BP 18: Membranes and vesicles I (joint BP/CPP)

Time: Tuesday 9:30–12:30 Location: H 1028

Invited Talk BP 18.1 Tue 9:30 H 1028

Multifaceted BAR-domain proteins to shape cell membranes — Coline Prévost[1], Mijo Simunovic[1,2], Henri-François Renard[1], Emma Evergren[3], Harvey McMahon[3], Ludger Johannes[1], Jacques Prost[1], Andrew Callan-Jones[4], and •Patricia Bassereau[1] — [1]Institut Curie, Paris, France — [2]University of Chicago, USA — [3]MRC, Cambridge, UK — [4]University Paris-Diderot, France

Cell plasma membranes are highly deformable and are strongly curved upon membrane trafficking or during cell motility. BAR-domain proteins with their intrinsically curved shape and their interaction with the actin cytoskeleton are involved in many of these processes.We have used in vitro experiments to study the interaction of BAR-domain proteins with curved membranes for understanding how inverted-BAR domain proteins such as IRSp53 are involved in the generation of filopodia and how the BAR-domain protein endophilin A2 can scission tubules induced by Shiga toxin internalization. We have pulled membrane nanotubes of controlled curvature from Giant Unilamellar Vesicles (GUVs) using optical tweezers and micropipette aspiration. With this approach coupled to theoretical modeling, we have evidenced for IRSp53 a protein phase separation along the nanotube occurring at low protein density for weakly curved membranes. It can explain the in vivo local clustering of the protein, a primary step in filopodia generation that precedes the recruitment of other partners. We have also shown that endophilin A2 scaffolds and stabilizes tubes in static conditions but induces scission when the tube is dynamically extended.

BP 18.2 Tue 10:00 H 1028

Measuring the composition-curvature coupling in binary lipid membranes by computer simulations — •Israel Abraham Barragán Vidal and Marcus Müller — Institut für Theoretische Physik, Georg-August-Universität, Friedrich-Hund-Platz 1, 37077 Göttingen, Germany

This manuscript contributes to the field of biophysics, in particular, the formation of local composition inhomogeneities in model membranes (rafts). We present a simple phenomenological model to describe the effective coupling between curvature and composition in a two-component lipid bilayer. Beside the elastic contribution to the free energy and an intrinsic coupling between curvature and composition, our model also includes contributions from a composition- and curvature-dependent free energy of mixing.

Using an implicit-solvent model we extract the intrinsic composition-curvature coupling from computer simulations with planar and highly curved cylindrical bilayers. Beside the effective curvature-composition coupling, our computational strategy offers an alternative to obtain the spontaneous curvature from moments of the stress profile across a bilayer membrane. We expect this strategy will find further applications.

BP 18.3 Tue 10:15 H 1028

New Strategy to Study a Single SNARE Mediated Membrane Fusion Event — •Jose Nabor Vargas[1], Kewin Howan[2], Andrea Gohlke[2,4], Ralf Seemann[1,3], Jean-Baptiste Fleury[1], and Frederic Pincet[2,4] — [1]Experimental Physics, Saarland University, Saarbrücken, Germany — [2]Laboratoire de Physique Statistique, Ecole Normale Supérieure, 75005 Paris, France — [3]Max Planck Institute for Dynamics and Self-Organization, Göttingen, Germany — [4]Department of Cell Biology, School of Medicine, Yale University, CT 06520 New Haven, USA

We present an approach to explore the properties of a single SNARE mediated membrane fusion event in a microfluidic chip. In a first step, a single free standing lipid membrane is generated at a defined position with the Droplet Interface Bilayer technique (DiB). In a second step, we inject a solution of divalent cations (Calcium, Ca2+) and small unilamellar vesicles functionalized with T-SNARE proteins (T-SUVs) around the planar membrane using a volume controlled flow. The presence of calcium mediates the direct fusion of the vesicles with the planar membrane, which is incorporating the proteins into the membrane. In a third step, we remove the calcium and the T-SUVs with a buffer solution. After this washing step, a solution of small unilamellar vesicles functionalized with V-SNARE proteins (V-SUVs) is injected around the planar membrane. And finally, we study single fusion event with good optical and electrical access.

BP 18.4 Tue 10:30 H 1028

Mechanics of the cell membrane coupled to the actomyosin cortex — •Jochen A. M. Schneider and Guillaume Salbreux — Max Planck Institute for the Physics of Complex Systems, Dresden, Germany

The cell membrane is the outer layer of a biological cell. It consists of lipids which form a two-dimensional fluid bilayer structure and is attached via linker proteins to the underlying actomyosin cortex, a thin network of actin filaments and myosin motors. In the past years, research has mainly focused on the physical description of the cell membrane and cytoskeleton independently. However, little is known on how they mechanically interact in the cell.

Here, we present a model for the interaction of membrane and cytoskeleton based on the assumption that the anchored membrane is attached to the underlying actomyosin cortex, subjected to active tension arising from myosin activity. Cell pressure results in membrane protrusions which can equilibrate their surface tensions by exchange of lipids. Using this physical description, we characterize how excess membrane area distributes around the cell. Based on a few fundamental cell parameters, the cortex tension, the membrane bending stiffness and the anchoring strength, we find a phase diagram with regions corresponding to a homogeneous distribution of membrane, to the pulling of membrane tubes and to the formation of one or several blebs. We finally use this result to discuss potential consequences for the mechanics of the cell.

BP 18.5 Tue 10:45 H 1028

Using Markov state models to obtain free energies of (de)mixing — •Djurre H. de Jong and Andreas Heuer — University of Münster, Münster, Germany

Obtaining free energies of demixing in multicomponent systems, for example lipid bilayers, would greatly benefit many (theoretical) studies. For such systems the initial state, i.e. the mixed configuration, is often thermodynamically highly unstable. This can render standard techniques like umbrella sampling problematic.

We show that application of Markov state models to several short and independent simulations allows one to extract the free energy gain upon demixing very reliably. Here it is important that the temporal evolution of an appropriately defined order parameter displays local fluctuations. Specifically, this method is applied to a two component Ising model and a binary Lennard-Jones system.

15 min break

BP 18.6 Tue 11:15 H 1028

The Mechanism of Phagocytosis: Two Stages of Engulfment — •David M. Richards and Robert G. Endres — Imperial College London, UK

Despite being of vital importance to the immune system, the mechanism by which cells engulf relatively large solid particles during phagocytosis is still poorly understood. From movies of neutrophil phagocytosis of polystyrene beads, we measure the fractional engulfment as a function of time and demonstrate that phagocytosis occurs in two distinct stages. During the first stage, engulfment is relatively slow and progressively slows down as phagocytosis proceeds. However, at approximately half-engulfment, the rate of engulfment increases dramatically, with complete engulfment attained soon afterwards. By studying simple mathematical models of phagocytosis, we suggest that the first stage is due to a passive mechanism, determined by receptor diffusion and capture, whereas the second stage is more actively controlled, perhaps with receptors being driven towards the site of engulfment. We then consider a more advanced model that includes signalling and captures both stages of engulfment. This model predicts that there is an optimum ligand density for quick engulfment. Further, we show how this model explains why non-spherical particles engulf quickest when presented tip-first.

BP 18.7 Tue 11:30 H 1028

No spatial spreading of chemotactic signaling in amoeboid cells upon receptor stimulation — •Matthias Gerhardt,

Michael Walz, and Carsten Beta — Institut für Physik und Astronomie, Karl-Liebknecht-Strasse 24/25, 14476 Potsdam, Germany

Recently we have shown that in chemotactic Dictyostelium discoideum cells stimulation of a confined membrane region with cAMP leads to confined signaling of PIP3, PTEN, and filamentous actin. A consequence of this observation is that cAMP stimuli cannot trigger spatial spreading of intracellular signaling. However, in the absence of an extracellular cAMP stimulus, components of the signal transduction system where observed to form traveling waves that show all hallmarks of an excitable system. This excitable system is characterized by PIP3-rich membrane regions circumscribed by actin segments propagating together as a composite wave across the substrate attached membrane of a Dictyostelium cell. Since cAMP stimulation causes depletion of such waves, we concluded there must be an intracellular switch, which determines whether the signal transduction is excitable or not. Since earlier observations show that a [betagamma]G knockout remarkably enhances PI3K activity, we conjecture that the PI3K is a suitable candidate to take on the role of an intracellular switch which controls excitability.

BP 18.8 Tue 11:45 H 1028

Recognition Force Spectroscopy on Lamellar Body Surfactants collected from Primary Alveolar Cells Type II — •Patrick Paul[1], Nina Hobi[2,3], Susanne Rappl[1], Thomas Haller[3], Manfred Frick[2], and Kay E. Gottschalk[1] — [1]Institute of Experimental Physics, Ulm University, Ulm, Germany — [2]Institute of General Physiology, Ulm University, Ulm, Germany — [3]Department of Physiology and Medical Physics, Division of Respiratory Cell Physiology, Medical University of Innsbruck, Innsbruck, Austria

Type II pneumocytes produce and secrete pulmonary surfactant into the alveoli of the lung. Surfactants lower the surface tension between the air-liquid interface within the alveoli. Surfactant consists of multilayers of lipids, mainly phosphatidylcholine, and specific, embedded surfactant proteins (SP-B and SP-C). Physiological studies demonstrated that these proteins play a major role in the stability of the surfactant [1]. However, the precise nature and exact structure of how these proteins are arranged within the lipids is yet unknown.

Hence, we imaged the structure of SP-B and SP-C assembly within a single-lipidlayer surfactant with single molecule force spectroscopy.

Reference: [1] Jesús Pérez-Gil, Structure of pulmonary surfactant membranes and films: The role of proteins and lipid-protein interactions, 2008, Biochimica et Biophysica Acta 1778, 1676-1695

BP 18.9 Tue 12:00 H 1028

Local viscosities near plasma membranes of living cells — •Felix Jünger and Alexander Rohrbach — Lab for Bio- and Nano-Photonics, University of Freiburg, Georges-Koehler-Allee 102, 79110 Freiburg, Germany

The molecular processes of particle binding and endocytosis are influenced by the locally changing mobility of the particle nearby the plasma membrane of a living cell. Close to different cellular interfaces, the viscous drag γ changes strongly with the distance to the interface. In our work we use photonic force microscopy (PFM) to investigate how γ changes when an optically trapped 1μm polystyrene bead approaches the plasma membrane of different biological cells. The bead's temporal fluctuations are tracked interferometrically in three dimensions with nanometer precision and on a microsecond time scale. The autocorrelation of the bead's motion reveals the friction coefficient γ(d) as a function of bead-membrane distance d. We find a simple exponential decay for γ(d) with a hydrodynamic decay length Λ(d) that fits well to the obtained experimental data. We investigated different cell types (J774, HT29, MDCK) and a giant unilamellar vesicle (GUV). We find that all values Λ(d) measured at biological membranes are significantly longer than those of a rigid glass coverslip, giving rise to the conclusion that the deformable shape of the membrane influences the hydrodynamic interaction.

BP 18.10 Tue 12:15 H 1028

Fluorescence Imaging of Light Induced Reactive Oxygen Species (ROS) in Plant Cell Tissue — •Franz-Josef Schmitt[1], Vladimir Kreslavski[2], Galina N. Schirshikova[2], Csongor Keuer[1], Sergei K. Zharmukhamedov[2], Suleyman I. Allakhverdiev[2], and Thomas Friedrich[1] — [1]Institute of Chemistry, Bioenergetics, TU Berlin, Berlin, Germany — [2]Institute of Basic Biological Problems, RAS, Pushchino, Moscow Region, Russia

UV-radiation in combination with toxic compounds like polyaromatic hydrocarbons (PAHs) lead to generation and accumulation of reactive oxygen species (ROS) in animal and plant cells. ROS generation by naphthalene (Naph), a lipophilic PAH, was studied with fluorescence microscopy employing the ROS sensitive dye dichlorofluorescein (DCF). Under high light illumination, Naph-treated leaves of Arabidopsis thaliana showed the spread of ROS waves across the tissue with a period time of 20 min. The reduction of PSII activity at the presence of Naph was accompanied by transient generation of hydrogen peroxide as well as swelling of thylakoids and distortion of cell plasma membranes. It could be shown that Naph treated leaves of Arabidopsis thaliana show enhanced DCF fluorescence in the thylakoid membrane. The comparison of short term and long term exposure to different PAHs revealed that at short term exposure, the PAHs with high water solubility lead to the strongest reduction of PS II activity while after long term exposure the effect of PAHs with low water solubility is stronger.