BP 4: Membranes and Vesicles I

Time: Monday 15:00–17:00 Location: HÜL 386

Invited Talk BP 4.1 Mon 15:00 HÜL 386

Role of membrane elasticity in clathrin-mediated endocytosis — Sandrine Morlot[1], Saleem Mohammed[1], Nicolas Chiaruttini[1], Valentina Galli[1], Marius Klein[3], Luìs Dinis[4], Martin Lenz[5], Giovanni Cappello[3], and •Aurélien Roux[1,2] — [1]Biochemistry Department, University of Geneva, CH-1211 Geneva, Switzerland — [2]Swiss National Centre for Competence in Research Programme Chemical Biology, CH-1211 Geneva, Switzerland — [3]Institut Curie, Centre de Recherche; CNRS, UMR 168, Physico-Chimie Curie; Université Pierre et Marie Curie, F-75248 Paris, France — [4]Departamento de Fìsica Atòmica, Molecular y Nuclear, Facultad de Ciencas Fìsicas, Universidad Complutense de Madrid, ES-28040, Madrid, Spain — [5]James Franck Institute, University of Chicago, IL-60637 Chicago, U.S.A.

In Clathrin-mediated endocytosis, Clathrin assembles into a soccerball-like structure at the plasma membrane that was proposed to deform the membrane by scaffolding. However, controversies in the community have appeared on the exact role of Clathrin: does its polymerization force is sufficient to curve the membrane, or deformation by other means (protein insertion) is required? We studied the formation of Clathrin buds from Giant Unilamellar Vesicles, and found that the pits can be flattened when membrane tension is increased. This suggested that the Clathrin polymerization force could be counteracted by membrane tension, which we further proved by directly measuring Clathrin polymerization force: by pulling a membrane tube out of a GUV aspirated in a micropipette, we can measure the force required to hold the tube through an optical tweezer system. When Clathrin is added, it polymerizes onto the GUV predominantly, and the force drops. From these measurements, we can deduce that the polymerization strength of Clathrin is in the range of a few hundred micronewtons per meter. This value confirms that clathrin polymerization can be counteracted efficiently by membrane tension. To finalize endocytosis, the clathrin-bud needs to be separated from the plasma membrane. Membrane fission requires the constriction and breakage of a transient neck, splitting one membrane compartment into two. The GTPase Dynamin forms a helical coat that constricts membrane necks of Clathrin-coated pits to promote their fission. Dynamin constriction is necessary but not sufficient, questioning the minimal requirements for fission. Here we show that fission occurs at the edge of the Dynamin coat, where it is connected to the uncoated membrane. At this location, the specific shape of the membrane increases locally its elastic energy, facilitating fission by reducing its energy barrier. We predict that fission kinetics should depend on tension, bending rigidity and the Dynamin constriction torque. We verify that fission times depend on membrane tension in controlled conditions in vitro and in Clathrin-mediated endocytosis in vivo. By numerically estimating the energy barrier from the increased elastic energy, and measuring the Dynamin torque, we show that: 1- Dynamin torque, about 1nN.nm, is huge but necessary to achieve constriction, and 2- Dynamin work sufficiently reduces the energy barrier to promote spontaneous fission.

BP 4.2 Mon 15:30 HÜL 386

Measuring local viscosities near membranes of living cells with photonic force microscopy — •Felix Jünger and Alexander Rohrbach — Lab for Bio- and Nano-Photonics, University of Freiburg, Georges-Koehler-Allee 102, 79110 Freiburg, Germany

The diffusive motion of a particle in the vicinity of a boundary surface is relevant from a biological point of view, since the viscous drag γ changes significantly with the distance to the interface, e.g. a cell membrane. 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 4.3 Mon 15:45 HÜL 386

Artificial DNA Membrane Nanopores — •Kerstin Göpfrich[1], Jonathan Burns[2], Vivek Thacker[1], Thomas Zettl[1], Silvia Hernandez-Ainsa[1], Eugen Stulz[3], Stefan Howorka[2], and Ulrich Keyser[1] — [1]Cavendish Laboratory, University of Cambridge, UK — [2]Department of Chemistry, University College London, UK — [3]Department of Chemistry, University of Southampton, UK

Membrane nanopores are essential components of biological and artificial cells. Our group has shown that we can create artificial nanopores using DNA origami self-assembly (N.A.W. Bell, *Nanoletters*, 2012; S.M. Hernandez-Ainsa, *ACS nano*, 2013) and anchor them in lipid membranes (J. Burns, K. Göpfrich, *Angewandte Chemie*, 2013).
Insertion of negatively charged DNA pores into a hydrophobic membrane is achieved by attaching functional hydrophobic groups in strategic positions on the DNA nanopores. Pore formation in lipid vesicles is studied for different nanopore designs and hydrophobic modifications via fluorescent imaging (V.V. Thacker, *Applied physics letters*, 2012). Single-channel current recordings of our artificial DNA nanopores are performed using a high-throughput lipid nanobilayer system that has recently been introduced by our group (K. Göpfrich, *Langmuir*, 2013; J.L. Gornall, *Nano letters*, 2011). Pore architecture and functionality of our DNA nanopores can be easily adapted, opening the pathway to design novel membrane channels.

BP 4.4 Mon 16:00 HÜL 386

Nanometer-resolved radio-frequency absorption and heating in bio-membranes — •Stephan Gekle[1] and Roland Netz[2] — [1]Physikalisches Institut, Universität Bayreuth — [2]Fachbereich Physik, Freie Universität Berlin

Radio-frequency (RF) electromagnetic fields are readily absorbed in biological matter and lead to dielectric heating. To understand how RF radiation possibly influences biological function, a quantitative description of dielectric absorption and heating at nanometer resolution beyond the usual effective medium approach is crucial.

We report an exemplary multi-scale theoretical study for biomembranes that combines i) atomistic simulations for the spatially resolved absorption spectrum at a single planar DPPC lipid bilayer immersed in water, ii) calculation of the electric field distribution in planar and spherical cell models, and iii) prediction of the nanometer resolved temperature profiles under steady RF radiation.

For a spherical cell model, we find a strongly enhanced RF absorption on an equatorial ring, which gives rise to temperature gradients inside a single cell under radiation.

BP 4.5 Mon 16:15 HÜL 386

Induced phagocytic particle uptake into a giant unilamellar vesicle using optical tweezers — Andreas Meinel, Benjamin Tränkle, and •Alexander Rohrbach — University of Freiburg, Georges-Köhler-Allee 102, 79110 Freiburg

Phagocytosis, the uptake and ingestion of solid particles into living cells, is a central mechanism of our immune system. Due to the complexity of the uptake mechanism, the different forces involved in this process are only partly understood. Therefore the usage of a Giant Unilamellar Vesicle (GUV) as the simplest biomimetic model for a cell allows to investigate the influence of the lipid membrane on the energetics of the uptake process. Here, we use a photonic force microscope (PFM) to approach an optically trapped 1 um latex bead to a immobilized GUV to finally insert the particle into the GUV. By analysing the mean displacement and the position fluctuations of the trapped particle during the uptake process in 3D with nanometre precision, we are able to record force and energy profiles, as well as changes in the viscous drag and the stiffness. The measured energy profiles, which are compared to a Helfrich energy model for local and global deformation, show a good coincidence with the theoretical results.

BP 4.6 Mon 16:30 HÜL 386

Cooperative wrapping of nanoparticles by membrane tubes — •Michael Raatz, Thomas R. Weikl, and Reinhard Lipowsky — Max Planck Institute of Colloids and Interfaces, Department of Theory and Bio-Systems, Potsdam, Germany.

The bioactivity of nanoparticles crucially depends on their ability to cross biomembranes. Recent simulations indicate the cooperative wrapping and internalization of spherical nanoparticles in tubular membrane structures. We systematically investigated the energy gain of this cooperative wrapping by minimizing the energies of the rotationally symmetric shapes of the membrane tubes and of membrane segments wrapping single particles. We found that the energy gain for the cooperative wrapping of nanoparticles in membrane tubes relative to their individual wrapping as single particles strongly depends on the ratio ρ/R of the particle radius R and the range ρ of the particle-membrane adhesion potential. For a potential range of the order of one nanometer, the cooperative wrapping in tubes is highly favorable for particles with a radius of tens of nanometers and intermediate adhesion energies, but not for particles that are significantly larger.

BP 4.7 Mon 16:45 HÜL 386

On the dynamic properties of giant unilamellar vesicles under flow - towards a model for shear force transduction in cells — •Bernhard Sebastian, Tobias Favero, and Petra Dittrich — ETH Zürich, Schweiz

We present a novel method for the study of external shear force transduction through vesicle membranes and their effect on the dynamics of the enclosed lumen in 3D using defocusing fluorescence microscopy.

Blood cells and endothelial cells are frequently exposed to mechanical strain induced by external flow, hence shear stress. External forces are transferred to the intracellular lumen through a process called mechanotransduction. We used giant unilamellar vesicles (GUVs) as a model system to investigate the effects of shear force induced mechanotransduction on the dynamics of the vesicle membrane as well as inside the lumen. Vesicles were trapped on a microfluidic chip. Fluorescent beads enclosed inside GUVs were used as flow tracer particles. A novel 3D tracking program allowed for visualization of the bead movement and its analysis in three dimensions at high spatial resolution using conventional fluorescence wide-field microscopy.

We observed bead movement along the GUV membrane in a bihemispheric pattern including rare crossing events between the hemispheres, independent of external flow speed. Detailed analysis of the bead trajectories revealed regions of high and low velocity that spatially depend on the magnitude of external flow. Flow in the GUV membrane was found to differ from that inside the lumen.