

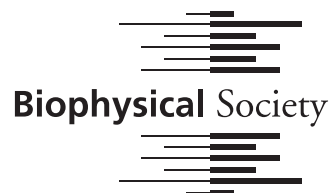
Liposomes, Exosomes, and Virosomes: From Modeling Complex Membrane Processes to Medical Diagnostics and Drug Delivery

Ascona, Switzerland | September 11–16, 2016



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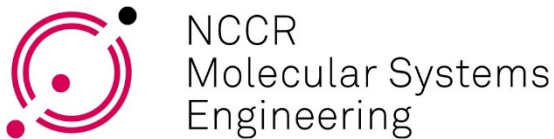
Organizing Committee

Daniel Müller, Eidgenössische Technische Hochschule Zürich

Lukas Tamm, University of Virginia

Horst Vogel, École Polytechnique Fédérale de Lausanne

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September 2016

Dear Colleagues,

We would like to welcome you to the Biophysical Society Thematic Meeting on ***Liposomes, Exosomes, and Virosomes: From Modeling Complex Membrane Processes to Medical Diagnostics and Drug Delivery***. This meeting is co-sponsored by Congressi Stefano Franscini of the ETH Zurich. We have assembled a stimulating program, with lectures focusing on many aspects and recent developments for investigating biochemical reactions and networks at, in, and across artificial and cell-derived vesicular membranes. The meeting will address themes that include: imaging of vesicles in cellular and extracellular trafficking processes, the role of nano-domains in membranes, exosomes as diagnostic biomarkers, and virosomes as vehicles of targeted drug delivery – just to name a few!

The program features 30 invited speakers, 19 short talks selected from contributed posters, and over 65 contributed posters. Over 120 participants will be in attendance to share and discuss their ideas. We hope that the meeting will not only provide a venue for exchanging recent exciting progress, but also promote fruitful discussions and foster future collaborations in the search of general principles of contemporary membrane and liposome science.

The mountains above Ascona, overlooking Lago Maggiore, along with the historic and architecturally notable (Bauhaus) meeting venue on Monte Verità, provide a stimulating ambiance for a meeting that will hopefully be remembered for many years. We thus encourage you to take part in social and cultural activities that will allow you to enjoy the multicultural spirit of the lake region that blends the Swiss canton of Ticino with the Italian region of Lombardia.

Thank you all for joining this meeting, and we look forward to enjoying this event with you!

Sincerely,

Daniel Müller, Lukas Tamm, Horst Vogel
The Organizing Committee

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GENERAL INFORMATION

Registration/Information Location and Hours

Registration will be located at the Registration Desk in the main entrance of Monte Verità. Registration hours are as follows:

Sunday, September 11	16:00 – 19:00
Monday, September 12	8:30 – 18:00
Tuesday, September 13	8:30 – 18:00
Wednesday, September 14	8:30 – 12:30
Thursday, September 15	8:30 – 18:00
Friday, September 16	8:30 – 13:00

Instructions for Presentations

(1) Presentation Facilities:

A data projector will be made available in the Auditorium. The Monte Verità will provide one (1) PC laptop and one (1) Macintosh laptop. Speakers who need to run a special program should bring their personal laptop. Speakers are advised to preview their final presentations before the start of each session.

(2) Poster Sessions:

- 1) All poster sessions will be held in Balint Salon.
- 2) A display board measuring 1189 mm (3' 10") high by 841 mm (2' 9") wide will be provided for each poster. Poster boards are numbered according to the same numbering scheme as in the E-book.
- 3) There will be formal poster presentations on Monday, Tuesday and Thursday. All posters will be available for viewing during all poster sessions.
- 4) During the assigned poster presentation sessions, 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.

Meals and Coffee Breaks

There will be a one-hour Welcome Reception on Sunday, September 11 from 17:30-18:30. This reception will be held on the Restaurant Terrace, weather permitting. Should weather conditions not be optimal, the reception will be held in the Spazio Roccia, which is located on the first floor of the main building.

Coffee breaks will be served in the Spazio Roccia.

Breakfasts, lunches, and dinners will be served in the dining hall, Sala Luce, which is located on the first floor of the main building.

Smoking

Please be advised that smoking is not permitted inside Monte Veritá or the meeting facilities. Smoking is permitted in outside areas.

Name Badges

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

Contact Information

If you have any further requirements during the meeting, please contact the meeting staff at the registration desk from September 11-16 during registration hours.

In case of emergency, you may contact the following:

Dorothy Chaconas, BPS Staff
dchaconas@biophysics.org

Front Desk, Monte Veritá
+ 41 91 785 40 55

Liposomes, Exosomes, and Virosomes: From Modeling Complex Membrane Processes to Medical Diagnostics and Drug Delivery

Ascona, Switzerland
September 11-16, 2016

PROGRAM

Sunday, September 11, 2016

16:00 – 19:00	Registration/Information	Main Entrance
17:30 – 18:30	Welcome Drink	Restaurant Terrace
19:00 – 20:00	Dinner	Sala Luce
Session I	Special Evening Lecture Daniel Müller, Eidgenössische Technische Hochschule Zürich, Switzerland, Chair	
20:00 – 20:50	Steven G. Boxer, Stanford University, USA <i>Disentangling Viral Membrane Fusion from Receptor Binding Using Synthetic DNA-Lipid Conjugates and a New Approach for Measuring Short-range Interactions in Membranes</i>	

Monday, September 12, 2016

8:30 – 18:00	Registration/Information	Main Entrance
8:30 – 8:45	Welcome Address Lorenzo Sonognini, Monte Verità	Auditorium
Session II	Suzanne Eaton, Max Planck Institute of Molecular Cell Biology and Genetics, Germany, Chair	
8:45 – 9:20	Kalina Hristova, Johns Hopkins University, USA <i>Probing the Early Stages of RTK Signaling in Plasma Membrane Vesicles</i>	
9:20 – 9:55	Dimitrios Stamou, University of Copenhagen, Denmark <i>Heterogeneities in Membrane Composition and Curvature of Liposomes, Two Non-stochastic Regulators of Biological Function</i>	
9:55 – 10:25	Coffee Break	Spazio Roccia
10:25 – 10:40	Kirsten Bacia, University of Halle, Germany* <i>Membrane Binding and Remodeling by the COPII Complex</i>	

* Contributed talks selected from among submitted abstracts

10:40 – 10:55	Shirley Schreier, University of São Paulo, Brazil* <i>Use of Model Membranes-Liposomes and Micelles of Variable Lipid Composition to Elucidate the Molecular Mechanism of Action of Pore-forming Proteins and Peptides</i>	
10:55 – 11:30	Helen R. Saibil, Birkbeck, University of London, United Kingdom <i>Membrane Pore-forming Proteins in the Molecular Arms Race between Host and Pathogen</i>	
11:30 – 12:05	Donald M. Engelman, Yale University, USA <i>pHLIP: Uses in Measuring Cell Surface pH, Imaging Tumors, and Delivering Therapeutics</i>	
12:30 – 14:30	Lunch	Sala Luce
Session III	Sarah L. Keller, University of Washington, USA, Chair	
14:30 – 15:05	Philippe Bastiaens, Max Planck Institute of Molecular Physiology, Germany <i>The Interdependence of Vesicular Membrane Dynamics and Signal Processing</i>	
15:05 – 15:20	Jean-Marie Ruyschaert, Université Libre de Bruxelles, Belgium* <i>Liposomes Activate Innate Immunity Cascades through Recognition of Toll-like Receptors</i>	
15:20 – 15:35	Matthew P. McDonald, Max Planck Institute for the Science of Light, Germany* <i>Fluorescence-free Imaging and Tracking of Individual Secretory and Transmembrane Proteins in a Living Cell</i>	
15:35 – 15:50	Marta Bally, Chalmers University of Technology, Sweden* <i>Artificial Cell Membrane Mimics to Study the Role of the Influenza Virus Matrix Protein M1 in Virus Budding</i>	
15:50 – 17:25	Coffee Break & Poster Session I	Balint Salon
17:25 – 18:00	Martin Hof, J. Heyrovský Institute of Physical Chemistry of the Czech Academy of Sciences, Czech Republic <i>Single Molecule Fluorescence Clarifies the Role of Monosialoganglioside GM1 and Sphingomyelin in the In-Membrane Oligomerization of β-Amyloid</i>	
19:00 – 20:00	Dinner	Sala Luce
Session IV	Special Evening Lecture Lukas Tamm, University of Virginia, USA, Chair	
20:00 – 20:50	Reinhard Jahn, Max Planck Institute for Biophysical Chemistry, Germany <i>Molecular Steps in SNARE-mediated Membrane Fusion</i>	

Tuesday, September 13, 2016

8:30 – 18:00	Registration/Information	Main Entrance
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* Contributed talks selected from among submitted abstracts

Session V	Donald M. Engelman, Yale University, USA, Chair	
8:45 – 9:20	Wolfhard Almers, Oregon Health & Science University, USA <i>Secretory Granules and Their Syntaxin Clusters in the Plasma Membrane</i>	
9:20 – 9:55	Horst Vogel, École Polytechnique Fédérale de Lausanne, Switzerland <i>Ligand-gated Ion Channels: Structures and Functions</i>	
9:55 – 10:25	Coffee Break	Spazio Roccia
10:25 – 10:40	Muhmmad Omar Hmeadi, Uppsala University, Sweden* <i>Plasma Membrane PI(4,5)P2 Is Critical for Secretory Granule Exocytosis</i>	
10:40 – 10:55	Christopher Stroupe, University of Virginia School of Medicine, USA* <i>Using Liposomes as a Model System for Probing the Biochemical Mechanisms of Intracellular Membrane Tethering</i>	
10:55 – 11:30	David Alsteens, Université Catholique de Louvain, Belgium <i>Imagining Individual Receptors while Extracting Kinetic and Thermodynamic Parameters Using FD-based AFM</i>	
11:30 – 12:05	Jay T. Groves, University of California, Berkeley, USA <i>Signal Transduction on Membrane Surfaces: The Roles of Space, Force, and Time</i>	
12:30 – 14:30	Lunch	Sala Luce
Session VI	Philippe Bastiaens, Max Planck Institute of Molecular Cell Biology and Genetics, Germany, Chair	
14:30 – 15:05	Anne K. Kenworthy, Vanderbilt University, USA <i>Targeting Proteins to Lipid Rafts: Mechanisms and Consequences</i>	
15:05 – 15:20	Nikhil R. Gandasi, Uppsala University School of Medicine, Sweden* <i>Assembly of the Secretory Machinery during Insulin Granule Docking</i>	
15:20 – 15:35	Joël de Beer, Eidgenössische Technische Hochschule Zürich, Switzerland * <i>Fusion of Synthetic Lipid Carriers to Exosomes Produces Hybrid Vesicles Harnessed for the Delivery of Biomolecules</i>	
15:35 – 15:50	Raya Sorkin, VU University, Netherlands* <i>Mechanics of Extracellular Vesicles from Plasmodium Falciparum Infected Red Blood Cells</i>	
15:50 – 17:25	Coffee Break & Poster Session II	Balint Salon
17:25 – 18:00	Anthony Hyman, Max Planck Institute of Molecular Cell Biology and Genetics, Germany <i>Phase Transitions: An Emerging Principle in Cytoplasmic Organization and Neurodegeneration</i>	
19:00 – 20:00	Dinner	Sala Luce

* Contributed talks selected from among submitted abstracts

Session VII	Special Evening Lecture Horst Vogel, École Polytechnique Fédérale de Lausanne, Switzerland, Chair
20:00 – 20:50	Gunnar von Heijne, Stockholm University, Sweden <i>Cotranslational Protein Folding</i>

Wednesday, September 14, 2016

8:30 – 12:30	Registration/Information	Main Entrance
Session VIII	Helen R. Saibil, Birkbeck, University of London, United Kingdom, Chair	
8:45 – 9:20	Wolfgang Meier, University of Basel, Switzerland <i>Biomimetic Block Copolymer Membranes</i>	
9:20 – 9:55	Andreas Kuhn, University of Hohenheim, Germany* <i>Reconstituted Membrane Insertion of Single Proteins in Real Time</i>	
9:55 – 10:25	Coffee Break	Spazio Roccia
10:25 – 10:40	Sonia Troeira Henriques, University of Queensland, Australia* <i>Cyclotides, Stable Drug Scaffolds Use Phosphatidylethanolamine Lipids as a Switch to Internalize Inside Cells</i>	
10:40 – 10:55	Burkhard Bechinger, University of Strasbourg/CNRS, France* <i>The Role of Membranes during Polyglutamine Self Aggregation of Huntingtin</i>	
10:55 – 11:30	Daniel Müller, Eidgenössische Technische Hochschule Zürich, Switzerland <i>Folding Steps of Single Polypeptides into Membrane Proteins</i>	
11:30 – 12:05	Christian Eggeling, University of Oxford, United Kingdom <i>Advanced (Super-Resolution) Optical Microscopy to Determine Plasma-Membrane Dynamics</i>	
12:30 – 14:00	Lunch	Sala Luce
Session IX	Daniel Müller, Eidgenössische Technische Hochschule Zürich, Switzerland, Chair	
14:00 – 14:50	Phyllis Hanson, Washington University, USA <i>Membrane Remodeling by ESCRT-III and Friends</i>	
14:50 – 18:30	Free Evening for Networking	
19:00 – 20:00	Dinner	Sala Luce

* Contributed talks selected from among submitted abstracts

Thursday, September 15, 2016

8:30 – 18:00	Registration/Information	Main Entrance
Session X	Gisou van der Goot, École Polytechnique Fédérale de Lausanne, Switzerland, Chair	
8:45 – 9:20	Lawrence Rajendran, University of Zurich, Switzerland <i>Role of Exosomes in the Production and Propagation of Amyloids in Neurodegenerative Diseases</i>	
9:20 – 9:55	Botond Roska, Friedrich Miescher Institute for Biomedical Research, Switzerland <i>Gene Therapy for Blindness</i>	
9:55 – 10:25	Coffee Break	Spazio Roccia
10:25 – 10:40	Stavroula Sofou, Rutgers University, USA* <i>Sticky Patches for Untargetable Cancer Cells: Triggered Ligand Clustering on Lipid Nanoparticles Enables Selective Cell Targeting and Killing</i>	
10:40 – 10:55	Karin Norling, Chalmers University of Technology, Sweden* <i>The Properties and Cellular Uptake Characteristics of Liposome-based Mucosal Vaccines</i>	
10:55 – 11:30	Anne Spang, University of Basel, Switzerland <i>Stress-dependent Prion-like Aggregate Formation Regulates Protein Sorting and Export at the Trans-Golgi Network</i>	
11:30 – 12:05	Lukas Tamm, University of Virginia, USA <i>The Role of Cholesterol in Virus Entry</i>	
12:30 – 14:30	Lunch	Sala Luce
Session XI	Anne K. Kenworthy, Vanderbilt University, USA, Chair	
14:30 – 15:05	Sarah L. Veatch, University of Michigan, USA <i>Phases and Fluctuations in Biological Membranes and Possible Implications for General Anesthesia</i>	
15:05 – 15:20	Susan Daniel, Cornell University, USA* <i>Membrane Protein Mobility and Orientation Preserved in Supported Bilayers Created Directly from Cell Plasma Membrane Blebs</i>	
15:20 – 15:35	Kelly K. Lee, University of Washington, USA* <i>Visualization and Sequencing of Membrane Remodeling Leading to Influenza Virus Fusion</i>	
15:35 – 15:50	Max Piffoux, Laboratoire Matière et Systèmes Complexes, France* <i>Monitoring Extracellular-Vesicles Dynamics at the Nanoscale by Liquid-Cell TEM</i>	
15:50 – 17:25	Coffee Break & Poster Session III	Balint Salon

* Contributed talks selected from among submitted abstracts

17:25 – 18:00	Suzanne Eaton, Max Planck Institute of Molecular Cell Biology and Genetics, Germany <i>Lipoproteins in Hedgehog Release and Signaling</i>	
19:00 – 20:00	Dinner	Sala Luce
Session XII	Special Evening Lecture Lukas Tamm, University of Virginia, USA, Chair	
20:00 – 20:50	Matthew Wood, University of Oxford, United Kingdom <i>Extracellular Vesicles for Trans-Blood Brain Barrier Drug Delivery</i>	

Friday, September 17, 2016

8:30 – 13:00	Information	Main Entrance
Session XIII	Jay T. Groves, University of California, Berkeley, USA, Chair	
8:45 – 9:20	Sarah L. Keller, University of Washington, USA <i>Colocalization of Lipid Domains across the Two Faces of a Membrane</i>	
9:20 – 9:55	Petra S. Dittrich, Eidgenössische Technische Hochschule Zürich, Switzerland <i>Microfluidic Methods to Study Lipid Membrane Permeation, Deformation, and Fusion</i>	
9:55 – 10:25	Coffee Break	Spazio Roccia
10:25 – 10:40	Wye Khay Fong, Monash University, Australia* <i>Dynamic Creation of Nanostructured Lipid Self-assembled Mesophases via Invertase Digestion Triggers Controlled Release of Encapsulated Drug</i>	
10:40 – 10:55	Roy Ziblat, Harvard University, USA* <i>Membrane Decoys as Anti-Viral Nanomedicine</i>	
10:55 – 11:30	Chen-Yu Zhang, Nanjing University, China <i>Exosomes Sufficiently Deliver Secreted Small RNA to Recipient Tissues</i>	
11:30 – 12:05	Gisou van der Goot, École Polytechnique Fédérale de Lausanne, Switzerland <i>Function and Dynamics of Protein Palmitoylation</i>	
12:05 – 12:30	Closing Remarks & Biophysical Journal Poster Awards Presentation Horst Vogel, École Polytechnique Fédérale de Lausanne, Switzerland CSF Poster Award	
12:30 – 14:30	Lunch	Sala Luce

* Contributed talks selected from among submitted abstracts

SPEAKER ABSTRACTS

Disentangling Viral Membrane Fusion from Receptor Binding Using Synthetic DNA-Lipid Conjugates and a New Approach for Measuring Short-range Interactions in Membranes

Steven G. Boxer¹, Robert Rawle^{1,2}, Frank Moss¹, Peter Kasson².

¹Stanford University, Stanford, CA, USA, ²University of Virginia, Charlottesville, VA, USA.

Enveloped viruses must bind to a receptor on the host membrane to initiate infection. Membrane fusion is subsequently initiated by a conformational change in the viral fusion protein. We present a method to disentangle the two processes of receptor binding and fusion using synthetic DNA-lipid conjugates to bind enveloped viruses to target membranes in the absence of receptor. We demonstrate this method by binding influenza virus to target vesicles and measuring the rates of individual fusion events using fluorescence microscopy. Influenza fusion kinetics are found to be independent of receptor binding. This approach should allow the study of viruses where challenging receptor reconstitution has previously prevented single-virus fusion experiments (e.g., HIV, Ebola and Zika).

The nanometer scale organization of the eukaryotic plasma membrane is presumed to be critical for signaling, viral budding, and other membrane phenomena. We use secondary ion mass spectrometry to probe the nanometer scale structure of supported lipid bilayers (SLBs) and monolayers by taking advantage of the intermolecular recombination of ions to form diatomic species that occurs in dynamic SIMS. As an example, we show that the efficiency of this atomic recombination to form secondary ¹³C¹⁵N⁻ ions depends on the distance between ¹³C and ¹⁵N atoms installed on lipid head groups. Likewise we can measure recombination of labels on opposite leaflets of a bilayer, putting an upper limit of about < 5 nm of the range of this method. We refer to this method of measuring nanometer-scale distances between isotopically labeled molecules as “a chemical ruler,” somewhat analogous to the use of FRET. While still in the calibration phase, this method may provide information on lipid-lipid, lipid-protein and protein-protein on a very short length scale.

Probing the Early Stages of RTK Signaling in Plasma Membrane Vesicles

Kalina Hristova

Johns Hopkins University, Baltimore, MD, USA

No Abstract

**Heterogeneities in Membrane Composition and Curvature of Liposomes, Two
Non-stochastic Regulators of Biological Function**

Dimitrios Stamou

University of Copenhagen, Copenhagen, Germany

No Abstract

Membrane Binding and Remodelling by the COPII Complex

Jan Auerswald, Sebastian Daum, Jan Ebenhan, Daniela Kruger, Mona Gross, Stefan Werner,
Kirsten Bacia.

University of Halle, Halle, Germany.

COPII vesicles are responsible for transporting cargo from the ER towards the Golgi apparatus in the secretory pathway. The small GTPase Sar1, which belongs to Sar1/Arf family of GTPases and the Ras-superfamily, is an essential component in COPII-vesicle formation. Upon activation with GTP, Sar1 binds to membranes, embedding an amphipathic helix into the proximal leaflet of the bilayer. The exact role of GTP presence versus GTP hydrolysis in the formation in COPII vesicle fission is still controversial. We study COPII vesicle formation in a bottom-up fashion on *S. cerevisiae* proteins using cryo-electron microscopy techniques, confocal imaging and biophysical techniques. Cryo-EM shows strongly different COPII coat morphologies under GTP hydrolyzing versus non-hydrolyzing conditions. More subtle differences are observed by cryo-EM among reconstituted COPII samples under non-hydrolyzing conditions. Membrane binding of the small GTPases Sar1 and Arf1 is studied using biophysical techniques. By combining fluorescence correlation spectroscopy (FCS) with a Langmuir film balance, the protein's footprint in the proximal membrane leaflet is revealed. Dual-color fluorescence cross-correlation spectroscopy analysis has been developed into a method for obtaining binding curves and testing binding models of protein binding to freely diffusing vesicles. The higher specificity makes fluorescence cross-correlation advantageous compared to previous fluorescence autocorrelation analysis also for small vesicle applications.

Use of Model Membranes-Liposomes and Micelles of Variable Lipid Composition to Elucidate the Molecular Mechanism of Action of Pore-forming Proteins and Peptides

Gustavo P. Carretero¹, Eduardo M. Cilli², Carlos Alvarez³, **Shirley Schreier**¹.

¹University of Sao Paulo, Sao Paulo, Sao Paulo, Brazil, ²State University of Sao Paulo, Araraquara, Sao Paulo, Brazil, ³University of La Habana, La Habana, Cuba.

Sticholysins I and II, cytolytic proteins purified from the sea anemone *Stichodactyla helianthus*, lyse biological and model membranes. The proposed mechanism of action consists of the formation of a toroidal pore involving the N-terminal domain. The interaction between peptides from the toxins' N-termini (StI1-31 and StI12-31 SELAGTIIDGASLTFEVLDKVLGELGKVSRL, and StII1-30 and StII11-30 ALAGTIIAGASLTFQVLDKVLEELGKVSRL) and model membranes – liposomes and micelles – was studied in order to contribute to the elucidation of the toxins mechanism of action at the molecular level. Peptides were used based on the hypothesis that protein fragments can mimic the structure and activity of the whole protein. An analogue containing the paramagnetic amino acid TOAC (N-TOAC-StII11-30) was also studied. Conformational studies made use of circular dichroism (CD), electron paramagnetic resonance (EPR), and fluorescence. Studies of structure prediction and molecular modeling were also performed. The peptides acquired α -helical conformation upon interaction with model lipid membranes, in agreement with the conformation found for these segments in the whole proteins. Studies with membranes of variable lipid composition, demonstrated that both electrostatic and hydrophobic interactions contribute to peptide binding. Fluorescence quenching of labeled lipids by paramagnetic TOAC and EPR spectra allowed us to locate the TOAC residue at the membrane-water interface, corroborating the proposed model of the toroidal pore. CD and EPR studies also allowed calculation of peptide-membrane binding constants. The peptides also mimicked the toxins function, as shown by assays of carboxyfluorescein leakage and hemolytic activity. Short peptides containing parts of StII1-30's sequence were synthesized with the aim of testing their antimicrobial activity. The peptides displayed low antimicrobial activity, as well as lack of hemolytic activity and toxicity against human cells.

Membrane Pore-forming Proteins in the Molecular Arms Race Between Host and Pathogen

Helen R. Saibil.

Birkbeck, University of London, London, United Kingdom.

Pathogens have evolved weapons to invade and damage our cells, and our immune system has evolved defences against these attacks. Among the weaponry used by both sides in this continual war are proteins that punch holes in cell membranes. Membrane perforation enables pathogens to take over host cells and resources for their own replication, and also enables host immune systems to kill invading pathogens. The membrane attack complex-perforin (MACPF)/cholesterol dependent cytolysin (CDC) superfamily of membrane pore-forming proteins is used by a wide range of pathogens as well as by host immune systems.

The objective of the work is to understand the mechanisms by which MACPF and CDC proteins convert from their soluble, monomeric forms into large, oligomeric arcs and rings that insert into membranes and perforate them, without any external energy source. The structures of several CDC and MACPF protein assemblies on liposomes have been determined by cryo electron microscopy and single particle analysis; the dynamics of pore formation were studied by atomic force microscopy. Fluorescence microscopy and cellular electron tomography have been used to study the actions of MACPF proteins *in situ*. The findings reveal common features of the pore forming mechanism in different members of the superfamily, involving the opening of a bent and twisted beta sheet and release of compact, helical clusters to assemble into an extended, transmembrane beta barrel. Displacement of a helical motif positioned near the bend in the beta sheet appears to be required to unlock the conformational change.

pHLIP®: Uses in Measuring Cell Surface pH, Imaging Tumors, and Delivering Therapeutics

Oleg A. Andreev^{2,3}, **Donald M. Engelman**^{1,3}, Yana K. Reshetnyak^{2,3}.

¹Yale, New Haven, CT, USA, ²University of Rhode Island, Kingston, RI, USA, ³pHLIP, Inc., Kingston, RI, USA.

Acidity is a general property of tumors, and may serve as a biomarker that is not susceptible to resistance by selection. The discovery of pHLIP®s (pH (Low) Insertion Peptides) provides a path to exploit this biomarker, and has led to the use of related peptides to study peptide insertion across bilayers, to selectively target cargoes to tumors and other acidic tissues *in vivo*, and to deliver molecules across tumor cell plasma membranes. A pHLIP® is unfolded on the surface of a membrane at normal pH, and folds to form a transmembrane helix when the pH is lowered.

Tumor acidity is expected to be enhanced at cell surfaces, and, by using pHLIP® to position pH-sensing dyes, it has been possible to document the lower surface pH, and to show that glucose further enhances acidity. Data will be presented on these key observations.

Imaging agents, such as fluorescent labels or PET isotopes, can be positioned at the surfaces of tumor cells. Accumulation of these labels may allow uses in diagnosis and image-guided surgery. Examples will be shown.

pHLIP® peptides also have the potential to target and deliver therapeutic molecules into tumor cells. A remarkable opportunity may be afforded to expand the chemical range of such molecules, since translocation succeeds for agents that are large and polar. We will show examples of the translocation of PNAs and of Amanitin, each of which is shown to inhibit tumor growth *in vivo*, and argue the case for a “new pharmacology”.

The Interdependence of Vesicular Membrane Dynamics and Signal Processing

Philippe Bastiaens.

Max Planck Institute of Molecular Physiology, Systemic Cell Biology, Dortmund, Germany.

Autocatalytic phosphorylation of receptor tyrosine kinases (RTKs) enables diverse, context-dependent responses to extracellular signals but comes at the price of autonomous, ligand-independent activation. Reactions in and on membranes play an important role in extracellular information processing by cells. The local concentration of signaling proteins is maintained by membrane dynamics to tightly control the qualitative response properties of signaling systems. In order to illuminate the relevance of this spatial dimension in signaling, I will describe how vesicular membrane dynamics control the autocatalytic activity of receptor tyrosine kinases such as EGFR and EphA2. Spontaneous RTK activation is suppressed by vesicular recycling and dephosphorylation by protein tyrosine phosphatases (PTPs) at the pericentriolar recycling endosome. This spatial segregation of catalytically superior PTPs from RTKs is essential to preserve ligand responsiveness of receptors at the plasma membrane. Ligand-induced clustering, on the other hand, promotes phosphorylation of c-Cbl docking sites and ubiquitination of the receptors, thereby redirecting them to the late endosome/lysosome. This switch from cyclic to unidirectional receptor trafficking thereby converts a continuous suppressive safeguard mechanism into a finite ligand-responsive signaling mode. By comparing the EGFR phosphorylation patterns upon siRNA-silencing and ectopic expression of PTPs using CA-FLIM we also identified which PTPs regulate EGFR phosphorylation when and where in the cell. EGFR phosphorylation patterns are regulated by the transit via endocytosis to different membrane compartments where the cytosolic phosphatase activity consists of different sets of PTPs. PTP localization thereby dictates when it interacts with the receptor. This spatially exerted control of PTPs on vesicular EGFR activity thereby shapes the finite response to growth factors.

Liposomes Activate Innate Immunity Cascades Through Recognition of Toll-like Receptors

Jean-Marie Ruyschaert.

Université Libre de Bruxelles, Brussels, Brabant, Belgium.

Toll-like receptors are major members of the Pattern Recognition Receptors (PRRs) from the innate immune system, which recognize bacterial or viral components (like lipopolysaccharides, flagellin, lipopeptides, single-stranded RNA) and transmit a signal to the cell that brings the immune system in a “state of emergency”, ready to react to a microbial invasion. Recently, apart their role in recognition of pathogen-associated patterns, we provided evidence that non-bacterial ligands like nanoliposomes do activate innate immunity cascades through recognition of Toll-like receptors. Using chimeric construction, molecular docking and site-directed mutagenesis we identified a new binding site which does not correspond to the known natural ligand binding site. This new concept that non-bacterial ligands do activate the innate system opened a new field that we will illustrate with a few examples. A lipid-based gene carrier which was supposed to be inert revealed immune-stimulatory activity, as evidenced by cytokine secretion (TNF- α , IL-12, IFN- β ,). Preliminary data showed that E.Coli cardiolipin activated the inflammatory responses whereas heart cardiolipin did not. These two types of cardiolipin differ exclusively by the degree acyl chain saturation. Cardiolipin from heart is largely unsaturated. Our experimental and modelling data provide evidence that acyl chain saturation is indeed a requirement for insertion into TLR binding pocket and explains the strong inflammatory activity of bacterial cardiolipin. An improved knowledge of the relationship between the lipid properties (nature of the hydrophilic moieties, hydrocarbon tails, mode of organisation) and the activation of the innate pathways opens the way to the design of new molecules tailored for specific applications in human cells (gene transport, adjuvant) and to therapeutic perspectives largely unintended until now.

Lonez, C., Vandenbranden, M., and Ruyschaert, J. M. (2012) *Adv. Drug Deliv. Rev.* 64, 1749-1758

Fluorescence-free Imaging and Tracking of Individual Secretory and Transmembrane Proteins in a Living Cell

Matthew P. McDonald, Katharina König, André Gemeinhardt, Richard W. Taylor, Susann Spindler, Vahid Sandoghdar.
Max Planck Institute for the Science of Light, Erlangen, Bavaria, Germany.

The cellular membrane plays a pivotal role in many biological and medical processes. As an example, proteins protruding from the membrane serve as signaling centers to nearby cells and extracellular biomolecules. Intercellular communications and secretions are also mediated by the membrane through endosome-membrane fusion. Here, we present our recent efforts towards understanding this ubiquitous dynamic system. Using a novel optical interferometric scattering detection technique (iSCAT), we observe real-time secretion events of single label-free proteins ejected from a living cell. Importantly, iSCAT functions by way of mixing the weak analyte's scattering signature with a relatively strong reflected plane wave. Even the tiniest nanoparticles are, therefore, observed via the interference between these two signals. In addition, we perform analogous measurements to track gold nanoparticle labeled transmembrane proteins and lipids in three dimensions as they diffuse across living cells and giant unilamellar vesicles. Temporal and spatial resolutions of $\sim 50 \mu\text{s}$ and $\sim 1 \text{ nm}$ are routinely achieved, allowing for an unprecedented look into membrane-protein diffusion dynamics. The developed method thus has the potential to solve a wide range of problems in cellular physiology, such as intercellular signaling, immunology, and cancer malignancy.

Artificial Cell Membrane Mimics to Study the Role of the Influenza Virus Matrix Protein M1 in Virus Budding

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The influenza virus egresses from its host by deforming the plasma membrane into a bud before pinching off by membrane fission. The viral matrix protein M1, a protein that forms a layer underneath the viral membrane connecting it to the viral genetic material, is believed to play an important role in the virion formation process. Nevertheless, the mechanisms underlying virus assembly and budding are still poorly understood. In this project, we take advantage of minimal cell-membrane models to study the interactions between the matrix protein and lipid membranes. Specifically, we aim at providing fundamental understanding on the role of M1 in virus assembly and egress as well as in virus uncoating during entry.

Giant unilamellar vesicles (GUV) are used to study the protein's ability to deform membranes into a bud. Binding studies performed with fluorescently-labelled proteins reveal that M1 alone is capable of deforming membranes: the interaction leads to membrane inward tubulation, creating vesicle-enclosed lipid structures greatly enriched in matrix protein.

GUV experiments are further complemented with investigations using supported lipid bilayers (SLBs) in combination with surface-sensitive techniques. Quartz crystal microbalance experiments make it possible to characterize the protein's binding affinity and specificity to negatively charged membranes. Our data reveal that protein binding is pH, salt and membrane-charge dependent. Further imaging of the SLB using fluorescence microscopy, surface-enhanced ellipsometry contrast and atomic force microscopy indicates that the protein can locally recruit negatively charged lipids, shedding light on the protein's propensity to self-aggregation at the bilayer surface.

Taken together, our study illustrates the unique potential of cell membrane mimics in providing fundamental biophysical insights into the properties of protein-membrane interactions and into the mechanisms leading to membrane deformation.

Single Molecule Fluorescence Clarifies the Role of Monosialoganglioside GM1 and Sphingomyelin in the In-Membrane Oligomerization of β -Amyloid

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Oligomers of the β -amyloid ($A\beta$) peptide are thought to be implicated in Alzheimer's disease. The plasma membrane of neurons may mediate the oligomerization of $A\beta$ present in brain. Using the single-molecule sensitivity of fluorescence, we address the oligomerization of $A\beta$ monomers on lipid bilayers containing essential components of the neuronal plasma membrane. We find that Sphingomyelin triggers the oligomerization of $A\beta$ and that physiological levels of GM1, organized in nanodomains, do not seed oligomerization. Moreover, GM1 prevents oligomerization of $A\beta$ counteracting the effect of Sphingomyelin. Our results establish a preventive role of GM1 in the oligomerization of $A\beta$ suggesting that decreasing levels of GM1 in brain, e.g. due to aging, could lead to reduced protection from the oligomerization of $A\beta$ and contribute to Alzheimer's onset.

In addition to the new insights into the molecular mechanism(s) that may be involved in Alzheimer's disease, it should be pointed out that this work contains a further important novel finding. We uncovered the existence of nanoscopic heterogeneities (radius 8-26 nm) in microscopically homogenous membranes. This was achieved by a combination of Monte Carlo Simulations, FLIM-FRET and FCS techniques using recently developed fluorescent ganglioside analogues. Such nano-heterogeneities are unresolvable by standard and super-resolution microscopy.

Molecular Steps in SNARE-mediated Membrane Fusion

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

In our own work, we have focused on understanding the mechanisms of SNARE assembly and SNARE-induced fusion using structural and biochemical approaches and in-vitro fusion reactions with native and artificial membranes. Furthermore, we have recently extended our work towards SNARE-“mimetics”, including SNARE-like synthetic molecules with artificially designed adhesion domains as well as membrane proteins of bacterial pathogens that are capable of substituting for endogenous SNAREs. We hope to achieve a better understanding of the energy landscape of the fusion pathway, thus shedding more light on a reaction fundamental to all eukaryotic cells.

Secretory Granules and Their Syntaxin Clusters in the Plasma Membrane

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50-70 copies of each, Syntaxin-1, SNAP-25 and Munc18, cluster in the plasma membrane where a granule has docked. We observed single clusters labeled with syntaxin-1-GFP (Stx) together with their associated granules. A clusters' assembly was induced by the docking of the granule (see also Gandasi & Barg, 2014, Nat Commun. 5, 3914). In single molecules studies, free Stx and Stx in clusters seemed at equilibrium. Maximally about 100 Stx molecules could be recruited by a granule. Apparently syntaxin is recognized by a (yet unidentified) ligand on the granule membrane that exists in limited amounts. Which features are required for syntaxin to enter clusters? All syntaxin mutants that inhibit entry into a complex with Munc18 in vitro, (Burkhardt, P et al., 2008, EMBO J 27, 923) were also deficient in entering clusters. Mutating residues facing out from the complex had no effect. We suggest that Stx acts in a complex with Munc18. Only 3 of 33 syntaxin residues known to contact Munc18 were essential for entry into clusters. Their locations suggest that Hc and SNARE domains are in close proximity, and that syntaxin is in a closed conformation. The recruitment of Stx to granules was halved when SNAP-25 was cleaved by Botulinum neurotoxin E (BoNT-E), and quartered when Munc18-1 and 2 was lacking. But when all but 1% of VAMP/synaptobrevin was cleaved by BoNT-G, recruitment was diminished by only 25%. Hence among the 3 proteins, synaptobrevin was the least important. PC-12 cells were examined by ultrastructure, and the fraction of granules docked to the plasma membrane was measured. Munc18-1 and 2 knockdown diminished docking threefold, synaptobrevin cleavage had no effect. Hence synaptobrevin alone cannot provide the anchor that docks a granule to the plasma membrane.

Ligand-gated Ion Channels: Structures and Functions

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No Abstract

Plasma Membrane PI(4,5)P2 Is Critical for Secretory Granule Exocytosis

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Phosphoinositides, which include PI(4)P (phosphatidylinositol- 4-phosphate), PI(4,5)P2 (phosphatidylinositol- 4,5-bisphosphate) and PI(3,4,5)P3 (phosphatidylinositol-3,4,5-trisphosphate), are involved in multiple signalling cascades but their role in the regulation of exocytosis is not fully understood. Recent studies indicate that phosphoinositides, in particular PI(4,5)P2 and PI(3,4,5)P3, are enriched near docked secretory granules in cell free systems. Here we have studied phosphoinositide dynamics near secretory granules in live pancreatic β -cells using TIRF microscopy. Phosphoinositides were detected with EGFP-labelled PH domains of phospholipase C (PLC) δ 1 (high affinity PI(4,5)P2 sensor), PLC δ 4 (low affinity PI(4,5)P2 sensor), Grp1 (PI(3,4,5)P3), and EGFP-labelled P4M domains (PI(4)P). Secretory granules were labelled with NPY-mCherry or NPY-td-Orange2. In live rat insulin secreting (INS1) cells we found all tested phosphoinositide markers to be evenly distributed across the cell membrane, without discernible clustering or accumulation at granule docking sites. However, when the cells were permeabilized with alpha-toxin, the phosphoinositide markers formed a punctate pattern with partial co-localization with docked secretory granules. In live cells, exposure to insulin promoted synthesis of PI(3,4,5)P3, but did not influence secretory granule distribution or exocytosis. Chemically-induced recruitment of a 5'-phosphatase to the plasma membrane decreased PI(4,5)P2 levels, and resulted in an 85% decrease in secretory granule exocytosis. Our data indicate that plasma membrane PI(4,5)P2 does not influence secretory granule recruitment or distribution. However, since PI(4,5)P2 is the predominant phosphoinositide, we hypothesize that it plays a role in the priming of secretory granules prior to exocytosis.

Using Liposomes as a Model System for Probing the Biochemical Mechanisms of Intracellular Membrane Tethering

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Membrane tethering is a physical association of two intracellular membranes prior to fusion of their lipid bilayers. Many proteins and protein complexes have been proposed to act as membrane tethering factors, but the biochemical mechanisms by which these factors mediate inter-membrane associations remain murky. Here, we have used large and small unilamellar liposomes as models to investigate membrane tethering mediated by the conserved HOPS/Class C Vps complex, an effector for the yeast vacuolar Rab GTPase Ypt7p. To assay tethering, we quantified co-localization of red- and green-labeled liposomes in a confocal fluorescence microscope. We found that for HOPS to tether large liposomes (diameter ~120 nm), Ypt7p is required on both apposed membranes. In contrast, HOPS can tether Ypt7p-free small liposomes (diameter ~55 nm) via a direct interaction between these highly-curved membranes and a curvature-sensing ALPS (amphipathic lipid packing sensor) motif on the Vps41p HOPS subunit. Finally, we found that HOPS can interact directly with the autophagosomal protein Atg8p (the yeast homolog of mammalian LC3B) to tether membranes bearing Atg8p to membranes bearing Ypt7p. Thus, we have shown here, for the first time, how a Rab effector engages in protein-protein and protein-lipid interactions to tether intracellular membranes. This study therefore demonstrates the power and flexibility of using liposomes as chemically-defined models of intracellular membranes in order to address hitherto unexplored questions in cell biology. Furthermore, HOPS is required for cellular entry of the Ebola virus, while autophagosome fusion is involved in diverse pathological states, including cancer, ischemia-reperfusion injury, and protein misfolding diseases. Thus, this study shows how liposomes can be used as platforms for understanding conditions of critical importance in biomedical research.

Imaging Individual Receptors While Extracting Kinetic and Thermodynamic Parameters Using FD-based AFM

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Currently, there is a growing need for methods that can quantify and map the molecular interactions of biological samples, both with high-force sensitivity and high spatial resolution. Force-distance (FD) curve-based atomic force microscopy is a valuable tool to simultaneously contour the surface and map the biophysical properties of biological samples at the nanoscale. This presentation reports the use of advanced FD-based technology combined with chemically functionalized tips to probe the localization of specific sites on single native proteins and on living cells at high-resolution. Using biochemically sensitive tips, we are able to locate specific interaction sites on native protein at unprecedented resolution. We also introduce experimental and theoretical developments that allow force-distance curve-based atomic force microscopy (FD-based AFM) to simultaneously image native receptors in membranes and to quantify their dynamic binding strength to native and synthetic ligands. These binding strengths provide kinetic and thermodynamic parameters of individual ligand-receptor complexes.

Signal Transduction on Membrane Surfaces: The Roles of Space, Force, and Time

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Most intracellular signal transduction reactions take place on the membrane surface. The membrane provides much more than just a surface environment on which signaling molecules are concentrated. There is a growing realization that multiple physical and chemical mechanisms allow the membrane to actively participate in the signaling reactions. Using a combination of single molecule imaging and spectroscopic techniques, my research seeks to directly resolve the actual mechanics of signaling reactions on membrane surfaces both in reconstituted systems and in living cells. These observations are revealing new insights into cellular signaling processes as well as some unexpected functional behaviors of proteins on the membrane surface.

Targeting Proteins to Lipid Rafts: Mechanisms and Consequences

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How proteins and lipids cooperate to form functional domains remains a major question in biology. Here, I will discuss our recent studies of how proteins interact with raft-associated lipids and the functional consequences of these interactions. First, I will discuss how transmembrane proteins are targeted to rafts, focusing on the 99 residue transmembrane C-terminal domain (C99) of amyloid precursor protein as a model. Association of C99 with cholesterol-rich membrane raft domains is thought to promote cleavage of C99 to release the Alzheimer's disease-promoting amyloid- β ($A\beta$) polypeptide. By reconstituting C99 into model membrane systems, we are currently testing the hypothesis that C99 has an intrinsic affinity for raft-like liquid ordered domains imparted by its ability to specifically bind to cholesterol. Second, I will discuss how the bacterial toxin cholera toxin targets rafts as a mechanism to enter cells via endocytosis. Using cell membrane-derived systems and living cells, we are currently examining the role of toxin-induced raft crosslinking in the induction of membrane curvature and toxin uptake.

Assembly of the Secretory Machinery During Insulin Granule Docking

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The assembly of the secretory machinery at the plasma membrane is a poorly understood prerequisite for regulated exocytosis. For example, it is not known whether the required proteins are preassembled at the release site or instead recruited and assembled after vesicle docking. Current models propose that docking of the vesicle occurs through binding to either raft-like clusters of SNARE proteins or to structural proteins such as RIM1, in both cases implying at least partial assembly of the secretory machinery prior to docking. However, direct evidence for this is lacking. Using high resolution live cell microscopy we showed that the transition from a loosely tethered to the stably docked state occurs within seconds after vesicle arrival by recruitment of syntaxin and munc18. Here we extend on this work and present quantification of several exocytosis proteins at insulin granule release site during docking, priming and exocytosis. We find that the Rab3 interacting protein RIM1 and Rabphilin were enriched at docking sites prior to vesicle tethering and docking. A slow increase in RIM1 fluorescence was seen during granule maturation into the releasable pool (priming), suggesting roles for RIM1 in both docking and priming. None of the other proteins were present before granule arrival, but these were instead recruited during docking or later during priming. Granules that successfully docked carried Rab3 and Rabphilin, whereas those that only temporarily tethered did not. In contrast, Rab27 and its effector Granuphilin were present on both types of granules. We conclude that sites enriched in RIM1 at the plasma membrane may facilitate docking by weakly tethering the incoming granule through interaction with rab3/rabphilin. Successful docking requires acute clustering of syntaxin/munc18, and we propose that this cluster then nucleates assembly of the exocytosis machinery.

Fusion of Synthetic Lipid Carriers to Exosomes Produces Hybrid Vesicles Harnessed for the Delivery of Biomolecules

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The release of native extracellular vesicles (EVs) by virtually all cells in the body, has emerged as a crucial mechanism for communication between neighboring and distant cells. Researchers are trying to harness this natural way of communication to deliver therapeutics into cells of interest.

Using these natural intercellular communicators as potent drug carriers requires the ability of loading therapeutic cargo into the vesicles. The current loading methods raise safety and/or scalability concerns as they involve bioengineering or vigorous membrane manipulation. Conversely, synthetic vesicles made by modern nanotechnological methods have a high therapeutic loading. Thus a method has been devised combining the loading capacity of synthetic vesicles with the complex surface structure of EVs in a hybrid carrier by fusion. The assembly of this hybrid carrier, termed “hybridosome”, was demonstrated in bulk (lipid mixing, light scattering) and at a single particle level (fluorescence cross-correlation spectroscopy, nanoparticle tracking). The underlying fusion mechanism is simultaneously inducible and self-limiting, offering a self-assembly method that is predictable and independent of bioengineering. All synthetic membrane constituents are non-toxic and FDA approved or currently in advanced clinical trials.

An in vitro proof-of-concept based on hybridosomes from glioblastoma-derived EVs and GFP reporter gene encapsulating synthetic vesicle, showed fast and functional gene delivery into glioblastoma cells. Hybridosome versatility was demonstrated by encapsulating nucleic acids, proteins and gold nanoparticles. Moreover, hybridosomes were decorated with PEG, antibody fragments and peptides, emphasizing that hybridosomes can additionally be tailored for cellular targeting and crossing biological membranes. The findings were extended to EVs derived from primary human platelets and neutrophils.

By presenting functional hybridosomes encapsulating both therapeutic and diagnostic agents, this new delivery platform allows legitimate prospects in several biomedical applications.

Mechanics of Extracellular Vesicles from Plasmodium Falciparum Infected Red Blood Cells

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Malaria is a life-threatening disease caused by parasites that are transmitted through the bites of infected mosquitoes, with Plasmodium falciparum (Pf) causing the most severe form of malaria (1). Very recently it was discovered that Pf infected red blood cells (iRBC) directly transfer information between parasites within a population using exosome like-vesicles that are capable of delivering genes (2). This communication promotes parasite differentiation to sexual forms and is critical for its survival in the host and transmission to mosquitoes.

Efficient DNA transfer via extracellular vesicles (EVs) occurs mainly at the early ring stage within the blood-stage asexual cycle, and it can be inhibited by addition of actin polymerization inhibitors. This suggests that actin polymerization is required for cell-cell communication (2). We expect, therefore, that mechanical properties of vesicles at different stages of the life cycle will be optimized for their function, and more specifically, the stiffness of the ring stage EVs is likely better optimized for efficient DNA-dependent communication. With the aim to understand how mechanical properties of EVs effect their efficiency of cargo delivery, we use Atomic Force Microscopy for mechanical characterization of extracellular vesicles secreted from Pf infected RBCs. We perform a detailed comparison between the mechanical properties (bending modulus values) of these vesicles and those secreted from healthy RBCs, as well as compare their size and morphology. We also prepare and characterize synthetic vesicles (SVs) with varying mechanical strength to determine how stiffness of SVs affects their adhesion to cells and cellular uptake rate, to gain a broader understanding of the link between mechanical properties and efficient cellular uptake.

1. WHO <http://www.who.int/mediacentre/factsheets/fs094/en/>.
2. N. Regev-Rudzki et al., Cell 153, 1120 (May 23, 2013).

Phase Transitions: An Emerging Principle in Cytoplasmic Organization and Neurodegeneration

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No Abstract

Cotranslational Protein Folding

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We have developed a technique based on the use of so-called translational arrest peptides to measure forces acting on a nascent chain during cotranslational processes such as membrane translocation and folding. In contrast to hard-core biophysical methods, the technique can be used both in vitro and in vivo. Recent results on cotranslational folding of cytoplasmic and membrane proteins will be presented.

Biomimetic Block Copolymer Membranes

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Similar to conventional lipids, suitable amphiphilic block copolymers self-assemble in aqueous media to membrane-like superstructures. The physical properties of these membranes can be controlled to a large degree via the chemical constitution, the molecular weight and the hydrophilic-to-hydrophobic block length ratio of these polymers. Compared to conventional low molar mass building blocks (e.g. lipids), membranes based on amphiphilic copolymers self-assembly, have both the advantage of superior stability and toughness, and offer various possibilities of tailoring physical, chemical and biological properties by multifunctionality, which can be implemented in one single macromolecule. Well-defined functions such as molecular recognition, cooperativity, regulation, replication, catalysis and antibiotics can be introduced by combining these synthetic superstructures with suitable functional units from Nature, e.g., by incorporation of integral membrane proteins or encapsulation of enzymes.

By introducing appropriately functionalized polymers we succeeded to immobilize intact block copolymer vesicles or induce the formation of planar block copolymer membranes on solid support. Interestingly by Fluorescence Correlation Spectroscopy we could quantify the lateral mobility of block copolymer molecules as well as inserted proteins inside the block copolymer membranes. In comparison to biological phospholipid membranes the block copolymer membranes indicate systematic differences in mobility.

In addition systematic experiments indicate that mixtures of phospholipids and block copolymers can form membranes in aqueous media consisting of phase separated phospholipid and block copolymer domains. Depending on their composition, the thickness of the polymer and/or phospholipid domains and their viscosity, we observed a systematic influence on insertion of proteins into a preferred domain and their local mobility.

Reconstituted Membrane Insertion of Single Proteins in Real Time

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The membrane insertase YidC inserts newly synthesized proteins into the plasma membrane. While defects in YidC homologs in animals and plants cause diseases, YidC in bacteria is essential for life. Membrane insertion and assembly of a functional ATP synthase and respiratory complexes is catalysed by the 6-spanning YidC protein of *E. coli*. To investigate how YidC interacts with the membrane-inserting proteins, we generated single cysteine mutants in YidC and in the model substrate, the Pf3 coat protein. The insertion process was then followed in a reconstituted system with purified components. The YidC protein was labeled with a fluorescent probe and assembled with lipids to form proteoliposomes. Single molecule FRET technology shows that the inserting Pf3 coat protein closely binds YidC at the cytoplasmic face of YidC with its C-terminal domain. Then, the N-terminal domain is translocated within milliseconds contacting the periplasmic domain of YidC at the outer face of the membrane. Observation of membrane-inserting complexes *in vivo* of single cysteine mutants of both the Pf3 coat protein and YidC was possible after coexpression and the analysis of disulfides formed during the first 30 sec of synthesis. The results show that the substrate contacts different YidC residues in 4 of the 6 transmembrane regions. The residues are located either in the region of the inner leaflet, in the membrane center as well in the periplasmic leaflet. These contacting sites show a consecutive transmembrane stack in the recently published crystal structure of YidC suggesting a substrate can slide in between TM3 and TM5 of YidC. A model will be presented how the Pf3 protein moves into the membrane bilayer by interaction with YidC.

Cyclotides, Stable Drug Scaffolds Use Phosphatidylethanolamine Lipids as a Switch to Internalize Inside Cells

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Cyclotides, a large family of peptides from plants, are exceptionally stable and characterized by a cyclic structure and three disulfide-bonds arranged in a cystine knot. Recently we have found that cyclotides can be internalized into human cells at non-toxic concentrations. Their exceptional stability, bioactivities and cell-penetrating properties make cyclotides exciting templates in drug design and delivery. Using surface plasmon resonance, nuclear magnetic resonance spectroscopy, mass spectrometry, confocal microscopy and flow cytometry, we have shown that the prototypic cyclotide, kalata B1, can enter cells via both endocytosis and by direct membrane translocation. Both pathways are initiated through binding to the cell membrane by targeting phosphatidylethanolamine-phospholipids at the cell surface and inducing membrane curvature. This unusual approach to initiate internalization might be employed to deliver drugs into cells and, in particular, into cancer cells with a higher proportion of surface-exposed phosphatidylethanolamine-phospholipids. Our findings highlight the potential of using cyclotides in the development of stable delivery systems for the manipulation of traditionally ‘undruggable’ targets, such as intracellular protein-protein interactions in cancer pathways.

The Role of Membranes During Polyglutamine Self Aggregation of Huntingtin

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The accumulation of aggregated protein is a typical hallmark of many human degenerative disorders, amongst which is Huntington's disease. Misfolding of the amyloidogenic proteins give rise to self-assembled aggregates. The huntingtin protein is characterized by a segment of consecutive glutamines (Qn), responsible for its fibrillation. It has been demonstrated that the 17 residue N-terminal domain of the protein, located upstream of its polyglutamine tract, strongly influences its aggregation behavior, and thereby the correlated development of the disease. We have shown that this Htt-17 domain can act as a reversible membrane anchor when at the same time it markedly increases polyglutamine aggregation rates. Here we characterize in quantitative detail how cross-talk between Htt17, the polyglutamine tract and liposomes strongly affects polyQ aggregation kinetics.

Furthermore, we investigated membrane interactions, structure, topology and aggregation of Htt-17-polyQ in great detail. The structural transitions of Htt17 upon membrane-association result in an in-plane aligned stable α -helical conformation. The membrane binding of Htt17 was analyzed and is strongly dependent on lipid composition, whereas the helical tilt angle is constant in all investigated membranes. The activity of the Htt17 membrane anchor is pivotal in bringing together the polyglutamine domains, facilitating their oligomerization into β -sheet fibrils. Our study shows how the membrane interactions of protein domains influences the supramolecular organization of amino acid polymers in a complex chemical environment and more specifically the role of the membrane interface in the folding and amyloid formation of polyglutamines. Such quantitative biophysical and structural data may also help to successfully design new therapeutic concepts and molecules targeting the N-terminal regions of huntingtin as well as proteins of other polyglutamine-related diseases.

Folding Steps of Single Polypeptides into Membrane Proteins

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No Abstract

Advanced (Super-Resolution) Optical Microscopy to Determine Plasma-Membrane Dynamics

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Molecular interactions are key in cellular signalling. They are often ruled or rendered by the mobility of the involved molecules. We present different tools that are able to determine such mobility and potentially extract interaction dynamics. Specifically, the direct and non-invasive observation of the interactions in the living cell is often impeded by principle limitations of conventional far-field optical microscopes, specifically with respect to limited spatio-temporal resolution. We depict how novel details of molecular membrane dynamics can be obtained by using advanced microscopy approaches such as the combination of super-resolution STED microscopy with fluorescence correlation spectroscopy (STED-FCS), of fast beam-scanning with FCS (scanning (STED-)FCS), of fluorescence recovery after photobleaching (FRAP), or of single-particle tracking. Their performance on investigating different diffusion modes of plasma membrane proteins and lipids in the living cell are compared, and we highlight how these modes give novel details of membrane bioactivity such as in immune cells. It is often optimal to gather complementary information from all techniques.

Membrane Remodeling by ESCRT-III and Friends

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No Abstract

Role of Exosomes in the Production and Propagation of Amyloids in Neurodegenerative Diseases

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No Abstract

Gene Therapy for Blindness

Botond Roska

Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

No Abstract

Sticky Patches for Untargetable Cancer Cells: Triggered Ligand Clustering on Lipid Nanoparticles Enables Selective Cell Targeting and Killing

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The majority of breast cancer patients (70%) have tumors designated as ‘HER2-negative’ (<1⁺ HER2-expression evaluated by immunohistochemistry or < 200,000 HER2-copies per cell). For these patients there are no targeted therapeutic options utilizing the HER2 receptor. The ability of conventionally targeted nanoparticles for specific targeting stops to hold on cancer cells expressing less than 200,000 copies of HER2 per cell or less than two receptors per nanoparticle's projected area (for particles of 100 nm in-diameter). This geometry corresponds to the limit of multivalent interactions (avidity) loosely defined as multiple contacts between neighboring same-cell receptors with ligands from a single nanoparticle. An alternative therapeutic approach is needed, therefore, to enable selective targeting and effective killing of cancer cells with low or too low HER2 expression. Towards this goal we designed targeted lipid nanoparticles (vesicles) that contain HER2-targeting short peptides densely conjugated (for high local multivalency) within sticky patches. Sticky patches are phase-separated raft-like lipid-domains of high local multivalency which is induced by preferential partitioning of peptide-functionalized lipids. To enable selectivity in binding, sticky patches are exclusively triggered to form in mildly acidic environments matching the tumor interstitium. Lipid phase-separation with lowering pH is a result of the interplay of decreasing (pH-tunable) electrostatic repulsion and attractive hydrogen bonding among the domain-forming lipids. We show that lipid nanoparticles with sticky patches selectively associate with and kill HER2-negative and triple negative breast cancer cells (MCF-7 and MDA-MB-231, respectively, with 60,000 and 90,000 HER2-copies per cell) while do not affect cardiomyocytes and breast normal cells. Systematic studies of association, dissociation and internalization rates of nanoparticles by cells will be presented, and a mechanistic mathematical model will be discussed with the aim to explain the observed high avidity.

The Properties and Cellular Uptake Characteristics of Liposome-based Mucosal Vaccines

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Liposomes have attracted attention as promising pharmaceutical carrier candidates. As vaccine vectors, they have the advantage of possessing inherently adjuvanting properties (1). Their physicochemical properties are easily customized; however, little is currently known about how the properties of antigen-carrying liposomes affect their processing by immune cells and how that in turn influences the elicited immune response. Our aim is to correlate the physicochemical properties of vaccine particles to their efficacy *in vivo* and in extension to use this knowledge in the development of an effective mucosal influenza vaccine. Hence we produce and characterize liposomal vaccine particles with regards to size, charge and antigen content. Furthermore, we develop *in vitro* assays to study the processing by dendritic cells. Currently, we are developing microscopy-based methods for studying two parts of the processing: the particle uptake and antigen presentation. For the uptake-assay, cells are grown on a topographically micro-patterned substrate in order to ensure the particles access to the basal membrane so that TIRF microscopy can be used to visualize particle attachment and uptake. The trajectories exhibited by the particles during this process are analyzed using single particle tracking in order to quantify and categorize different modes of uptake. In order to assess how the packaging of the antigen, and mode of uptake, affects the extent of the presentation, an antibody against the antigen peptide-MHC class II complex is used to visualize the amount of antigen functionally presented on the cell surface. Hopefully these new tools will help us make more informed vaccine design choices as well as identify promising vaccine formulation candidates at an early stage.

1. Torchilin, V. P. (2005) Recent advances with liposomes as pharmaceutical carriers. *Nature Reviews Drug Discovery*, 4 (2) 145-160.

Stress-dependent Prion-like Aggregate Formation Regulates Protein Sorting and Export at the Trans-Golgi Network

Claudia Stohrer, Martina Horanova, **Anne Spang**.

Organisms have to respond to environmental stress such as dehydration. To mount a rapid response, sensors are present in the plasma membrane serving as a signal relay station. Polycystin2 (PKD2)-related TRP channels act as mechanosensors and are activated under hypotonic conditions. The yeast PKD2-related Flc2 is present at the plasma membrane and the ER. The localization of the protein is dependent on the presence of functional ER-plasma membrane contact sites and constant cycling between the trans-Golgi Network (TGN) and the plasma membrane. Regulated export of Flc2 to the plasma membrane is achieved by the exomer adaptor complex. While Flc2 is plasma membrane-localized in iso- and hypotonic medium, it is rapidly endocytosed and stored in the TGN under hyperosmotic stress. Flc2 contains a short N-rich unstructured stretch in the C-terminus, and TGN retention is at least partly dependent on the prion-like domain of Pin2. This prion-like domain dependent retention mechanism prevents untimely discharge of the proteins at the plasma membrane and equally protects it from degradation. Upon stress removal, Flc2, similar to Pin2, quickly regained plasma membrane localization, in a process involving the Batten disease protein Btn2, which has been implicated in resolving prion aggregates. Thus Pin2 and Flc2 might be part of a stress-stabilized liquid droplet domain at the TGN that is resolved by Btn2 when the stress subsides.

The Role of Cholesterol in Virus Entry

Lukas Tamm

University of Virginia, Charlottesville, VA, USA

No Abstract

Phases and Fluctuations in Biological Membranes and Possible Implications for General Anesthesia

Sarah L. Veatch.

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The thermodynamic properties of plasma membrane lipids play a vital role in many functions at the mammalian cell surface. Some functions are thought to occur, at least in part, because plasma membrane lipids have a tendency to separate into two distinct liquid phases. We propose that these lipid mediated functions occur because plasma membrane composition is tuned close to a miscibility critical point at physiological temperature. This hypothesis is supported by our observations of micron-sized and dynamic critical composition fluctuations in isolated plasma membranes near room temperature. In this talk, I will discuss some of our ongoing efforts to probe for the existence and consequences of criticality in the plasma membranes of intact cells using super-resolution imaging of intact cellular membranes and functional studies of lipid mediated processes. I will focus on recent results showing a striking correlation between the effects of compounds on critical temperatures in isolated plasma membrane vesicles and their function as general anesthetics in cells and organisms. I will present our current thinking of how at least some aspects of anesthetic functions could be attributed to lipid heterogeneity.

Membrane Protein Mobility and Orientation Preserved in Supported Bilayers Created Directly from Cell Plasma Membrane Blebs

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Membrane protein interactions with lipids are crucial for their native biological behavior, yet characterization methods are often carried out on purified protein in the absence of native lipids. We present a simple method to transfer membrane proteins expressed in mammalian cells to an assay-friendly, cushioned, supported lipid bilayer (SLB) platform. The SLB platform is planar and compatible with many surface characterization tools. However, its full potential has yet to be reached because of the challenges associated with integration of membrane proteins, namely maintaining protein fluidity, orientation, and function. We developed a method for the delivery of proteins to SLBs via cell blebs. Cell blebs are sections of the cell membrane that bud off into a proteoliposome during local detachment of the membrane from the cytoskeleton. Native membrane travels with the proteins to the SLB, so crucial lipid interactions can be preserved. Cell blebs, expressing either GPI-linked yellow fluorescent proteins or neon-green fused transmembrane P2X2 receptors, were induced to rupture on glass surfaces using PEGylated lipid vesicles, which resulted in planar supported membranes with over 50% mobility for multi-pass transmembrane proteins and over 90% for GPI-linked proteins. Individual proteins were tracked and diffusion was characterized as free or confined, revealing details of the local lipid membrane heterogeneity surrounding the protein. A particularly useful result of our bilayer formation process is the protein orientation in the SLB. For both the GPI-linked and transmembrane proteins used here, an enzymatic assay revealed that protein orientation in the planar bilayer results in the extracellular domains facing towards the bulk, and that the dominant mode of bleb rupture is via the “parachute” mechanism. This work is the first experimental confirmation of the parachute mechanism, to our knowledge.

Visualization and Sequencing of Membrane Remodeling Leading to Influenza Virus Fusion

Long Gui^{1,2}, Jamie L. Ebner¹, Alexander Mileant², **Kelly K. Lee**^{1,2}.

¹University of Washington, Seattle, WA, USA, ²University of Washington, Seattle, WA, USA.

Protein-mediated membrane fusion is an essential step in many fundamental biological events including enveloped virus infection. The nature of protein and membrane intermediates and the sequence of membrane remodeling during these essential processes remain poorly understood. Here we used cryo-electron tomography (cryo-ET) to image the interplay between influenza virus and vesicles with a range of lipid compositions. By following the population kinetics of membrane fusion intermediates imaged by cryo-ET, we found that membrane remodeling commenced with the hemagglutinin fusion protein spikes grappling to the target membrane, followed by localized target membrane dimpling as local clusters of hemagglutinin started to undergo conformational refolding. The local dimples then transitioned to extended, tightly apposed contact zones where the two proximal membrane leaflets were in most cases indistinguishable from each other, suggesting significant dehydration and possible intermingling of the lipid head groups. Increasing the content of fusion-enhancing cholesterol or bis-monoacylglycerophosphate in the target membrane, led to an increase in extended contact zone formation. Interestingly, hemifused intermediates were found to be extremely rare in the influenza virus fusion system studied here, most likely reflecting the instability of this state and its rapid conversion to postfusion complexes, which increased in population over time. By tracking the populations of fusion complexes over time, the architecture and sequence of membrane reorganization leading to efficient enveloped virus fusion were thus resolved.

Monitoring Extracellular-Vesicles Dynamics at the Nanoscale by Liquid-Cell TEM

Max Piffoux¹, Amanda Brun¹, Florence Gazeau¹, Damien Alloyeau².

¹Laboratoire matière et systèmes complexes, Paris, France, ²Laboratoire matériaux et phénomènes quantiques, Paris, France.

Introduction:

Exosomes and microvesicles are promising biotherapies that could potentially replace conventional cell therapies. On the road toward the routine use of these nano-objects in clinics, the nanoscale characterization of these complex soft materials in liquid environment has to be improved. Transmission electron microscopy has been a method of choice to image microvesicles embedded in amorphous ice. Nevertheless the effects of freezing processes on the membrane remains unclear and make impossible dynamic observations. Here we describe the use of liquid-cell transmission electron microscopy (LCTEM) for the dynamic characterization of extracellular vesicles (EV) in PBS media.

Results:

LCTEM consists in imaging the dynamics of nano-objects in an encapsulated liquid solution within an electron-transparent microfabricated cell. We demonstrate that this recent in situ technique allows the observation of EV in their native state without any prior staining and provides the unique opportunity to explore their behavior and structural characteristics in real time with nanometer resolution. We determined relevant parameters for EV based therapy, such as their size distribution, concentration, phosphatidyl-serine content (using gold labeled annexin V) and magnetic nanoparticle loading. Besides, the morphological analyses of EVs in liquid, through the measurement of their reduced volume, allow studying their size-dependent physical properties. Using real-time imaging (up to 25 frames/s), EV motion and dynamics in liquid is analyzed. We reveal that their Brownian motion is slowed by a 100 fold, due to electrostatic/covalent interactions with the membranes of the liquid-cell. Remarkably, the detection of fusion events shows that LCTEM could also open up a new way to study membrane dynamics. Further works are in progress to investigate sample purity and other dynamical processes, such as EV labeling or osmotic shocks, by controlling the composition of the media.

Lipoproteins in Hedgehog Release and Signaling

Suzanne Eaton

Max Planck Institute for Biophysical Chemistry, Goettingen, Germany

No Abstract

Extracellular Vesicles for Trans-Blood Brain Barrier Drug Delivery

Matthew Wood.

Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, United Kingdom.

Extracellular vesicles (EVs) are natural nanovesicles whose function is the intercellular transport of protein and RNA components. Given the urgent requirement for more effective application and delivery of biotherapeutic agents in the treatment of neurological disorders we have sought to exploit the natural properties of EVs and EV-based technologies for this purpose. We have therefore begun to develop a range of EV-based platform technologies including; EV display of brain-targeting for trans-blood brain barrier delivery; EV delivery nucleic acid-based therapeutic agents; EV display of biotherapeutic agents; novel methods for EV isolation. EVs comprising these drug cargoes or for biotherapeutic display have been evaluated *in vitro* and *in vivo*, to evaluate biodistribution, brain penetration and efficacy in animal disease models. In particular we have begun to study gene silencing applications for neurodegenerative disease and methods for modulating neuroinflammation. Future prospects for developing and exploiting EV-based technologies for regenerative medicine applications will be discussed.

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- 2: El-Andaloussi S, Lee Y, Lakhali-Littleton S, Li J, Seow Y, Gardiner C, Alvarez-Erviti L, Sargent IL, Wood MJ. Exosome-mediated delivery of siRNA *in vitro* and *in vivo*. *Nature Protocols* 2012 Dec;7(12):2112-26
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Colocalization of Lipid Domains Across the Two Faces of a Membrane

Sarah L. Keller, Matthew C. Blosser.

The wide diversity of lipid and protein types within a cell's plasma membrane can leave researchers both enthralled by the membrane's richness and overwhelmed by the membrane's complexity. Our laboratory investigates giant unilamellar vesicles as a model of plasma membranes in cells. We find that many physical behaviors reported in complex plasma membranes occur in our model, protein-free membranes. For example, the distribution of lipids on one face of the membrane strongly affects the distribution on the other. In other words, the two opposing leaflets of the membrane are strongly coupled, even when no proteins are present. More recently, we have measured the strength of this coupling by shearing membranes within a microfluidic device, which causes the upper leaflet of the membrane to slide over the lower leaflet, moving domains out of registry. We convert our experimental results into an energy penalty per unit area for misregistered domains. This value sets a lower limit on the size of sub-micron domains in each leaflet that should align across the two faces of a membrane due to coupling of the lipids alone. Alignment of smaller domains in each leaflet would require stronger coupling due to, for instance, transmembrane proteins.

Microfluidic Methods to Study Lipid Membrane Permeation, Deformation and Fusion

Petra S. Dittrich,

ETH Zurich, Zurich, Switzerland.

Microfluidics is nowadays an established technology and provides a huge toolbox for analytical and bioanalytical methods. Microfluidic platforms facilitate precise metering and handling of fluid volumes down to a few hundred picoliters, positioning of cells and vesicles, creating of chemically defined liquid environments, and tailoring mechanical or physical conditions. In this presentation, our recent microfluidic methods to study processes and properties of membranes are introduced and discussed. For studies with vesicles, we use an array of hydrodynamic traps to immobilize the vesicles. Each trap is positioned in a small microchamber that is defined by a valve. The valve can be opened and closed quickly to allow for a complete fluid exchange in the chamber. On these platforms, we investigated the passive permeation of small organic molecules and peptides across the membranes. A modified design with larger hydrodynamic traps enabled the immobilization of two or more vesicles and hence, studies of membrane fusion. Induced by electrical fields or by means of fusogenic peptides, the fusion process including hemifusion could be observed in great detail. In addition, we could gain new insights into the properties of membranes, when exposed to mechanical forces. For these studies, we used vesicles with phase-separated domains and deformed them while they are trapped in the microfluidic device and we could show that lipid sorting occurs, i.e., domains fuse, upon the increase of tension.

Occasionally, we even observe budding of the liquid disordered phase. In further experiments, we exposed the GUVs to shear stress of defined strength and were able to visualize the changes of the domains and their relaxation after stopping the shear stress. Together, these studies may reveal in more detail the role of the membrane in the cellular response to mechanical strains.

Dynamic Creation of Nanostructured Lipid Self-assembled Mesophases via Invertase Digestion Triggers Controlled Release of Encapsulated Drug

Wye Khay Fong^{1,2}, Francesco Orтели¹, Wenjie Sun¹, Sánchez-Ferrer Antoni¹, Ben Boyd², Raffaele Mezzenga¹.

¹Monash University, Parkville, Victoria, Australia, ²ETH Zürich, Zürich, Switzerland.

The development of enzymatically responsive matrices has been of recent interest in the drug delivery field as it can take advantage of enzymes that are over-expressed in diseases such as cancer in order to trigger the release of encapsulated molecules. These ‘smart’ materials have the potential to provide early detection of disease and provide site selective drug release, thereby minimising exposure of healthy tissues to toxic drug, whilst maximising drug effectiveness.

In this study, geometrically ordered lipid nanoparticles with internal bicontinuous cubic phase structure were dynamically formed via enzymatic digestion with invertase. A simple liposome forming lipid mixture containing a mixture of a non-digestible lipid (phytantriol) and a digestible sugar ester (sucrose laurate) was transformed to the cubic phase via enzymatic hydrolysis of the sugar head group. The digestion of the headgroup results in the exit of a monosaccharide from the interface and consequently the alteration of the lipid packing in the liposomes, resulting in crystallization of the cubic-phase internal nanostructure. Time-resolved small-angle X-ray scattering (SAXS) revealed the kinetics of the order-to-order transition, with HPLC and an enzymatic method used to quantify the digestion kinetics of the sugar ester.

Controlled release was then demonstrated with this invertase responsive matrix. A model dye, fluorescein, was encapsulated into the vesicles and release was triggered upon digestion with invertase. This designer approach to creating specialised drug delivery systems, whereby an amphiphile susceptible to digestion by a specific enzyme results in a phase transition and therefore drug release, presents a highly sought after enzymatic method for triggered release.

Membrane Decoys as Anti-Viral Nanomedicine

Roy Ziblat¹, Sarah Stubbs², Xuling Zhu², Sean Whelan², Priscilla Yang², David Weitz^{1,3},
¹Harvard University, Cambridge, MA, USA, ²Harvard University, Boston, MA, USA, ³Harvard
University, Cambridge, MA, USA.

Humans are locked in an evolutionary struggle with viruses. As population density rises the risk of new pandemics is ever increasing and there is high demand for anti-viral drugs. Viruses carefully choose host cells, infiltrate through their membranes, and disrupt cell activity by hijacking their protein machinery for viral replication. The main therapeutic approaches are by interrupting the viral life cycle using bio-molecular inhibitors, or by vaccination, which helps the immune system to fight off the virus. Vaccines, however, are not effective against rapidly mutating viruses and have little therapeutic value to patients already infected. In this study, a novel therapeutic approach is proposed whereby viruses are induced to fuse with a “decoy” membrane before ever encountering the host cell.

Lipids, from which the cell membranes are composed, differ significantly according to cell type and organelles they encompass. The selectivity of the virus to fuse with the specific host cell is lipid dependent. When designing a decoy membrane, the challenge is, therefore, to identify for every type of virus its matching lipid compositions. To address this challenge, we have developed a unique microfluidic setup that enables high-throughput screening over a large library of membranes to identify a lipid selectivity profile for any target protein, including the viral proteins that interact with the cell membrane. The library is composed of over 200 membranes and is 7-fold larger than any previously reported library. Using this methodology, we generated lipid selectivity profiles for dengue, ebola and influenza viruses, and in each case were able to identify distinct lipid compositions that are recognized with high affinity and selectivity even without the presence of a receptor protein. We demonstrate that by using the nano-decoys, the infectivity of cells exposed to the virus is diminished significantly.

Exosomes Sufficiently Deliver Secreted Small RNA to Recipient Tissues

Chen-Yu Zhang

Nanjing University, Nanjing, Jiangsu, China

No Abstract

Function and Dynamics of Protein Palmitoylation

Gisou van der Goot

École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

No Abstract

POSTER ABSTRACTS

POSTER SESSION I

Monday, September 12, 2016

15:55 – 17:30

Balint Salon

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

Allen-Benton, Maxwell	1-POS	Board 1
Aydin, Deniz	4-POS	Board 2
Beales, Paul	7-POS	Board 4
Bocskei-Antal, Barnabás	10-POS	Board 5
Bozó, Tamás	13-POS	Board 7
Catte, Andrea	16-POS	Board 8
Deng, Nan-Nan	19-POS	Board 10
Frey, Shelli	22-POS	Board 11
Gutierrez, Mary Gertrude	25-POS	Board 13
Henrich, Erik	28-POS	Board 14
Hobiger, Kirsten	31-POS	Board 16
Khan, Sanobar	34-POS	Board 17
Krainer, Georg	37-POS	Board 19
Liang, Binyong	40-POS	Board 20
Monticelli, Luca	43-POS	Board 22
Obeid, Sameh	46-POS	Board 23
Panatala, Radhakrishnan	49-POS	Board 25
Rossi, Giulia	52-POS	Board 26
Seelheim, Patrick	55-POS	Board 28
Silva, Liana	58-POS	Board 29
Vacha, Robert	61-POS	Board 31
Zhang, Xianren	64-POS	Board 32

Posters should be set up on the morning of Monday, September 12 and removed by 18:00 on
Thursday, September 15.

1-POS Board 1

Construction of A Multi-Stage Micro-Reactor In A Droplet Interface Bilayer System

Maxwell Allen-Benton.

King's College London, London, London, United Kingdom.

Droplet interface bilayers (DIBs) are a stable, convenient and flexible platform for the formation of artificial lipid bilayers. Formed from the contact of two lipid monolayer encapsulated aqueous droplets in a surrounding oil phase, they are compatible with trans-membrane protein insertion and support the formation of asymmetric bilayers. As well as being a promising platform for studying membrane biophysics and membrane transport proteins, droplet interface bilayers are finding uses in synthetic biology. The stability of DIBs allows large networks of bilayer-connected droplets to be constructed, with the incorporation of proteins pores facilitating controlled movement of substances throughout the aqueous phase of the network.

I aim to use the droplet interface bilayer system to construct a multi-chambered, enzymatically driven micro-reactor. The aqueous interiors of the droplets will serve as the chambers of the reactor, with porins and membrane transport proteins facilitating the movement of reactants and products across the lipid bilayers that separate the chambers. The efficacy of the system will be assessed using fluorometric and colorimetric indicators. As such, a spatially separated enzymatic cascade reaction will be created in a synthetic system.

4-POS Board 2

Interaction of Atypical Kinase ADCK3 with Inner Mitochondrial Membrane Models

Deniz Aydin, Matteo Dal Peraro.
EPFL, Lausanne, Switzerland.

Mitochondria perform diverse fundamental roles in human health besides operating as “powerhouses” of cells, as they harbour a unique proteome that remains largely unexplored. A growing number of inherited metabolic diseases are associated with mitochondrial dysfunction, which necessitates the structural and functional elucidation of mitochondrial proteins. ADCK3, a member of the mitochondrial coenzyme Q synthesis machinery, is a protein that is structurally characterized but remains functionally elusive. It has a poorly understood connection to coenzyme Q biosynthesis and an inherited neurodegenerative disease (ARCA2). Molecular dynamics (MD) simulations are a powerful tool to understand and visualize the molecular mechanisms of such structurally characterized proteins. Coarse-grained (CG) force fields are specifically useful in studying protein-lipid systems owing to their higher efficiency in sampling. In this work, CG-MD simulations are performed to determine the interaction surface of ADCK3 with the inner mitochondrial membrane, which gives hints about possible interaction surfaces of ADCK3 with other members of the coenzyme Q synthesis machinery. Further identification of specific residues involved in membrane interaction, mostly through electrostatic interactions with cardiolipins, enable future experimental validation of this interaction surface through mutagenesis experiments. The identified interaction surface exposes the substrate binding pocket to the membrane, which has implications about how ADCK3 mediates functional interactions with lipids.

7-POS Board 4

Enhanced Functional Durability of Membrane Proteins in Hybrid Lipid-Block Copolymer Vesicles

Sanobar Khan¹, Mengqiu Li², Lars Jeuken², **Paul A. Beales**¹.

²University of Leeds, Leeds, United Kingdom. ¹University of Leeds, Leeds, United Kingdom,

Membrane proteins make up approximately one third of all proteins and are the targets of around 60% of drugs currently on the market. Their broad range of functions from transmembrane transport and signal transduction to adhesion and catalysis also makes membrane proteins attractive constituents within novel bio-/nano- technologies. However membrane proteins are (in general) difficult to handle and, when in reconstituted systems, tend to lack the long-term stability that is often required for technological applications. With the aim of improving the functional durability of reconstituted membrane proteins, we are investigating the use of hybrid lipid-block copolymer vesicles. Hybrid vesicles have been reported to synergistically combine the natural biocompatibility of phospholipid bilayers with the mechanical robustness and stability of polymersome membranes. Using cytochrome *bo3* as a model protein, we demonstrate that this membrane protein can be reconstituted into the membrane of hybrid vesicles. We find that cytochrome *bo3* retains favourable activity when reconstituted in hybrid vesicles with moderate polymer content compared to the activity in traditional proteoliposomes. The advantage of hybrid vesicles over simple liposomes becomes evident from the long term stability of membrane protein function: cytochrome *bo3* loses all functional activity in liposomes within 3-4 weeks, while significant (~50%) protein activity was retained within hybrid vesicle formulations after >6 weeks. Therefore hybrid vesicles show great promise for durable formulation of membrane proteins for applications that require extended functional lifetimes.

10-POS Board 5

Studying of Reactive Oxygen Species Production Activity and Membrane Destruction Ability of Porphyrins on Cell Membrane Models

Barnabás Bocskei-Antal, Nikoletta Kósa, Ádám Zolcsák, István Voszka, Gabriella Csík, Levente Herényi.
Semmelweis University, Budapest, Hungary.

Photo dynamic therapy (PDT) is a widespread medical treatment based on the light-triggered generation of reactive oxygen species (ROS) by porphyrin derivatives. ROS may cause oxidative damage to membranes as well as to DNA and, in consequence, ultimately kill cells. Hence, the binding ability, the ROS production of porphyrins and the occurred cell membrane damage through the lipid peroxidation are of outstanding interest.

Here we investigated the light-triggered production of ROS by two mesoporphyrin IX derivatives (MPCl, MPE) and the produced membrane damage on small multilamellar vesicles (SUV). The monocomponent vesicles were formed of various phosphatidylcholines.

The amount of generated oxygen radicals was studied in the aqueous medium of the vesicles and in the lipid bilayer environment. ROS in the aqueous media was measured on the basis of the produced tri-iodide from potassium iodide in the presence of molybdenum catalyst, which was followed by absorption spectrophotometry. The ROS in the lipophilic membranes and in near-membrane regions was measured with a dihydrorhodamine derivative by fluorescence spectroscopy. The damage of the lipid bilayers induced by the ROS was measured with fluorescence correlation spectroscopy (FCS). For control measurement we used a hydrogen-peroxide treatment of the SUVs measured by dynamic light scattering (DLS).

In aqueous media (without liposomes) MPCl was highly effective in ROS formation whereas in case of MPE no similar effect was observed. Liposome-incorporated MPCl produced ROS in much higher amounts than the MPE in the aqueous medium of the liposomes. In near-membrane regions MPE produced ROS in the same amount as MPCl. The direct membrane destruction ability of the light-triggered ROS was observed in the change of the size distribution of the vesicles.

13-POS Board 7

Antichaotropic Salts Can Provoke the Aggregation of PEGylated Liposomes

Tamás Bozó¹, Tamás Mészáros², Judith Mihály⁴, Attila Bóta⁴, Miklós Kellermayer^{1,3}, János Szebeni², Benedek Kálmán².

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Polyethylene glycol (PEG) and ammonium sulfate (AS) are ingredients widely used to produce remote loaded, sterically stabilized liposomes (SSL-s). We found that at concentrations about three times higher than that is used for remote loading, AS can provoke the precipitation of PEGylated vesicles. The aim of this study was to elucidate this phenomenon by studying the effect of AS and other salts on PEGylated vesicles.

Precipitation of liposomes was examined by dynamic light scattering and turbidimetry. Phase contrast and atomic force microscopy imaging were used to assess the morphology of supraventricular structures. Zeta potential and FTIR experiments were used to assess the surface charge of the liposomes and conformation of PEG molecules, respectively.

Aggregation of the liposomes was found AS concentration dependent and reversible up to about 1.5 M AS, however at even higher concentrations vesicle fusion occurred. Other antichaotropic salts (eg. sodium sulfate, magnesium sulfate) also evoked aggregation in a concentration-dependent manner, while the chaotropic guanidinium-HCl did not precipitate the vesicles.

Aggregation is not surface charge driven as change of zeta potential of liposomes upon AS addition showed no correlation with it. Threshold concentration of aggregation shifted to lower values increasing PEG coverage of the liposomes, while PEG-free liposomes did not precipitate upon addition of AS. FTIR experiments showed that AS reduced the ratio of *trans* and *gauche* configurations of the C-O-C bonds of the of the PEG molecules.

In conclusion, antichaotropic salts may reduce the hydration of PEG polymer chains leading to aggregation and (at higher concentrations) fusion of sterically stabilized vesicles. Controlled aggregation of PEGylated liposomes may have both technological and therapeutical consequences.

16-POS Board 8

Multiscale Molecular Dynamics Simulations of Antimicrobial Peptides Chrysopsin-3 in Lipid Bilayers

Andrea Catte, Vasily S. Oganessian.

School of Chemistry, University of East Anglia, Norwich, United Kingdom.

Antimicrobial peptides (AMPs) are small cationic proteins able to destabilize the lipid bilayer structure through different mechanisms of interaction. In this study, we investigate the processes of peptide aggregation and pore formation by chrysopsin-3 peptides in lipid bilayers and vesicles using multiscale molecular dynamics (MD) simulations. The combination of the long timescale of coarse grained (CG) MD simulations with the high resolution of all atom (AA) MD simulations allows us to study the formation and the structure of pores. In 50 μ s CG MD simulations chrysopsin-3 peptides spontaneously interact with the lipid membrane forming distorted toroidal pores and aggregates in palmitoylcholine (POPC), dipalmitoylcholine (DPPC), and palmitoylcholine (POPE):palmitoylcholine (POPG) lipid bilayers and vesicles. Moreover, chrysopsin-3 peptides are also found adsorbed on the lipid membrane. All these different modes of binding of chrysopsin-3 peptides are in agreement with experimental results (1). The AA MD simulation of a POPC lipid bilayer with 50 chrysopsin-3 peptides fine grained from a 16 μ s CG structure shows the penetration of water into the lipid bilayer and a reduction of the α -helical content of chrysopsin-3 peptides in agreement with previous computational studies (2). The free energy profile of the insertion of a peptide into the lipid bilayer indicates that the transition from surface adsorbed to transmembrane peptides is associated with a high energy barrier.

References

1. Wang, K. F., R. Nagarajan, and T. A. Camesano, *Biophys. Chem.* 2015, 196, 53-67.
2. Thøgersen, L., B. Schiøtt, T. Vosegaard, N. C. Nielsen, and E. Tajkhorshid, *Biophys. J.* 2008, 95, 4337-4347.

19-POS Board 10

Monodisperse Multicompartment Liposomes

Nan-Nan Deng, Wilhelm Huck.
Radboud University, Nijmegen, Netherlands.

Liposomes are self-assembled phospholipid vesicles with great potential in fields ranging from drug delivery to synthetic biology. However, high throughput, controlled fabrication of monodisperse liposomes with controllable compartments has not been achieved thus far. Here, we report on a surfactant-assisted microfluidic route to assemble oil-free monodisperse liposomes with single- or multi-compartments via precise control over interfacial energies of complex water-in-oil-in-water (W/O/W) emulsion systems. The use of various emulsion templates, in combination with the spontaneous dewetting afforded by the surfactant, allows for the creation of uniform, single bilayer liposomes with multiple inner compartments. This method is compatible with complex bioreactions as exemplified by the production of eGFP in liposomes via cell-free gene expression. We believe this is the example where liposome formation from microfluidically prepared double emulsion templates is fully controlled. The high yield, high throughput, and uniformity of structures produced has potential for future designs of cell-like bio-devices or as coupled microreactors.

22-POS Board 11

Polystyrene Nanoparticles Alter the Structure and Stability of Model Cell Membranes

David R. Van Doren, Luke Cuculis, **Shelli L. Frey**,
Gettysburg College, Gettysburg, PA, USA.

Unique material properties of nanoparticles (NPs) contribute to a diversity of applications that range from increasing transparency and protection of sunscreen to transporting drugs across cell membranes without damaging the cell itself. Because the interactions of NPs with biological membranes have not been fully characterized to correlate surface physical and chemical characteristics with mode of action, model cell membranes exposed to NPs were monitored with fluorescence microscopy. Giant unilamellar vesicles (GUVs) composed of 1:1:1 dipalmitoylphosphatidylcholine (DPPC)/1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC)/cholesterol were prepared with 0.8% mol fluorescent TR-DHPE, exposed to NPs either during the vesicle electroformation process or afterwards, and then imaged. The effects of varying concentrations of 40, 60, or 100 nm functionalized polystyrene NPs were measured through morphology changes and vesicle size distributions. Aminated polystyrene particles reduced the size of stable GUVs with the magnitude of the effect scaling with NP concentration. Additionally, the distribution of aminated nanoparticles within the membrane resulted in lipid tubule formation that extended from the vesicle structure; the membrane bending may be attributed to a NP crowding mechanism. Carboxylated particles produced less dramatic effects compared to the control system. In both cases, high NP concentration completely prevented GUV formation, indicating a saturation concentration effect. Understanding the features impacting NP-membrane interactions may help elucidate health and environmental implications of these associations and direct the design of better NP-based technology.

25-POS Board 13

G Protein-Coupled Receptors Incorporated Into Rehydrated Diblock Copolymer Vesicles Retain Functionality

Mary Gertrude Gutierrez¹, Farzad Jalali-Yazdi¹, Justin Peruzzi², Carson T. Riche¹, Richard W. Roberts¹, Noah Malmstadt¹.

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We successfully incorporated the human serotonin receptor, G Protein Coupled Receptor (GPCR), 5-HT_{1A}R, in polymeric giant unilamellar protein-vesicles (pGUPs) made of diblock copolymer. By utilizing an agarose rehydration technique for protein incorporation, the GPCR is inserted in the biased, physiological orientation with the C-terminus cytosolic and N-terminus extracellular. The GPCR is fully functional within the polymeric bilayer, exhibiting physiological responses to various ligands. The entire population of GPCRs in pGUPs remains fully functional after lyophilization for 120 hours.

28-POS Board 14

Customized Lipid Bilayers in Cell-Free Synthetic Biology: From Mechanisms to Applications

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In contrast to soluble proteins, membrane protein research is often complicated by the hydrophobic character of the target protein. Cell-free synthetic biology provides new platforms for the efficient production of membrane proteins by adjusting and refining artificial hydrophobic expression environments for the functional folding of synthesized membrane proteins. We demonstrate the synergistic combination of nanodiscs and cell-free production, which allows for systematic lipid screening of detergent sensitive membrane proteins to generate high quality samples. Using *MraY* translocase homologues from various pathogenic bacteria, we demonstrate that *MraY* has very strict lipid requirements for its activity. The co-translational insertion of *MraY* into preformed nanodiscs enables the complete *in vitro* reconstitution of designed biosynthetic pathways for essential cell-wall precursors by comprising *MraY* homologues as well as whole sets of soluble enzymes in detergent free environments. Selective inhibition at different pathway steps demonstrates the potential of these synthetic *in vitro* pathways as a new drug screening platform. Furthermore, nanodisc based cell-free expression was combined with non-covalent LILBID mass spectrometry to characterize insertion mechanisms as well as oligomeric state formation of membrane proteins in nanodiscs. We have analyzed the complex formation of a variety of membrane proteins including proteorhodopsin, the multidrug transporter *EmrE* and the enzymes *MraY* and *LspA*. By implementing isotope labelling, we show monomer and up to hexamer formation of membrane proteins in nanodiscs, and we identify parameters suitable to trigger complex assembly. We furthermore give evidence that *MraY* dimer formation depends on its lipid environment and is essential for enzymatic activity. Moreover, we show the first details of the molecular mechanisms of membrane protein insertion into the size restricted nanodisc bilayers.

31-POS Board 16

PTEN and VSPs: On the Way to Identify the Structural Origin for Their Substrate Specificity

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The phosphatase and tensin homolog deleted on chromosome ten (PTEN) is one of the most crucial tumor suppressor proteins in mammals. It counteracts the PI3-kinase signaling cascade by cleaving 3'-site phosphate groups from membranous phosphoinositides (PIs). In doing so, PTEN prevents unlimited cell proliferation and tumor genesis. The phosphatase is a soluble protein that transiently binds to the membrane surface for catalysis. This property hardens the experimental access to PTEN's activity for direct interventions. Voltage-sensitive phosphatases (VSPs) overcome this limitation. The enzymes share high sequential and structural homology to PTEN. However, their enzymatic activity is coupled to the transmembrane potential, which allows a reversible depletion of PIs under experimental control. By substituting the catalytic domain of the VSP from *Ciona intestinalis* (Ci-VSP) against that one of PTEN, we created a voltage-switchable VSP-chimera with the 3'-site PI-specificity of PTEN. Because VSPs prefer to dephosphorylate the 5'-site position of PI-substrates, we are currently studying native VSPs in comparison to the VSP-chimera to identify the structural origin for the substrate specificity of the phosphatases. For this purpose, we use an approach that combines molecular dynamics simulations and phosphatase activity assays in mammalian cells and in vitro. First experiments revealed the importance of the membrane environment for the regulation of the substrate specificity. In particular, the reaction toward one PI-substrate seems to be controlled by an allosteric mechanism that is mediated by at least one of the flexible loops that surround the active site. Since this regulation process depends on the properties of the membrane used for the phosphatase assay, we are currently searching for the optimal membrane system that will help us to identify the structural determinants for the substrate specificity of PTEN and its homologs.

34-POS Board 17

Formation and Characterization of Hybrid Lipid – Biodegradable Block Copolymer Vesicles

Sanobar Khan¹, Paul A. Beales¹, Kathryn Hill².

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Vesicular nanoparticles have proven to be a successful formulation strategy to modify drug pharmacokinetics and enhance therapeutic index, with numerous examples in current clinical use. The majority of these successes have been in the form of liposomes, often with a non-fouling polymer-brush coating. Block copolymer vesicles have also attracted interest for drug delivery applications due to their broader range of physicochemical properties. Recently, hybrid vesicles formed from a blend of lipids and block copolymers have shown promise for synergistically combining the natural biocompatibility of liposomes with the enhanced mechanical robustness of polymersomes, further expanding the tuneable parameter space for vesicle engineering. Here we will present an investigation of hybrid vesicles formed from mixtures of the phospholipid POPC with a range of different biodegradable diblock copolymers. This will encompass different vesicle formation protocols, comparison to the unitary liposome and polymersome systems and characterization of vesicle size distributions, hydrophilic encapsulation efficiency, thermal phase transitions, lateral mixing behaviour and temporal stability. The aim of this work is to identify promising hybrid vesicle blends for biomedical applications in drug formulation and delivery.

37-POS Board 19

Single-Molecule FRET Reveals Structural Basis for Conformational Misfolding of a Cystic Fibrosis Mutation in CFTR

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Cystic fibrosis (CF) is the most common lethal genetic disease among the Western population caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR). The development of effective therapeutic agents against CF requires a molecular understanding of how mutation-related structural alterations lead to impaired functioning of CFTR. The main cause for CF are processing mutations frequently found within CFTR-transmembrane (TM) segments which affect co-translational folding and insertion. However, the underlying molecular mechanisms for misfolding remain obscure, mainly because of the unavailability of high-resolution structures and a lack of methods suitable for studying membrane protein structure-function relationships.

Devising a divide-and-conquer approach in combination with single-molecule FRET (smFRET), we present here a strategy to gain novel insights into the structural basis for conformational misfolding in CFTR. Using this approach, we study the CF-phenotypic polar mutation V232D located within the fourth TM helix (TM4) of CFTR. *In vivo* folding and insertion of TM4 is functionally coupled with TM3 where both TM segments are co-translationally inserted into the membrane as a TM helix-loop-helix hairpin motif (TM3/4). Utilizing this minimal folding unit, we employ smFRET as a molecular ruler to readout structural alterations imposed on the helical packing of the TM3/4 hairpin upon mutation. In contrast to earlier findings that suggested a TM lock between TM3 and TM4 by a non-native H-bond, our results reveal that V232D TM3/4 associates with membranes in an open conformation. This implies that the charged residue imposes an energy penalty on membrane insertion of the hairpin. We propose a model for CF pathogenesis where partitioning of V232D TM3/4 into the interfacial region leads to misfolding of CFTR during co-translational membrane insertion and inhibits maturation by trapping the protein as a partially folded intermediate.

40-POS Board 20

Interaction of Complexin with SNARE Proteins Depends on the Lipid Environment of the Membrane

Binyong Liang^{1,2}, Alex J. Kreuzberger^{1,2}, Volker Kiessling^{1,2}, Rafal Zdanowicz^{1,3}, David S. Cafiso^{1,3}, Lukas K. Tamm^{1,2}.

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Fusion of synaptic vesicles with the presynaptic membrane is mediated by SNAREs and several SNARE-interacting proteins. Complexin is a soluble protein, which binds strongly to fully assembled neuronal SNARE complexes. Complexin is required for Ca²⁺-triggered fast fusion at nerve terminals; however, its mechanism of action remains elusive and controversial. Inhibitory and fusion-promoting effects have been reported and attributed to different domains of complexin. In addition, preferential binding of complexin to charged and highly curved membranes has been postulated to also contribute to its regulatory effect.

We have used solution NMR and EPR spectroscopy, isothermal titration calorimetry, fluorescent anisotropy, and TIRF microscopy to probe multiple interactions between complexin and SNARE proteins in different complexes in a number of membrane-mimetic systems. Based on the molecular details revealed by NMR and EPR, and thermodynamic and kinetic measurements performed by ITC and fluorescence anisotropy, respectively, we present a model of how complexin effects SNARE-mediated membrane fusion in a physiological lipid environment.

43-POS Board 22

Effect of Hydrocarbons and Alcohols on Lipid Phase Separation – A Simulation Perspective

Jonathan Barnoud¹, Giulia Rossi², Siewert-Jan Marrink¹, **Luca Monticelli**³.

³CNRS, Lyon, France. ¹University of Groningen, Groningen, Netherlands, ²University of Genoa, Genoa, Italy,

Cell membranes have a complex lateral organization featuring domains with distinct composition, also known as rafts, which play an essential role in cellular processes such as signal transduction and protein trafficking. In vivo, perturbation of membrane domains (e.g., by drugs and anesthetics) has major effects on the activity of raft-associated proteins and on signaling pathways [1]. In live cells, membrane domains are difficult to characterize because of their small size and highly dynamic nature, so model membranes are often used to understand the driving forces of membrane lateral organization. Studies in model membranes have shown that some lipophilic compounds can alter membrane domains, but it is not clear which chemical and physical properties determine domain perturbation. The mechanisms of domain stabilization and destabilization are also unknown. Here we describe the effect of hydrocarbons and alcohols of different chain length on the lateral organization of phase-separated model membranes consisting of saturated and unsaturated phospholipids and cholesterol [2]. Using molecular simulations at the coarse-grained level, we predict that aliphatic compounds promote lipid mixing by distributing at the interface between liquid-ordered and liquid-disordered domains. Long-chain alcohols also destabilize phase separation, although they do not act as linactants. Short-chain alcohols, instead, appear to have little effect on membrane lateral organization, at physiologically relevant concentrations. We predict that relatively small concentrations of hydrophobic species can have a broad impact on domain stability in model systems, which suggests possible mechanisms of action for hydrophobic pollutants in vivo.

References

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46-POS Board 23

On Chip Biodetection, Analysis and Nanometrology of Cell-Derived Vesicles

Sameh OBEID¹, Gaël Belliot², Benoit Le Roy de Boiseaumarié¹, Adam Ceroi³, Guillaume Mourey³, Philippe Saas³, Wilfrid Boireau¹, Céline Elie-Caille¹.

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Extracellular vesicles (EVs) are small vesicles (10-4000 nm) shed from different cells types upon activation or apoptosis. EVs, including exosomes, microparticles and apoptotic bodies, are recognized to play an important role in various biological processes and are also recognized as potential biomarkers of various health disorders¹.

Currently, the technic of choice used for the detection of EVs is flow cytometry (FC)², but the main drawback of FC is the lower detection limit ($\approx 300\text{nm}$ at best); consequently, a big fraction of vesicles cannot be detected.

Our work aims to use the potential of surface plasmon resonance (SPR) method to detect, and qualify, specifically and without labeling, the EVs in their complex media, via their (immune)capture onto the biochip-surface using different specific ligands.

Using two calibration standards of known size and concentration (Virus-Like Particles “VLP” (50nm) & modified melamin resin MF beads (920nm)), we have shown that the capture level of these entities by their specific ligands is linearly related to their concentration in the sample. Dynamic ranges of concentrations (8×10^{10} to 2×10^{12} VLPs/ml and $3,25 \times 10^7$ to $3,25 \times 10^8$ beads/ml) have been established for 2 min injection time. These concentration ranges match the typical range of EVs natural concentrations in plasma^{3,4}. Additionally, by varying the injection time, the dynamic range increases, which provides more sensibility for the detection of MPs in a wide concentration range.

Moreover, a combination between SPR and atomic force microscopy (AFM) approaches appears to be suitable for the metrological analysis of captured EVs, which allows identification of their size subpopulations. Furthermore, an on-chip proteomic study was also engaged to discover specific proteomic profiling of EVs in different conditions.

At last, we have adjusted this analytical platform based on a gold biochip to qualify and compare resting and activated platelet MPs.

49-POS Board 25

Membrane Nucleoporins Form Porous Proteoliposomes with Nuclear Pore-Like Selectivity

Radhakrishnan Panatala^{1,2}, Suncica Vujica^{1,2}, Yusuke Sakiyama^{1,2}, Roderick Lim^{1,2},
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Switzerland,

Eukaryotic cells maintain order and function by the selective accumulation of essential macromolecules in the nucleus. This process is regulated by soluble nuclear transport receptors (NTRs) that gain exclusive access through 50 nm channels known as nuclear pore complexes (NPCs). Here, we have engineered nanoporous proteoliposomes that harbor key membrane proteins of the NPC. Using fluorescence-based translocation assays, we observed that these proteoliposomes show to discriminate against non-specific macromolecules above a certain size-threshold whereas specific transport factors are transported across the pores. Imported NTRs are subsequently entrapped within the proteoliposome lumen using wheat germ agglutinin (WGA), a known NPC blocking agent. In conjunction, we performed morphological characterization of the nanopores by high-speed atomic force microscopy (HS-AFM), which revealed that the permeability barrier, composed of FG-nucleoporins, resembles to that of NPCs from *X. laevis* oocyte nuclei. Eventually, we'd like to apply such a macromolecular logistics system to sort and encapsulate specific molecular modules from a complex environment leading to the construction of a molecular factory.

52-POS Board 26

Monolayer-Protected Gold Nanoparticles Walk Into Model Lipid Membranes Step-By-Step

Giulia Rossi¹, Federica Simonelli¹, Davide Bochicchio^{1,2}, Riccardo Ferrando¹.

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The design of ligand-protected metal nanoparticles (NPs) with biomedical applications relies on the understanding, at the molecular level, of their interactions with cell membranes. Here we study, via unbiased molecular dynamics simulations and free energy calculations, the kinetics and the thermodynamics of the interaction between anionic ligand-protected gold NPs and model lipid membranes. We model the NP-membrane complex at both coarse-grained and atomistic level. We show that the NP-membrane interaction is a three-step process: electrostatics-driven adhesion to the membrane surface, hydrophobic contact and final embedding in the membrane core via anchoring of the charged ligands to both membrane leaflets. Our free energy calculations show that anchoring is highly favorable and not reversible. Furthermore, we show that the interaction pathway of NPs can be influenced by the spatial arrangement of ligands on the NP surface. NPs with homogeneous surface arrangement of anionic and hydrophobic ligands interact with membranes via two metastable configurations: adsorbed at the membrane surface, and membrane-embedded. Patched, heterogeneous ligand arrangements, instead, lead to the stabilization of a third, intermediate metastable configuration, resulting in a much slower kinetics of interaction with the membrane [1].

[1] F. Simonelli, D. Bochicchio, R. Ferrando and G. Rossi, *J. Phys. Chem. Lett.* 2015, 6, 3175–3179

55-POS Board 28

The Outer Membrane Protein OprG of *Pseudomonas aeruginosa* Facilitates the Transport of Small Amino Acids

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Pseudomonas aeruginosa is an opportunistic human pathogen that is responsible for a growing number of nosocomial infections and the main cause of death in patients with cystic fibrosis. Its very stable outer membrane lacks unspecific porins and poses an efficient permeation barrier to both hydrophilic and lipophilic compounds rendering *P. aeruginosa* resistant to many common antibiotics.

The x-ray crystal structure of the *P. aeruginosa* outer membrane protein OprG revealed a tall 8-stranded β -barrel extending far into the extracellular space and featuring a proline-rich gate near the membrane interface. It has been speculated that this proline gate is involved in the transport of small hydrophobic compounds across the outer membrane.

However, we found that OprG facilitates the transport of small, uncharged amino acids both in vitro and in vivo as shown by liposome swelling assays and *P. aeruginosa* growth curves. When determining the NMR solution structures of wild-type OprG and the transport incompetent P92A mutant OprG, we found shorter β -barrels with long disordered extracellular loops in both proteins. However, the P92A mutation led to an asymmetric elongation of the barrel and changes in the loop dynamics implying that unrestricted motion of loop 3 is required for amino acids transport.

58-POS Board 29

Lipid-Gel Domains in Living Cells: Tackling the Biological Significance of Atypically Ordered Ceramide-Domains

Ana E. Ventura^{1,2,3}, Sandra Pinto², Ana R. Varela^{1,2,3}, Giovanni D'Angelo⁴, Anthony Futerman³, Manuel Prieto², **Liana C. Silva**¹.

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

Acknowledgments: This work was supported by Fundação para a Ciência e Tecnologia (FCT), Portugal: PTDC/BBB-BQB/0506/2012, PTDC/BBB-BQB/3710/2014, SFRH/BD/110093/2015 to AEV and Investigador FCT2014 to LCS

[1]. Castro, B. M., Prieto, M. & Silva, L. C. Ceramide: A simple sphingolipid with unique biophysical properties. *Prog. Lipid Res.* 54, 53–67 (2014).

61-POS Board 31

Peptide Features Determining Its Translocation and Pore Formation

Ivo Kabelka¹, Daan Frenkel², **Robert Vacha**¹.

¹Masaryk University, Brno, Czech Republic, ²University of Cambridge, Cambridge, United Kingdom.

Amphiphilic peptides can interact with phospholipid membrane and severely affect its barrier function by translocation or pore formation. This is particularly important for antimicrobial and cell-penetrating peptides as it can determine their lethality or ability to act as drug delivery systems against bacteria or pathological cells. However, the necessary peptide properties and conditions for membrane translocation and pore formation are not well understood. Using coarse-grained simulations, we have calculated the free energy of pore formation and translocation of amphiphilic helical peptides under various conditions. We found that the most effective in pore formation are peptides with length similar to membrane thickness. Moreover, the preferred peptide orientation in the pore and during the translocation was found to agree well with the hydrophobic mismatch rationalization. Long peptides were thus observed to orient parallel to membrane plane forming a ‘double-belt’ pore. The obtained understanding of peptide behavior at the membrane may be useful for the rational design of peptides that are more effective and specific against given target cells or bacteria.

64-POS Board 32

Simulation of Nanoparticle-Membrane Interaction

Xianren Zhang,

Beijing University of Chemical Technology, Beijing, China.

Nanoparticles are widely used in biomedical fields, such as gene and drug delivery, nanoparticle-based sensing and imaging etc. In these applications, the efficient uptake of nanoparticles (NPs) into cells becomes a critical issue, because NPs are required to be capable of transporting through cell membranes. On the other hand, nanoparticles adhering on cells may cause damage to cell membranes and induce adverse biological effects, with the potential to create cytotoxicity. In this regard, understanding of the mechanism of NP uptake is essential to bio-applications of nanoparticles. I will summarize our recent simulation works on the interaction between cell membrane and nanoparticles are addressed. The internalization pathways of nanoparticles, including endocytosis and penetration, depends on the size, shape and rigidity of nanoparticles.

POSTER SESSION II

Tuesday, September 13, 2016

15:55 – 17:30

Balint Salon

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

Amaro, Mariana	2-POS	Board 1
Balobanov, Vitalii	5-POS	Board 3
Beltrano, Peter	8-POS	Board 4
Bouchet, Ana	11-POS	Board 6
Brunaldi, Kellen	14-POS	Board 7
Cebecauer, Marek	17-POS	Board 9
Carravilla, Pablo	20-POS	Board 10
Georgiev, Vasil	23-POS	Board 12
Harris, Nicola	26-POS	Board 13
Herlo, Rasmus	29-POS	Board 15
Jones, Stephen	32-POS	Board 16
Kiessling, Volker	35-POS	Board 18
Kuhn, Jason	38-POS	Board 19
Lopez-Montero, Ivan	41-POS	Board 21
Mulvihill, Estefania	44-POS	Board 22
Obeid, Sameh	47-POS	Board 24
Prunotto, Alessio	50-POS	Board 25
Sanganna Gari, R. Reddy	53-POS	Board 27
Sergeeva, Oksana	56-POS	Board 28
Singh, Rohit	59-POS	Board 30
Yang, Sung-Tae	62-POS	Board 31
Ziblat, Roy	65-POS	Board 33

Posters should be set up on the morning of Monday, September 12 and removed by 18:00 on
Thursday, September 15.

2-POS Board 1

Laurdan and NBD-Lipids in Membranes: What Are They Actually Probing?

Mariana Amaro, Piotr Jurkiewicz, Martin Hof.
J. Heyrovský Institute of Physical Chemistry of A.S.C.R., v.v.i., Prague, Prague, Czech Republic.

Fluorescence methods are versatile tools for obtaining dynamic and topological information about biomembranes. Laurdan and nitrobenzoxadiazole (NBD)-labelled lipids are popular fluorescent membrane probes. However, common misunderstandings of important aspects of the photophysics of both probes lead to inaccurate interpretations of experimental results.

The laurdan generalized polarization (GP) function is commonly used to assess the order/hydration of model and cell membranes. Laurdan GP is often found in literature as an indicator of “extent of water penetration”. However, GP is an empirical steady-state ratiometric parameter from which one cannot separately resolve the influence of order and hydration. Only time-dependent fluorescence shift (TDFS) measurements can reveal independently information on polarity and mobility of laurdan’s solvation shell. Here we present laurdan TDFS data which demonstrate that GP reflects predominantly the mobility of the hydrated *sn*-1 carbonyls (order) and not the extent of hydration of a bilayer in the liquid crystalline phase.

The photophysical characteristics of NBD make it a sensitive probe and NBD-lipid analogues have found wide use in the study of biomembranes. An interesting application of NBD-lipids in membranes is the sensing of solvent relaxation by the red edge excitation shift (REES) phenomenon. REES of NBD-lipids is typically interpreted as reflecting restricted mobility of solvent surrounding the fluorophore, and thus used to infer on membrane order. Here we detail REES, TDFS and lifetime data of NBD-lipids which demonstrate that the photophysical behaviour of NBD cannot be explained by restrictions to solvent relaxation. We show that the origin of REES is the heterogeneous transverse location of the NBD probes and, therefore, that REES is not an adequate method for probing solvent relaxation/membrane order.

Amaro, M.; et al., *Biophys. J.*, (2014) 107, 12
Amaro, M.; et al., *PCCP.*, (2016) 18

5-POS Board 3

Influence of Composition and Charge of Phospholipid Membranes on the Conformational State of a Protein: A Case of Apomyoglobin

Vitalii Balobanov, Nelly Ilyina, Natalia Katina, Ivan Kashparov, Valentina Bychkova.
Institute of protein research RAS, Pushchino, Russian Federation.

Investigation of protein-membrane interaction is very important for understanding of many aspects of protein life. This is evident for insertion of proteins into membranes or their translocation across membranes, and also for functioning proteins or involvement them into toxic action of amyloid structures in the cell. It was found that negatively charged phospholipids influence significantly on the protein structure. Thus, it is critical to estimate what conditions promote these interactions. In this work influence of negatively charged phospholipid membranes and ratio of protein-to-phospholipid concentrations on kinetics of protein-membrane interaction is studied. Apomyoglobin which properties are well studied was used as an example. Conformational states of the protein were tested by tryptophan fluorescence and far UV circular dichroism spectra. It is shown that in the range of the protein-phospholipid concentrations ratios of 5-20% substantial acceleration of protein-membrane interaction was observed, while in the range of 20-100 % negatively charged phospholipids the effect is practically similar. Below 5% content of negatively charged phospholipids in membrane the process of protein-membrane interactions sharply slows down. The rate of this interaction also depends on the protein structure stability. The importance of the obtained results for understanding of influence of membrane surfaces on the conformational state of proteins, on the folding of membrane proteins and their role in the protein aggregates toxicity is discussed. Static experiments was supported by the MCB program of RAS. Studying kinetic of interaction was supported by the Russian Scientific Foundation.

8-POS Board 4

**Large Area Model Biomembranes (LAMBs) to Understand Membrane Mechanics,
Structure, and Function**

Peter J. Beltramo, Nicole Schai, Jan Vermant.
ETH Zürich, Zürich, Switzerland.

The development of model cellular membranes is crucial for fundamental investigations of biological phenomena, ranging from antimicrobial peptide pore formation and biomolecule transport to bilayer elasticity and lipid raft formation. In this talk, a versatile platform to generate free-standing, planar, phospholipid bilayers with millimeter scale areas will be introduced. The technique relies on an adapted thin-film balance apparatus allowing for the dynamic control of the nucleation and growth of a planar black lipid membrane in the center of an orifice surrounded by microfluidic channels. A unique advantage in this system for the study of membrane mechanics is control of the membrane tension in a planar geometry, which is demonstrated by measuring the elasticity modulus of bilayers with varying composition. Simultaneous fluorescence microscopy enables monitoring the lateral heterogeneity in ternary lipid mixtures undergoing phase separation. In particular, the effect of interdigitation on phase separation in mixtures containing hybrid lipids (“linactants”) is shown by comparing bilayer to monolayer experiments. Independent control of the solution conditions on either side of the bilayer allows for mimicking the response of biological membranes to external stimuli, such as the introduction of antimicrobial peptides. In this realm, the destabilizing effect of a model antimicrobial peptide, magainin 2, due to pore formation and altered membrane tension on bilayers reconstituted from bacterial cell lipids is analyzed. Together, the results demonstrate a new paradigm for studying the mechanics, structure, and function of model biomembranes.

11-POS Board 6

Modulation of the Membrane Properties by an Ether-Lipid Derivate and Its Consequences in Metastasis Development

Ana Bouchet^{1,5}, Fernando Herrera², Charlotte Sevrain^{3,5}, Hèlène Couthon-Gourvès^{3,5}, Paul-Alain Jaffrès^{3,5}, Aurélie Chantôme^{1,5}, Marie Potier-Cartereau^{1,5}, Jose Requejo-Isidro⁴, Christophe Vandier^{1,5}.

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Specific properties of lipid organisation (fluidity, phase separation, thickness) regulate the structure and activity of membrane proteins, including ion channels. Their role in the regulation of several diseases has been pointed out during the last decade. Our previous data have shown that the SK3 channel (a calcium-activated potassium channel) is involved in the migration of various cancer cells (1). Interestingly an ether-lipid derivate named Ohmline modulates the activity of this channel. The decrease in channel activity by Ohmline is associated with a reduction of cancer cell migrations but does not affect the migration of non-cancer cells (2). Many antecedents indicate that during cell migration a change in membrane fluidity occurs in the plasma membrane of cancer cells providing evidence of a pivotal role of biophysical adaptation in this biological process that ultimately lead to metastatic development.

In the present work, we characterise the physicochemical properties of Ohmline in various lipid environments. Molecular dynamic simulations suggest that Ohmline molecules organize differently depending on the lipid bilayer nature. Fluorescence spectroscopy on model lipid membranes confirm that Ohmline modulates the bilayer order. Imaging the lipid-packing state upon treatment of live cells with Ohmline supports the model in-situ.

The consequences of these observations will be discussed in terms of therapeutic prospectives in tumour development and more particularly in metastasis development.

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- (1) Girault A, et al. (2012) *Curr. Med. Chem.*, 19, 697-713
- (2) Girault A, et al. (2011) *Current Cancer Drug Target*, 11, 1111-1125

14-POS Board 7

Pepducins Rapidly Bind and Diffuse Across Membranes

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The objective of this study was to evaluate the transmembrane transport of pepducins containing a palmitate or myristate linked to peptides corresponding to the third loop of PAR1 (protease activator receptor 1) and to the first loop of CXCR1/CXCR2 (chemokine receptors). Large unilamellar vesicles (LUV) of egg-phosphatidylcholine (egg-PC) were either encapsulated with the pH sensitive fluorescent probe pyranin or labeled in the outer leaflet of the lipid bilayer with the charge sensitive fluorescent probe FPE (fluorescein phosphatidylethanolamine). HEK293 cells were also labeled with FPE. Pepducins at final concentrations from 0.3 to 1.25 μM (< 1 mol% with respect to egg-PC) produced less than 2% of calcein leakage. Therefore, the transport assays were carried within these concentrations. Pepducins added to LUVs or HEK293 cells labeled with FPE or LUVs with pyranine produced a fast ($t_{1/2} < 2$ s) and dose-dependent increase in the fluorescence. The peptide alone (not acylated) did not affect the fluorescence, confirming the importance of acyl chain in anchoring the pepducins in the lipid bilayer. The fast FPE response reflected a net positive change in the interface of the zwitterionic egg-PC (partitioning) and as expected, PAR1 pepducins (3+ positive net charges) produced higher levels of FPE fluorescence than CXCR pepducins (1+ positive net charge). The increase in pyranine fluorescence indicated alkalization of the intravesicular compartment, probably induced by the basic residues that had reached the inner leaflet of the lipid bilayer. We concluded that pepducins were able to bind rapidly to the external leaflet of lipid bilayers and flip to the inner leaflet by simple diffusion. The fatty acid anchor was mandatory for these processes to take place. These data give support to the biological effects of pepducins explained by their interaction with the intracellular loops of GPCRs.

17-POS Board 9

Modulation of Membrane Viscosity by Integral Membrane Proteins

Marie Olsinova, Lukasz Cwiklik, Piotr Jurkiewicz, Jan Sykora, Martin Hof, **Marek Cebecauer**,
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Integral proteins of the plasma membrane are involved in a variety of biological processes. Such proteins frequently form transient nanoclusters with poorly understood function. Transmembrane domains (TMDs) form the closest contact of integral proteins with surrounding lipids, potentially influencing their properties. We have investigated LW21, an artificially-designed, transmembrane helical peptide with amino acid sequence GLLDSKKWWLLLLLLLLLALLLLLLLWKKFSRS in unilamellar vesicles composed of mono-unsaturated lipid, dioleoylphosphatidylcholine (DOPC). Mobility of peptides and lipids (tracers) was determined in the absence and presence of cholesterol. We observed that the presence of low and physiological concentrations of LW21 peptide (1-3 mol%) caused significant reduction in mobility of both, lipids and peptides. This effect was even more pronounced in the presence of 25 mol% cholesterol. Interestingly, indistinguishable diffusion of lipids and peptides was measured in the presence of cholesterol, supporting transbilayer effect of cholesterol in lipid membranes. Our observations cannot be explained solely by currently preferred models of membrane organisation. No peptide aggregation, lipid segregation or anomalous diffusion due to obstacles were detected. LW21 exhibits no hydrophobic mismatch in DOPC membranes, irrespective of the presence of cholesterol. Rather, our data indicate that the peptide increases local microviscosity in lipid membranes. We support this prediction with quantitative MD simulations and experimentally, showing that the peptide increases rigidity of the membrane at the level of phospholipid carbonyl groups, as well as acyl chains. In summary, we re-emphasise the impact of integral membrane proteins on the mobility of membrane components and propose a simple model how dense protein clusters may locally alter membrane properties.

20-POS Board 10

Imaging the Packing State and Lateral Organization of the HIV-1 Membrane Lipids by Two-Photon and Atomic Force Microscopy

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The chemical composition of the human immunodeficiency virus type 1 (HIV-1) envelope is critical for fusion and entry into cells suggesting that preservation of a functional lipid bilayer organization may be required for efficient infection. The HIV-1 acquires its envelope from host cell plasma membranes at sites enriched in raft-type lipids. Furthermore, infectious particles display aminophospholipids in their surface, indicative of dissipation of the inter-leaflet lipid asymmetry metabolically generated at plasma membranes. Here, using membrane lipids extracted from infectious HIV-1 particles, we have correlated information on lipid-packing obtained using two-photon Laurdan fluorescence imaging and lipid organization on the nanoscale using atomic force microscopy. We have established the roles played by the different HIV lipid components and obtained unprecedented insights into its phase state that are critical to the complete description of the viral entry mechanism.

23-POS Board 12

Light-Triggered Morphological Transformations in Giant Vesicles

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Regulation of the lipid membrane structure and morphology are critical for many cellular processes such as the fission-fusion sequence in vesicular transport or endo- and exocytosis. A well-known membrane mimetic system for exploring such cellular processes is giant unilamellar vesicles (GUVs). Photosensitive molecules offer a way to modulate the membrane morphology. Here, we studied the behavior of GUVs in the presence of two photosensitive molecules, tetrafluoroazobenzene (F-azo) and azobenzene-trimethylammonium-bromide (azoTAB). Upon irradiation with light (green or UV and blue), the F-azo molecules undergo reversible trans-cis isomerization [Bléger et al. JACS 134:20597-20600, 2012]. UV and blue light irradiation of the vesicles mixed with F-azo induce reversible morphological transformations such as budding and bud readsorption. The molecule partitioning and orientation in the membrane was probed with molecular dynamics simulations. The energy gain of insertion and the barrier for flipping suggest that F-azo partitions in the membrane and that the observed GUV morphological transitions result from the F-azo isomerization. The associated area increase in the GUVs was measured via vesicle electrodeformation for two different F-azo concentrations. The influence of molecule isomerization on phase separated vesicles was also examined. The appearance of liquid-disordered domains within the preexisting liquid ordered phase was observed. Differently from the action of F-azo molecules, under UV irradiation, azoTAB molecules cause the GUVs to rupture. A potential application of such system includes the development of drug delivery systems with light-triggered release of solutes. These results suggest that the photosensitive molecules provide us with a handle to modulate the membrane morphology and stability.

We acknowledge S. Santer (Potsdam University) for the azoTAB molecules and IMPRS for the financial support.

26-POS Board 13

Investigating the Insertion and Folding of Membrane Proteins Into Lipid Bilayers Using a Cell Free Expression System

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Membrane proteins play a vital role in many biological processes, and yet due to their instability *in vitro* remain poorly understood. This project aims to investigate the insertion and folding of membrane proteins into lipid bilayers, using a commercial cell free expression system (PURExpress) in combination with synthetic liposomes of defined lipid composition. These studies will aid understanding of cooperative folding, folding intermediates, and the effects of the lipid bilayer on folding and insertion. We have chosen model *E.coli* proteins as they can offer important insights into other proteins, and thus facilitate the study of more biologically relevant targets. We have found that the *E.coli* rhomboid protease GlpG, and the Major Facilitator Superfamily (MFS) transport proteins LacY, XylE, GalP and GlpT, spontaneously insert into liposomes without the aid of an insertase such as SecYEG. This indicates that the innate hydrophobicity of these membrane proteins is sufficient for insertion into a bilayer. Spontaneously inserted GlpG is functional, as measured by its ability to cleave BODIPY-labeled casein, indicating that it is correctly folded. We have found that spontaneous insertion is highly influenced by the lipid composition of the liposomes. All the proteins tested to date prefer at least 50 mol% DOPG, and high DOPC has been found to be unfavourable for spontaneous insertion. On-going and future work will utilise rare codons to alter the rate of translation, and assess the effect this has on the final folded structure of membrane proteins. We will also examine whether the two helical domains of MFS transporters fold independently or cooperatively when expressed as two separate polypeptides. Preliminary results indicate that they fold independently. This work aids understanding into the folding and stability of membrane proteins.

29-POS Board 15

Membrane Curvature Sensing, Membrane Deformation and Lipid Binding Assayed Simultaneously for N-BAR Proteins in Newly Developed Single-Vesicle Assay: FlowSLiC

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The expanding sub-group of BAR (Bin/Amphiphysin/Rvs) domain proteins, the N-BARs, contain an N-terminal amphipathic helix (AH). AHs can sense membrane curvature, and have accordingly been identified as the membrane curvature sensing (MCS) segment of N-BARs. The insertion of amphipathic helix into the lipid membranes has also been demonstrated to deform membranes (MemDef), often through low-throughput bulk vesiculation assays, but the interplay between the helix and the scaffolding BAR domain dimer is still subject to debate. In addition, the lipid binding for N-BARs often includes an oligomerization regime, potentially involving an AH-mediated lattice formation.

Here we have identified a novel amphipathic helix in PICK1 from the Arfaptin group of BAR proteins, and characterized the lipid interaction regimes through our previously developed Single Liposome Curvature-sensing (SLiC) assay. We show how these parameters control the localization and activity of the PICK1. We thereafter utilize the knowledge of the lipid binding capacities of this protein, as well as the bona fide N-BAR protein Endophilin, to develop a new Flow Cytometry-based assay to explore the interaction between proteins and liposomes. The assay we hence named; FlowSLiC.

FlowSLiC is high-throughput, advantageously assaying all three cardinal features of lipid binding (MCS, MemDef, oligomerization) simultaneously. Here, we use it to delineate the concentration- and time-dependent components in the binding regimes of these two N-BARs, but the generality of the assay makes it suited to assay the binding capacities of lipid binding proteins wide across biology.

32-POS Board 16

Feedback-Controlled pH-Switching Within Vesicle Nanoreactors

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Within the field of bottom-up synthetic biology, vesicular nanoreactors can be utilised in the compartmentalisation of a variety of different entities. Such entities, depending on their biological or chemical function, can be used to define these systems within a specific field of technological advancement, e.g., therapeutics, biosensing, protocell technology, etc. A significant challenge for the therapeutic application of vesicle technology is to improve the communication between a vesicle and its environment, allowing for smart, regulated release of active compounds in response to a variety of complex biological cues. The primary challenge in designing an effective and efficient drug delivery system, capable of self-mediating its own permeability and eliciting a controlled drug-release profile, is the incorporation of self-regulation. Undoubtedly, to achieve this, some form of feedback control is required. One option, which complies with the biocompatibility of these therapeutic systems, is enzymatic feedback. Towards this goal, we will demonstrate a urea-urease base-catalysed feedback-controlled pH-switch within vesicular confinement. By varying initial reaction conditions, membrane physicochemical properties, and vesicle sizes (ranging from the nano- to the microscale), we will demonstrate tuneable temporal and spatial control of the pH-switching behaviour. By understanding feedback-controlled reactions within self-assembled vesicle compartments, our long term aim is to engineer systems that can regulate a desired drug release profile, in response to changing environmental signals.

35-POS Board 18

Probing Conformational States of the SNARE Fusion Machinery Using Supported Lipid Bilayers and Site Directed FLIC Microscopy

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The fusion of synaptic vesicles with the presynaptic membrane is a fast and highly regulated process that is catalyzed by the neuronal SNAREs synaptobrevin-2 (VAMP-2), SNAP-25, and syntaxin-1a (Syx1a). In addition to the SNAREs a number of protein players that regulate the fusion process have been identified and characterized. Despite tremendous progress in this field we are still missing a molecular timeline that leads from docking of synaptic vesicles to the plasma membrane, to priming of the fusion machinery and eventually to fusion of the two membranes once an action potential reaches the synaptic terminal. One of the difficulties in approaching these questions arises from the fact that the lipid environment plays an active previously under-appreciated role in this protein driven process.

Reconstituting SNAREs into supported lipid bilayers allows us to characterize the state of the SNARE and regulatory proteins in different defined lipid environments. Using site-directed fluorescence interference contrast (sd-FLIC) microscopy we measure distances of specific residues in Syx1a and VAMP-2 from the membrane surface – and thus conformational transitions of these proteins – at nm-resolution during proposed steps of the priming pathway. Here we report how the conformational states of Syx1a and VAMP-2 change upon binding to regulatory proteins such as Munc18.

38-POS Board 19

Targeting Liposomes for Uptake into CEACAM-Expressing Human Cells Using a Bacterial Membrane Protein

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The pathogenic bacteria *Neisseria gonorrhoeae* and *N. meningitidis* induce their own phagocytosis into human host cells prior to replication. Bacterial cell entry is promoted by the binding of Neisserial outer membrane opacity-associated (Opa) proteins to human carcinoembryonic antigen-like cell adhesion molecule (CEACAM) receptors, a widely-distributed class of cell receptors in the human body. Binding of Opa proteins to either CEACAM1, CEACAM3, CEACAM5, or CEACAM6 generates intracellular signalling events which lead to bacterial internalization by both phagocytes and epithelial cells. We are interested in determining whether Opa proteins reconstituted in liposomes retain the ability to promote entry of liposomes into epithelial cells similar to *Neisseria*. Because CEACAM receptors targeted by Opa may demonstrate tissue-specific expression or increased expression in various cancers, the ability to target Opa-proteoliposomes to CEACAM⁺ cells could prove valuable in therapeutic delivery. Our results indicate that Opa-proteoliposomes target CEACAM⁺ cells for internalization using an active-uptake mechanism. Additionally, Opa-proteoliposome uptake into CEACAM⁺ cells correlates with both cell size and CEACAM expression levels. With uptake established, CEACAM selectivity is currently being investigated.

41-POS Board 21

H₂ Fueled ATP Synthesis on an Electrode: Mimicking Cellular Respiration

Ivan Lopez-Montero^{1,2}, Oscar Gutiérrez-Sanz³, Paolo Natale^{1,2}, Ileana Marquez³, Marta C. Marques⁴, Sonia Zacarias⁴, Marcos Pita³, Ines A. Pereira⁴, Antonio L. De Lacey³, Marisela Velez³.

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ATP, the molecule used by living organisms to supply energy to many different metabolic processes, is synthesized mostly by the ATPase synthase using a proton gradient generated across a lipid membrane. We present evidence that a modified electrode surface integrating a NiFeSe hydrogenase and a F₁F_o-ATPase in a lipid membrane can couple the electrochemical oxidation of H₂ to the synthesis of ATP. This electrode-assisted conversion of H₂ gas into ATP could serve to generate this biochemical fuel locally when required in biomedical devices or enzymatic synthesis of valuable products.

44-POS Board 22

Directly Observing the Lipid-Dependent Self-Assembly and Pore-Forming Mechanism of the Cytolytic Toxin Listeriolysin O

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Listeriolysin O (LLO) is the major virulence factor of *Listeria monocytogenes* and a member of the cholesterol-dependent cytolysin (CDC) family. Gram-positive pathogenic bacteria produce water-soluble CDC monomers that bind cholesterol-dependent to the lipid membrane of the attacked cell or of the phagosome, oligomerize and insert into the membrane to form transmembrane pores. Although different models have been proposed to explain how CDCs bind and form pores into the membranes, this process is still not well understood. Using electron microscopy and time-lapse atomic force microscopy, we show that wild-type LLO binds to membranes, depending on the presence of cholesterol and phospholipids composition. LLO oligomerizes into arc- or slit-shaped assemblies, which merge into complete rings. With increasing cholesterol content of the membrane, the number of transmembrane pores increases. The dynamic fusion of arcs, slits and rings into larger rings and pores does not involve a height difference between prepore and pore. Our results reveal new insights into the pore-forming mechanism and introduce a dynamic model of pore formation by LLO.

47-POS Board 24

Circulating Microparticles as Intercellular Communication Tools Between Platelets and Cancer Cells: A Complex Relationship Investigated by an "On Chip" Approach

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¹FEMTO-ST, Besançon, France, ²Graduate Institute of Biomedical materials and Tissue Engineering, Taipei Medical University, Taipei, Taiwan.

In cancer, the interaction of the abnormal malignant cells with their microenvironment is essential for tumor development, later progression and the development of metastatic disease. The tumor microenvironment is a collective term that includes the tumor's surrounding and supportive stroma, the different effectors of the immune system, blood platelets, hormones and other humoral factors. A better understanding of the interplay between the tumor cells and its microenvironment can provide efficient tools for cancer prevention, screening and risk assessment protocols. Platelets are key players in hemostasis and thrombosis, and they also have much broader roles in balancing health and disease. Platelets also interact with inflammatory and immune cells, mediating between inflammation and thrombosis. Moreover, complex interactions between tumor cells and circulating platelets play an important role regulating tumor growth, dissemination and angiogenesis^{1,2}. Platelet-derived microparticles (PMPs) also share a pathological function in this complex interaction. Microparticles (MPs) are small (50 to 1000 nm) plasma membrane remnants shed from cells upon their activation or apoptosis. They are an important intercellular communication tool and may modify cellular behavior in certain conditions. MPs load consists of proteins, lipids, and nucleic acids, including microRNA, which may be transferred horizontally between cells³. In cancer, oncogenic pathways drive production of MPs, and also stimulate production of MPs harboring tissue factor (TF) that contributes to cancer-induced thrombosis⁴. MPs also correlate with processes related to cell aggressiveness including tumor growth and metastasis. In this particular MPs-cancer context, we propose to deeper characterize the MPs subpopulations produced by cancer cells alone and in the presence of platelets. Our "on chip" strategy, based on Surface Plasmon Resonance and Atomic Force Microscopy coupling, enables the determination of MPs cell origin and size subpopulations.

50-POS Board 25

The Role of Cardiolipins in Uncoupling Proteins 1/2 Functionalities

Alessio Prunotto, Matteo Dal Peraro.

École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland.

Uncoupling Proteins (UCP) 1/2 are mitochondrial proteins that play a fundamental role in the energetic economy of this organelle. These proteins are actually designated to pump protons out of the inner mitochondrial matrix. As a matter of fact, for the generation of ATP, the cell necessitates to generate an electrochemical potential within the mitochondria, which is guaranteed by the electron transport chain, which accumulates protons inside the mitochondrial matrix. Such accumulation has to be dissipated by the UCP1 and UCP2, in order to prevent damages to the mitochondria functions. UCP1/2 are considered attractive targets for antidiabetes and antiobesity drugs. UCP2 has been solved by NMR. Based on this structure, we built the UCP1 using comparative modeling, thanks to the high identity between the two primary sequences (57%). The mechanism of action of these proteins is still largely unknown; for example, it is debated whether they assume a dimeric or tetrameric form, as well as how the transportation of protons is carried on. From this point of view, cardiolipins were proven to act as structure stabilizers and to enhance the proton transport activity of these proteins. Molecular Dynamics simulations of UCP1/2 were conducted at both atomistic and coarse-grain level, in order to understand the influence of the cardiolipins in the oligomerization state of these proteins. To assess this, we ran several simulations at different cardiolipins concentrations, in order to depict the consequences that the absence (or reduction) of this particular kind of lipid has on UCP1/2.

53-POS Board 27

Quiet OmpG Nanopores for Small Molecule Detection

Raghavendar Reddy Sanganna Gari, Patrick Seelheim, Lukas Tamm.
University of Virginia, Charlottesville, VA, USA.

Interest in nanopore technology has been growing due to their unique capabilities in small molecule sensing, measurement of protein folding, and low cost DNA and RNA sequencing. The heptameric α -hemolysin (α HL) nanopore has been used successfully for these applications. However, modifications of α HL pores for optimizing the performance are challenging due to its heptameric nature. The *E. coli* outer membrane protein OmpG is an excellent alternative to α HL because its pore is formed by a single polypeptide chain. OmpG is a 14-stranded β -barrel protein with seven long extracellular flexible loops. It is known that loop 6 controls the pore access in a pH-dependent manner and its dynamics result in spontaneous gating of the open pore current. This phenomenon eventually limits the applications of OmpG in biosensing. Our goal is to develop quiet OmpG nanopores that are free of spontaneous gating and also insensitive to pH. In this work, we achieved quiet pores by altering the gating loop 6 and residues nearby. Here, we present NMR studies of these quiet pores in lipid micelles to assess their loop dynamics and conformational changes along with electrophysiology measurements. Our engineered OmpG nanopores are approximately 50 times quieter than the wildtype protein and are more robust to pH changes. We further modified the inside of these quiet OmpG derivatives with a copper chelating moiety to facilitate the detection of small molecules such as catecholamines and amino acids.

56-POS Board 28
Dissecting Exosome Biogenesis and Uptake via Anthrax Toxin

Oksana A. Sergeeva¹, Katrin Volkmann², Laurence Abrami¹, Prisca Liberali², Gisou Van der Goot¹.

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Exosomes are endosomal vesicles that can be trafficked from cell to cell. While they have been implicated in a variety of diseases and linked to biomarkers, understanding how exosomes are made from cells, let alone from diseased cells, has been a challenge. Because most laboratories use specific purification techniques and cell types to study exosome biogenesis and uptake, little is known about general exosome endogenous properties. Recently, we found that lethal factor (LF) of anthrax toxin can be transferred to naïve cells via exosomes. Usually, LF enters the cell using protective antigen and anthrax receptors and then eventually makes its way into the cytoplasm, where it can cleave MAPKKs and cause apoptosis. Using anthrax toxin as a tool allows us to observe and probe exosome biogenesis and uptake without purification of the exosomes or major perturbations. We are investigating what genes and factors are required for exosome biogenesis and release from primary cells and then uptake by naïve cells. We have been screening a thousand genes that may be implicated in the exosome pathway for their involvement, and are following up on the most interesting candidates. Additionally, we are unraveling the mechanism behind ER stress and GPI-anchored proteins playing a role in exosome uptake. Overall, we are gaining general knowledge of exosome biology via our unique system, which can then be used to address exosome biomarkers and disease.

59-POS Board 30

Single Particle Tracking in Cushioned, Blebbed Supported Lipid Bilayers Enables Studies of Transmembrane Protein Diffusion

Rohit R. Singh¹, Martin I. Malgapo², Maurine E. Linder², Susan Daniel¹,
¹Cornell University, Ithaca, NY, USA, ²Cornell University, Ithaca, NY, USA.

Supported Lipid Bilayers (SLB's) are effective models for studying some biomembrane phenomena. A thin layer of water between the substrate and the bilayer engenders 2D fluidity and enables studies of lipid diffusion and peripheral membrane protein diffusion. However, the water layer is not thick enough to prevent friction between most transmembrane proteins and the substrate. Because of this, it is difficult to study the diffusive properties of proteins that protrude significantly from the membrane. Here, we study several related cushioning strategies that are easy to construct and support the mobility of most transmembrane proteins. All cushions make use of PEGylated lipids to lift the bilayer away from the substrate. The concentration and length of the PEGylated lipids can be varied to maximize mobility for a protein of choice. The PEGylated lipids can also be biotinylated to allow for a double cushion strategy. In this approach, streptavidin is first used to passivate the substrate and will form bonds with the PEGylated lipids to anchor them in place. The bilayer can be formed by vesicle fusion, allowing us to incorporate membrane proteins from cell blebs without using detergents or other artifactual methods. The efficacy of the different cushioning strategies was assessed through single particle tracking (SPT) of fluorescently tagged DHHC20, a ~40 kDa acyltransferase with 4 transmembrane domains. We will discuss the biological implications of these results and the applications of this platform to studying cytoskeleton-mediated confinement of plasma membrane components. We will also briefly discuss a complementary method for carrying out Brownian dynamics simulations to model protein diffusion. The results of these simulations will be used to put forth a model for the mechanism of cytoskeletal confinement based on hydrodynamic interactions with immobilized membrane proteins.

62-POS Board 31

Line Tension at Lipid Phase Boundaries as Driving Force for HIV Fusion Peptide-Mediated Membrane Fusion

Sung-Tae Yang, Volker Kiessling, Lukas K. Tamm.
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Lipids and proteins are organized in cellular membranes in clusters, often called “lipid rafts”. Although raft-constituent ordered lipid domains are thought to be energetically unfavorable for membrane fusion, rafts have long been implicated in many biological fusion processes. For the case of HIV gp41-mediated membrane fusion, this apparent contradiction can be resolved by recognizing that the interfaces between ordered and disordered lipid domains are the predominant sites of fusion. Here we show that line tension at lipid domain boundaries contributes significant energy to drive gp41 fusion peptide-mediated fusion. This energy, which depends on the hydrophobic mismatch between ordered and disordered lipid domains, may contribute tens of kBT to fusion, i.e., it is comparable to the energy required to form a lipid stalk intermediate. Line-active compounds such as vitamin E lower line tension in inhomogeneous membranes, thereby inhibit membrane fusion, and thus may be useful natural viral entry inhibitors.

65-POS Board 33

Determining the Specificity of Insoluble Protein Trans-Membrane Domains to Lipid Compositions

Roy Ziblat¹, Laura Arriaga², Shaorong Chong³, David Weitz¹.

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The specific interactions between proteins and lipid compositions are a key feature in the architecture and function of biological membranes. However, most proteins interacting with lipids are insoluble in water. Therefore, studying their specificity to lipids is very challenging, especially when focusing on their trans-membrane domains that span through the hydrophobic membrane. We introduce here a method that overcomes the solubility limitation and identifies the specific binding of trans-membrane domains to a membrane library composed of over a hundred different lipid compositions. This study reveals that trans-membrane domains are highly selective and that the specific interactions of trans-membrane domains to lipids depend not only on the presence of a single lipid, but on a combination of a few. Comparison of five human trans-membrane domains indicates that each has a unique affinity profile, demonstrating the complexities of protein-lipids interactions and indicating the importance of studying the specificity of trans-membrane domains to lipids.

POSTER SESSION III

Thursday, September 15, 2016

15:55 – 17:30

Balint Salon

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

Aubin-Tam, Marie-Eve	3-POS	Board 2
Barrera, Francisco	6-POS	Board 3
Biner, Olivier	9-POS	Board 5
Bozó, Tamás	12-POS	Board 6
Carvalho, Vanessa	15-POS	Board 8
Chopineau, Joel	18-POS	Board 9
Engberg, Oskar	21-POS	Board 11
Goers, Roland	24-POS	Board 12
Heath, George	27-POS	Board 14
Hidalgo, Alberto	30-POS	Board 15
Karabadzak, Alexander	33-POS	Board 17
Kósa, Nikoletta	36-POS	Board 18
Kulik, Andrzej	39-POS	Board 20
Meers, Paul	42-POS	Board 21
Natali, Alberto	45-POS	Board 23
Pace, Hudson	48-POS	Board 24
Ramirez-Alvarado, Marina	51-POS	Board 26
Sarria, Marisa	54-POS	Board 27
Shi, Xiaojun	57-POS	Board 29
Sanders, Michael	60-POS	Board 30
Yang, Sung-Tae	63-POS	Board 32

Posters should be set up on the morning of Monday, September 12 and removed by 18:00 on
Thursday, September 15.

3-POS Board 2

Functionalized SMA-Nanodiscs for Conjugation of Membrane Proteins to Dyes and Surfaces

Simon Lindhoud, Vanessa Carvalho, Jochem Pronk, **Marie-Eve Aubin-Tam**.
Delft University of Technology, Delft, Netherlands.

The integration of membrane proteins into biophysical and biosensing assays have lagged considerably in comparison to soluble proteins owing to the extreme difficulties associated with purifying, handling and conjugating membrane proteins. I will present newly developed functionalization procedures to interface membrane proteins with surfaces, while preserving their native membrane environment.

Nanodiscs are soluble scaffolds for membrane proteins, which consist of nanometer-sized discoidal phospholipid bilayers surrounded by an amphipatic polymer, such as the membrane scaffold protein [1] or styrene-maleic acid (SMA) co-polymer [2]. Membrane proteins embedded in lipid nanodiscs maintain their membrane-integrated state in this soluble complex, and can therefore be handled similarly to soluble proteins. SMA co-polymers are highlighted as agents for detergent-free purification of membrane proteins, which can solubilize membrane proteins in presence of their native lipid membrane environment. To facilitate conjugation of SMA-nanodiscs to surfaces, nanoparticles and fluorophores, we exploit the reactivity of maleic anhydride moieties in SMA towards amines to modify the polymer with cysteamine [3]. Thus, we equip the polymer with a sulfhydryl group (SMA-SH). This sulfhydryl group is then modified with thiol-reactive probes, such as maleimide derivatives of fluorophores and biotin. SMA-SH nanodiscs are characterized with gel filtration, dynamic light scattering and electron microscopy.

We find that SMA-SH enables the functionalization of membrane proteins with a variety of different probes, while not requiring any mutation or chemical modification of the protein itself. We anticipate that this versatile approach will find application in membrane protein purification, in biosensing, and in biophysical assays.

1. Bayburt TH, Grinkova YV, Sligar SG. *Nano Lett.*, 2, 853-856, 2002
2. Scheidelaar, S., et al. *Biophys. J.*, 108, 279-290, 2015
3. Lindhoud S, Carvalho V, Pronk JW, Aubin-Tam ME, *Biomacromolecules*, 17, 1516-1522, 2016

6-POS Board 3

Rational Design of a Novel Soluble Peptide with pH-Responsive Membrane Insertion

Vanessa P. Nguyen, Daiane S. Alves, Haden L. Scott, Forrest L. Davis, **Francisco N. Barrera**.
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Several diseases, such as cancer, are characterized by acidification of the extracellular environment. Acidosis can be employed as a target to specifically direct therapies to the diseased tissue. We have used first principles to design an acidity-triggered rational membrane (ATRAM) peptide with high solubility in solution that is able to interact with lipid membranes in a pH-dependent fashion. Biophysical studies show that the ATRAM peptide binds to the surface of lipid membranes at pH 8.0. However, acidification leads to the peptide inserting into the lipid bilayer as a transmembrane α -helix. The insertion of ATRAM into membranes occurs at a moderately acidic pH (with a pK of 6.5), similar to the extracellular pH found in solid tumors. Studies with human cell lines showed a highly efficient pH-dependent membrane targeting, without causing toxicity. Here we show that it is possible to rationally design a soluble peptide that selectively targets cell membranes in acidic environments.

9-POS Board 5

**Fusion of Oppositely Charged Proteoliposomes as a Method for Membrane Protein
Co-Reconstitution**

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In order to investigate the functional interplay of several membrane proteins (MP) on a molecular basis, they have to be extracted from their complex native environment and reconstituted into a well-defined membrane mimicking system such as liposomes. A good example for a functional interplay is oxidative phosphorylation in bacteria and mitochondria by the members of the respiratory chain (complex I-IV). Since every MP requires its own reconstitution protocol, we split the procedure in two steps. First, we reconstitute each purified MP into liposomes and in a second step, fuse the proteoliposomes. Different strategies to achieve liposome fusion have been described such as the use of SNARE proteins, fusogenic peptides, DNA oligomers, or oppositely charged lipids.

We recently successfully applied SNARE-mediated fusion of proteoliposomes, but this method is limited to one round of fusion and therefore only interactions of two MPs can be studied. To investigate more complex systems, more than one round of liposome fusion might be necessary. We are therefore currently testing liposome fusion by oppositely charged lipids (DOPG/DOTAP) as an alternative to SNARE-dependent fusion. Using fluorescent lipid mixing assays and liposome size determination, we established protocols for charge mediated liposome fusion in our hands and applied the optimised conditions to fuse liposomes containing respiratory chain enzymes. Using this strategy, it was possible to co-reconstitute different terminal oxidases and the *E. coli* ATP synthase, imitating the last step of oxidative phosphorylation. The oxidase thereby creates an electrochemical proton gradient that energizes the ATP synthase, requiring an intact (proton tight) lipid bilayer after the fusion process.

We applied the same technology to incorporate purified ATP synthase into inverted membrane vesicles from an ATP synthase deficient *E. coli* strain, successfully restoring respiratory driven ATP synthesis in these vesicles.

12-POS Board 6

Evolution of Cochleate Membrane Rolls From Unilamellar Liposomes

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Addition of calcium ions to liposomes with negatively charged phospholipid headgroups may lead to the formation of membrane rolls, the so-called cochleates. These structures have been intensively studied as potential micro- and nanosized vehicles for drug delivery. However, little is known about how they evolve from liposomes and what factors modulate their generation.

The aim of our study was to follow and quantify the formation of cochleates from unilamellar liposomes. Samples were analyzed by using phase contrast and atomic force microscopy (AFM) imaging and force spectroscopy.

When divalent cations were added to dioleoyl phosphatidylserine liposomes, they first aggregated in form of multilamellar vesicles. Cochleates could be identified only hours later and it took a few days to weeks until they become the dominant phase of the sample. Partially (un)rolled particles were found occasionally, indicating that membrane layers may flow over each other. A particle counting method was utilized to quantify the efficiency of cochleate formation at various preparation conditions. The results indicate that the rate of calcium addition has a key role in cochleate formation. Nanomechanical perturbations revealed that cochleates are made up of solid-like membrane layers which are mechanically independent from each other, thus allowing for shape changes and slow dynamics.

15-POS Board 8

Biochemical and Structural Studies of FtsH, a Membrane Anchored Degradation Machine

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Proteases are responsible for elimination of non-functional proteins, controlling protein levels, or modification of protein function. An important group of proteases are part of the ATPases associated with various cellular activities proteases (AAA+) family. AAA+ proteases are degradation machines, which exploit energy from ATP hydrolysis to unfold protein substrates and translocate unfolded polypeptides through a central pore, down towards a degradation chamber. In particular, FtsH is a membrane-anchored AAA+ protease, which play crucial roles in membrane protein quality control, protein transport across the membrane and dislocation of specific transmembrane segments. Although cytoplasmic structures are described, the full-length structure and the route by which soluble or integral membrane proteins translocate into the FtsH central pore to be unfolded, remains unclear. Structural characterization of full-length FtsH solubilized with either detergent or styrene maleic acid (SMA) nanodiscs provides insights on this mechanism and on FtsH integration in the lipid bilayer.

We optimise expression and purification protocols, using the full-length sequence that encodes *Aquifex Aeolicus* FtsH. The results of the detergent solubilized FtsH, in negative staining and cryo-electron microscopy single particle analysis, show the first structure of the full-length FtsH. Proteolytic and ATPase activities are also measured. We also use SMA as solubilisation agent, which enables the formation of SMA-FtsH-nanodiscs such that FtsH remains in its native membrane environment.

18-POS Board 9

Investigation of the Use of Exosomes as Drug Delivery Systems

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Exosomes, as natural vectors of biomolecules, has been described as interesting drug delivery systems for mainly nucleic acids, but also lipids or proteins. We propose to use pharmaceutical and chemical methods (post-insertion, click chemistry,...) to allow drug loading as well as surface modification to increase the pharmacokinetics behavior and targeting efficiency with “easy to handle” processes which should facilitate the development of new exosome-based therapeutics. The efficient exosome-mediated delivery in vivo requires targeting vesicles for uptake by specific recipient cells, as the half life of non targeted exosomes was close to 2min after systemic administration in mice (Morishita et al., 2015; Smyth et al., 2015; Wiklander et al., 2015). As a supplementary hurdle, frequently encountered targeting process used to modify exosomes, require transfection of cells and lead to low production yield (Alvarez-Erviti et al., 2011; Hung et al., 2015). In this context, we choose to modify human mesenchymal stem cells derived exosomes in a post-production manner, by various physico-chemical methods. We are currently working on the surface modification of exosomes (PEGylation, ligand attachment). These modifications are assessed by physico-chemical characterization (size, surface charge), and quantification of the grafted molecules (PEG quantification (KI/I2), ELISA,...) at the exosome surface. In parallel, the loading ability is evaluated for different kind of molecules (chemicals, protein, nucleic acids), and different process. The efficiency of surface modification will be evaluated in vitro in regards to interaction with plasma proteins as well as with immune cells (THP1 monocytes) thanks to a fluorescent tracking (FACS, confocal microscopy). The interaction with cells which produced exosomes and other cell lineage (tumoral cell line) will be evaluated.

21-POS Board 11

Characterization of How Cholesterol's Affinity for Different Phospholipids Affect Lateral Segregation

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Cholesterol is known to influence lateral domain formation in model membranes, which likely resembles the formation of nanodomains in biological membranes. Lateral segregation is also likely affected by cholesterol's preference for saturated acyl chains over monounsaturated, and especially polyunsaturated ones. Here we have investigated how cholesterol influenced the lateral segregation of saturated and unsaturated phospholipids (PLs), for which cholesterol had a varying degree of affinity. The formation of ordered domains ((gel or liquid-ordered (lo)) was detected by measuring the fluorescence lifetime of trans-parinaric acid (tPA) in bilayers composed of different unsaturated phosphatidylcholines, and dipalmitoyl-phosphatidylcholine (DPPC) or N-palmitoyl-sphingomyelin (PSM), with and without cholesterol. The tPA experiments showed that cholesterol facilitated lateral segregation, which was dependent on stoichiometry of the mixture of unsaturated and saturated PLs. The facilitated lateral segregation could be explained by correlating the relative affinity of cholesterol for the different PLs in the bilayers. In addition, differential scanning calorimetry (DSC) and ²H nuclear magnetic resonance (NMR) showed that cholesterol increased the thermostability of both the lo- and gel-domains. The acyl order in the lo-domains was increased when the degree of unsaturation was increased in the unsaturated PLs, likely by enriching the ordered domains in saturated lipids and cholesterol. This agreed with the conclusions from the tPA-experiments, and gave insight into how cholesterol facilitated lateral segregation. Our data suggests that knowledge of the relative affinity of cholesterol for the different PLs in a bilayer could predict in which biological membranes cholesterol is most probable to promote lateral domain formation.

24-POS Board 12

From Model to Product: Using Proteorhodopsin to Drive a Molecular Hoover

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The creation of biomimetic reaction compartments is part of the bottom-up approach in synthetic biology. In order to fulfil a desired task, these systems often require transport of substrates and products to/from their interior. Passive diffusion can be enabled by pores, whereas active and controllable transport requires energy. Light-driven proton pumps such as proteorhodopsin (PR) generate a fundamental electrochemical gradient (proton motif force) upon illumination. Modern detergent-mediated membrane protein reconstitution procedures allow the integration of membrane proteins into synthetic membranes, however, they usually lack control over the final orientation of the proteins which is especially crucial for directional transporters like PR. Furthermore, this process relies on the self-assembly of the membrane components without direct control. Predetermined outcomes are only achieved by changing the starting conditions, which requires detailed knowledge about key parameters.

We bypassed this issue by fusing green fluorescent protein (GFP) to PR, as the hydrophilic nature of GFP drives its orientation upon reconstitution into preformed lipid and polymer vesicles. Statistical modelling ‘Design of experiments (DoE)’ was used to identify significant factors and further allows their optimization towards a desired outcome. We applied this methodology rationally to find conditions, which lead to proper formation of proteolipo- and proteopolymersomes.

The fluorescence of GFP allowed us to detect PR-GFP inside the membrane by fluorescence correlation spectroscopy. It turned out that lipid and polymer membranes require different treatments for successful reconstitution. Subsequently, the parameter space was narrowed down by setting boundary conditions for the highest pumping activity. The internal pH of the proteovesicles was measured via fluorescence spectroscopy and the final model was able to predict the activity with a high precision. The model allows optimization of the process and provides the relevant knowledge of key parameters at the same time.

27-POS Board 14

Assembling Double and Multi-layered Lipid Membranes to Study Electron Transfer Pathways

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Multilayer lipid membranes perform many important functions in biology, such as electrical isolation (myelination of axons), increased surface area for biocatalytic purposes (thylakoids and mitochondria), and sequential processing (golgi cisternae). Here we develop a simple layer-by-layer methodology to form lipid multilayers via vesicle rupture onto existing supported lipid membranes using poly-l-lysine (PLL) as an electrostatic polymer linker. The assembly process was monitored at the macroscale by quartz crystal microbalance with dissipation (QCM-D) and the nanoscale by atomic force microscopy (AFM) for up to six lipid bilayers. By varying buffer pH and PLL chain length, we show we can control the separation between the membranes. By incorporating functional membrane proteins into these multilayers using either protein reconstitution into proteoliposomes or by mixing vesicles with membrane extracts we show how this technique can be used to multiply the function of membrane proteins normally limited to a single bilayer. We demonstrate this using cyclic voltammetry of lipid multilayers on gold using two different membrane proteins, a hydrogenase that catalyzes the oxidation of H₂ and Cytochrome bo₃ which catalyzes O₂, both to produce protons.

This approach also provides a route to creating complex gram negative bacterial membrane mimics, allowing the study of the electron transfer pathways between a number of inner and outer membrane proteins. Our study focuses on *Shewanella oneidensis* MR-1, a bacterium which can reduce poisonous heavy metal ions, a better understanding of which may further microbial biotechnologies such as microbial fuel cells and electrosynthesis. By reconstituting the membrane proteins thought to be key to the MR-1 electron transfer pathway and assembling the double membrane on gold we show how this still not fully understood pathway can be investigated for the first time using a bottom up approach.

30-POS Board 15

Pulmonary Surfactant: A Shuttle to Deliver Drugs into Lung Airspaces

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

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

33-POS Board 17

Lipid Influences on Peptide Insertion Across Bilayers

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The study of polypeptide insertion into biological membranes can enhance our understanding of membrane protein stability and folding, and also has potential practical applications. pH Low Insertion Peptides (pHLIPs) are a family of water soluble peptides that bind to membrane surfaces in a mainly unstructured form. Acidic pH triggers them to fold into helices and insert as transmembrane helices. Due to their pH dependent properties, pHLIPs can target acidic tissues such as tumors. Here we present a study of pHLIPs' interaction with both natural and artificial membrane bilayers designed to model the various lipid compositions of healthy and cancerous cell membranes. We constructed liposomes with varied ratios of cholesterol, phosphatidylserine lipids (PS), and saturated phospholipids to mimic the plasma membranes of cancer cells. We employed biophysical methods: fluorescence, CD and a centrifugal separation assay to study pHLIP's association with different membranes at different conditions. Results show that lipid composition, liposome size, and the buffer environment affect pHLIPs' partition/association with membranes. As pHLIP-based tumor-targeting agents move towards the clinic, understanding the influence of lipid composition on their membrane insertion activity is an important step in navigating the complex biodiversity in cancer cell membranes. This work was supported by National Institute of Health grant GM 073857

36-POS Board 18

Newly Synthesized Liposomal Antituberculosic Compounds and Effects on In Vitro Model Systems

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Introduction. Liposomes are widely investigated nanocarriers, which are capable for incorporation of both lipid-soluble and water-soluble drugs. WHO aimed to eradicate TB disease, which is caused by *Mycobacterium tuberculosis* bacteria.

Aim. Preparation and encapsulation of newly synthesized antituberculosic compound into liposomes. To enhance the encapsulation efficiency and to target intracellular bacteria in the macrophages.

Methods. We have prepared two types of liposomes.

Type I. consists of dipalmitoyl phosphatidylcholine (DPPC).

Type II. consists of: dioleoylphosphatidylethanolamine (DOPE), cholesteryl hemisuccinate (CHEMS) and pegylated distearoyl phosphatidylethanolamine (DSPE-PEG).

We used thin lipid film technology to prepare multilamellar vesicles (MLV) and henceforward both types were treated with extrusion technique to get small unilamellar vesicles (SUV). The size distribution of liposomes was determined/measured with dynamic light scattering (DLS). The change of diameter of vesicles shows the rate of aggregation. The used antituberculosic compounds were: TB 501, TB 504, TB 505 and TB 515 (chemical structure not published yet). Encapsulation efficiency was determined by measuring absorbance after size exclusion chromatography (SEC). Cellular uptake of liposomal compounds and non-encapsulated drugs was measured by flow cytometry on MonoMac6 human monocytic cell line. Intracellular fluorescence intensity and forward-scattered light (FSC) of MonoMac6 cells was monitored (488 nm (Coherent Sapphire, 22 mW) laser, which is proportional to the cellular uptake.

Results. The extrusion method resulted rather uniform and stable vesicles. Encapsulation efficiency was influenced by the physico-chemical properties of antituberculosic compounds. The in vitro activity of liposomal antituberculosic compounds was determined on *M. tuberculosis* H37Rv culture. Considering that *M. tuberculosis* is an intracellular pathogen the effect of the compounds was studied on *M. tuberculosis* H37Rv infected MonoMac6 human monocytes.

This work was supported by Hungarian Research Fund 104275.

39-POS Board 20

**Study of Lipid - Light Harvesting Complex II (LHC II) Model Membranes Using
Nanoscale Infrared Spectroscopy**

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LHCII proteins are the most abundant trans-membrane protein on Earth, responsible for light harvesting in the photosynthetic apparatus of plants. Two types of pigment-protein complexes were analyzed: LHCII extracted from spinach leaves grown in dark condition and LHCII-HL extracted from pre-illuminated spinach leaves. Both proteins were embedded into artificial bi-layer lipid membranes.

LHCII proteins interact to each-other forming characteristic pillars, contrary to LHCII-HL which are phosphorylated, and were randomly scattered.

We applied here nanoscale Infrared (IR) Spectrometer (nanoIR) which uses tunable pulsed IR laser for excitation and AFM tip for detection. When particular wavelength is absorbed, sample is heated-up and expands. Thermal expansion of the sample is 'kicking' AFM cantilever, vibrating at resonant frequency to increase sensitivity of the method. Amplitude of vibrations is proportional to local IR absorption. Tuning the laser one can obtain IR absorption spectrum with spatial resolution down to 10 nm. Alternatively, one can get functional image of the absorption at single wavelength. It is very useful to study inhomogeneous thin samples.

Using nanoIR method we could directly observe and confirm formation of the pillars (increasing efficiency of photosynthesis) in samples containing LHCII and random distribution of LHCII-HL, extracted from the leaves exposed to light. Using phosphorylation of LHCII complexes, nature is adjusting efficiency of photosynthesis to light conditions.

1. Janik E. et al., PLANT CELL, 25, 6, 2155-2170 (2013)
2. Gruszecki W.I. et al. NANOSCALE, 7, 35, 14659-14662 (2015)

42-POS Board 21

Membrane-Active/Fusogenic Activity of the Outer Membrane Vesicles from *Lysobacter Enzymogenes C3*

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Gram negative bacteria produce small ~50-200 nm outer membrane vesicles (OMV) from their outer envelope. OMV have been implicated in activities such as transmission of virulence factors, horizontal gene transfer and development of biofilms. We have found that *Lysobacter enzymogenes C3* produces OMV that may be used to disseminate OMV-membrane-associated antifungal antibiotics produced in a polyketide pathway. We used defined, model liposomal membranes and fluorescent lipid probe assays to investigate the apparent fusogenic activity of these OMV. *Lysobacter* (and *E. coli*) OMV appeared to be essentially spontaneously fusogenic with the bare membranes of liposomes composed of fluid lipid mixtures of several compositions. Support for a fusogenic mechanism of interaction was shown by the ability of the same liposome compositions to transfer encapsulated large fluorescently-labeled dextrans (40 kDa) to an OMV density fraction. Under the same conditions, no apparent fusion of liposomes with whole bacterial cells or other liposomes was observed. Heat treatment of the OMV (70 °C) did not inhibit apparent fusion with liposomes or the antifungal activity, suggesting neither is enzymatically driven. When OMV themselves were fluorescently labeled with carobcyanine lipid probes and then incubated with yeast cells, an apparent transfer of the probes to yeast cell membranes could be seen. We also tested the ability of the OMV to mediate transfer of fluorescent dextrans from liposomes to live *Lysobacter* cells. The presence of OMV greatly enhanced dextran transfer from liposomes to the whole cell fraction, suggesting the possible involvement of fused liposome-OMV products. These results may implicate OMV as a potential interfering factor but also as a potential liposomal fusion target if the goal is to disseminate therapeutics to various secondary targets such as bacterial biofilms, where OMV typically are found and may participate in intercellular transport.

45-POS Board 23

**Light-Harvesting Complexes (LHC) Cluster Spontaneously in Membrane Environment
Leading to Shortening of Their Excited State Lifetimes**

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Photosynthetic organisms evolved the capacity to harvest the energy of solar radiation and store it into chemical compounds. In vascular plants and green algae, sunlight is absorbed by a series of membrane proteins called light-harvesting complexes (LHC). The most abundant of these pigment-protein complexes is LHCII. The LHCII have a dual function: in low light conditions they absorb solar energy and efficiently transfer the excitation energy to the reaction center; in high light they additionally play a role in photoprotection by dissipating the energy absorbed in excess as heat. This last process called Non-Photochemical Quenching (NPQ) leads to a decrease of the excited state lifetime of Chlorophyll a (Chl a), limiting the possibility of Chl triplet formation and thus the production of singlet oxygen. The dual function of LHCII has been extensively studied in detergent micelles, but recent results have indicated that the properties of this complex differ in a lipid environment. In this work, we checked these suggestions by studying LHCII in liposomes. By combining bulk and single molecule measurements, we monitored the fluorescence characteristics of liposomes containing single complexes up to densely packed proteoliposomes. We show that the natural lipid environment per se does alter the properties of LHCII, which for single complexes remain very similar to that in detergent. However, we show that LHCII has the strong tendency to cluster in the membrane and that protein interactions and the extent of crowding modulate the lifetimes of the excited state in the membrane.

48-POS Board 24

Polymer-Supported Lipid Bilayers Derived from Native Cell Membranes as Platforms for Studying Biological Nanoparticle-Cell Interactions

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The ability to produce polymer-supported lipid bilayers (pSLBs) that contain native membrane components which retain transmembrane protein mobility and activity offers a new paradigm in the study of nanoparticle-cell surface interactions. These pSLBs are created using native cell membrane vesicles (NMVs) (i.e., plasma membrane preparations or ghost cells) which allows these biomimetic surfaces to display the complex composition of the plasma membrane in a format amenable to a wide range of surface-sensitive analytical techniques. Characterization of the mobility and functionality of membrane proteins contained within these pSLBs will be discussed. Additionally, compositional and physical characterization of the NMVs will be presented.

The utility of this platform will be presented in the context of two systems: 1) the study of Herpes Simplex Virus (HSV) interactions with the pSLBs derived from monkey kidney (VERO) cells and 2) the study of exosomes isolated from glioma cell cultures and their interactions with pSLBs derived from endothelial cells. The HSV/VERO system was used in investigating the potency of viral-binding inhibitors, while the exosome/endothelial system was used to investigate the role of exosomes in angiogenesis. Total Internal Reflection Microscopy (TIRFM) in combination with single-particle tracking of fluorescently labeled biological nanoparticles provided the ability to investigate single-particle binding kinetics in both systems. These systems illustrate the potential of single-particle tracking on NMV-derived pSLBs for the comprehensive investigation of the interaction characteristics of individual biological nanoparticles with native cell membranes.

51-POS Board 26

Urinary Exosomes Allow for the Identification of Pathogenic Light Chains in Light Chain Amyloidosis Tissues

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Immunoglobulin light chain (AL) amyloidosis is a potential fatal complication of B-cell clonal proliferation. Currently, the best biomarker for treatment monitoring is serum free light chain (FLC) assay but it cannot distinguish monoclonal FLC from polyclonal FLC once it drops below the lower limit of normal for FLC.

Urinary EXs have previously been found to display different characteristics among patients with AL amyloidosis compared to controls. High molecular weight LC oligomers are found only in patients with active AL amyloidosis (1).

We hypothesize that urinary EXs can be used as a biomarker to assess organ response in cases where the patient had reached hematologic complete response (CR) but continue to exhibit organ progression.

Exosomes were extracted and fractionated as previously reported (1). Intact immunoglobulin light chains were identified in patient plasma, EX, and kidney biopsy amyloid deposits using mass spectrometry as the detection method (2).

Oligomeric LCs species were only found in urinary EXs of patient AL-ex11 (progressive renal failure). New patient AL-ex12 urinary EXs do not present oligomeric LC species. AL-ex13 and AL-ex14 were in hematologic and organ CR and their EXs did not present any oligomeric species.

The monoclonal LC in AL-ex11 urinary EXs at the time of hematologic CR was identified as a lambda 6a (IGLV 6-57). The LC found in the urinary exosomes has the same molecular mass and sequence of the protein found in the kidney amyloid biopsy and the cDNA from the plasma cell clone. The urinary EXs enrich the pathogenic protein and allowed for the identification of the pathogenic FLC protein by mass spectrometry and cDNA sequencing.

1. Ramirez-Alvarado M, et al. PloS one. 2012;7(6):e38061.

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54-POS Board 27

PEGylated Chol-Phospholipids-Based Nanoparticles for Encapsulation of Marine Toxins to Cancer Therapy

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Cancer remains still among the most difficult pathologies to surmount. Tumor cells tend to mutate and develop resistance to available drugs. The search for improved cytotoxic agents continues to be adamant for the discovery of novel anticancer therapies. Owing to their potent toxicity, prospecting for novel anticancer agents among marine phyto-derived toxins seems a promising and unexplored path to follow, and even more, if nanoscale vehicles are considered for their targeted delivery towards cancer cells and tissues, to make the most of their interesting features, while reducing in vivo toxicity, limited effectiveness and eventual resistance. In this context, we investigated innovative stealth (including, pH-sensitive) liposomal nanoformulations for marine phycotoxins encapsulation in order to target deliver and control release their well-known potent cytotoxicity. PEGylated liposomes (mean size inferior to 120 nm) composed of Chol-phospholipids were prepared both by thin-film hydration and ethanol injection protocols, coupled with extrusion. Size, polydispersity and surface net-charge were evaluated via dynamic light scattering and Z-potential analysis, respectively. For the non pH-sensitive liposomes, high shelf-stability and conservation of the physicochemical properties along time were obtained, independently of the nanofabrication method. The encapsulation efficiency, releasing profile and serum stability were characterized. To boost the identification of the limits within which these nanosystems can be applied safely, preserving the bioavailability of the loading, in vitro validation is being pursued.

57-POS Board 29

Probing Lipid-Protein Interactions in Situ with Single-Molecule Sensitivity

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Proteins interact with PIP lipids in the plasma membrane (PM). These interactions are ubiquitous in cell signaling pathways and regulate a wide variety of cellular processes. The kinetic and thermodynamic details of these interactions, however, are difficult to probe directly in biological membranes. Here we investigate the behavior of PIP lipids in supported lipid bilayers (SLBs) in response to protein binding with two single-molecule time-resolved fluorescence techniques: pulsed interleaved excitation fluorescence cross-correlation spectroscopy (PIE-FCCS) and single particle tracking (SPT). PIP lipids are introduced to the top leaflet of the supported lipid bilayers using a novel method recently developed in our group. In this way we can make background-free SLBs with a range of PIP concentrations that are suitable for both SPT and PIE-FCCS measurements of lipid-protein interaction at the membrane interface. We probe the interactions of PIP with adsorbed proteins by characterizing changes in PIP mobility caused by protein binding. We then measure the co-diffusion of proteins with PIP lipids as direct evidence of lipid-protein complexation. PIE-FCCS allows us to characterize the size and stability of the lipid-protein complex, and the dependency on charge interactions in different experimental conditions (such as charge density of the lipid head group and solvent conditions). We will report on recent work with model peptides and as well as with the kinase domain of EphA2. Interactions between the cytoplasmic domain of receptor tyrosine kinases and the inner leaflet of the PM have been implicated in receptor autoinhibition. Our assay is an ideal platform to investigate the affinity and specificity of lipid-protein interactions in a uniquely quantitative way.

60-POS Board 30

In Vitro Folding of the Membrane Transporter LeuT in Detergent and Lipid Bilayer Environments

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Little is understood about the process that governs the folding of multi domain α -helical integral membrane proteins from an amino acid sequence to a three dimensional functional structure[1]. The neurotransmitter sodium symporter (NSS) protein family is an example of α -helical transporter proteins. The NSS family encompasses a wide range of prokaryotic and eukaryotic ion-coupled transporters that regulate the transport of neurotransmitter molecules whose dysfunction has been implicated in several neurological and psychiatric disorders [2]. The prokaryotic orthologue of the NSS family LeuT comes from the organism *Aquifex aeolicus*. LeuT is responsible for the uphill transport of Leucine as a part of a symport mechanism driven by a sodium electrochemical gradient.

In Vitro folding of α -helical membrane proteins has been studied mostly within protein detergent complexes or unfolded protein has been refolded into a lipid bilayer [3]. Here we exploit reversible chemical unfolding to determine the unfolding free energy of LeuT both as part of a protein-detergent complex and within a lipid bilayer. In dodecyl maltoside micelles, LeuT reversibly unfolds in Urea, whereas in a lipid bilayer LeuT only reversibly unfolds in the presence of both Urea and the nonionic detergent Octyl- β -Glucoside without physically changing the state of the lipid bilayer. Chemical denaturation causes a significant reduction in the α -helical content and loss of transport activity, full recovery of helical content and substrate transport occurs when the denaturants are removed allowing the free energy to be determined.

This study gives insight into the comparative free energy landscape of protein folding between detergent and lipid environments as well as the effect of protein-lipid interactions on membrane protein function and stability.

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Fine-Tuning of HIV Entry Sites by Recognition of Cellular Receptors in Heterogenous Cell Membranes

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Pathogens have evolved sophisticated strategies for the attachment to target cells to evade host immune responses and to establish infection. Lipid rafts of host cell membranes serve as ubiquitous entry sites; however, it remains largely unknown why pathogens, including HIV, seem to prefer nanoscopic ordered lipid domains over more disordered fluid membrane regions for infection. This study provides surprising answers to the long-standing question about the roles of lipid rafts in HIV entry using giant plasma membrane vesicles (GPMVs), which are phase-separated into large-scale liquid-ordered (Lo) and liquid-disordered (Ld) membrane domains. We show that HIV does not enter cells from within lipid rafts but rather at the boundaries between raft and non-raft regions of the plasma membrane. The HIV receptor CD4 is substantially sequestered into the Lo phase while the coreceptor CCR5 localizes preferentially in Lo/Ld interfaces on GPMVs, suggesting that HIV particles initially bind to lipid rafts and then move to their boundaries, which seem to be prone to HIV entry by constituting energetically favorable spots for cell entry by membrane fusion. Lo/Ld phase coexistence is not required during the HIV attachment stage, but recognition of a membrane phase boundary is a prerequisite for successful HIV membrane fusion. We propose that the coalescence of lipid rafts plays a key role in many vital cellular processes including adaptive immune responses to combat infectious pathogens and that HIV preferentially hijacks membrane phase boundaries to gain entry into the cells.