-sterol membrane domains. However, in vitro experiments have not been able to recapitulate such partitioning between liquid ordered (lo)/liquid disordered (ld) phases (I), suggesting that more complex physical principles influence Ras sorting into membrane domains in vivo.

We employed our single liposome assay¹ to study the recruitment by membrane curvature of the minimal anchor of the N-Ras isoform for ld or lo phase-state systems. This confocal microscopy based study revealed membrane curvature as a novel modulator of N-Ras lipid anchor partitioning (II) and that membrane curvature was essential for enrichment in raft-like lo phase (III).² Additionally we used microscopic molecular theory to elucidate that the curvature dependent lo enrichment was driven by increased relief of the lateral pressure in curved lo versus ld membranes.²

Ordered phases and curvature often coexist in vivo and recruit lipidated proteins, i.e. in trafficking vesicles or caveolae invaginations. Our results suggest that membrane curvature can allow cells to regulate lateral partitioning of lipidated proteins into such ordered membrane domains.

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Biophysical Studies of Myristoylated Unique and SH3 Domains of Src Kinase and their Interaction with Lipid Membranes

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C-Src is a member of the Src family of non-receptor tyrosine kinases, which are involved in many signaling pathways. It is composed of the N-terminal, the SH3, SH2, kinase and C-terminal domains, and is anchored to membranes via cooperative electrostatic and hydrophobic interactions. Indeed the N-terminal intrinsically disordered region (Unique Domain UD) is myristoylated. Weak interactions with lipids in the Unique and SH3 domains and intramolecular interactions between them were recently found in the non-myristoylated form. Our objective consisted in characterizing the myristoylated form of the Unique + SH3 domain (MyrUSH3). Binding kinetics to liposomes was followed using surface plasmon resonance (SPR) and revealed two MyrUSH3 populations, a dominant form binding with fast association and dissociation, and a minor persistently bound (PB) population not described earlier, found to possibly be MyrUSH3 dimers. In a construct in which the SH3 domain was replaced by the GFP protein, single molecule photobleaching experiments of these PB species bound to liposomes were conducted. A major population of dimers over the bilayer surface was detected. Lower affinity lipid binding regions in the UD and SH3 domains were studied by nuclear magnetic resonance (NMR), as well as their intermolecular interactions. The latter were conserved in presence of the myristoyl chain, but secondary lipid binding regions were found to behave differently in presence or absence of the acyl moiety. The SPR and fluorescence studies revealed autoassociation tendencies of the myristoylated N-terminal domain of c-Src upon binding to liposomes. NMR data highlighted the interplay between the lipid binding regions of UD and SH3 and the intermolecular interactions and revealed a myristoyl interacting loop in SH3. Thus, the myristoylated intrinsically disordered UD may act in c-Src regulation at the lipid bilayer interface.

QCM-D: A Powerful Surface Analysis Tool for Protein Studies

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No Abstract

Oligomerization of Pore Forming Proteins in Membranes at the Single Molecule Level**Ana J. Garcia Saez.**

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Pore forming proteins share the ability to pierce holes in the host membranes. They are usually synthesized in an inactive form which is monomeric and soluble. Activation includes membrane binding, where they undergo a conformational change followed by oligomerization and pore formation. The assembly pathway is a key step in the molecular mechanism of membrane permeabilization, but the underlying principles remain poorly understood. Here, I will present single molecule approaches that provide new insight into the assembly pathway of the apoptotic protein Bax and the sea anemone toxin Equinatoxin II, as representative examples of pore forming toxins.

Nanolipoprotein Particles Confined within Nanoporous Sol-gel or Bound to GUVs**Marjorie Longo.**

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Nanolipoprotein Particles (NLPs) are disc-shaped nanometer-sized lipid bilayer patches stabilized by a belt of scaffold proteins. NLPs have an average thickness of 5 nm, with a diameter ranging from 10-25 nm depending on the stoichiometric ratios and types of lipids and scaffold proteins being used. This allows NLPs to be compatible with the pore size (5-50 nm) of mesoporous silica. Therefore, we perform entrapment of NLPs using a quick, simple sol-gel processing technique for TMOS that includes evaporation of the majority of the methanol after the hydrolysis reactions. To ensure proper functioning of silica sol-gel entrapped NLPs, we have investigated the phase behavior of the lipids in addition to the secondary structure, localization, and environmental polarity of the scaffold proteins. Our results indicate that silica gel-entrapped NLPs remain intact, with only slightly altered lipid and scaffold protein structure and dynamics. We will briefly discuss the potential to entrap NLPs containing embedded integral membrane proteins (IMPs) for various applications such as biosensing, affinity chromatography, high-throughput drug screening, and bio-reaction engineering.

Further, scaffold proteins can bear polyhistadine tags, which are capable of chelating to Cu^{2+} metal ions. Lipid-phase specific, iminodiacetic acid (IDA) functionalized lipids are also capable of chelating Cu^{2+} , providing a mechanism for phase-targeted binding of NLPs. We investigate this binding via fluorescence microscopy and characterize interaction with phase-separated supported bilayers and giant unilamellar vesicles (GUVs). The thermodynamics (enthalpy of lipid mixing and steric pressure of protein crowding) and morphology of binding are also examined. Targeted binding of NLPs bearing functional IMPs and/or other biomolecules to supported lipid bilayers has a variety of applications, including development of nano-array technologies and biosensors.

PH-Triggered Conformational Signaling on Membrane Interfaces**Alexey Ladokhin.**

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The conversion of a protein structure from a water-soluble to membrane-inserted form is one of the least understood cellular processes. Examples include the cellular action of various bacterial toxins and colicins, tail-anchor proteins and multiple proteins of the Bcl-2 family, bearing pro-apoptotic and anti-apoptotic functions. In our lab we study Bcl-2 proteins as well as the diphtheria toxin (DT) T-domain, which undergoes conformational change in response to endosomal acidification, inserts into the lipid bilayer and translocates its own N-terminus and the attached catalytic domain of the toxin across the membrane. Our goal is to describe at the molecular level the mechanisms of pH-triggered conformational switching of the DT T-domain and apoptotic regulator Bcl-xL, which serve as models for membrane insertion/translocation transitions of structurally related proteins. Here we present our progress toward this objective, including structural, kinetic and thermodynamic characterization of the insertion pathway of the DT T-domain and Bcl-xL using both experimental and computational approaches. Our results indicate that insertion pathway of the T-domain contains several staggered pH-dependent transitions and that several key protonatable residues play unique roles in conformational switching. We find that physicochemical properties of the lipid bilayer modulate membrane interactions of Bcl-xL, suggesting that changes in lipid composition can play a role in apoptotic regulation.

Structural and Nanomechanical Features of Reconstructed Spectrin - Actin Membrane Cytoskeletons for Artificial Cell Realizations: an Atomic Force Microscopy Characterization

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The possibility to fabricate giant unilamellar vesicles (GUVs) composed of native membranes opens exciting opportunities for artificial cell synthesis. In particular, GUVs can be artificially prepared by electrosweeling from native erythroid membranes (erythroGUVs). Erythroid membranes are naturally furnished with a spectrin cytoskeleton that supports their mechanical resilience upon blood stream. Previously, we have shown a method to reconstruct spectrin skeletons onto erythroGUVs when incubated with ATP. Here, we present a detailed nano-structural study of artificial erythroGUV skeletons adsorbed onto glass cover slides performed with a combination of Atomic Force Microscopy (AFM) and fluorescence optical microscopy imaging. Three different kinds of filaments have been identified depending on the ATP concentration. At low ATP (μM), separate actin- and spectrin-enriched filaments can be observed. At high ATP concentrations (mM), a highly-connected network is formed, the links between the nodes being complex filaments composed by actin and spectrin. From nano-mechanical AFM measurements, a value of the Young modulus $E_{\text{sp-act}} = 0.4 \text{ MPa}$ is found for the complex filaments, whereas single spectrin- and actin-enriched fibers are found stiffer ($E_{\text{sp}} = 5$ and $E_{\text{act}} = 15 \text{ MPa}$, respectively). Upon further ATP, a reconstructed network emerges as a protein cytoskeleton that supports increased membrane rigidity in erythroGUVs.

Solvent Models in Protein Adsorption Simulations: Explicit, Implicit vs Experiments

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Molecular dynamics (MD) simulations with three different solvation models, atomic force microscopy (AFM) in liquid and single-molecule force spectroscopy are combined to access the suitability of these models in describing the adsorption of ImmunoglobulinG (IgG) antibodies over a hydrophobic surface modeled with a three-layer graphene slab. The MD simulations produce two contradicting results. On one hand, two different implicit solvation models based on the generalized Born methods predict that the IgG adsorption occurs with a severe protein unfolding in less than 40ns. On the other hand, explicit solvation models predict that the IgG antibodies are strongly adsorbed, do not unfold, retain their secondary and tertiary structure upon deposition. This conundrum, widely spread on the literature, is solved here by resorting to the conclusive experimental evidence. AFM measurements of the protein height and inter-domain distances only complies with the explicit solvent simulations. In addition, single-molecule force spectroscopy demonstrate that once adsorbed the IgG is still bioactive, which is in contradiction with the severe unfolding of the IgG in the implicit solvent simulations. Therefore, these findings, clearly demonstrate the inadequacy of widely used implicit solvent in modeling the protein adsorption process.

Peptides Forming Beta-Sheets on Hydrophobic Surfaces Cooperatively Promote Insulin Amyloid Aggregation.

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LMGP, Grenoble, France.

Protein stability and aggregation is a concerning issue for pharmaceutical industry. Insulin is one of the 20 human proteins know to form amyloid fibrils. For insulin, this kind of aggregation in physiological conditions is dependent on surface adsorption. In particular hydrophobic and charged material surfaces to which insulin is exposed during its dissolution, formulation and storage can trigger amyloid fibril formation. The typical kinetic of this aggregation is divided into 3 steps: the lag phase, during which surface-adsorbed aggregation nuclei are formed, the growth phase (fast aggregation phase) and a plateau (end of aggregation phase). We study the mechanism of surface-dependent aggregation in vitro and use small adsorbed peptides as mediators (enhancers or inhibitors) of aggregation. In particular, we have shown that peptides adopting a beta-sheet structure on hydrophobic surfaces are able to accelerate insulin aggregation in a cooperative manner. The cooperativity observed is likely based on the formation of small peptide patches on the surface. These peptide patches stabilize insulin adsorption as well as their own and therefore enhance the formation of aggregation nuclei and reduce the lag time. These results may lead to a better understanding of the formation of material-surface triggered amyloid formation and can have direct applications in developing new ways of preventing therapeutic proteins from aggregation in vitro.

Harnessing Antibodies at an Electrode Surface: Electrical Control of IgG Conformation and Functional Activity

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We have devised a supramolecular edifice involving His-tagged protein A and antibodies to yield surface immobilized, uniformly oriented IgG layers with Fab fragments exposed off a gold electrode surface. We demonstrate here that we can affect the conformation of immobilized IgGs, likely pushing/pulling electrostatically Fab fragments towards/from the electrode surface. This result is achieved by the action of a potential applied to the electrode with respect to solution that acts on IgGs' positively charged aminoacids (Lys and Arg). Such an action results, on its turn, in a modulation of the accessibility of the specific recognition regions of Fab fragments by antigens in solution. As a consequence, antibody binding affinity to antigens turns out to be affected by the sign of the applied potential: a positive potential, pushing Fab fragments towards solution, enables an effective capture of antigens; a negative one pulls the fragments towards the electrode, where steric hindrance caused by neighboring molecules largely hampers the capture of antigens. A bunch of different yet concurrent experimental techniques has been used to measure binding kinetics and surface coverage, to evaluate the effect of the applied electric field on IgGs, and to point out the key role of positively charged residues in determining the phenomenon described here. Those techniques include EC-QCM, EIS, Fluorescence confocal microscopy and ECAFM.

The reported findings expand the concept of electrical control on biological reactions and can be used to gate electrically specific recognition reactions with far reaching consequences in biosensors, bioactuators, smart biodevices, and nanomedicine in general [1].

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Ubiquitin Adsorbs on the TiO₂ (100) Surface by an Anchor-Lock Mechanism

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Proteins that unfold from their native states when adsorbed to inorganic surfaces can potentially cause neurodegenerative diseases, including Parkinson's and Alzheimer's disease. Conversely, proteins that retain their folded state when adsorbed to inorganic surfaces can act as favorable binding sites to other biomolecules, depending on orientation and whether that orientation stays fixed. When no unfolding occurs, the surface-adsorbed protein structure can be described by a translation and subsequent rotation of the native folded state. We used atomistic and coarse-grained molecular dynamics simulations to analyze the adsorption of ubiquitin – one of the most common proteins in living organisms – to the fully hydrated TiO₂ (100) surface – one of the most common engineered nanoparticle surfaces. Our simulations show that ubiquitin is a rigid body during the adsorption process. We extracted the orientation angles for ubiquitin from the optimal rigid body rotation matrix with regard to a known reference structure. The analysis revealed that the tail of ubiquitin acts as an "anchor" for the otherwise rigid protein, and attaches to the first solvation layer at the TiO₂ surface. The protein can rapidly "lock" into its preferred adsorbed orientation once the anchoring is complete. The atomistic results were in agreement with the full map of adsorbed orientations determined from coarse-grained modeling of TiO₂-protein interactions. The simulations emphasize the importance of strongly bound surface waters for the adsorption behavior of solvated proteins at polar interfaces.

Control of Protein Adsorption and Protein-induced Membrane Deformation by Phospholipid Unsaturation

Bruno Antonny.

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Membrane bound organelles differ by their shape and lipid composition. Gradients of phospholipids with different fatty acid profiles are observed along the secretory pathway (endoplasmic reticulum > Golgi > plasma membrane) and in differentiated structures (e. g. axons). Using a combination of biochemical and biophysical measurements, of cell biology observations and of molecular dynamics simulations, we are exploring the role of phospholipid unsaturation on the physicochemical properties of cellular membranes. Our work reveals that membrane curvature and lipids with monounsaturated acyl chains cooperate for the formation of large lipid packing defects that can be recognized by peripheral proteins with bulky hydrophobic residues. In contrast, polyunsaturated phospholipids dampen the effect of membrane curvature on large lipid packing defects, owing to their ability to adopt various bent conformations. As a result, membrane fission is facilitated. These findings give a rationale for the high levels of monounsaturated phospholipids in membranes with biosynthetic functions (endoplasmic reticulum) and the high levels of polyunsaturated phospholipids in membranes undergoing very fast shape changes (synaptic vesicles).

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Membrane Curvature Induces Scaffolding by BAR-Domain Proteins

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Cell plasma membranes are highly deformable and are strongly curved upon membrane trafficking or during cell motility. BAR-domain proteins with their intrinsically curved shape and their interaction with the actin cytoskeleton are involved in many of these processes. We have used in vitro experiments to study the interaction of BAR-domain proteins with curved membranes for understanding how the BAR-domain protein endophilin A2 can scission tubules, which are formed for instance upon Shiga toxin internalization. We have pulled membrane nanotubes of controlled curvature from Giant Unilamellar Vesicles (GUVs) using optical tweezers and micropipette aspiration. With this approach coupled to theoretical modeling, we have shown that endophilin A2 scaffolds and stabilizes tubes in static conditions but induces scission when the tube is dynamically extended. We have also shown that, with our tube assay, scission is independent of the presence of N-terminal amphipathic helices on their BAR domain, in contrast with previous studies on small vesicles.

Many Weak Interactions Make a Difference – from the Self Assembly of Intrinsically Disordered Proteins to Superselective Targeting**Ralf Richter**^{1,2,3}.¹CIC biomaGUNE, San Sebastian, Spain, ²Université Grenoble Alpes - CNRS, Grenoble, France, ³Max-Planck-Institute for Intelligent Systems, Stuttgart, Germany.

Multivalent interactions are frequent in biological systems. They are key to the regulation of many biomolecular recognition events and to the self-organization of biomolecules into materials. This is particularly so at surfaces and interfaces, because these naturally provide a platform for the multivalent presentation of binding partners. Despite their importance, multivalent interactions remain poorly understood. In this lecture, I shall present results of our efforts to better understand the role of multivalent interactions in two biological systems: (i) the nuclear pore permeability barrier, a meshwork of intrinsically disordered proteins that fills the nuclear pores and makes nucleo-cytoplasmic transport selective, and (ii) the interface between polysaccharide-rich extracellular matrix and the cell surface which is key to the communication of cells with their environment. In order to study these systems directly on the supramolecular level, we have developed an unconventional approach that draws on knowledge from several scientific disciplines. Exploiting surface science tools, we tailor-make model systems by directed self-assembly of purified biomolecules (proteins, lipid and polysaccharides) on solid supports. With a toolbox of biophysical characterization techniques, including QCM-D, ellipsometry, AFM and RICM, these model systems are then investigated quantitatively and in great detail. The experimental data, combined with soft matter physics theory, allow us to develop a better understanding of how the properties of the individual molecules and interactions translate into supramolecular assemblies with distinct physico-chemical properties. The insights gained help us to uncover physical mechanisms underlying biological functions (e.g. ‘superselective targeting’ of the cell surface by the polysaccharide hyaluronan) and may also lead to novel applications in the life sciences.

The Role of the Membrane in Protein Pattern Formation

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The Min protein system of the bacterium *Escherichia coli* is a beautiful example of how protein self-organization and pattern formation occurs in the fluid phase and on membranes via reaction-diffusion. Reconstituted onto supported or free-standing membranes, and supplied with energy in the form of ATP, the proteins MinD and MinE, which are in live cells responsible for positioning the cell division machinery, self-organize into parallel concentration waves that can be faithfully directed by structuring the membrane laterally or topologically. In my talk I will discuss the role of membrane binding in the emergence of patterns and protein gradients, and highlight the archetypical role that switchable membrane anchors, such as lipidation, may have in many polarity and/or pattern forming systems in biology. I will further discuss the role of membrane structure and topology on the emergence of Min patterns, and briefly explore the role of membrane charge and local lipid order. Finally, I will develop our concept of reconstituting a minimal version of cell division based on the essential modules of the bacterial divisome.

GM₁ Nanodomains Inhibit the Oligomerization of Membrane Bound β -amyloid Monomers

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Oligomers of β -amyloid (A β) are thought to spark neuronal dysfunction, cell death and Alzheimer disease onset.¹ The monosialoganglioside GM₁ has been suggested to seed aggregation of A β and thus enhance the formation of A β 's cytotoxic oligomers.² However, such studies are commonly performed in system with high concentrations of GM₁, sphingomyelin, cholesterol, and amyloid peptides, thus their pertinence to the physiological oligomerization of A β on cellular membranes might be low. In this work, we strive to emulate more physiological conditions.

The oligomerization of A β on lipid bilayers containing essential components of the neuronal plasma membrane is addressed using the single molecule sensitivity of fluorescence. The oligomerization of A β is characterized by changes of its lateral diffusion coefficient and by Cross-Correlation Fluorescence Correlation Spectroscopy.³ A novel Fluorescence Lifetime Förster Resonance Energy Transfer approach⁴ is used to characterize heterogeneities on the nanometer scale in lipid bilayers.

We find that sphingomyelin is a key trigger for the in-membrane oligomerization of A β monomers. Physiological levels of GM₁, organized in nanodomains, do not seed A β 's oligomerization. Moreover, GM₁ counteracts the effect of sphingomyelin and prevents the oligomerization of A β . This molecular evidence for GM₁ as an inhibitor of the oligomerization of A β supports the idea of GM₁ as a protective factor⁵, rather than GM₁ as an enhancer of the toxic oligomerization of A β .

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Synaptic Vesicle Lipids Reveal Structured State and Antagonistic Allosteric Mechanisms of Intrinsic Disorder within Synaptotagmin I**Michael E. Fealey**^{1,2}, Anne Hinderliter².¹University of Minnesota, Minneapolis, MN, USA, ²University of Minnesota Duluth, Duluth, MN, USA.

Synaptotagmin I (Syt I) is a vesicle-localized integral membrane protein responsible for sensing the calcium influx that triggers neurotransmitter release. Syt I consists of a transmembrane helix, a cytosolic 60 residue tether region, and two C2 domains that bind calcium and acidic phospholipids. Until recently, the role of the 60 residue tether region in Syt I function received little attention. We noticed that this tether region has features of intrinsic disorder and hypothesized that it exerts allosteric control over the adjacent calcium binding C2 domains to tune protein function. In testing this hypothesis, we first assessed the impact of local lipid environment on the structure of the isolated tether region. Using differential scanning calorimetry (DSC) and nuclear magnetic resonance, we found that a lipid composition mimicking a synaptic vesicle selects for ordered conformers in the intrinsically disordered sequence. A simple binary lipid mixture did not have any apparent ordering impact. Knowing the intrinsically disordered region was influenced by a membrane that mimics its native organelle, we next assessed its allosteric impact on the first C2 domain (C2A) using DSC. Strikingly, we found that discrete regions of the disordered tether had opposite effects on C2A unfolding. Calcium binding to C2A in the presence of the entire disordered region shifts the unfolding transition to low temperature. In contrast, when the 13 most N-terminal residues (mostly lysines) are removed, calcium binding dramatically increases the temperature of the unfolding transition. In both cases, calcium binding stabilizes the protein but the extent differs as do the contributions of each thermodynamic parameter underlying the transition. These results indicate that the combine intrinsic disorder and synaptic vesicle lipids have the ability to evoke antagonistic responses of Syt I to calcium ligation.

Scanning Protein Surface with a Solvent Mimetic Probe: an NMR Approach

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Changes in the solvent accessible surface area (SASA) of proteins underlie protein folding, molecular recognition phenomena, and assembly of complexes. Nevertheless, this fundamental parameter becomes elusive for experimental scrutiny. Methylene carbene (MC) reacts with polypeptides allowing an estimate of the magnitude and nature of SASA. MC arises from the photocleavage of diazirine (DZN), a tiny heterocycle similar in size and shape to water, thus able to exert solvent mimicry. MC is an extremely reactive species that inserts readily into X-H bonds (X=C, O, S or N). Coupled to radioactivity or mass spectrometry detection, DZN labeling becomes useful to probe folding and interactions. By contrast, multidimensional NMR does not demand cleavage of the polypeptide, potentially opening a rich panorama on structure. General methylation was assessed in *E. coli* thioredoxin (TRX), where the insertion of MC at multiple sites across the surface was verified. ¹H-NMR spectra of progressively reacted TRX show a significant enrichment in the aliphatic region, supporting the dominant insertion event into C-H bonds belonging to amino acid side-chains. Remarkably, buried residues such as Val 16 remain unmodified. Consistently, ¹H,¹³C-HSQC spectra uncover new cross-peaks corresponding to water exposed methyl groups, while concurrently protein methylene signals disappear. On the other hand, ¹H,¹⁵N-HSQC spectra reveal the impact of side-chain methylation on backbone amide environments. Discrete alterations occur at indole groups of partially exposed Trp 28 and 31. By contrast, backbone methylation itself is a rare occurrence. Because of its mild reaction conditions and strong focus on side-chain modification, DZN labeling adds its unique value to current footprinting methods, such as H/D exchange and hydroxyl radical reactions. Collectively, NMR analysis of MC modified proteins offers a fertile source of information upon which a full map of solvent accessibility can be built.

Dynamic Interactions of Protein Elements of the Bacterial Division Machinery Evidenced in Phospholipid Bilayer Nanodiscs

Victor Hernandez-Rocamora^{1,3}, Silvia Zorrilla¹, Carlos Alfonso¹, Allen Minton², Miguel Vicente¹, **German Rivas**¹.

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The first molecular assembly of the bacterial division machinery is the proto-ring, which in *E. coli* is formed as a result of the anchoring of FtsZ (a self-assembling GTPase ancestor of cytoskeletal tubulin) to the cytoplasmic membrane by the action of FtsA (an amphitropic protein) and ZipA (a bitopic membrane protein) [1]. We have studied the activities, interactions and assembly properties of FtsZ in ZipA-containing nanodiscs by means of analytical ultracentrifugation and fluorescence-based techniques, combined with electron microscopy and biochemical assays [2]. Nanodiscs are structures formed by a membrane scaffold protein encircling a phospholipid bilayer, which can incorporate membrane proteins preserving their natural properties while behaving as soluble entities [3]. These results have been exploited to optimize the reconstitution of proto-ring elements in giant vesicles [4,5] to gain new insights into the precise functions of protoring elements in cell division events.

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What Cholesterol is Doing in the Plasma Membrane

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The molecular basis for the essential role of specific sterols in supporting particular cell growth (for example, cholesterol in mammalian cells and ergosterol in yeast cells) has long been the object of intense interest. Cholesterol modulates the function of particular mammalian membrane proteins critical to cellular function. Ergosterol modulates the activity of particular yeast membrane proteins. Experimental data support primarily two mechanisms for this modulation by sterols. In one mechanism, the requirement of "free volume" by integral membrane proteins for conformational changes as part of their functional cycle is antagonized by the presence of high levels of cholesterol in the membrane. This results from the membrane ordering promoted by cholesterol. In the other mechanism, the sterol modulates membrane protein function through direct sterol-protein interactions. Sterols bind to the membrane protein and act as effectors modulating protein activity. Biochemical experiments show binding of cholesterol to some membrane proteins. Recent X-ray crystal structures of some of these same proteins reveal the details of the cholesterol binding site. This mechanism provides an explanation for the modulation of the activity of important membrane proteins and for the essential requirement of a structurally-specific sterol for cell viability.

Spatiotemporal Organization of Receptors in Living Cell Membrane Surfaces

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A hot topic in cell biology is to understand the specific nanometer-scale organization and distribution of the surface machinery of living cells and its role regulating the spatiotemporal control of different cellular processes. Cell adhesion, pathogen recognition or lipid-mediated signaling, all fundamentally important processes in immunology, are governed by molecular interactions occurring at the nanoscale. From the technical point of view, 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 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 constraints 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. 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. 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. For nanoscale imaging, we have recently pushed the limits of spatial resolution by demonstrating dual colour imaging of individual fluorescent molecules with true 20nm spatial resolution and sub-nanometre localization accuracy using antenna probes. In parallel, we have recently demonstrated that photonic antennas allow the recording of individual lipid diffusion on living cell membranes in regions as small as 20nm in size.

Protein Structural Dynamics at Surfaces as Studied by Infrared Nanospectroscopy

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Membrane proteins are the target of more than 50% of all drugs and are encoded by about 30% of the human genome. Electrophysiological techniques, like patch-clamp, unravelled many functional aspects of membrane proteins but suffer from structural sensitivity. We have developed Surface Enhanced Infrared Absorption Spectroscopy (SEIRAS) to probe the structure and function of solid-supported biomembranes and membrane proteins on the level of a monolayer. In a new approach, we monitored the expression, insertion and folding of membrane proteins by SEIRAS. A cell-free assay to express bacteriorhodopsin (bR) was used and insertion and folding of the nascent polypeptide into surface-tethered lipidic nanodiscs was followed in-situ and time-resolved by SEIRAS to resolve this complex reaction via the analysis of the amide I vibration of the peptide backbone (C=O stretching vibration).

The structure of the native environment of bR in the purple membrane was probed by scanning near-field IR microscopy. Mapping of the protein structure with 30 nm spatial resolution and sensitivity to individual protein complexes by Fourier transform infrared nano-spectroscopy (nano-FTIR) was demonstrated. The first broadband infrared spectra of purple membranes were recorded indicating their local α -helical structure.

High-speed Atomic Force Microscopy: The Dynamics and Interaction of Protein and Membrane Surfaces

Simon Scheuring, Lorena Redondo, Atsushi Miyagi, Felix Rico, Ignacio Casuso.
INSERM / Aix-Marseille University, Marseille, France.

The advent of high-speed atomic force microscopy (HS-AFM; [1]) has opened a novel research field for the dynamic analysis of single bio-molecules [2,3,4,5]. The endosomal sorting complex required for transport (ESCRT) mediates membrane remodeling in cells. We used HS-AFM to study the ESCRT-III complex, i.e. Snf7. HS-AFM movies reveal Snf7 complex formation from filaments to matured assemblies: Interfilament dynamics provide basis for a mechanistic understanding of tension generation for membrane fission [6]. Annexin-V (A5) binds to negatively charged lipid bilayers in the presence of Ca²⁺ for membrane healing. Using a HS-AFM coupled to a buffer exchange flow system, we found two classes with different apparent affinity in the reversible association-dissociation of A5 to the membrane [7].

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Spatial Organization of Na⁺/K⁺-ATPase and Lipid Domains in Free-standing Membranes Captured on a Solid Support

Tripta Bhatia¹, John Hjort Ipsen¹, Flemming Cornelius², Ole G. Mouritsen¹, **Adam Cohen Simonsen¹**.

¹University of Southern Denmark, Odense M, Denmark, ²Aarhus University, Aarhus C, Denmark.

Imaging the lateral organization of membrane proteins and coexisting lipid phases at the nanoscale holds the key to establish a link between membrane structure and function. A dilemma has been that available high-resolution imaging techniques are often only applicable to supported membranes while information on the free-standing analog is wanted. As a possible solution, we demonstrate a general methodology to fixate and image giant unilamellar vesicles (GUVs) at sub-optical length scales. Individual GUVs are rapidly transferred to a solid support forming planar bilayer patches. These are taken to represent a fixated state of the free-standing membrane, where in-plane spatial structures are kinetically trapped.

High-resolution images of domain patterns in the liquid-ordered (lo) and liquid-disordered (ld) co-existence region in the phase-diagram of ternary lipid mixtures are revealed by Atomic Force Microscopy (AFM) scans of the patches. Macroscopic phase separation as known from fluorescence images is found, but with superimposed fluctuations in the form of nanoscale domains of the lo and ld phases. The size of the fluctuating domains increases as the composition approaches the critical point, but with the enhanced spatial resolution, such fluctuations are detected even deep in the coexistence region.

Likewise, we demonstrate the reconstitution of the Na⁺/K⁺-ATPase pump in GUVs and the subsequent collapse to planar patches. AFM imaging reveals the spatial organization of pumps with single molecule resolution both in host-membranes with one fluid phase and in membranes containing two coexisting fluid phases. We comment on the localization of pumps with respect to the domain border and the influence of pump activity on the stability of the patches.

Single-Molecule Visualisation of the Mechanisms of Autoinhibition and Dysregulation of the EGFR on Living Cell Membranes

Laura C. Zanetti Domingues¹, Sarah R. Needham¹, Christopher J. Tynan¹, Dimitrios Korovesis¹, Selene K. Roberts¹, David T. Clarke¹, Michela Perani², Peter J. Parker^{2,3}, Daniel J. Rolfe¹, Marisa Martin-Fernandez¹.

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EGFR family receptors are involved in a variety of epithelial tumours. While the solution structure is well-known, the mechanisms that regulate its activity at the membrane level are less well understood. In particular, the conformational linkage between extracellular and intracellular domains and the effect of inhibitors on full-length structure are yet to be fully elucidated.

By combining multicolour Single-Particle Tracking (SPT), fluorescence localisation imaging with photobleaching (FLImP) at a resolution of <7 nm, and fluorescent resonance energy transfer (FRET) on a stably transfected CHO cell model, we have probed the geometry and the ability of EGFR to oligomerise and activate under perturbations that disrupt regulation mechanisms proposed by studies of isolated EGFR domains.

We have observed conformational coupling across the membrane in intact cells, and confirmed a role for the extended conformation activation, as suggested by crystallographic studies.

Moreover, we have confirmed the existence of ECD-mediated quasi-dimers in the basal state, and the inhibitory role of plasma-membrane/kinase domain interactions.

Finally, we have uncovered an intermediate ligand-independent conformation in which the kinase domains and C-terminus associate to reduce the energy of activation, which results in hyper-activation in presence of EGF. This conformation is achieved by blocking protein-lipid interactions or inhibitor treatment.

In summary, our investigations have managed to assemble a coherent view of how intact EGFRs are regulated in their native membrane environment.

Furthermore, we have identified an intermediate oligomeric association driven by tyrosine kinase inhibitors, which might be relevant for resistance mechanisms.

High Resolution Imaging Atomic Force Microscope Study of Interactions at the Membrane-Fluid Interface

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The cell membrane is essential for all living systems, serving as a barrier between cells and their environment. It is typically composed of a lipid bilayer, containing embedded and/or anchored proteins that mediate different biological function such as energy conversion, signal transduction and solute transport [1]. To elucidate the basic structure of biological membranes it is necessary to make direct experimental observations of the molecular organization of the protein and lipid bilayer under physiologically relevant conditions. Using a bespoke high resolution Atomic Force Microscopy (AFM) it is possible to characterize the structure and function of both native and model lipid membranes with embedded proteins [2]. During this study we focus our attention on transmembrane MvP voltage-gated potassium channels embedded in a lipid bilayer, which are activated by changes in transmembrane potential [3]. High resolution AFM images of the membrane channel reveal the predicted tetrameric channel structure of the ion channel. Interestingly, we observed the formation of an asymmetric depression in the supporting lipid membrane surrounding the channel. This may be due to an alteration in the lipid bilayer structure to accommodate the ion channel. Using AFM is possible to investigate the interactions between proteins and membrane- liquid interface [4], [5] aiding our understanding and leading to future therapeutic application.

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Germanium Catches Proteins in Action

Jonas Schartner, Jörn Güldenhaupt, Konstantin Gavriljuk, Andreas Nabers, Klaus Gerwert, Carsten Kötting.
Biology & Biotechnology, Bochum, Germany.

The attenuated total reflection fourier transform infrared (ATR-FTIR) spectroscopy allows a detailed analysis of surface attached molecules, including their secondary structure, reaction mechanism, orientation, and interaction with small molecules.[1] This technique reveals vibrational changes in the attached molecules. We recently developed a universal immobilization technique for the specific immobilization of N-Ras and Photosystem I on a silane modified germanium surface.[1]

We now present a new approach employing thiol chemistry on germanium.[2] On one hand germanium crystals provide a great signal-to-noise ratio in ATR-FTIR. On the other hand protein immobilization via thiol chemistry is well-established because it is standard for modifications of gold surfaces e.g. in surface plasmon resonance. Here we combine the best of both worlds and report on germanium surface functionalization with different thiols which allowed for specific immobilization of histidine-tagged proteins with over 99% specific binding. The great advantages of using thiols in comparison with silanes are that a huge variety of thiols with functional groups is commercially available and the monolayer stability is very high. Nativity of protein folding was confirmed by secondary structure analysis. Stimulus induced difference spectra were obtained for immobilized Channelrhodopsin 2, the small GTPase N-Ras and the phosphocholine-transferase AnkX, which demonstrated protein function at atomic level.[3] To further improve the S/N ratio the establishment of a 3D-surface was achieved by tethering dextran-polymers to germanium. Proteins were immobilized in multilayers with a distance of about 9 nm as shown for mCherry and GFP.[4] The difference signal was increased by more than factor three when tris-ANTA was employed for catching proteins in action.

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POSTER ABSTRACTS

POSTER SESSION I

Tuesday, October 13, 13:30 – 15:00
Hall of the main building of Biology Faculty

All posters are available for viewing during all three poster sessions; however, below are the formal presentation times when presenters are required to remain in front of their poster boards to meet with attendees.

Aguilar, Mibel	1-POS	Board 1
Binette, Vincent	4-POS	Board 4
Carravilla, Pablo	7-POS	Board 7
Dos Santos Cabrera, Marcia	10-POS	Board 10
Ganesan, Sai Janani	13-POS	Board 13
Goni, Felix	16-POS	Board 16
Inaba, Takehiko	19-POS	Board 19
Knowles, Michelle	22-POS	Board 22
Lubart, Quentin	25-POS	Board 25
Raso, Ana	28-POS	Board 28
Sayar, Mehmet	31-POS	Board 31
Stüber, Jakob	34-POS	Board 34
Truong, Khuong	37-POS	Board 37

Posters should be set up on the morning of October 13 and removed by 18:00 October 15.

1-POS Board 1

Helix 8 of the Angiotensin-II Type 1a Receptor Interacts With Phosphatidylinositol Phosphates and Modulates Membrane Insertion

Daniel J. Hirst¹, Tzong-Hsien Lee¹, Walter G. Thomas², **Mibel Aguilar**¹.

¹Monash University, Clayton, Australia, ²The University of Queensland, St Lucia, Vic, Australia.

The carboxyl-terminus of the type 1 angiotensin II receptor (AT1A) regulates receptor activation/deactivation and the amphipathic Helix 8 within the carboxyl-terminus is a high affinity interaction motif for plasma membrane lipids. We have used dual polarisation interferometry [1] to examine the role of phosphatidylinositides in the specific recognition of Helix 8 in the AT1A receptor. A synthetic peptide corresponding to Leu305 to Lys325 (Helix 8 AT1A) discriminated between PIPs and different charges on lipid membranes [2]. Peptide binding to PtdIns(4)P-containing bilayers caused a dramatic change in the birefringence (a measure of membrane order) of the bilayer. Kinetic modelling showed that PtdIns(4)P is held above the bilayer until the mass of bound peptide reaches a threshold, after which the peptides insert further into the bilayer. This suggests that Helix 8 can respond to the presence of PI(4)P by withdrawing from the bilayer, resulting in a functional conformational change in the receptor.

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4-POS Board 4

Probing the Anchoring of the Huntingtin N-Terminal on a Phospholipid Bilayer Using All-Atom Simulations

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Huntington's disease is characterized by motor dysfunctionalities and the loss of cognitive function and associated with the aggregation of the huntingtin protein into amyloid fibrils. The exon1 of huntingtin is crucial because it is sufficient to reproduce the phenotypes and aggregation features of the Huntington disease. It is composed of a 17 amino acids sequence (Htt17) at its N-terminal, a polyglutamine repeat (Q_N) domain and a proline rich domain (C38). Huntingtin's aggregation is triggered when its Q_N domain surpasses the threshold of 36 glutamines. Htt17 is particularly important because of its potential role as a membrane anchor that could accelerate the fibrillation process. Recent solution and solid-state NMR experiments have unveiled the structure and the orientation of Htt17 in DPC micelles and POPC bilayer [1]. Here, we use all-atom explicit solvent molecular dynamics (MD) and Hamiltonian replica exchange (HREX) simulations to refine the experimental picture focusing, in particular, on the characterization of the dynamic and thermodynamic of the proposed NMR model inside a phospholipid bilayer. We find that the fully formed α -helix is more stable in the membrane than the proposed NMR model in micelles and that Htt17's hydrophobic plane is almost parallel to the membrane. In this position, key nonpolar residues are deeply inserted and hidden from the solvent. Simulations also reveal localized membrane perturbation around Htt17 due to the extension of neighbor phospholipid acyl chains to cover the nonpolar surface of Htt17. These types of membrane deformations were shown to promote dimerization. Htt17 dimerization could therefore be initiated by electrostatic interactions as the charged residues stay mostly accessible to the solvent. Htt17 dimers could form large aggregates that radically change the membrane properties and permeation.

1. Michalek, M. et al., *Biochemistry*, (2013)

7-POS Board 7

Cholesterol-dependent Membrane Fusion Induced by the HIV-1 GP41 MPER-TMD and Blocking by Antibodies Functioning at Membrane Surfaces

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Anti-HIV antibodies 4E10 and 10E8 neutralize practically all viral strains and isolates tested in standard assays. These ‘pan-neutralizing’ antibodies bind to the gp41 membrane proximal external region (MPER)-transmembrane domain (TMD) junction at the membrane surface of virions poised for fusion and block the process. The resulting broad neutralization underscores the conservation and functionality of the MPER-TMD region. In recent work, we have described that peptides representing this region have potent membrane-destabilizing effects. Here, based on the outcome of NMR structural studies, vesicle assays, atomic force microscopy characterization and molecular dynamics simulations, we propose a mechanism for the involvement of the MPER-TMD region in HIV-1 fusion, which is dependent on the high cholesterol content accumulated in the viral envelope. In addition, we provide evidence that underpins the potential use of its activity as a new target for inhibitor and immunogen development.

10-POS Board 10

Amphiphilic Derivatives of Chitosan Hold Similarities with Antimicrobial Peptides in Their Mechanism of Action

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Chitosans have been modified and functionalized to improve their action as antimicrobials. Antimicrobial chitosans may target the phospholipid matrix of cell membranes and this has been considered a target towards which resistance is hardly developed. In this work two chitosan derivatives were designed to gain hydrophobic and cationic character at different ratios. They were synthesized and evaluated for their features as amphiphilic compounds and for their interaction with three model membranes of different lipid composition, mimicking bacterial, fungal and erythrocyte membranes. Antimicrobial and cytotoxic activity was also assessed, demonstrating that both derivatives present antimicrobial activity but just the one with higher cationic character showed cytotoxicity towards human cervical carcinoma cells. The strategy used provided chitosan derivatives with intense lytic activity, inducing more than 70% dye leakage and similar behavior for both derivatives in the anionic model membranes. The less charged compound exhibits a slightly improved selectivity in relation to the anionic over the zwitterionic bilayers. This effect indicates that it is possible to balance the hydrophobic/hydrophilic character to improve the efficiency of chitosan derivatives. The disturbance of the lipid acyl chain order was also observed, and together with zeta potential measurements we hypothesize that the “charge cluster mechanism” ascribed to some antimicrobial peptides could be applied to these chitosan derivatives.

13-POS Board 13

Coarse Graining to Investigate Membrane Induced Peptide Folding of Anticancer Peptides

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Information about membrane induced protein folding mechanisms using all-atom molecular dynamics simulations is a challenge due to time and length scale issues. On the same hand, coarse-grained (CG) modeling has made a significant impact on our understanding of multiple processes, from self assembly of lipid bilayers to amyloid-fibril formation. However, there is a lack of a low resolution, transferable model to study mechanisms of peptide folding in a membranous environment. We recently developed a low resolution Water Explicit Polarizable PROtein coarse-grained Model (WEPPROM) by adding oppositely charged dummy particles inside protein backbone beads. These two dummy particles represent a fluctuating dipole, thus introducing structural polarization into the coarse-grained model. With this model, we were able to achieve significant α/β secondary structure content de novo, without any added bias. We extended the model to zwitterionic and anionic lipids, by adding oppositely charged dummy particles inside polar beads, to capture the ability of the head group region to form hydrogen bonds. Our models have their roots in the MARTINI force field. We use zwitterionic POPC (Palmitoyl Oleoyl Phosphatidyl Choline) and anionic POPS (Palmitoyl Oleoyl Phosphatidyl Serine) as our model lipids, and a cationic antimicrobial peptide with anticancer activity, SVS1, as our model peptide. In this work, we characterize the driving forces for SVS1 folding on lipid bilayers with varying anionic and zwitterionic lipid compositions. We use SVS1 mutants that do not fold as our negative peptide control. Peptides are used as model systems to understand protein behaviour. Based on our results, membrane induced peptide folding is driven by both (a) cooperativity in peptide self interaction and (b) cooperativity in membrane-peptide interaction. This work compares and contrasts the relationship between lipid composition and its role in peptide folding.

16-POS Board 16**Ceramide Increases Free Volume Voids in DPPC Membranes**

Felix Goni, Eneko Axpe, Aritz B. Garcia-Arribas, Jon I. Mujika, Alicia Alonso, Jesus M. Ugalde, Fernando Plazaola.
Universidad del Pais Vasco, Leioa, Spain.

Positron annihilation lifetime spectroscopy (PALS) can measure changes in local free volume voids in lipid bilayers. PALS has been applied, together with differential scanning calorimetry (DSC) and molecular dynamics (MD) simulations, to study free volume voids in DPPC and DPPC : ceramide (85 : 15 mol : mol) model membranes in the 20–60 °C range. The free volume void average size clearly increases with the gel–fluid phase transition of the lipid, or lipid mixture. Ceramide increases void size at all temperatures, particularly in the range causing the gel–fluid transition of the mixture. A parallel study of PALS and calorimetric data indicates that, for the complex thermotropic transition of the DPPC–ceramide mixture, PALS is detecting the transition of the DPPC component, while calorimetry changes indicate mainly the melting of the ceramide-enriched domains. Molecular dynamics calculations provide a clear distinction between ceramide-rich and poor domains, and show that the voids are predominantly located near the membrane nodal plane. The ceramide-induced increase in void volume size occurs as well at temperatures when both phospholipid and ceramide are in the fluid state, indicating that the effect is not the result of phospholipid–ceramide domain coexistence. The above observations may be related to hitherto unexplained properties of ceramide, such as the increase in membrane permeability, and the induction of transmembrane (flip-flop) lipid motion.

19-POS Board 19**Phospholipase C β 1 Induces Membrane Tubulation in a Phosphatidylethanolamine-dependent Manner.**

Takehiko Inaba¹, Takuma Kishimoto¹, Takuya Tajima², Shota Sakai¹, Mitsuhiro Abe¹, Motohide Murate¹, Asami Makino¹, Nario Tomishige¹, Reiko Ishitsuka¹, Shinji Takeoka², Toshihide Kobayashi¹.

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Lipid membrane curvature plays important roles in various physiological phenomena. Using darkfield microscopy, we performed non-biased screening of a protein that induces deformations of non-labeled liposomes. We identified that phospholipase C β 1 (PLC β 1) induces tubulation of the liposome containing phosphatidylethanolamine and phosphatidylserine. Interestingly, the characteristic C-terminal sequence of PLC β 1, but not the conserved regions of PH domain or catalytic domains, is essential for the tubulation of liposomes. Our results indicate that hybrid structure of PLC β 1 controls its enzyme activity through sensing and/or modulation of the membrane curvature by the C-terminal domains.

22-POS Board 22

The Membrane Binding of C-reactive Protein Depends on Conformational State

Aml Alnaas, Carrie L. Moon, Mitchell Alton, **Michelle Knowles**.
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C-reactive protein (CRP) is an immune system protein that serves to protect the body by binding to damaged membranes. CRP is actively involved with the clearance of apoptotic cells and oxidized LDL by binding to the phosphatidylcholine (PC) head group in a calcium dependent manner. How CRP recognizes only damaged membranes, as opposed to healthy PC-containing membranes, is an interesting question that we aim to address in our research. Previous work on CRP suggests that there are at least two conformational states and these have different physiological function. In our work, we tested the membrane binding capabilities of two conformers, pentameric CRP and monomeric CRP. Monomeric CRP (mCRP) binds strongly to curved membranes in an in vitro supported lipid bilayer that has sites of curvature. Pentameric CRP (pCRP) avoids these sites of curvature. Curved membranes were created using a nanoparticle-patterned, supported lipid bilayer. Fluorescent nanoparticles (diameter = 40-200 nm) were deposited and subsequently coated with lipids then imaged by confocal microscopy.

The downstream function of CRP is to bind to proteins in the complement immune response, which ultimately leads to the removal of apoptotic cells and oxidized LDL. Our fluorescence anisotropy work demonstrates that mCRP readily binds C1q, a complement protein, but pCRP does not. When mCRP is present on regions of membrane curvature, it recruits C1q. This work demonstrates that mCRP can bind to curved membranes and recruit complement immune proteins without the presence of lipids that are often part of damaged membranes, such as oxidized lipids and lysoPC.

25-POS Board 25

Phosphatidylinositol-4,5-bis(phosphate)-induced Moesin Adsorption on Supported Lipid Bilayers : Role of Moesin Phosphorylation

Quentin Lubart^{1,2}, Fabien DALONNEAU¹, Laurent BLANCHOIN², Catherine PICART¹.

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Moesin protein from the ezrin/radixin/moesin family (ERM) link the cellular membrane to the cytoskeletal actin filaments via the phosphatidylinositol-4,5-bis(phosphate) (PIP2). This link induces many cellular events, such as the immunological synapse and microvilli formation. ERM can be in a closed or in an open conformation. The open protein can interact with PIP2 and can be phosphorylated at one or two threonine (T) residues. This phosphorylation helps to maintain the open ERM conformation, which enables the proteins to be involved in other cellular events and plays a role in the formation of protein dimers.

Our aim was to reconstitute in vitro the interactions between the plasma membrane and moesin and to study the role of the specific phosphorylations.

To this end, wild-type moesin and double phosphomimetic mutant, where T235 and T558 were replaced by aspartic acid (moesin-DD), were produced. We used PIP2-containing large unilamellar vesicles (LUVs) and supported lipid bilayers (SLBs) to mimic the cell membrane. Cosedimentation assays were used to quantify the affinity constant between the proteins and PIP2-LUVs. Quartz crystal microbalance with dissipation monitoring (QCM-D) was used to investigate in real time the formation of PIP2-SLB and to quantify moesin adsorption. The oligomerization of moesin was studied by analytical ultracentrifugation.

The affinity constant obtained by QCM-D and by cosedimentation experiments were in agreement. The moesin-DD mutant formed more dimers in solution than wild-type moesin, had a higher affinity for PIP2-containing membranes and interacted in a cooperative manner with the PIP2-SLB.

These results suggested that phosphorylation may play a role in stabilizing moesin in an open conformation at the plasma membrane. Our findings may be relevant for the formation in vivo of the immunological synapse and microvilli.

28-POS Board 28

FtsZ Polymers Tethered to the Membrane by ZipA Are Susceptible to Spatial Regulation by Min Waves

Ana Raso^{1,2}, Ariadna Martos², Mercedes Jiménez¹, Zdenek Petrášek³, Germán Rivas¹, Petra Schwille².

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Bacterial cell division is driven by an FtsZ ring in which the FtsZ protein localizes at mid-cell and recruits other proteins, forming a divisome. In *Escherichia coli*, the first molecular assembly of the divisome, the proto-ring, is formed by the association of FtsZ polymers to the cytoplasmic membrane through the membrane-tethering FtsA and ZipA proteins. The MinCDE system plays a major role in the site selection of the division ring because these proteins oscillate from pole to pole in such a way that the concentration of the FtsZ-ring inhibitor, MinC, is minimal at the cell center, thus favoring FtsZ assembly in this region. We show that MinCDE drives the formation of waves of FtsZ polymers associated to bilayers by ZipA, which propagate as antiphase patterns with respect to those of Min as revealed by confocal fluorescence microscopy. The emergence of these FtsZ waves results from the displacement of FtsZ polymers from the vicinity of the membrane by MinCD, which efficiently competes with ZipA for the C-terminal region of FtsZ, a central hub for multiple interactions that are essential for division. The coupling between FtsZ polymers and Min is enhanced at higher surface densities of ZipA or in the presence of crowding agents that favor the accumulation of FtsZ polymers near the membrane. The association of FtsZ polymers to the membrane modifies the response of FtsZ to Min, and comigrating Min-FtsZ waves are observed when FtsZ is free in solution and not attached to the membrane by ZipA. Taken together, our findings show that the dynamic Min patterns modulate the spatial distribution of FtsZ polymers in controlled minimal membranes. We propose that ZipA plays an important role in mid-cell recruitment of FtsZ orchestrated by MinCDE.

31-POS Board 31

Conformation and Aggregation of Peptides in Bulk vs. Interface

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¹Koc University, Chemical and Biological Engineering Department, Istanbul, Turkey, ²Koc University, Computational Science and Engineering Program, Istanbul, Turkey.

Conformation and assembly of proteins and peptides are not solely determined by their sequence, but are also strongly dependent on their environment. Interfaces, whether they are macroscopic (e.g. air/water interface, membrane), or molecular (e.g. surrounding molecules hydrophobic surfaces) strongly influence the conformation of biopolymers by forcing them to partition their hydrophobic and hydrophilic residues. In this study we investigate the conformation of two different peptides in bulk and at the air/water interface. The first molecule is a 14 residue LK model peptide with a sequence composed of leucine and lysine residues. The second molecule is the N-terminal 17 amino acid sequence in huntingtin (htt^{NT}) which plays an important role in Huntington's disease. By using molecular dynamics and enhanced simulation techniques, we analyzed the conformational behavior of these peptides in bulk water, at the air/water interface, and finally in the presence of other peptides in bulk water and at the air/water interface. We demonstrate the role of the interface in altering both the preferred conformation and the self-assembly behavior of these peptides. By analyzing the potential of mean force curves between two peptides in different environments, we demonstrate that both the conformation and self-assembly behavior for such peptides strongly depends on the interplay between electrostatic forces, hydrophobic effect, and their strong tendency to form backbone hydrogen bonds.

34-POS Board 34

Insights into HER2 Biology Through Structure-based Protein Engineering

Jakob C. Stüber, Christian Jost, Iwo König, Yann Waltenspühl, Annemarie Honegger, Benjamin Schuler, Andreas Plückthun.
University of Zurich, Zürich, Switzerland.

Biparatopic Designed Ankyrin Repeat Proteins (DARPin)s binding the oncoprotein HER2 outperform clinically approved antibody drugs in tissue culture experiments by a novel, apoptosis-inducing mechanism of cytotoxicity. X-ray structures of the DARPin moieties in complex with single extracellular subdomains require us to postulate previously unreported HER2 orientations to be induced by DARPin binding.

Our work aims at elucidation of the molecular mechanism of biparatopic DARPin)s by three approaches: (1) Specific receptor labeling on live cells for fluorescence-based methods such as superresolution microscopy and fluorescence cross-correlation spectroscopy for dynamic and structural information; (2) overexpression and purification of HER2 from insect cells for structural investigations by electron microscopy; and (3) protein engineering to deliberately alter the DARPin properties and limit degrees of freedom within the DARPin-receptor complexes. Our current data are indeed in agreement with the adoption of non-native HER2 conformations upon DARPin binding, and furthermore suggest the formation of higher-order HER2 oligomers. These results might contribute to the formulation of a new therapeutic principle in receptor tyrosine kinase targeting.

37-POS Board 37

Pore Forming of Fragaceatoxin C into Membrane Studied by Coarse-grained Molecular Dynamics Simulations**Khuong Truong**^{1,2,3}, Myunggi Yi^{1,2,3}.²Center of Marine-integrated Biomedical Technology (BK21+), Pukyong National Uni, Busan, South Korea, ¹Interdisciplinary Program of Biomedical, Electrical & Mechanical Engineering, Pukyong National Uni, Busan, South Korea, ³Marine Integrated Bionics Research Center, Pukyong National Uni, Busan, South Korea, ⁴Department of Biomedical Engineering, Pukyong National Uni, Busan, South Korea.

Pore-forming toxins (PFT) are water-soluble proteins. They have ability to self-assemble on the membrane. They form oligomeric transmembrane pores and this causes cell damage. Many studies have determined crystal structures of fragaceatoxin C, a PFT protein, at different states, monomer in water, monomer with lipid-bound, dimer on membrane and transmembrane pore. However, the mechanisms of lipid binding and conformational changes of fragaceatoxin C are still unclear. We carried out 4 coarse-grained molecular dynamics simulations of fragaceatoxin C in different states (monomer, dimer, oligomer (2 systems: pre-pore and pore)) with lipid bilayer. The bilayer was composed of a mixture of Sphingomyeline (SM) and DOPC lipid at molar ratio 1:1. Long time simulations were performed to observe self assembly of fragaceatoxin C. Including monomer binding to membrane, two monomers bound to membrane making dimer then forming oligomer (pre-pore) and finally pore forming into membrane. We will present these self assemblies and corresponding conformational changes. These simulations provide molecular insight into pore forming of PFT into membrane.

POSTER SESSION II

Wednesday, October 14, 13:45 – 15:30
Hall of the main building of Biology Faculty

All posters are available for viewing during all three poster sessions; however, below are the formal presentation times when presenters are required to remain in front of their poster boards to meet with attendees.

Albert, Arlene	2-POS	Board 2
Bridges, Andrew	5-POS	Board 5
Chung, Dominic	8-POS	Board 8
Flores Romero, Hector	11-POS	Board 11
Gimenez-Romero, David	14-POS	Board 14
Gupta, Preeti	17-POS	Board 17
Kadir, Mohammad	20-POS	Board 20
Kumar, Vinay	23-POS	Board 23
Márquez, Iliana	26-POS	Board 26
Ros, Uris	29-POS	Board 29
Sobrinos Sanguino, Marta	32-POS	Board 32
Suckling, Richard	35-POS	Board 35
Ugarte-Uribe, B.	38-POS	Board 38

Posters should be set up on the morning of October 13 and removed by 18:00 October 15.

2-POS Board 2**Palmitoylation and Cholesterol Effects on the Kinetic Stability of the GPCR, Rhodopsin**

Arlene Albert, Scott Corley, Madan Katragadda.
University of Connecticut, Storrs, USA.

The photoreceptor, rhodopsin is a kinetically stable G-Protein Coupled Receptor that is located in rod outer segment disk membranes. Differential scanning calorimetry (DSC) studies have shown that rhodopsin exhibits an irreversible scan rate dependent endothermic transition (T_m) at approximately 72 °C. The activation energy for thermal denaturation (E_{act}) calculated from the scan rate dependence of the T_m is sensitive to the integrity of the lipid bilayer. It is also sensitive to proteolytic cleavage of the extramembraneous loops of rhodopsin. Here we investigate the influence of palmitoylation and membrane cholesterol on rhodopsin kinetic stability. DSC experiments were performed using a MicroCal VP-DSC microcalorimeter. Samples were scanned at 15, 30, 60 and 90 °/hr. Because the protein transitions are irreversible, a second scan was used to determine the baseline. Rhodopsin palmitoylation at cys 322 and cys 323 anchors the C-terminus to the membrane. The E_{act} for rhodopsin in disk membranes treated with hydroxylamine to remove these palmitate groups was approximately 35 Kcal/mole less than that of native rhodopsin. The E_{act} for rhodopsin was also determined at different membrane cholesterol levels. These data indicated a decrease in stability both above and below native membrane cholesterol levels.

5-POS Board 5**Septin Filaments Recognize Micron-Scale Positive Plasma Membrane Curvature**

Andrew Bridges, Patricia Occhipinti, Amy Gladfelter.
Dartmouth College, Hanover, USA.

Septins are conserved filament forming GTPases that maintain cell polarity by restricting diffusion of proteins in the plasma membrane and endoplasmic reticulum while acting as a molecular scaffold for cytosolic proteins. Septin higher-order structures form on and associate with the plasma membrane and cells carrying mutated septin genes display irregular cell polarity, abnormal cell shape, are defective at cytokinesis, and are generally inviable. Though it has been shown that Cdc42 drives the accumulation of septins at incipient sites of cell polarity, properties of the plasma membrane that influence and maintain their localization have not been established. Here, we show that septins preferentially bind the plasma membrane in numerous cell types at sites of micron-scale positive curvature, a common topology of polarized cells. In regions of the cell devoid of positive curvature, septin filaments preferentially minimize interacting with negative plasma membrane curvature. Using phospholipid bilayer coated glass microspheres we show that curvature recognition is an intrinsic property of septin filaments. This work demonstrates that septins are a filamentous system capable of sensing plasma membrane shape on the micron-scale. Utilizing this property, septins respond to large scale changes in cell shape and communicate these changes to the cytoplasm.

8-POS Board 8

High Density Lipoprotein Modulates Thrombosis by Preventing Von Willebrand Factor Self-Association

Dominic W. Chung^{1,2}, Junmei Chen¹, Minhua Ling¹, Xiaoyun Fu^{1,3}, Barbara A. Konkle^{1,3}, Ying Zheng⁴, José A. López^{1,2,3}.

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Von Willebrand factor (VWF) is a multimeric glycoprotein in plasma that plays an important role in hemostasis by mediating platelet binding to sites of vascular injury. In recent studies, VWF has also been implicated in microvascular thrombosis, in part because of its unique ability to self-associate in response to shear stress and form hyperadhesive strands of enormous sizes attached to the endothelial surface. These surface-bound VWF strands, if not removed by the metalloprotease ADAMTS13 in plasma, can bind platelets efficiently and form occlusive thrombi in the microvasculature. Using several experimental systems, we show that VWF self-association was responsible for (1) adsorption of purified VWF onto plastic or glass surface under static conditions, (2) adsorption of VWF in plasma onto surfaces under shear stress, (3) assembly of secreted VWF molecules into VWF strands on the endothelial surface in flow chambers, and (4) incorporation of fluid-phase VWF molecules onto endothelial VWF fibers in synthetic microvessels. Importantly, we also found that VWF self-association in each of these instances could be markedly attenuated by high density lipoprotein (HDL) particles or its major apolipoprotein, ApoA-I. Platelet adhesion to VWF strands or fibers was also reduced in proportion to the reduction in self-associated VWF. In a mouse model of thrombotic microangiopathy, HDL attenuated the thrombocytopenia induced by injection of high doses of VWF. Consistent with its antithrombotic properties, the level of ApoA-I in patients with hyperadhesive forms of VWF, such as thrombotic thrombocytopenic purpura and sepsis, was significantly reduced. These results suggest that regulation of VWF self-association may be another mechanism by which HDL protects against cardiovascular disease and interference with VWF self-association would be a new approach to treating thrombotic disorders.

11-POS Board 11

Investigating the Membrane Topology of Proapoptotic BAX

Hector Flores Romero, Juan Garcia-Valero, Olatz Landeta, Ane Landajuela, Itsasne Bustillo-Zabalbeitia, Miguel Garcia-Porras, Oihana Terrones, Gorka Basañez.
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Mitochondrial outer membrane (MOM) permeabilization is the point of no return in many forms of apoptotic cell death. The BCL2 family member BAX is a crucial effector of MOM permeabilization that adopts an inactive conformation in the cytosol of healthy cells. It is well established that functional BAX activation is a complicated process, initiated by translocation of BAX to the MOM and culminating with assembly of BAX into a protein-permeable apoptotic pore. However, most of our current structural knowledge of BAX originates from studies in membrane-free environments. Given the inherent complexity of the cellular apoptotic network, here we used the single-cysteine accessibility methodology applied to in vitro reconstituted systems (MOM-like liposomes and isolated mitochondria) to gain structural and mechanistic information about BAX in a membrane environment.

14-POS Board 14

Apoptose Recognition by Anti-TRIM21 Autoantibodies in Systemic Lupus Erythematosus

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Systemic lupus erythematosus (SLE) is related to autoimmune diseases that share several genetic and phenotypic features. TRIM21 is a characteristic autoantigen in SLE patients, although it has not been addressed how this protein regulates this pathology. To investigate this association, we studied the recombinant human antigen TRIM21-IgG molecular recognition.

QCM-based studies indicated TRIM21-IgG interactions in SLE patients occurs by an antibody bipolar bridging mechanism. These studies also confirms that the anti-TRIM21 serum only accelerates the formation of the resulting complex, remaining unchanged the TRIM21 biological function in SLE patients and control subjects. The peptide array-based epitope mapping indicates that the major linear human epitope of anti-TRIM21 serum spans the amino acid sequence LRRKQELAE. This epitope has been confirmed by computational prediction of major histocompatibility complex class II binding regions in antigenic protein sequences, which exclusively focused on solvent-accessible residues. For that, the structure homology modelling for TRIM21 was made.

In view of that, the mechanism by which TRIM21 may link to the SLE is clarified. We hypothesized that circulating autoantibodies interact with apoptose of the TRIM21 protein when this one translocates from the cytoplasm to cell surface. The anti-TRIM21 serum only reinforces the “sensor” (immune signal induction) function of TRIM21 in translocated cells through the TRIM21-anchored Ub-63Ub chains, leading to tissue inflammation and systemic autoimmunity. According to our experimental data, we can relate the presence of anti-TRIM21 autoantibodies in SLE patients with skin, heart and brain disorders, where this protein has been observed in plasma membrane-localized form in many studies.

Acknowledgements

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17-POS Board 17

Structural, Morphological and Kinetic Studies of Human Carbonic Anhydrase II Aggregation

Preeti Gupta. Shashank Deep.
Indian Institute of Technology, Delhi, New Delhi, India.

Investigation of physico-chemical factors that modulate protein aggregation is important not only to understand and mitigate amyloid-related pathologies, but also for the manufacture, storage, and administration of protein based therapeutics. In the present work, we investigated the effect of two different environmental determinates i.e. an organic solvent, trifluoroethanol (TFE) and salt (NaCl) ions on the aggregation propensity of human carbonic anhydrase II (HCA II). Along with aggregation kinetics, we also examined the morphological properties of aggregates induced at above-mentioned solution conditions.

Our studies with TFE indicate that HCA II undergoes a transition from β -sheet to α -helix on addition of alcohol. TFE exhibited a bell-shaped dependence of aggregation on the cosolvent concentration. At intermediate [TFE], protein adopts partially structured, extended non-native β -sheet conformation with maximum aggregation propensity. Also, protein aggregates induced by TFE possess amyloid-like features as revealed by ThT binding and TEM studies. Our results suggest that TFE concentration and polypeptide backbone conformation are critical for protein aggregation.

The second condition explored was the effect of salt ions on the heat-induced aggregation kinetics of HCA II. The aggregation kinetic trace of protein displayed a typical single-transition (sigmoid) profile suggesting a nucleation dependent polymerization. Strikingly, on addition of salt, the biphasic aggregation kinetic behaviour with two distinct transitions was observed. The extent as well as rate of aggregation of both the transitions was extremely receptive to NaCl concentration. The biphasic aggregation pattern was also observed with other salts (NH_4Cl , MgCl_2), but they affect two transitions differently. Protein solubility studies and SEM analysis suggest the assemblage of monomeric protein into small, coalesced spherical particles during first transition, whereas there is only fusion of existing spheres into less packed aggregate clusters during second transition without any recruitment of protein from the solution.

20-POS Board 20**Visualization of Structural Changes Accompanying Activation of Kainate Receptors Using Fast-Scan Atomic Force Microscopy Imaging**

Mohammad F. Kadir, J M. Edwardson.
University of Cambridge, Cambridge, United Kingdom.

Ionotropic glutamate receptors are believed to undergo twisting and shortening upon activation. We used fast-scan atomic force microscopy (AFM) imaging to examine the conformational change of the GluK2 kainate receptor, integrated into a lipid bilayer, in response to activation by the agonist glutamate, either added to the imaging chamber or generated by UV photolysis of caged glutamate. In both cases, the height of the extracellular domain of the receptor fell by 0.8 nm upon activation. In contrast, there was no significant height change in response to glutamate in the presence of the GluK2 antagonist CNQX. Our study represents the first demonstration of the effect of activation on the conformation of GluK2 receptors under near-physiological conditions.

23-POS Board 23**The Binding Affinity of Metal Ions and Leukadherin-1 to CD11bA is Mutually Regulated.**

Vinay Kumar, Shashank Deep.
INDIAN INSTITUTE OF TECHNOLOGY, DELHI, Delhi, India.

The binding affinity of leukadherin-1(LA1) with CD11bA is regulated by metal ions and LA-1 also regulates the binding of metal ions with CD11bA. It has been well established that in general, Mn^{2+} or Mg^{2+} uniformly facilitate whereas Ca^{2+} inhibit integrin-ligand interactions in vitro. We measured the affinity of these cations to the inactive and active states of the cation binding αA -domain (CD11bA) from integrin CD11b/CD18 in the absence and presence of the Leukadherin-1(LA-1). Leukadherins are new class of anti-integrin compounds that are integrin agonists, the only true agonists known so far.

The information obtained from such studies can be aid in the development of pharmacological compounds as well in the elucidation of factors that determine the specificity of an interaction. ITC and fluorescence measurements showed that Mg^{2+} and Ca^{2+} exhibit equivalent affinities to inactive CD11bA but Mn^{2+} was found to have highest affinity. Binding affinity of Mn^{2+} or Mg^{2+} to CD11bA increased substantially on addition to active CD11bA, with no change in that of Ca^{2+} binding affinity. It was observed that Leukadherin-1 induced a dramatic increase in the binding affinity of all three metal ions (enthalpy driven) to either form of CD11bA and on the other hand, metal ions also increase the binding affinity of LA1 (enthalpy driven) with either form of CD11bA. The increase in the binding affinity of metal ions with CD11bA in the presence of LA1 is consistent with the electronegativity of metal ions in a similar manner as without compound LA1.

In the present work, we observed that the compound LA1 plays a critical role in regulation of binding affinity of metal ions to CD11bA and the metal ions itself regulate the binding affinity of Leukadherin-1 to CD11bA.

26-POS Board 26**Formation of Supported Lipid Bilayers of Charged E. coli Lipids on Modified Gold by Vesicle Fusion.**

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ICP - CSIC, Madrid, Spain.

We describe a simple way of fusing E. coli lipid vesicles onto a gold surface. Supported lipid bilayers on metal surfaces are of great interest for several reasons: transducing a biological signals to an electric readout, using surface analytical tools such as Surface Plasmon Resonance (SPR), infrared reflection absorption spectroscopy, neutron reflectivity or electrochemistry. The most widely used method to prepare supported lipid membranes is fusion of preexisting liposomes. This fusion of lipid vesicles is quite efficient on hydrophilic surfaces such as glass, mica or SiO₂, but it remains a challenge on metal surfaces such as gold, titanium oxide or indium tin oxide, particularly for vesicles containing charged lipids, as is the case of bacterial lipids. We describe a method based on modifying the gold surface with a Self-Assembled Monolayer (SAM) of mercaptopropionic acid and partial detergent solubilization of the liposomes. The formed bilayers were characterized using a Quartz Crystal Microbalance, Atomic Force Microscopy and Fluorescence Recovery After Photobleaching.

29-POS Board 29

Differences in Activity of Actinoporins are Related with the Hydrophobicity of Their N-Terminus

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Actinoporins are pore-forming toxins (PFT) produced by sea anemones with molecular weight around 20 kDa and high affinity for sphingomyelin. The most studied actinoporins are sticholysins I and II (StI/StII) from *Stichodactyla helianthus*, equinatoxin II (EqII) from *Actinia equina*, and fragaceatoxin C (FraC) from *Actinia fragacea*. Their N-terminal comprise an amphipathic alpha-helix preceded by a more or less hydrophobic segment, depending on the toxin, of around 10 amino acid residues. Although it is clear that the N-terminal is the most variable sequence in this protein family, the role of their hydrophobic segment is not fully understood. Here we show a comparison of StI, StII, EqII, and FraC activities with that of their respective N-terminal synthetic peptides. The hemolytic and permeabilizing activity of the peptides reproduce qualitatively the behavior of their respective parental proteins and are particularly related to the hydrophobicity of the corresponding 1-10 segment. Furthermore, the dendrogram analysis of actinoporins' N-terminal sequence allows relating differences in alignment with differences in activity among the four toxins. We have also evaluated the penetration depth of the N-terminal segment of StI and StII by using Trp-containing peptide-analogues. Our data suggest that the N-terminus of StII is more deeply buried into the hydrophobic core of the bilayer than that of StI. We hypothesize that the highest activity of StII could be ascribed to a larger hydrophobic continuum, an uninterrupted sequence of non-charged mainly hydrophobic amino acid residues, of its N-terminus promoting a highest ability to partially insert in the membrane core. Moreover, as we show for four related peptides that a higher hydrophobicity contributes to increase the activity, we reinforce the notion that this property must be taken into account to design new potent membranotropic agents.

32-POS Board 32

Reconstitution of Interactions between Bacterial Division Proteins in Lipid Coated Microbeads

Marta Sobrinos Sanguino¹, Silvia Zorrilla López¹, Allen P. Minton², Begoña Monterroso Marco¹, Germán Rivas Caballero¹.

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A new quantitative method based on lipid coated microbeads using fluorescence spectroscopy has been optimized for the detection and characterization of interactions between proteins involved in bacterial division. In *Escherichia coli*, the proto-ring is the first multi-protein complex formed at the beginning of division. This complex consists of three essential proteins. Among them, we have focused on FtsZ, a GTPase that polymerizes in the presence of GTP, and ZipA, a membrane protein that anchors the cytoplasmic FtsZ to the membrane. Here we have analyzed the complexes of FtsZ and ZipA using lipid coated microbeads and other complementary methods to specifically address the impact of the density of ZipA receptors at the bilayer on the interactions with FtsZ. With this aim, silica microbeads have been functionalized by coating them with a mixture of phospholipids from *E.coli* containing different amounts of DGS-NTA lipids to which soluble mutants of ZipA, lacking the transmembrane domain, were anchored through a poly-histidine tag. The interaction between FtsZ and ZipA immobilized onto the microbeads at different densities was characterized through sedimentation, monitoring the fluorescence intensity of labeled FtsZ after microbeads pelleting. The effect of the nucleotide (GTP or GDP), on the interaction between immobilized ZipA and FtsZ was addressed. Preliminary results show that variations in the surface density of ZipA induce significant changes in the interaction with FtsZ. We also show that the fluorescence assay based on lipid coated microbeads can detect inhibitors of the FtsZ/ZipA interactions such as peptides derived from the C-terminal of FtsZ. Therefore, the lipid coated microbead assays proved to be useful for the characterization of interactions between division proteins and may also be applied for the systematic screening of inhibitors of these interactions, potentially useful for the development of new antibiotics.

35-POS Board 35

Structures of the N-Termini of Human Notch Ligands and Their Binding to Phospholipids.

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Notch receptors and their ligands are core components of a cell-cell signalling pathway which plays a role in metazoan development, the importance of which is illustrated by several genetic disorders associated with aberrant Notch signalling. We recently identified the calcium dependent phospholipid binding C2 domain at the N-terminus of the human ligand Jagged1 (Chillakuri et al., 2013). A recent structure of the Notch ligand DLL4 in complex with Notch1 (Luca et al., 2015), has shown that EGF12 of the Notch receptor (1-4 in mammals) interacts with a loop at the side of the C2 domain of DLL4 showing that this domain can bind to both Notch and phospholipids simultaneously. The N-terminal C2 domain is conserved across the Notch ligands, and we have recently obtained crystal structures of other Notch ligands showing that the loops within the putative lipid binding site of the C2 domains are considerably variable between ligands, presumably highlighting their differing lipid binding specificity. We are interested in further elucidating the specificity of the different Notch ligands for different lipids, and in understanding the role of this interaction in the Notch signaling pathway.

Chillakuri CR, Sheppard D, Ilagan MX, Holt LR, Abbott F, Liang S, Kopan R, Handford PA, Lea SM. (2013) Structural analysis uncovers lipid-binding properties of Notch ligands. *Cell Rep.* 5(4):861-7.

Luca VC, Jude KM, Pierce NW, Nachury MV, Fischer S, Garcia KC. (2015) Structural basis for Notch1 engagement of Delta-like 4. *Science.* 347 (6224):847-53.

38-POS Board 38**Drp1 Polymerization Stabilizes Curved Tubular Membranes Similar to Those Found in Mitochondria**

B. Ugarte-Uribe¹, Coline Prévost², Patricia Bassereau², Ana J. García-Sáez¹.

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Drp1, an 80-kDa mechanochemical GTPase of the dynamin superfamily, is required for mitochondrial fission in mammals in a process dependent on Drp1 self-assembly and coupled to GTP hydrolysis. Although dysfunction of Drp1 has been linked to human disease, there are still some clues missing regarding the molecular mechanism of action of Drp1 on membranes that remain to be elusive. As dynamin, Drp1 is thought to form helical coat circling a membrane tubule leading to membrane deformation. To study the effect of Drp1 in membrane curvature we used preformed tubes pulled from GUVs under near-physiological conditions. In the presence of GTP a fast Drp1 polymerization along the preformed tube was observed, where Drp1 coating preserved the membrane tube within it. In addition, Drp1 rearrangement on membranes from homogeneous binding to the formation of nucleation points was shown to be enough to stabilize a narrow range of radii akin to Drp1 nucleation foci found in cell mitochondria. This suggests that Drp1 could play a role as curvature stabilizer which sets it apart from other dynamin homologs and may be relevant for the molecular mode of action of Drp1 in the context of the cell.

POSTER SESSION III

Thursday, October 15, 13:45 – 15:30
Hall of the main building of Biology Faculty

All posters are available for viewing during all three poster sessions; however, below are the formal presentation times when presenters are required to remain in front of their poster boards to meet with attendees.

Bhatia, Tripta	3-POS	Board 3
Busch, Karin	6-POS	Board 6
Cruz, Antonio	9-POS	Board 9
Frachon, Thibaut	12-POS	Board 12
Gomez-Fernandez, Juan	15-POS	Board 15
Haglin, Elizabeth	18-POS	Board 18
Kim, Songmi	21-POS	Board 21
Lee, Yuno	24-POS	Board 24
Mori, Takaharu	27-POS	Board 27
Sanchez-Barrena, Maria	30-POS	Board 30
Stein, Matthias	33-POS	Board 33
Sukharev, Sergei	36-POS	Board 36
Valbuena, Alejandro	39-POS	Board 39

Posters should be set up on the morning of October 13 and removed by 18:00 October 15.

3-POS Board 3**Fluctuation Analysis of Nonequilibrium Membranes Reveals Lipid-Protein Interactions**

Tripta Bhatia¹, Allan Hansen¹, Michael Lomholt¹, Flemming Cornelius², Ole Mouritsen¹, John Ipsen¹.

¹MEMPHYS, University of Southern Denmark, Odense, Denmark, ²Aarhus University, Aarhus, Denmark.

The flicker data is analyzed on the basis of a phenomenological model of membrane fluctuations with Na/K pump activity. The description allows us to determine the coupling mechanism between protein activity and membrane fluctuations.

6-POS Board 6**Local pH Differences at Respiratory Active Mitochondrial Membrane Proteins in Cellula**

Bettina Rieger¹, Iven Winkelmann^{2,1}, Daria Shalaeva^{1,3}, Armen Mulkidjanian^{1,3}, Wolfgang Junge¹, **Karin Busch**^{4,1},

⁴University of Münster, Münster, Germany, ¹University of Osnabrück, Osnabrück, Germany, ³Lomonosov University, Moscow, Russian Federation, ²University of Stockholm, Stockholm, Sweden,

On central task of bioenergetics is the production of ATP by use of ion gradients across membranes. In mitochondria, oxidative phosphorylation (OXPHOS) is such a coupled process. Complex IV, cytochrome c oxidase, pumps protons from the N- to the positively charged P-side of the inner membrane. Complex V, the FOF1 ATP synthase, generates ATP by allowing protons to flow back across two half channels in its FO subunit. We show by use of site-directed fusion of the sensor pHluorin, a pH-sensitive GFP derivative, that a lateral proton gradient exists between the proton source CIV, and the proton sink CV in the steady state. Furthermore, we characterize the nano-environment of active cytochrome c oxidase, CIV, in living cells, in more detail. By use of subunit-attached pH probes we report a lower pH value at a subunit of CIV next to the membrane than at a subunit oriented towards the bulk phase. In addition, the fluorescent lifetime of pHluorin near the membrane was reduced indicating a different nano-environment. Together our data obtained from in situ measurements under physiological conditions suggest that a diffusion-controlled lateral proton gradient exists between two integrated membrane proteins, one operational as proton source and the other as proton consumer. Furthermore, for the first time, we experimentally demonstrate a second pH gradient between membrane and bulk phase. The Mitchell concept of a constant proton motive force in a single microcompartment is challenged by these results.

9-POS Board 9

Biosurfaces and Drug Delivery: Evaluating the Effect of Tacrolimus, an Immunosuppressive Drug, on the Structure and Performance of Pulmonary Surfactant Films

Alberto Hidalgo¹, Francesca Salis², Guillermo Orellana², **Antonio Cruz**¹, Jesús Pérez-Gil¹.

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The respiratory surface of the mammalian lung is covered by a thin aqueous layer, and on top of it, by a lipid-protein surface active material, the pulmonary surfactant (PS). To prevent pulmonary collapse during breathing, PS must adsorb very rapidly into the air-liquid interface and spread efficiently along it. Therefore, it offers unique opportunities to vehiculize different drugs, while hiding and protecting them from clearance.

It has been well established that surfactant proteins ensure a proper PS behaviour and are essential to maintain the integrity of PS. Alterations, deficiencies, or the lack of surfactant proteins produce severe dysfunctions in the respiratory process. The interaction of inhaled entities with surfactant films may perturb structure and performance of surfactant layers, compromising the respiratory function. Therefore, it is crucial to analyse whether each particular molecule delivered to the lung, or transported by surfactant, could somehow affect structure-function determinants in PS.

In the present work we have evaluated the effect of Tacrolimus, an immunosuppressive drug, on interfacial DPPC films and on interfacial layers made of a porcine PS extract (containing 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. In parallel, we evaluated the drug transporting capabilities of PS along the air-water interface, using a novel setup designed in our laboratory.

Tacrolimus affects the lateral structure of surfactant films, but after repetitive compression-expansion cycles, this effect was apparently reverted, suggesting that the surface was progressively depurated from the drug. The results suggest that PS could be optimized as a drug-delivery agent with the potential of liberating the drug once subjected to compression-expansion dynamics at the distal airways.

12-POS Board 12

Fast Insulin Amyloid Aggregation at the Air-Liquid-Solid Triple Interface

Thibaut Frachon^{2,1}, Marianne Weidenhaupt¹, Quentin Le masne², Franz Bruckert¹.
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With the growing use of therapeutical proteins and the development of medical delivery devices, protein stability becomes a major concern in pharmaceutical industrial processes. Proteins can lose their native conformation by unfolding and reach a new free energy minimum by forming insoluble aggregates. Insulin is one example of such an aggregation-prone protein, which easily forms amyloid fibrils on hydrophobic surfaces under agitation. Within a device containing a protein solution, hydrophobic interfaces are found at the border between hydrophobic materials and liquid and between air and liquid. These different interfaces come into close proximity in protocols, frequently used in industry, involving intermittent wetting.

We designed a model experiment, where we observe by optical microscopy the formation of insulin amyloid fibers as a protein solution moves repetitively back and forth in a microfluidic channel. Thioflavin T fluorescence was used to monitor the formation of amyloid deposits and reflection interference contrast microscopy was used to monitor the morphology of the liquid film after receding. The growth of protein aggregates on the surface was characterized. We demonstrate that insulin fibers mainly form in regions of intermittent wetting, but not in regions exposed to hydrodynamic shear stress alone, remaining always wet. This clarifies the role of interfaces in insulin aggregation, showing that wall shear stress alone is insufficient to rapidly trigger amyloid fiber formation. We study the influence of temperature, channel size, fluid velocity, duration of the recession phases and nature of the materials surfaces. Our experiments illustrate how the formation of a triple solid/liquid/air interface in industrial processes may lead to protein instability. The careful design of fluid movement in devices handling protein solutions can therefore improve their stability.

15-POS Board 15

Edelfosine Shows Affinity for Cholesterol and Disorganizes Liquid Ordered Structures

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Edelfosine is an alk1-lysophospholipid with antineoplastic and immunomodulating effects. We compare here the effect of edelfosine on the organization, at different temperatures, of liquid ordered membrane-like structures, with four other asymmetric phospholipids, PAF, PAPC, lysoPAF and lysoPC. When a POPC/SM/cholesterol (1:1:1 mole ratio) ternary mixture was studied by DSC no phase transition was detected, but the addition of 20 mol % edelfosine to produced a phase transition $L\beta$ to $L\alpha$. This new transition should be attributed to the association of edelfosine to cholesterol capable of neutralizing its effect. Such a transition was not produced by the addition of the other four asymmetric phospholipids. Results from SAXD showed that edelfosine, PAF and PAPC at 5 and 15°C produced spacings that suggest lamellar crystalline structure, while at 45°C results indicated a bilayer structure in fluid state. In contrast, lysoPC produces a crystalline structure at 5 and 15°C, while at 45°C was in a bilayer in fluid state. Furthermore, the spacing of the sample with lysoPAF at 5°C indicates that it has two phases, probably both of lamellar type. At 15°C the pattern displays a spacing indicating that one of the two phases disappeared, while at 45°C two spacings indicate that the lamellar phase that remains is a bilayer in fluid state. 31P-NMR showed that edelfosine forms bilayers when combined with just cholesterol, being more capable of doing so than the other compounds studied. These bilayers were characterized by microscopy. The results obtained in this study support the theory that the antitumor drug edelfosine acts through reorganization and modification of lipid rafts in liquid ordered state (L_o) and therefore supports that these domains are a promising therapeutic target against cancer.

18-POS Board 18

Signaling-related Structural Changes of Chemoreceptor Nano-Arrays

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In bacteria, 200-nm extended networks of membrane protein nano-arrays cooperatively sense and adapt to environmental stimuli in order to direct swimming patterns. Upon ligand binding to the chemoreceptors, a small 2Å downward piston motion is able to promote inactivation of the histidine kinase, CheA more than 250Å away. Understanding the mechanism of this phenomenon could lead to development of novel antibiotics capable of disrupting bacterial chemotaxis, thus inducing nutrient starvation and death.

We assemble native-like functional chemoreceptor arrays by binding a His-tagged cytoplasmic fragment of the Asp receptor to templating vesicles in the presence of the other protein components, a coupling protein CheW and kinase CheA. Electron cryotomography (ECT) demonstrates that these *in vitro* arrays consist of extended hexagonal assemblies with each hexagon formed by six trimers of receptor dimers, with the same 12-nm spacing seen for intact receptors in cells. Assembly density has been shown to alter kinase and methylation activities in an inverse fashion, analogous to the physiological signaling states. We hypothesize that the kinase-off/methylation-on signaling state has an expanded membrane-proximal region relative to the kinase-on/methylation-off signaling state. To test this hypothesis, we are investigating the membrane-proximal area of each signaling state by measuring protein binding and activities for arrays assembled on vesicles of known surface area. Preliminary results indicate that greater crowding is only possible by restricting the surface area during, rather than after array assembly. This suggests the kinase-on array is highly stable and restricts access of additional protein into the array. For example, sterically restricting access of CheR to the receptor methylation sites is a potential mechanism for controlling methylation activity. Concurrently, we are using solid state NMR to measure dimer-dimer distances to characterize the trimer of dimers structure and quantify the proposed signaling-related receptor expansion.

21-POS Board 21**Understanding Structural Mechanism of β_2 -Adrenergic Receptor Based on Water Molecule Using All-Atom Molecular Dynamics (MD) Simulation**

Songmi Kim, Changbong Hyeon.

Korea Institute for Advanced Study, Seoul , South Korea.

G protein-coupled receptors (GPCRs) are membrane proteins and responsible for various cell response including vision, smell, hormones, odorants, neurotransmitter and other factors. The GPCRs have conformational change when binding ligand or signal molecule to transmit signals from extracellular to intercellular regions. Several GPCR crystal structures revealed that the activation of GPCRs is mediated by structural water molecules. To understand structural mechanism focused on water molecules within β_2 -adrenergic receptor (β_2 AR), we constructed systems for different states. We have performed 1 μ s all-atom MD simulation for each system in membrane environment. The dynamics of water molecule in β_2 AR and structural analysis have been carried out by time correlation function of contacts between residue and water molecules and by calculating the water penetration.

24-POS Board 24**DMSO Disorders Water Structure and Enhances Water Diffusion Near Phospholipid Bilayer Surfaces**

Yuno Lee, Changbong Hyeon.

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Dimethyl sulfoxide (DMSO) prevents ice formation by disrupting the hydrogen bond network among water molecules. Despite its broad use as a cryoprotectant and long-lasting efforts to probe water dynamics in the presence of DMSO, the microscopic underpinnings by which DMSO prevents ice formation on cell surfaces are not fully understood. Here, using all atom molecular dynamics simulations of POPC/water systems at varying DMSO concentrations, we probe the structural and dynamical properties of water and DMSO in the vicinity of phospholipid bilayers. Our study is consistent with the recent studies pointing to DMSO-induced dehydration, but critically reveals the presence of fine structures in hydration layer near bilayer surfaces. DMSO has a unique property that the extent of depletion from solvent-bilayer interfaces, which leads to preserving the hydration layer, is more long-ranged and greater than other cosolvent such as sucrose. As a consequence, the mobility of surface water at increased DMSO concentrations is less affected than that of the bulk water in DMSO solution, enhancing the surface water diffusion relative to the bulk.

27-POS Board 27

Replica-Exchange Molecular-Dynamics Simulations of Mixed Lipid Bilayer Systems**Takaharu Mori**, Yuji Sugita.

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Conformational sampling is fundamentally important for simulating complex bio-molecular systems. The generalized-ensemble algorithm, especially the temperature replica-exchange molecular dynamics method (T-REMD), is one of the most powerful methods to explore structures of bio-molecules such as proteins, nucleic acids, carbohydrates, and also of lipid membranes. Recently, we have proposed a new generalized-ensemble algorithm for membrane systems, which we call the surface-tension REMD method (γ -REMD) [1]. Each replica is simulated in the NP γ T ensemble, and surface tensions in a pair of replicas are exchanged at certain intervals to enhance conformational sampling of the target membrane system. In this study, we carried out γ -REMD simulation for a pure DPPC lipid bilayer [1], and also T-REMD, γ -REMD, and γ T-REMD simulations for a mixed POPC/DMPC lipid bilayer [2]. In the DPPC simulation, we found that lateral diffusion of lipid molecules was enhanced compared with conventional MD simulation. In the POPC/DMPC simulations, lateral diffusion in the same lipid phase was enhanced in all REMD simulations. As for "mixing" of two lipid components, however, it was enhanced in T-REMD, while it was suppressed in γ -REMD. In γ T-REMD, both enhancement and suppression were observed, presumably because lateral diffusion is accelerated at high temperature while it is suppressed under high surface tension. We suggest that surface tension affects the degree of mixing of lipid components in biological membranes.

[1] T. Mori, J. Jung, and Y. Sugita, *J. Chem. Theory Comput.*, 9, 5629-5640 (2013).

[2] J. Jung, T. Mori, C. Kobayashi, Y. Matsunaga, T. Yoda, M. Feig, and Y. Sugita, *WIREs Comput. Mol. Sci.*, doi: 10.1002/wcms.1220 (2015).

30-POS Board 30

The Calcium Sensor NCS-1 as a Pharmacological Target for Synapse Regulation in Autism

Alicia Mansilla³, Antonio Chaves-Sanjuán¹, Nuria Campillo², Carmen Gil², Lourdes Infantes¹, Ana Martinez², Alberto Ferrus³, **Maria Jose Sanchez-Barrena**¹.

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Several forms of autism, including Fragile X syndrome (FXS), show an excess of synapses in brain cortex. Current strategies to counteract the excess of synapses focus in the design of drugs that antagonize postsynaptic glutamate receptors. This strategy, however, does not consider a general feature of neurons across nervous systems, which is the inverse correlation between the number of synapses and the probability of neurotransmitter release per synapse. Thus, it is most likely that the functional down-regulation of synapses will trigger an increase in synaptogenesis, which will lead to a spiral of ever increasing dosage in the pharmacological treatment to maintain the total synapse activity low. Recently we have described the mechanism by which these neuronal features are co-regulated by the myristoylated Ca²⁺-sensor NCS-1 and its binding partner Ric8a, a GEF that activates G protein complexes. Structural studies demonstrated the key residues on NCS-1 necessary for NCS-1/Ric8a complex formation. Altogether our data suggest that if we were able to disrupt the NCS1/Ric8 complex with small compounds, it would be possible to use them as therapeutic drugs to decrease the number of synapsis in mental disorders where synapse number is abnormal. Using virtual screening methods we have found an aminophenothiazine-class molecule that impedes complex formation and re-establishes synapse function in FXS model of *Drosophila*. Crystallographic data on the NCS-1/small compound complex shows the reason of the inhibition. This structure will permit us to improve the pharmacological properties of the molecule for a future use as therapeutic drug.

References:

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S Baños-Mateos, A Chaves-Sanjuán, A Mansilla, A Ferrús, MJ Sánchez-Barrena. *Acta Cryst F*, 2014.

33-POS Board 33

Posttranslationally-modified Rab5 Protein Binding to Different Model Membranes

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Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany.

Rab5 is a small GTPase and molecular switch that controls events in early endosome fusion by shuttling between its active GTP-bound and inactive GDP-bound state. After posttranslational modification, the protein is anchored to the plasma membrane via two C20 geranylgeranyl (GG) moieties close to its C-terminus. This CXC cysteine prenylation is required for Rab5 proteins to associate with cellular membranes. In the absence of this prenyl modification, the protein remains cytosolic and is unable to function properly.

Membrane-associated Rab5 is able to recruit further effector proteins to the membrane and form local protein-protein complexes. The hypervariable N- and C-terminal regions could not be resolved by protein X-ray crystallography.

We here present structural and dynamic properties of membrane-bound full-length Rab5 in the active and inactive states obtained from initial loop refinement and subsequent long-time all-atom Molecular Dynamics simulations.

Simulations of Rab5 in different model membranes were performed and revealed a highly dynamic behavior of the membrane-anchored protein. Independent from the GTPase activation state, the G-domain rapidly changes orientation from a rather perpendicular position towards a position close to the membrane surface with two alpha-helices almost parallel to the bilayer. In addition to the residues in the lipid-exposed helices certain protein-membrane contacts are established by basic and polar residues in the flexible N- and C-terminal regions. The two geranylgeranyl anchors show a high degree of torsional flexibility; however, their motions are strongly correlated. Furthermore, the overall anchor insertion depth shows a dependence on the membrane phospholipid composition, i.e. is slightly increasing with membrane thickness. The structural properties of the lipid bilayer seem to be rather unaffected by the protein anchor insertion.

36-POS Board 36

Peptide Binding to Membranes and Tension-dependent Penetration Are Involved in the Inhibition of GsMTx4 on Mechanosensitive Ion Channels

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GsMTx4, a 34 amino acid cysteine-knot peptide from spider venom, is highly specific at inhibiting cationic mechanosensitive ion channels (MSCs). GsMTx4 does not seem to interact stereospecifically with the channel protein, but acts from the boundary lipids. GsMTx4 has a hydrophobic face with tryptophans (Trp) surrounded by a ring of charged amino acids, including six lysines. To investigate the mechanism of MSC inhibition, we created lysine to glutamate mutants and tested them on Piezo1 channels in patches. Four mutants showed a 20-40% loss of activity. The free energy of binding to liposomes determined by isothermal titration calorimetry (ITC) varied with the mutation and was highest in the most functionally compromised K8E mutant. The binding affinity determined independently by aqueous Trp quenchers agreed with ITC. The penetration depth determined using brominated lipid quenchers indicated a shallow penetration, but the mutants generally penetrated deeper. ITC suggested there was more than one binding reaction, consistent with predicted shallow and deep modes. The effect of membrane tension on penetration depth was studied using lipid monolayers in a Langmuir trough. All peptides bound to expanded monolayers and occupied a large proportion of the area. Compression near the monolayer-bilayer equivalence pressure forced WT peptide from the monolayer, but the inactive mutants remained bound. These data place GsMTx4 near the polar interface of the outer monolayer at depth set by the lysines, and occupying a small fraction of membrane area at resting tensions. As the lateral pressure of lipids decreases during membrane stretching, the peptides penetrate deeper acting as “area reservoirs” effectively clamping the surface tension and preventing activation of MSCs.

39-POS Board 39

Quantification and Modification of the Equilibrium Dynamics and Mechanics of a Viral Capsid Lattice Self-assembled as a Protein Nanocoating**Alejandro Valbuena**, Mauricio García Mateu.

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Self-assembling, protein-based bidimensional lattices are being developed as functionalizable, highly ordered biocoatings for multiple applications in nanotechnology and nanomedicine. Unfortunately, protein assemblies are soft materials that may be too sensitive to mechanical disruption, and their intrinsic conformational dynamism may also influence their applicability. Thus, it may be critically important to characterize, understand and manipulate the mechanical features and dynamic behavior of protein assemblies in order to improve their suitability as nanomaterials. In this study, the capsid protein of the human immunodeficiency virus was induced to self-assemble as a continuous, single layered, ordered nanocoating onto an inorganic substrate. Atomic force microscopy (AFM) was used to quantify the mechanical behavior and the equilibrium dynamics (“breathing”) of this virus-based, self-assembled protein lattice in close to physiological conditions. The results uniquely provided: (i) evidence that AFM can be used to directly visualize in real time and quantify slow breathing motions leading to dynamic disorder in protein nanocoatings and viral capsid lattices; (ii) characterization of the dynamics and mechanics of a viral capsid lattice and protein-based nanocoating, including flexibility, mechanical strength and remarkable self-repair capacity after mechanical damage; (iii) proof of principle that chemical additives can modify the dynamics and mechanics of a viral capsid lattice or protein-based nanocoating, and improve their applied potential by increasing their mechanical strength and elasticity. We discuss the implications for the development of mechanically resistant and compliant biocoatings precisely organized at the nanoscale, and of novel antiviral agents acting on fundamental physical properties of viruses.