BP 1: Membranes, Vesicles, Synthetic Cells

Time: Monday 9:30–13:00 Location: BAR Schö

BP 1.1 Mon 9:30 BAR Schö

Bottom-up assembly of a synthetic glycocalyx on lipid vesicles — •Kevin Jahnke and David A. Weitz — Harvard University, Cambridge, MA, USA

The glycocalyx serves as physicochemical barrier that increases cellular rigidity and as interface for chemical cues to guide cell-cell communication. However, while preliminary results highlight the importance of the glycocalyx, the biophysical functioning remained elusive and mostly untested due to the complexity within natural cells. Recent advances in the membrane functionalization of giant unilamellar vesicles (GUVs) with macromolecules like DNA and proteins (Jahnke et al., ACS Nano 2022; Jahnke et al. Nat. Commun. 2021) pave the way for a systematic investigation of glycocalyx properties within a fully-controlled environment. Here, we engineer biomimetic glycocalyces to understand their effect on the biophysical properties of GUVs. The synthetic glycocalyx consists of polysaccharides functionalized with cholesterol that self-assemble in the lipid membrane. We employ fluorescence recovery after photobleaching and micropipette aspiration to assess the changes in diffusion and membrane rigidity of glycocalyx-decorated GUVs. The control over the type of polysaccharide, its molecular weight and density on the vesicle enable us to design and study a variety of synthetic glycocalyces. Additionally, we compare them to other common vesicle functionalizations like polyethyleneglycol and explore their potential for carbohydrate-specific adhesion. This work underpins bottom-up glycocalyx engineering as important tool for cellular biophysics and biotechnological applications.

BP 1.2 Mon 9:45 BAR Schö

Surface-induced phase separation of reconstituted nascent integrin clusters — •Chiao-Peng Hsu[1], Jonas Aretz[2], Reinhard Fässler[2], and Andreas Bausch[1] — [1]Center for Functional Protein Assemblies and Lehrstuhl für Zellbiophysik (E27), Physics Department, Technische Universität München, Garching, Germany — [2]Max Planck Institute of Biochemistry, Martinsried, Germany

Integrin adhesion complexes are essential membrane-associated cellular compartments for multi-cellular life. While biomolecular condensates organize specific functions in cells, cell membranes can regulate the positions and dynamics of many biomolecular condensates. Yet, the role played by membrane surfaces in the formation of initial integrin adhesion complexes still needs to be fully understood. Here, we report that phosphoinositides containing lipid membranes induce minimal integrin adhesion condensates composed of integrin β tails, kindlin, talin, paxillin, and FAK at physiological ionic strengths and protein concentrations. We show that the presence of phosphoinositides is key to enriching kindlin and talin on the membrane, forming first nascent integrin complexes, which in turn are necessary to further nucleate condensates. These results demonstrate that the biophysical properties of lipid membranes are key for inducing specific membrane-associated condensates throughout the cell.

BP 1.3 Mon 10:00 BAR Schö

Small-Angle and Inelastic Neutron Scattering from Polydisperse Oligolamellar Vesicles Containing Glycolipids — •Lukas Bange[1], Ingo Hoffmann[2], and Emanuel Schneck[1] — [1]Institute for Condensed Matter Physics, Technical University Darmstadt, Germany — [2]nstitut Laue-Langevin, Grenoble, France

Glycolipids are known to stabilize biomembrane multilayers through preferential sugar-sugar interactions that act as weak transient membrane crosslinkers [1, 2]. We use small-angle and inelastic neutron scattering on oligolamellar phospholipid vesicles containing defined glycolipid fractions in order to elucidate the influence of glycolipids on membrane mechanics and dynamics. Small-angle neutron scattering (SANS) reveals that the oligolamellar vesicles (OLVs) obtained by extrusion are polydisperse with regard to the number of lamellae, n, which renders the interpretation of the inelastic neutron spin echo (NSE) data [3] non-trivial. To overcome this problem, we propose a method to model the NSE data in a rigorous fashion based on the obtained histograms of n and on their q-dependent intensity-weighted contribution. This procedure yields meaningful values for the bending rigidity of individual lipid membranes and insights into the mechanical coupling between adjacent membrane lamellae, including the effect of the glycolipids.

References [1] Latza, Demé, Schneck, Biophys J., 2020, Volume 118, 7, P1602-1611 [2] Kav et al, Front. Mol. Biosci., 2021, 8, 754645 [3] Hoffmann, Hoffmann, Farago, Prevost, Gradzielski, J. Chem. Phys., 2018, 148, 104901

BP 1.4 Mon 10:15 BAR Schö

Controlling phase separations and reaction kinetics in microfluidically trapped droplets — •Sebastian W. Krauss, Paula Girones Paya, and Matthias Weiss — Experimental Physics I, University of Bayreuth, Germany

Droplet-based microfluidics is an efficient and versatile tool to study biomimetic reactions and self-organization in selected geometries and small volumes. It is also frequently used to perform high-throughput experiments, e.g. as selection platforms for directed evolution or for personalised medicine. While elaborate techniques are available for the production of picoliter-sized droplets, there is an increasing demand for subsequent manipulation and control of the droplet interior after production. Here we report on a straightforward method to rapidly and reversibly adjust the size of single to several hundred double-emulsion droplets in a microfluidic sieve by varying the osmotic pressure, leading to a change in concentration of enclosed molecules. We show that this approach allows for driving reversible demixing transitions of a biomimetic binary fluid which can be used to control the kinetics of enclosed enzymatic reactions. We also show that changing droplet sizes can be exploited for a reversible denaturing of double-stranded DNA, which may eventually allow for an osmotically driven PCR in small droplets.

BP 1.5 Mon 10:30 BAR Schö

Predicting membrane turnover during cytokinesis — •Felix Frey[1] and Timon Idema[2] — [1]Institute of Science and Technology Austria, Klosterneuburg, Austria — [2]Department of Bionanoscience, Kavli Institute of Nanoscience, Delft University of Technology, Delft, The Netherlands

When animal cells divide, they split into two equal parts. Since the volume of the cell is typically conserved during cell division, the projected area of the cell membrane has to increase to allow for the change of shape. The membrane area is controlled by exocytosis, resulting in an increase in membrane area and its counterpart endocytosis, resulting in a decrease in membrane area. However, it is unclear how exo- and endocytosis need to adapt to enable successful division. To address this question, we developed a kinetic model in which membrane gain and loss depend on membrane curvature and tension [1]. We apply this model to a series of calculated vesicle shapes, which we take as a proxy for the shape of dividing cells. We find that the ratio of membrane gain and loss changes non-monotonically during cytokinesis due to the complex interplay between membrane area and shape. Our results suggest that controlling membrane turnover is critical for the successful division of both biological and artificial cells. [1] Felix Frey and Timon Idema, Phys. Rev. E 106, 024401 (2022).

BP 1.6 Mon 10:45 BAR Schö

Antimicrobial peptides: Revealing the Penetration Mechanism of Melittin in the Outer Membrane of Gram-Negative Bacteria — •Justus C. Stephani[1], Luca Gerhards[1], Ilia A. Solov'yov[1], and Izabella Brand[2] — [1]Dept. of Physics, Carl von Ossietzky Universität, Germany — [2]Dept. of Chemistry, Carl von Ossietzky Universität Oldenburg, Germany

Studying the interaction between antimicrobial peptides (AMPs) and bacterial membranes might aid to find new treatments against bacterial pathogens and even drug-resistant bacteria. The AMP melittin can target the complex structure of a cell membrane leading to membrane permeabilization via hole formation or disruption. We report on the results of electrochemical experiments, aided by modern, all-atom molecular dynamics simulations that reveal the role of lipopolysaccharides (LPS) in the outer membrane of gram-negative bacteria in melittin binding and penetration. We demonstrate that certain amino acid residues play a key role in the binding of melittin to the membrane and thereby stabilizing and preserving the confirmation of the peptide. With a combined method of polarization modulation infrared reflection-absorption spectroscopy (PM IRRAS) and the statistical analysis of C=O bond orientation in the peptide, we determine

the orientation of melittin on the membrane and observed penetration of the N-Terminus of the peptide into the membrane and a formation of hydrogen bonds between the N-Terminus and carboxylate and phosphate of the LPS.

15 min. break

Invited Talk BP 1.7 Mon 11:15 BAR Schö

Cell-free expression of membrane proteins and control of their spatial organization in synthetic lipid membranes — •Jan Steinkühler — Northwestern University, Evanston, USA — Georg-August-Universität Göttingen, Germany

Cell-free expression (CFE) is a powerful tool for synthesizing proteins outside of living cells, including membrane proteins. In this talk, we will discuss the factors that affect the yield of synthesized membrane proteins in CFE systems, including ribosome stalling and the balance between peptide-membrane association and peptide aggregation rates. We will also present a quantitative kinetic model that can be used to rationalize the engineering of protein N-terminal domain sequences and membrane composition for improved membrane protein synthesis. In addition to covering the synthesis of natural membrane proteins, we will show how CFE of de novo membrane protein designs can be used to study the role of membrane-protein hydrophobic mismatch in protein integration and organization in synthetic lipid membranes. [DOI:10.1101/2022.06.01.494374]. Our findings provide insight into protein organization in biological membranes and a framework for building up of synthetic cell membranes with new functions.

BP 1.8 Mon 11:45 BAR Schö

Entry of Microparticles into Giant Lipid Vesicles Induced by Optical Force — •Fessler Florent, Sharma Vaibhav, Muller Pierre, Thalmann Fabrice, Marques Carlos, and Stocco Antonio — Institut Charles Sadron, Strasbourg, France

Interactions between micro- or nano-sized objects and lipid membranes are crucial in many processes such as entry of viruses in host cells, microplastics pollution, drug delivery or biomedical imaging. Here, we investigated the physical principles of particle crossing of lipid membranes using microparticles and giant unilamellar vesicles (GUVs) in the absence of strong irreversible binding and for low membrane tensions. In these conditions, we observed that organic as well as inorganic particles can always penetrate inside GUVs provided that an external picoNewton force is applied. In the limit of a vanishing particle-membrane affinity, we pointed out the role of membrane area reservoirs and showed that a force barrier minimum exists when the particle size is comparable to the bendocapillary length.

BP 1.9 Mon 12:00 BAR Schö

Simulating transient pores in hemifused membranes — •Russell Spencer and Marcus Müller — Georg-August Universität Göttingen, Institute for Theoretical Physics, 37077 Göttingen, Germany

Transportation of material from one side of a hemifused membrane to another can be facilitated through the formation of transient pores, which open, allow the material to pass and then close. One important example of this occurs in the 'kiss and run' (KAR) mechanism for transporting material from inside a vesicle in a cell to outside of the cell. The vesicle first fuses with the membrane, forming a hemifusion diaphragm (HD). A pore then opens in the HD allowing the material out, then the pore closes and the vesicle detaches. This process occurs in the release of neurotransmitters from a synapse into the post-synaptic cleft. This work uses self-consistent field theory and the string method to calculate the minimum free energy path for membrane fusion and the opening and closing of the transient pore. We also study the effects of proteins, which facilitate the process by altering the thermodynamics of pore formation.

BP 1.10 Mon 12:15 BAR Schö

Lipid movement in monolayers at the air/water interface: hydrodynamic coupling to the subphase — Florian Gellert, Heiko Ahrens, and •Christiane A. Helm — Institute of Physics, University of Greifswald, Germany

Domain nucleation and growth in the liquid expanded/liquid condensed (LE/LC) phase transition in erucic acid monolayers at the air/water interface is studied. Dendritic domains are observed at high compression speeds and seaweed domains at low compression speeds. The different domain types are distinguished by fractal dimension, tip width, and the spacing of the side arms. A local, normalized supersaturation describes the hydrodynamic coupling of lipids in the LE phase moving towards the domain border to subphase movement. The coupling differs for the growth regimes. Additionally, the shape and symmetry of the domains are affected by barrier movement. The downstream side of the domains grows faster than the upstream side, as shown by directionality diagrams and FFTs. We suggest that the flow direction disturbs the diffusion direction of the lipids within the LE phase but not the coupling to the subphase.

BP 1.11 Mon 12:30 BAR Schö

X-ray studies of bidirectional switching in phospholipid membranes containing photoswitchable glycolipids — •Svenja C. Hövelmann[1,2,3], Jonas E. Warias[1], Rajendra P. Giri[1], Jule Kuhn[1], Karin Hansen[1], Lukas Petersdorf[1], Nicolas Hayen[1], Philipp Jordt[1], Andrea Sartori[1], Chen Shen[3], Franziska Reise[4], Olaf M. Magnussen[1], Thisbe K. Lindhorst[4], and Bridget M. Murphy[1,3] — [1]Institute of Experimental and Applied Physics, Kiel, Germany — [2]Deutsches Elektronen-Synchrotron DESY, Hamburg, Germany — [3]Ruprecht Haensel Laboratory, Kiel, Germany — [4]Otto Diels Institute of Organic Chemistry, Kiel, Germany

Lipid molecules not only play an essential role in the structure and geometry in biomembranes but also in the functionality and self-assembly of membrane proteins and channels. Their dynamic is under intense investigation owing to their applications in biosensor engineering and drug delivery. To understand the interaction between lipid and functional molecules, we investigate photoswitchable glycoconjugates embedded in a 1,2-dipalmitoyl-phosphatidylcholine (DPPC) model systems in the form of Langmuir films and vesicles. The glycoconjugates change reversibly between their trans- and cis-conformation by illumination with visible and UV light inducing a reversible change in the surrounding molecular arrangement. These structural changes, their evolution and time scales are characterised with multiple measurement techniques including X-ray scattering. Studies performed on mixed monolayers and vesicles with varying glycoconjugates identify bidirectional switching in the in DPPC monolayers.

BP 1.12 Mon 12:45 BAR Schö

Structure/Friction relationship in solid supported phospholipid layers — •Swen Helstroffer, Pierre Muller, and Thierry Charitat — Institute Charles Sadron, Strasbourg, France

Stacks of phospholipid bilayers adsorbed on biological rubbing surfaces lubricate remarkably well under severe conditions. However, the energy dissipation pathway allowing ultra low friction is still unknown. To elucidate the mechanism, we propose here to study an experimental model consisting of hydrated phospholipid layers deposited at the air/solid interface. Our system presents a well-controlled geometry in which we can fine-tune the hydration level of the lipid heads. By combining neutron reflectometry and ellipsometry studies with macroscopic tribology experiments, we demonstrated a negative correlation between hydration and friction coefficient. Using the Eyring model, we obtained microscopic activation volumes characteristic of slip. Our results suggest that the hydration level is a key parameter for lubrication. We believe that the sliding plane is located in the confined water layers.