Tethered Membranes: Fundamentals and Applications (TETHMEM)

752. WE-Heraeus-Seminar

29 Aug - 01 Sep 2021
hybrid at the Physikzentrum Bad Honnef

WILHELM UND ELSE
HERAEUS-STIFTUNG

Introduction

The Wilhelm und Else Heraeus-Stiftung is a private foundation that supports research and education in science with an emphasis on physics. It is recognized as Germany's most important private institution funding physics. Some of the activities of the foundation are carried out in close cooperation with the German Physical Society (Deutsche Physikalische Gesellschaft). For detailed information see https://www.we-heraeus-stiftung.de

Aims and scope of the 752. WE-Heraeus-Seminar:

Supported and tethered lipid bilayers have emerged as one of the most popular mimics of the cell membrane. While initially serving as model systems in research aimed at gaining fundamental insights about biophysical and physicochemical properties of cellular membranes, they have in recent years gained increasing acceptance in the context of studying complex biological processes as well as in various pharmaceutical and diagnostic applications. The aim of the TETHMEM WE-Heraeus seminar is to provide an exchange platform for scientists of various backgrounds and career levels working with lipid assemblies as cell membrane mimics. To exploit the full potential of these lipid membranes, we will discuss the latest progress in the production and characterization of these complex molecular architectures with current state-of-the-art and newly developed biophysical techniques. This will be followed by a survey of potential applications in fundamental biology, but also in diagnostics and drug discovery. Accordingly, we expect participants from a variety of fields including physics, chemistry, biology, biotechnology and medical science to gather around a common interest. The seminar will provide a comprehensive overview of the different aspects of this steadily growing field. Leading international experts will review the present status of supported and tethered membranes as well as their applications in keynote and invited talks. Ample time will be devoted to exchange and discussion, providing the opportunity for established and young researchers to maintain and create new connections, thereby cementing the TETHMEM community. Participants are invited to present their current research in poster sessions. Poster flashes will be given.

Scientific Organizers:

Prof. Dr. Claudia Steinem — Georg-August-Universität, Göttingen, Germany
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Prof. Dr. Marta Bally — Umeå University, Sweden
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Prof. Dr. Fredrik Höök — Chalmers University of Technology, Gothenburg, Sweden
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Introduction

Administrative Organization:

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Registration:

Martina Albert (WE Heraeus Foundation)
at the Physikzentrum, reception office
Sunday (17:00 h – 20:00 h) and Monday morning

Program

Program

Sunday, 29 August 2021

17:00 – 20:00	Registration	
from 18:00	*BUFFET SUPPER / Informal get together*	
20:00 – 21:00	Wolfgang Knoll	**Happy 40th Anniversary - from "Lipid Monolayers on Alkylated Planar Glass Surfaces" to Supported/Tethered Membranes**

Monday, 30 August 2021

07:30 – 08:30	*BREAKFAST*	
08:30 – 08:45	Scientific organizers	**Welcome and Organization**
08:45 – 09:00	Stefan Jorda	**About the Wilhelm and Else Heraeus Foundation**

Session I: Chair: Fredrik Höök

09:00 – 09:45	Marite Cardenas (online)	**Model cellular membranes: From flat to strongly curved structures**
09:45 – 10:30	Giovanna Fragneto	**The structure of model membranes**
10:30 – 11:00	*COFFEE BREAK* and posters	
11:00 – 11:45	Stephen Evans (online)	**Membranes at solid and quasi-solid interfaces models for new biosensors**
11:45 – 12:30	Claudia Steinem	**Pore spanning membranes: Recent developments and applications**
12:30	*LUNCH*	

Program

Monday, 30 August 2021

Session II: Chair: Claudia Steinem

13:45 – 14:30	Erik Reimhult	**Hybrid lipid and block copolymer membranes and vesicles**
14:30 – 15:15	Raya Sorkin	**Membrane remodeling in synaptic vesicle fusion and migrasome formation**
15:15 – 15:35	*COFFEE BREAK*	
15:35 – 16:15	**Poster flashes** **Chair: Marta Bally**	
16:15 – 17:00	**Online poster session I**	
17:00 – 17:45	**Online poster session II**	
17:45 – 18:30	Atul Parikh (online)	**Structured fluids: Transient assembly of lipid amphiphiles into myelin figures**
18:30 – 19:15	Steven Boxer (online)	**Tethered proteins, vesicles and viruses**
19:15	*DINNER*	

Program

Tuesday, 31 August 2021

08:00	*BREAKFAST*	

Session III: Chair: Susan Daniel

09:00 – 09:45	Andela Saric	**ESCRT-III filaments in reshaping and splitting cells across evolution**
09:45 – 10:30	Stephen Block	**Probing enzymatic activity and multivalent interactions using optical microscopy**
10:30 – 11:00	*COFFEE BREAK* and posters	
11:00 – 11:45	Marta Bally	**Virus diffusion at the cell surface: from cell-surface mimics to live cell microscopy**
11:45 – 12:30	Peter Kasson (online)	**Using tethered membranes to uncover the physical determinants of enveloped virus entry**
12:30	*LUNCH*	

Session IV: Chair: Andreas Janshoff

13:45 – 14:30	Susan Daniel	**Re-creating cellular protein synthesis and posttranslational modification on-chip for the cell-free bionanomanufacturing of therapeutics**
14:30 – 15:15	Peter Jönsson (online)	**Protein binding across immune-cell contacts and its influence on T-cell activation**

Program

Tuesday, 31 August 2021

15:15 – 15:45	*COFFEE BREAK* and posters	
15:45 – 16:30	Ralf Richter	**The glycocalyx – tailored model systems to reveal mechanisms of assembly and function of the sweet coats around our cells**
16:30 – 17:15	Neha Kamat	**Harnessing Bilayer Membranes and Cell-Free Systems to Investigate and Control Biological Processes**
17:15 – 19:15	**Social activity** (a hike in the vicinity)	
19:30	*HERAEUS DINNER at the Physikzentrum* *(cold & warm buffet, with complimentary drinks)*	

Program

Wednesday, 1 September 2021

08:00	*BREAKFAST*	

Session V: Chair: Erik Reimhult

09:00 – 09:45	Antreas Kalli (online)	**Understanding the function of mechanosensitive channels using multiscale molecular dynamics simulations**
09:45 – 10:30	Nam-Joon Cho (online)	**Solvent-assisted lipid bilayer formation: beyond vesicle fusion**
10:30 – 11:00	*COFFEE BREAK* and posters	
11:00 – 11:45	Fredrik Höök	**Supported cell-membrane mimics on porous silica for studies of vesicle fusion and cargo delivery**
11:45 – 12:30	Andreas Janshoff	**The role of membranes in cell deformation**
12:30 – 12:40	Scientific organizers	**Closing remarks and poster awards**
12:40	*LUNCH*	

End of Seminar / Departure

Posters

Poster Session I Monday 16:15 – 17:00 CET

1	Peter Adams (online)	**Lipid nanodiscs as a platform to study the timescale of energy transfer between Light-Harvesting membrane proteins and lipid-linked chromophores**
2	Nima Aliakbarinodehi	**Quantitative Characterization of lipid nanoparticle interactions with endosomal membrane mimics using single nanoparticle analytics**
3	Jakob Andersson	**Expanding the utility of tethered membrane systems**
4	Fouzia Bano	**Supported lipid bilayers for investigating the role of sulphation pattern on the interactions between human papillomavirus and cell surface glycosaminoglycans**
5	Jörn Dietz	**Colloidal Probe Force Spectroscopy - Probing membrane protein interaction and separate fusion intermediates in the context of SNARE-mediated membrane fusion**
6	Gonen Golani	**Fusion of virus and host membranes – the role of virus geometry and matrix proteins**
7	Tim Heißenberg	**A new approach to measure the ezrin-actin binding affinity to investigate the influence of ezrin on minimal actin cortices**
8	Toni Hentrich	**Polyphilic interactions of the small GTPase Arf1 with membranes**
9	David Kleinheinz	**Functional reconstitution of the insect odorant receptor coreceptor in a novel model membrane architecture**

Poster Session I Monday 16:15 – 17:00 CET

10	Zachary Manzer	**Cell-free synthesis of fully assembled and functional transmembrane proteins into supported lipid bilayers**
11	Sophie Meredith (online)	**A comprehensive comparison of the photophysics of membrane-based fluorophores using "in-membrane electrophoresis" and patterned supported lipid bilayers**
12	Sarmini Nageswaran	**Structure and mechanics of strained membrane-bound vimentin filaments**

Poster Session II Monday 17:00 – 17:45 CET

13	Erik Olsén	**Quantification of the nanoparticle size contribution to the diffusivity when tethered to a lipid bilayer**
14	Alexis Ostwalt	**Investigating the role of membrane composition on lipid bilayer fluidity and transmembrane protein expression**
15	Hudson Pace	**Cell-Membrane Derived Supported Lipid Bilayers**
16	Ulrich Ramach	**Direct measurement of current transduction across energy converting cell membranes using a novel membrane-on-a-chip system**
17	Dominik Ruppelt	**Preparation of pore-spanning membranes based on porous alumina for the investigation of the antimicrobial peptide lugdunin**
18	Kerstin Seier (online)	**Looking closer at Ebola and Marburg Glycoprotein Pseudotypes**
19	Jan Steinkühler	**Modelling cell-free membrane protein expression**
20	Nikolas Teiwes	**Characterization of giant plasma membrane vesicles: Towards a native-like in vitro system**
21	Judith Thoma (online)	**Supported Membranes for Phenotyping the Influence of Clinical Agents on Stem Cell Dynamics: Connecting Experiments and Theory**

Poster Session II Monday 17:00 – 17:45 CET

22	Konrad Thorsteinsson	**Binding kinetics of human noroviruses to histo-blood group antigens determined using plasma membrane mimics**
23	Sarah Verbeek	**Effects of arginine derivatives and oligopeptides on negatively charged model membranes**
24	Stefanie Wedepohl	**SARS-CoV-2 surrogates for binding and inhibition studies**
25	Akihisa Yamamoto (online)	**Supported Membranes for Discriminating Cancer Progression of Human Gastric Cells**

Abstracts of Lectures

(in alphabetical order)

Virus diffusion at the cell surface: from cell-surface mimics to live cell microscopy

M. Bally

Department of Clinical Microbiology, Umeå, Sweden
Wallenberg Centre for Molecular Medicine, Umeå, Sweden

Viruses are small pathogenic particle that rely on hijacking a cellular host to replicate and spread. The initial recruitment of a virus particle to the cell surface is a complex dynamic multistep process that requires diffusion of the virus particle through the glycocalyx, the sugar coat covering cells, to reach the cell membrane where it may further diffuse laterally in search of a suitable point of entry. Many viruses, including herpes simplex virus (HSV), initiate their recruitment on the host cell by binding to carbohydrates found on the cell surface and in the glycocalyx, in particular sulfated glycosaminoglycans (GAGs), heparan sulfate for example. This initial recognition is crucial in the viruses' life cycle as it leads to infection. Equally important is however, the capability of the virus to overcome these interactions upon egress to ensure virus propagation. A tight regulation of such interactions is also essential in the context of virus transport at the cell surface since binding a high amount of receptors with high affinity might trap the virion at the cell surface before it meets entry receptors, while a too weak binding may not be enough for attachment of the particle or to ensure sufficient residence time for entry.

In our work, we study the molecular mechanisms modulating HSV binding, release and diffusion at the cell surface. To do so, our experimental approach is based on probing interactions between individual virus particles and the cell surface using a combination of minimal cell-surface mimics and live cell microscopy. A minimal model of the cell's carbohydrate coat based on the end-on immobilization of GAG chains makes it possible to study the details of virus-GAG interactions. [1-3] These findings can be further correlated with the behavior of the virus on live cells, where single particle investigations provide fine details on the different steps leading to viral entry. [4]

Taken together, our research contributes to a better understanding of the mechanisms regulating the interaction between a virus and the surface of its host. Such insights will without doubt facilitate the design of more efficient antiviral drugs or vaccines.

References

[1] Altgarde, N., et al., Journal of Biological Chemistry **290(35)**, 21485 (2015)
[2]. Peerboom, N., et al., Biophysical Journal **113(6)**, 1223, (2017)
[3] Delguste, M. et al. ACS Chemical Biology, **14(3)**, 534 (2019)
[4] Abidine, Y., et al., Submitted.

Probing Enzymatic Activity and Multivalent Interactions Using Optical Microscopy

Stephan Block[1]

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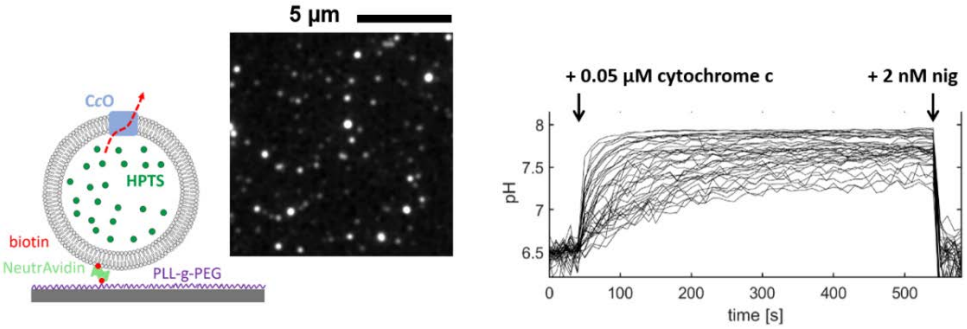
Abstract

Proteins supply a variety of different functionalities in biological systems such as specific molecular recognition, enzymatic activity, and molecular transport. Many of these processes take place at lipid bilayers, acting for example as compartmentalizing hydrophobic barrier, but also allowing for a selective molecular transport through the barrier based on specialized, bilayer-embedded membrane proteins. In this talk, I will demonstrate that optical microscopy can be used to massively parallelize the quantification of protein function such as enzymatic activity and specific binding to attachment factors.

A first example addresses the *enzymatic activity of heme-copper oxidases* [1], such as the cytochrome *c* oxidase (C*c*O), which are transmembrane proteins that take up electrons and protons and thereby generate an electrochemical gradient across the hosting membrane that drives ATP synthesis. I will show that reconstituting single C*c*Os into 100 nm liposomes filled the pH-sensitive dye pyranine enables to quantify proton uptake of a single C*c*O and for 100s of single C*c*Os in parallel (see figure). Such measurements reveal that C*c*O exhibits populations with different protonation dynamics and enable to study the impact of lipid composition and pH gradient on the proton uptake dynamics.

In addition, I will demonstrate that weak and/or complex interactions can also be probed by optical microscopy [2-3]. By employing the interaction of interest to link bionanoparticles to an interface, it is possible to quantify this interaction by tracking single bionanoparticle motion at interfaces. The concept enables to probe the equilibrium *binding dynamics of viruses* interacting with lipid bilayers (serving here as artificial cell membranes), which typically form multiple interactions to membrane-embedded attachment factors/receptors in parallel and therefore generate a complex (multivalent) overall interaction with the membrane. It will be shown that quantifying the mobility of membrane-bound viruses provides information on the recruitment of attachment factors/receptors by viruses. Application of this approach to quantify changes of virus-membrane interactions caused by addition of virus inhibitors will be discussed.



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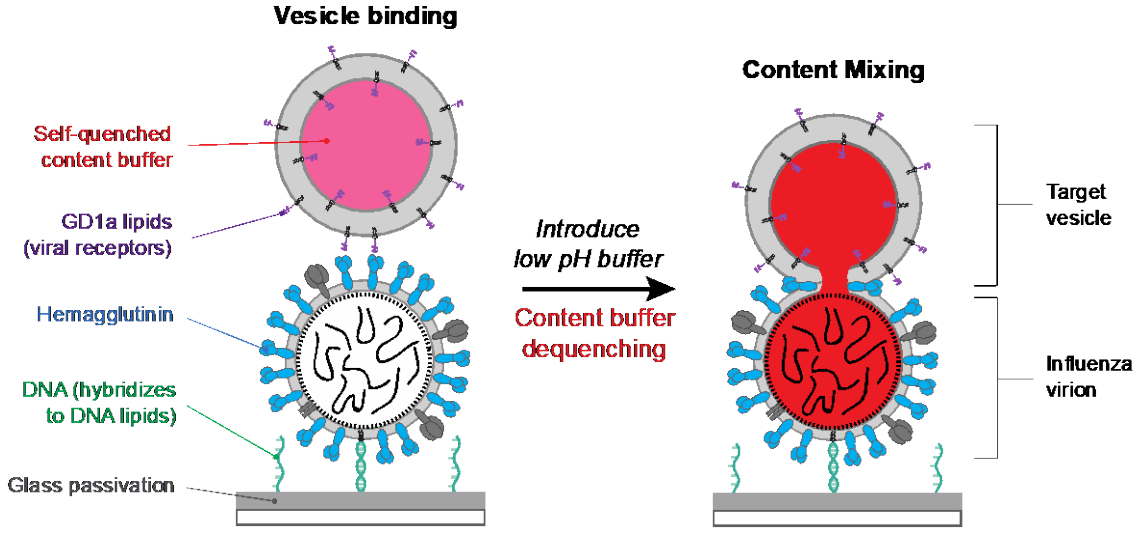
[3] Müller M, Block S et al. *Nano Letters* **19**, 1875-1882 (2019)

Tethered proteins, vesicles, and viruses

Steven Boxer, Stanford University

Supported membranes offer many advantages for imaging, however, the proximity to the solid support creates issues, the theme of this conference. For this reason, we have focused on vesicles or enveloped viruses tethered either to SLBs or passivated surfaces with specific binding sites. I will describe recent work from our lab related to studies of enveloped virus fusion, largely influenza (IVA). These viruses bind to sialic acid residues on the target membrane, and this can be modeled either using relatively simple glycolipids or more realistic synthetic mucin mimics. Viral fusion to an SLB can be useful for studying hemi-fusion, however, full content transfer requires a space on the other side of the bilayer to accommodate the content. Tethered vesicles or tethered virions can overcome this problem. A further issue is that most lipid or content mixing assays are based on dye dequenching but adding high concentrations of dyes to virus particles is problematic. Therefore, we have adopted an architecture in which unlabeled virions are tethered to a surface, then labeled target vesicles (either lipid or content or both and with adjustable composition) are tethered to the virions prior to a pH drop that initiates fusion. Detailed analysis of the efficiency and kinetics of viral fusion and its dependence on cholesterol content of the target membrane will be discussed.



Model cellular membranes: From flat to strongly curved structures

Marité Cárdenas
Malmö University

Cellular membranes are essential components of cells that compartmentalize cellular events, control communication between compartments and with the exterior, enable the formation of gradients of ions and other solutes, and provide a proper environment for the large percentage of proteins that are membrane bound or associated to it[1]. The ability to carry out this diverse array of essential functions requires a high degree of dynamic organization at the nanoscale. It is poorly understood how the different functions are compartmentalized into different domains by lipid-lipid and/or lipid-protein interactions, largely due to difficulties in the handling of cellular membranes and the requirement of sophisticated techniques to analyze them at the molecular level. The current state of the art on membrane organizations heavily focusses on planar membranes[2] despite these membranes often being highly curved as in dynamic processes such as invagination and vesiculation, but also in organelles such as the endoplasmic reticulum and the mitochondria. Neutron scattering combined with deuteration is an ideal technique to study the structure and dynamics of multicomponent systems where different parts of the system can be highlighted individually[3–5]. For example, Neutron Reflection (NR) allows to extract depth profiles of rather complex biointerfaces[2] and revealed the overall structure of lipid bilayers as a function of composition[6,7], the dependency of the core thickness on acyl chain type[7], and the position of cholesterol in the bilayer [8,9], the flip/flop [10], and the ability of membranes to exchange lipids across bilayers[3], among other parameters such as fluctuations on floating bilayers[11]. Similar depth profile information can be extracted for lipid vesicles from small angle neutron scattering (SANS) but the accuracy is lower as compared to NR due to the smearing by the inherent orientational averaging and lower dQ/Q. For example, using SANS the membrane thickness of photolipid vesicles was shown to be tuned in response to illumination by UV and blue light[12]. Both NR and SANS lack the possibility to extract lateral correlations, which is accessible to GISANS, such as local adhesion points, clustering of adhesion anchors[13] as well as lipid rafts[14]. In this talk I will present a range of examples that demonstrate the power of neutron scattering and selective deuteration for studying the structure and composition of model cellular membranes. I will then finally present a new approach based on diffracting scaffolds to study membrane structure as a function of curvature suited for a range of surface sensitive techniques.

References:

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Solvent-assisted lipid bilayer formation: beyond vesicle fusion

Nam-Joon Cho

Nanyang Technological University, Materials Science and Engineering, Singapore

Supported lipid bilayers (SLBs) are highly versatile cell membrane mimics that are widely used for nano-bio interface applications. While vesicle fusion is currently the most popular method for SLB fabrication, there remains broad interest in developing simple yet effective methods that work across a wider range of material substrates, are compatible with biologically relevant lipid compositions, and require less sample preparation. In this talk, I will present our group's recent efforts to develop the solvent-assisted lipid bilayer (SALB) method, which is a new SLB fabrication approach that involves simple deposition of phospholipids in a water-miscible organic solvent onto a target substrate, followed by an aqueous solvent-exchange step to form the desired SLB rapidly and with high quality. A key feature of the SALB method is that the SLB membrane forms in an energetically favored scenario from the bottom-up. Aided by lipid-substrate interactions, surface-adsorbed lipids in organic solvent are rapidly converted into a lamellar-phase SLB upon addition of aqueous buffer solution. Importantly, the process does not require pre-formed precursor vesicles, which enables versatile fabrication of SLBs composed of phospholipids and sterols in user-defined ratios that overcome previous compositional limitations associated with vesicle fusion. Owing to the technically minimal requirements of the SALB method, we have developed on-chip lipid microfluidics to form miniaturized biomembranes with a rich complexity of components reminiscent of natural cell membranes. In turn, all these capabilities are enabling fundamental and applied investigations related to membrane biology and pharmaceutical drug development.

Re-creating cellular protein synthesis and posttranslational modification on-chip for the cell-free bionanomanufacturing of therapeutics

Susan Daniel
RF Smith School of Chemical and Biomolecular Engineering, Cornell University

The market for therapeutic proteins is valued near $140 billion annually. Many of these therapeutics are glycoproteins, which require the addition of specific sugars, called “glycans” at an exact position on the protein through a process called glycosylation. The glycan affects protein folding and function and ensures it retains its therapeutic potency. In nature, glycoproteins are produced through a series of sequential reactions inside a cell in the ER and Golgi apparatus. Making therapeutic glycoproteins within cells is challenging for a variety of reasons, and extensive and costly purification steps are required to harvest the therapeutic material. Cell-free protein synthesis holds great promise for producing high-value, biotherapeutic nanomaterials without cell culture and benefitting from chemical manufacturing know-how. In this approach, raw materials and biological enzymes are mixed together to produce biological products. Shortcomings of this approach are competing reactions, side products, and low yields. Cells avoid these shortcomings by localizing reactions within subcellular compartments, such as the ER and Golgi apparatus, and orchestrating the reaction sequences. The biocatalysts that give the final molecule its essential posttranslational features are compartmentalized in these organelle membranes. But in a cell-free approach, handling enzymes outside of their native lipid environment can drastically reduce their activity. Thus, *in vitro*, sequential, bio-enzymatic reactions have never been achieved in a cell-free manner. In this work, we mimic the elegant compartmentalization strategies used by cells in a microfluidic biomembrane device that organizes biological reactions in proper spatial and temporal sequence, giving ultimate flexibility in optimizing individual reactions and constructing glycans with high specificity. This cell-free device enables facile optimization of glycosylated protein and lipid production, and provides a framework for understanding how experimental conditions affect product yield and quality that is broadly applicable to the bio-nanomanufacturing of virtually any posttranslationally-modified protein. Additional benefits of this manufacturing paradigm to society are reducing the cost of these drugs and providing scientists an avenue to design and develop synthetic drug compounds that may or may not exist in nature to treat disease.

Membranes at Solid and Quasi-solid Interfaces Models for New Biosensors

G. Heath, P. Bao, F. Armistead and S.D. Evans

University of Leeds, Leeds, LS2 9JT, UK

We are developing high-throughput approaches to studying membrane interactions using microfluidic deformation cytometry of liquid-crystal droplets coated with lipid layers. This talk will describe three pieces of work that combine to show the potential of the approach and our preliminary data.

In the first, we have studied the selective interaction of toxins with supported lipid membranes. In particular, we have used fast-scan atomic force microscopy (AFM) and QCMD to detail the attack of Smp43, an antimicrobial peptide (AMP) with a helical-hinge-helical topology isolated from the venom of the North African scorpion Scorpio maurus palmatus on both prototypical prokaryotic (DOPC:DOPG) and eukaryotic (DOPC:DOPE) model lipid membranes. We observe the dynamic formation of highly branched defects being supported by 2D diffusion models – of note the reaction is significantly slower in the PC/PE membranes than the PC/PG.[1]

In the second element of the work, we have adsorbed the lipid monolayers on mono-disperse lipid-coated nematic liquid crystal (LC) droplets, of diameter 16.7 ± 0.2 μm. When treated with Smp43 at concentrations within its biologically active range, the LC droplets undergo a dramatic change in appearance, associated with the transition from a typical radial configuration to a bipolar configuration, which is readily observed by polarizing microscopy.[2]

Finally, we implement deformation cytometry typically used for the analysis of single cells[3] to apply shear forces on the lipid coated droplets and demonstrate significant differences between the shear deformation of the two-lipid mediated different LC states. This opens a new high-throughput route for studying membrane interaction.

References

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The structure of model membranes

Giovanna Fragneto

Institut Laue-Langevin, Science Division, Grenoble, France

The role of membranes in cell deformation

Andreas Janshoff

Institute of Physical Chemistry, Tammannstr. 6, University of Goettingen, 37077 Goettingen

Cell cortices are responsible for the resilience and morphological dynamics of cells. Measuring their mechanical properties is impeded by contributions from other filament types, organelles, and the crowded cytoplasm. We established a versatile concept for the precise assessment of cortical viscoelasticity based on force cycle experiments paired with continuum mechanics. Apical cell membranes of confluent MDCK II cells were deposited on porous substrates and locally deformed. Force cycles could be described with a time-dependent area compressibility modulus obeying the same power law as employed for whole cells. The reduced fluidity of apical cell membranes compared to living cells could partially be restored by reactivating myosin motors. A comparison with artificial minimal actin cortices (MACs) reveals lower stiffness and higher fluidity attributed to missing cross-links in MACs.

Protein binding across immune-cell contacts and its influence on T-cell activation

V. Junghans[1], T. Dam[1], M. Chouliara[1], P. Jönsson[1]

[1]Lund University, Lund, Sweden

Binding between ligands and receptors across contacting cells is vital for several biological processes. This includes activation of T cells, where T-cell receptors (TCRs) bind to antigen presented by MHC complexes (pMHC). How strong this interaction needs to be to start an immune response is, however, unclear. I will here highlight some of our recent results on the affinity between TCRs and pMHC molecules displaying different peptides, from cognate to self. In addition, I will also show how the auxiliary binding pair CD2/CD58 both can stabilize and destabilize TCR binding pMHC [1]. I will also discuss how the weak interaction between the co-receptor CD4 and pMHC can influence T-cell signaling [2], and present a new method of analyzing binding affinities on a single cell level. I will finally discuss how nickel-chelating lipids, commonly used in model membranes for in vitro T-cell studies, can induce TCR-dependent cell signaling without influencing CD45 exclusion [3]. Altogether demonstrating that it is not only the protein-protein interaction per see that determines the binding magnitude in vivo.

References

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Understanding the function of mechanosensitive channels using multiscale molecular dynamics simulations

Dario De Vecchis[1], Jiehan Chong[1] and Antreas C Kalli[1,2]

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Responding to external mechanical stimuli is of vital importance for many organisms as mechanosensation plays a vital role in many biological processes. In cells, pressure will be converted to lateral tension in the cell membrane which mechanosensitive channels will respond to. Recent structural and functional data of a number of mechanosensitive channels demonstrated that lipids and physical properties of the cell membrane such as curvature play a key role in the function of these channels. Developments in biomolecular simulations allows us to simulate increasing large and complex membrane systems that mimic native membranes in which these proteins function. In this talk, I will discuss our studies in which we used molecular dynamics simulations at the coarse-grained and atomistic resolutions to study the dynamics and interactions of the Piezo1 mechanosensitive channel. Piezo1 is a critical mechanical sensor in a number of cells including endothelial cells, erythrocytes and other cell types. It has a highly curved membrane region which is thought to be important for its activation. To study how Piezo1 is activated and how it interacts with its lipid environment we simulated Piezo1 in complex model bilayers that mimic the lipid composition of the endothelial membrane in which this protein functions. Our simulations show that Piezo1 changes the curvature in its vicinity creating a dome in the membrane with a trilobed topology that extends beyond the radius of the protein. Negatively charged phospholipids e.g., PIP_2 and cholesterol were found to strongly interact with Piezo1 creating a unique lipid environment around Piezo1. Our studies have also shown that upon bilayer stretching, Piezo1 adapts to the stretched bilayer by flattening and expanding its blade region. Our results suggest novel models for the interactions of Piezo1 channel with lipids demonstrating how protein/membrane interactions and physical properties of the membrane may regulate its function.

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Harnessing Bilayer Membranes and Cell-Free Systems to Investigate and Control Biological Processes

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Membranes play a vital role in a variety of physiological processes. Recapitulating these processes outside of the cell may allow us to better understand them as well as design an entirely new class of materials that can sense, transport, or target important biological signals and molecules. In this talk, I will present our recent work using model membranes and cell-free expression systems to (1) uncover the role of membrane mechanical properties on the folding of a model mechanosensitive channel protein, the mechanosensitive channel of large conductance (MscL) and (2) design a membrane-based device that initiates encapsulated cell-free reactions. First, I will describe how the incorporation of non-natural amphiphiles, such as diblock copolymers, into artificial membranes allows for the generation of new membrane physical properties. We find that membrane structural and physical properties affect cotranslational folding of an alpha-helical membrane protein. Next, I will describe our recent work using membrane-bound DNA tethers to control vesicle fusion and content mixing between specific vesicles, initiating encapsulated cell-free reactions. We find that DNA-mediated vesicle fusion efficiency may be increased by introducing liquid-ordered and disordered lipid phases into vesicle membranes. Our approach, bridging synthetic biology techniques and model membrane assembly, provides an innovative yet simple method to probe the role of membrane composition on protein dynamics and to design new membrane-based devices.

Using tethered membranes to uncover the physical determinants of enveloped virus entry

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Enveloped viruses infect cells via a process of membrane fusion. Here, we report the use of engineered membrane substrates as well as tethered physiological membranes to unravel the physical determinants of enveloped virus entry. Using single-virus fusion kinetics, we show how membrane deformability rather than membrane curvature controls the early rate-limiting steps in viral membrane fusion. We further demonstrate that, at least for influenza virus, the endosomal membrane composition and curvature does not contain special permissive factors for entry. Two key platforms, engineered membranes on nanoparticle substrates and isolated physiological membrane-virus conjugates, together permit controlled manipulation of membrane properties and the ability to test their physiological consequences.

Happy 40th Anniversary – from lipid monolayers on alkylated glass substrates to tethered membranes

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In 1981, a paper on the „ Physical Properties of Lipid Monolayers on Alkylated Planar Glass Surfaces", published by Vinzenz von Tscharner and Harden McConnell from the Stauffer Laboratory at Stanford University (Biophys. J. 36 (1981) 421-42) marked a fundamental break in research on artificial lipid bilayer membranes: after many years of either structural studies with vesicles or work done with bimolecular lipid membranes (BLMs, "black" lipid membranes) as experimental platform on certain aspects of the functional properties of these model systems, all of a sudden the experimental basis was provided for simultaneous research on both, the structure and the function, and the correlation between them.

I will give a few examples, both historic and more recent, of the long the way it took from there until a novel model membrane system, the tethered lipid bilayer membrane (tBLM) was established.

STRUCTURED FLUIDS: Transient Assembly of Lipid Amphiphiles into Myelin Figures

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Phase behaviors of common amphiphiles, such as surfactants, lipids, and block copolymers, at thermodynamic equilibrium are now well-understood. But how these molecules organize within self-organizing dynamic morphologies – such as those that appear under far from equilibrium conditions while dissipating energy – is incompletely understood. An extra-ordinary example of self-organizing media is the class of multi-cylindrical aggregates, called myelin figures, which appear when dry mass of amphiphiles encounter water. Assembling at the material boundary, these transient, but long-lived, protrusions expend osmotic energy and organize into metastable cylindrical liquid crystals – tens of micrometers wide and hundreds of micrometers long – exhibiting a long-range smectic ordering of thousands of alternating layers of lipid bilayers and water in a multi-cylindrical geometry. This talk presents our recent findings of unusual spatial organization of amphiphiles in multicomponent myelins and considers the responsiveness of myelins to external assaults due to physical and chemical stresses. We find that when the hydrating lipid mass contains phase separating mixtures of an unsaturated lipid, cholesterol, and sphingomyelin, the molecules segregate differentially across different lamellae in single myelins. In stark contrast to intralamellar lateral phase separation, such as occurs in equilibrium multilamellae, these multicomponent myelin figures display a continuous, interlamellar radial gradients of concentrations of cholesterol (and sphingomyelin) and unsaturated lipid with the latter concentrating in the inner lamellae and the former accumulating at the highest concentrations in the outer lamellae. Moreover, we find that external stresses trigger novel instabilities producing characteristic buckled, bent, and helical surface morphologies. We propose that this emergent morphological diversity – reflecting higher-order spatio-temporal self-organization in non-equilibrium lipid self-assembly – are driven not by the equilibrium properties of these molecules. But rather kinetic differences in the information processing of hydration characteristics of individual molecules while expending energy dictates this emergent behavior of lipid mixtures.

Hybrid lipid and block copolymer membranes and vesicles

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Hybrid lipopolymer vesicles are membrane vesicles that can be self-assembled on both the micro- and nanoscale. They are potential novel smart materials for drug delivery systems that could combine the relative strengths of liposome and polymersome drug delivery systems without their respective weaknesses on the nanoscale. However, we still know little about their properties and how they could be tailored. Most methods of investigation are limited to the microscale or are performed on supported membranes.

In this context, I will present some of our work on membranes composed of phospholipids and polybutadiene-*b*-poly(ethylene oxide). We have investigated these self-assembled membranes as supported bilayer membranes, giant unilamellar vesicles, and large unilamellar vesicles. By probing them with confocal fluorescence microscopy, QCM-D, AFM (force spectroscopy), DLS, and encapsulation and release assays, we conclude, e.g., that nanoscale large unilamellar vesicles show different phase separation and permeability than giant vesicles, and that this kind of hybrid membranes does not show increased robustness in all aspects, as often proposed. Taking such results into account, we demonstrate how they are still exciting candidates for triggered drug release systems using, e.g., enzymatic or magnetothermal triggers.

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The glycocalyx – tailored model systems to reveal mechanisms of assembly and function of the sweet coats around our cells

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Virtually all vertebrate cells surround themselves with a glycocalyx, *i.e.*, a coat made of glycans and proteins that is attached to the plasma membrane. The glycocalyx accomplishes essential cellular functions, including in inter-cellular communication, cell adhesion and cell motility, but also in the control of pathogens. The molecular and physical mechanisms underpinning these various functions remain under-studied and rather poorly understood, in large part because tools to manipulate and analyse the glycocalyx and its components, *in vivo* and *in vitro*, are lagging behind what is currently possible inside cells.

This talk will present selected examples of efforts in the laboratory, and with external collaborators, to develop new biochemical and biophysical tools that help define mechanisms of glycocalyx assembly and function. Common to all these efforts is the use of model glycocalyces that are reconstituted from selected glycocalyx components through biomolecular self-organisation. The models are simpler than the original yet reproduce salient features and are well-defined in their composition and organisation. Their quantitative biophysical analysis, combined with theoretical models from polymer and soft matter physics, have provided new mechanistic insights and revealed intriguing phenomena such as ‘superselective’ recognition, ‘sliding’ bonds and protein-driven micro-phase separation. A better understanding of these processes, ultimately, opens new avenues to engineer novel biomaterials and glycocalyx functions in *vivo*.

ESCRT-III filaments in reshaping and splitting cells across evolution

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The molecular machinery of life is largely created via self-organisation of individual molecules into functional larger-scaled structures. Such processes are multi-scale in nature and constantly driven far from thermodynamic equilibrium. Our group develops minimal coarse-grained computer models for non-equilibrium organisation of macromolecules into functional nanomachinery that can produce mechanical work involved in key cellular processes.

Today I will discuss our recent research on physical modelling of active elastic ESCRT-III filaments that dynamically shift their geometries and mechanical properties to reshape and cut cell membranes. I will first present our model in the context of eukaryotic cell trafficking, supported by experimental data [1-2]. Then I will discuss the evolutionary origins of this physical mechanism and its role in cell division in the archaeal branch of the tree of life. Finally, I will show quantitative comparison between live cell imaging of dividing archaeal cells and our simulations of the whole cell division process [3-4].

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Membrane remodeling in synaptic vesicle fusion and migrasome formation

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Fundamental understanding of physiological processes that occur at biological membranes, such as membrane fusion, necessitates addressing not only the biochemical aspects, but also biophysical aspects such as membrane mechanical properties and membrane curvature. In this talk, I will show how we combine tethered membranes, optical tweezers and confocal fluorescence microscopy to study membrane remodelling by calcium sensor proteins, which are crucial in neuronal communication. Using this approach, we discovered surprising differences between the action mechanisms of two structurally similar proteins, Doc2b and Synaptotagmin1 (Syt1), as determined by quantifying the strength and probabilities of protein-induced membrane-membrane interactions and lipid and content mixing assays [1]. I will further describe how we use a similar approach to gain insight into a particular case of membrane shaping during: the formation of a newly-discovered organelle termed migrasome. Migrasomes are very recently discovered signalling vesicles are generated from cell retraction fibers as a consequence of cell migration [2]. It is of great interest to understand their formation mechanism, as migrasome mediated cellular communication may be a general process in various physiological functions that require localized communication between cells.

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Pore spanning membranes: Recent developments and applications

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Pore-spanning membranes (PSMs) are a valuable tool to study membrane-bound processes that require a planar membrane facing two aqueous compartments accessible by fluorescence microscopy techniques. In this talk, I will discuss recent advances in the preparation and characterization of PSMs. In addition, two examples will be highlighted to demonstrate the advantages and disadvantages of PSMs.

i) Lipid domains in plasma membranes act as molecular sorting platforms. In model membranes, such as GUVs and supported bilayers, lipid domains with defined chemical composition and lipid packing have been observed and certain characteristics have been reproduced. However, *in vivo*, the plasma membrane experiences a proteinaceous scaffold underneath, which can sort, compartmentalize and recruit components within the membrane. The influence of such scaffolds on the phase behavior of lipid membranes has been barely studied. We used PSMs to investigate the impact of a partial attachment of a membrane to a support on its phase behavior. Using two different fluorophores, PSMs were visualized by fluorescence microscopy allowing us to distinguish between different membrane phases, as a function of the cholesterol content. Our results demonstrate that the underlying porous mesh significantly alters the phase behavior of the phase-separated membranes.

ii) Neuronal fusion is a process by which two initially distinct membranes merge resulting in one interconnected structure to release neurotransmitters into the presynaptic cleft. To get access to the different stages of the fusion process, several *in vitro* assays have been developed. Among those assays, we developed a single vesicle assay based on PSMs with reconstituted t-SNAREs, which can overcome some of the drawbacks associated with the other membrane architectures. In this talk, I will discuss the fusion efficiency, the intermediate states and the kinetics of fusion as obtained by a simultaneous fluorescent readout of lipid mixing and content release using synthetic vesicles with reconstituted synaptobrevin 2 (v-SNARE) and synaptotagmin-1. The results are compared with those obtained if synthetic vesicles are replaced by natural chromaffin granules also harboring synaptobrevin 2.

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Abstracts of Posters

(in alphabetical order)

Lipid nanodiscs as a platform to study the timescale of energy transfer between Light-Harvesting membrane proteins and lipid-linked chromophores

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Nanodiscs are an idealized platform to study single membrane proteins assembled into a small patch of lipid bilayer. Light-Harvesting Complex II (LHCII) is a membrane protein containing a network of chlorophyll pigments, with the crucial role of absorbing the solar energy required to power photosynthesis in plants. LHCII provides strong absorption of blue and red light, however, it has minimal absorption in the green spectral region where solar irradiance is maximal. In a recent proof-of-principle study **[1]**, we enhanced the absorption in this spectral range by developing a biohybrid system where LHCII proteins together with lipid-linked Texas Red (TR) chromophores, were assembled into lipid membrane vesicles (proteoliposomes). This conference presentation will show new work **[2]** where we organised TR and LHCII into a lipid nanodisc, which provided a homogeneous, well-controlled platform to more deeply study the interactions between TR molecules and single LHCII complexes. Fluorescence spectroscopy determined that TR-to-LHCII energy transfer has an efficiency of at least 60%, resulting in a 255% enhancement of LHCII fluorescence, two-fold greater than in the previous system. Ultrafast transient absorption spectroscopy was employed to probe the picosecond timescales of energy transfer and revealed two time constants of 3.7 and 128 ps relating to two distinct populations of Texas Red molecules involved in energy transfer. Structural modelling and theoretical calculations allowed us to examine how different molecular configurations and dipole orientations would affect the experimentally observed energy transfer. These results indicated that these timescales correspond to TR-lipids that are loosely or tightly-associated with the protein, respectively, with estimated average TR-to-LHCII separations of ~3.5 nm and ~1 nm. Overall, we demonstrate that a nanodisc-based biohybrid system provides an idealised platform to explore the photo-physical interactions between extrinsic chromophores and membrane proteins with potential applications in understanding or exploiting photosynthetic systems.

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Quantitative Characterization of lipid nanoparticle interactions with endosomal membrane mimics using single nanoparticle analytics

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To optimize the efficiency of pH-sensitive ionizable lipid nanoparticles (LNPs) designed as mRNA delivery vehicles, it is crucial to gain in-depth understanding about their interaction with biological membranes. Despite the crucial role of the interaction between LNPs and the endosomal membrane for efficient cytosolic cargo delivery, most investigations have addressed how LNPs interact with the outer cell membrane. In this work we contribute with biophysical assays combining single LNP imaging using TIRF microscopy with endosomal membrane mimics to investigate how binding kinetics and fusion depends on pH. To this aim, negatively-charged lipid bilayer consisting of POPC:POPS:NBD-PE (93.5:6:0.5), was formed on planar glass and porous silica substrate as a reductionist endosomal membrane model. The pH-induced interaction between LNPs and the bilayer was studied utilizing TIRF microscopy to monitor the adsorption of both suspended and tethered LNPs (MC3:DSPC:Cholestrol:DMPE-PEG, 50:10:38.5:1.5) to the membrane. Specific focus was put on analysing the effect of protein corona formation on the LNPs, and how that process impact pH-induced binding and subsequent fusion events. We observed a significant serum-dependent inhibition of LNP binding to endosomal model membrane (Fig. 1, left), attributed to protein-corona formation causing a shift in the onset of binding towards lower pH. These results comply with live-cell imaging data, also demonstrating the crucial role of lipoproteins for cellular uptake and, specifically, a potentially hampering influence on endosomal escape. In addition, for endosomal membrane mimics formed on porous silica, efficient pH-induced LNP fusion was observed below pH 6.6. Time resolved imaging revealed markedly different rates of escape from the fusion sites for lipids and cargo (Fig. 1, right). While the lipids display monophasic kinetics, two phases could be identified for cargo release, thus revealing insights of potential importance for improved LNP design. (Figure 1-Right).

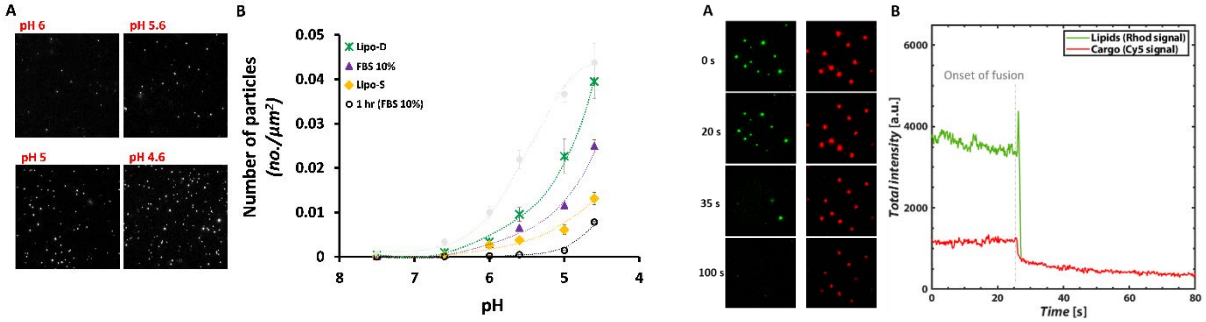

Figure 1. Left) The pH-dependent binding of the LNPs after incubation with untreated FBS 10%, Lipoprotein depleted (Lipo-D) or lipoprotein saturated (Lipo-S). A, TIRF images of the model system showing the binding of LNP (81.92×81.92 μm). B, the pH-dependent adsorption response of LNPs as a function of decreasing pH and at different protein corona conditions. Right) Separate analysis of lipids and cargo intensities with single particle resolution. A, Temporal evolution of lipids (Rhod) in green and cargo (Cy5) in red as pH dropped in the channel. B, Rhod and Cy5 intensities, identifying the behaviour of lipids and cargo of LNPs upon fusion. The drop of cargo and lipids' peak intensity determined the onset of fusion.

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Expanding the utility of tethered membrane systems

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In recent years, we have worked to expand the utility of tethered membrane systems by diversifying their structure. First, we expanded the sub-membrane space to allow for increased membrane fluidity[1], to allow for the formation of more fluid membrane systems to facilitate better protein incorporation and function. Next, we developed methods to assemble asymmetric tethered membrane systems resembling the outer membrane of Gram-negative bacteria[2] which we used to test novel antibiotic treatments[3]. We then used new membrane systems with increased sub-membrane space and fluidity to embed membrane-spanning DNA origami nanopores developed by the group of Prof. Stefan Howorka[4]. The nanopores are closed with a key sequence which can be designed such that it can open in the presence of specific DNA sequences, for example antibiotic resistance genes, acting as a new biosensing platform. Finally, we further developed these membrane systems by altering the substrate structure to increase the amount of sub-membrane space which allowed us to incorporate the OR22a-orco insect odorant receptor complex from *Drosophila Melanogaster* such that it opened reversibly and reproducibly upon addition of its natural ligand ethyl hexanoate and ethyl propionate (publication in progress). Taken together, these developments show the incredible potential of model membrane systems for both biosensing and to understand fundamental biological process such as olfactory sensing of insects.

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Supported lipid bilayers for investigating the role of sulphation pattern on the interactions between human papillomavirus and cell surface glycosaminoglycans

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The recruitment of viral pathogens to host cells is often facilitated by carbohydrates on the cell surface. For example, glycosaminoglycans (GAGs), negatively charged oligosaccharides, play a major role in the initial recruitment and accumulation of viruses at the cell surface. For instance, the degree and type of GAG sulfation has been shown to modulate the attachment/detachment behavior of enveloped viruses like herpes simplex virus type I [1]. However, our understanding about the biophysical properties of the interactions between non-enveloped virus and GAGs is still very limited, specifically when it comes to their dynamics. In this research study, we probe how the attachment, detachment and diffusion of a non-enveloped virus, human papillomavirus type 16 (HPV16), is regulated on the cell surface. HPV16 is a double-stranded DNA virus of critical medical importance, as it is the leading cause of cervical cancer [2]. Using combinatory approach based on (i) single particle tracking by total internal reflection fluorescence (TIRF), (ii) atomic force microscopy-based single molecule force spectroscopy (AFM-FS) [3,4], and (iii) surface-immobilized GAGs -a cell-surface mimic platform based on supported lipid bilayers that offers tight control on grafting density and presentation of GAGs- we study how specific sulfation patterns on highly sulphated GAGs like heparin influence the binding kinetics and diffusion behaviors of HPV type 16. Our TIRF data shows that HPV16 binds with higher affinity to parental heparin as compared to selectively N-Desulphated heparin with no major difference in their diffusion behavior. Our AFM-FS data in excellent agreement with TIRF shows higher binding probability and lower dissociation rate for the interaction of HPV16 and parental heparin further highlighting the role of N-sulphation on the binding behavior. Taken together, this implies that the type of sulphation of the GAGs has an important functional implication for the viral attachment which may influence entry.

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Colloidal Probe Force Spectroscopy - Probing membrane protein interaction and separate fusion intermediates in the context of SNARE-mediated membrane fusion

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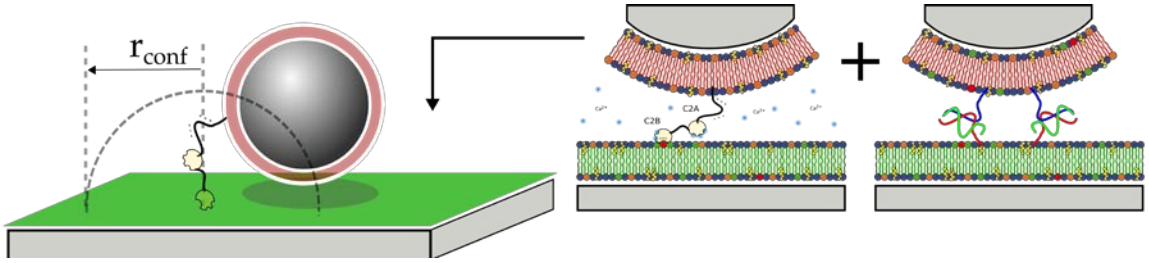

In connection with reconstituted biological model systems, we demonstrate how colloidal force spectroscopy allows us to investigate the behavior of a biological system like the membrane fusion of vesicles. We explore the use of colloidal particles (CPs) of various sizes, which, coupled to different springs, serve to measure forces as they probe nanometer-scale interactions of the micrometer-sized colloids coated with a membrane (mCPs) against solid supported membranes. Here mCPs facilitate regions of weak, specific protein-bilayer interactions in the context of SNARE-mediated membrane fusion by using protein-bilayer reconstitution. Experiments were performed in the presence and absence of bilayer-anchored f-Synaptotagmin-1 as Calcium ions were present in the solution. We conduct these experiments based on Atomic Force Microscopy (AFM), Optical tweezers (OT), and Holographic Video Particle Tracking (HVPT) as they enable us to first discriminate intuitively intermediate states from step heights and corresponding lifetimes of the fusion pathway. Based on this, we resolve them by colloidal particle motion fluctuation with complete detail. Our knowledge from AFM and OT allowed us to separate these from fusion events. We propose a mechanism for syt-1 to interact with anionic bilayers and promote fusion in the presence of SNARE proteins. We found that syt-1 binding to membranes depends on the PI(4,5)P2 content in the absence of Ca2+. The understanding that develops is that syt-1 remodels the membrane in the presence of calcium and PIP2, thereby enormously improving the efficiency of membrane fusion by avoiding stalled intermediate states.

Fusion of virus and host membranes – the role of virus geometry and matrix proteins

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Many medically important viruses, including influenza A, Ebola and corona viruses, are enveloped by a lipid membrane. Therefore, a crucial step during infection is the fusion of the viral and cellular membranes. The fusion pathway involves a series of non-bilayer intermediates configurations with three consecutive steps. First, the monolayers of the two opposing membranes merge to form a hemifusion connection, referred to as the hemifusion stalk. Second, radial expansion of the stalk brings the distal lipid monolayers of the bilayers together into a hemifusion diaphragm. Third, opening and expansion of a fusion pore within the hemifusion diaphragm completes the fusion process. The formation of hemifusion stalk and expansion of the fusion pore constitute the two major energy barriers in the fusion process, with magnitudes of dozens of k_BT. While formation of the hemifusion stalk is directly driven by the viral fusion proteins and was extensity studied in the last decades, pore formation and expansion is driven by the stress in the diaphragm and is less well understood. Here we use the theory of the membrane elasticity to compute the stresses in the hemifusion diaphragm and the resulting energy barrier to fusion pore formation and expansion. We analyze for the first time the effect of the virus geometry and virus membrane-matrix interaction on viral fusion rate. We also suggest a model for the role of interferon-induced transmembrane proteins (IFITMs) for inhibition of fusion by increasing the energy barrier of fusion pore expansion, effectively delaying the viruses in an intermediate hemifusion stage, and thus preventing infection.

A new approach to measure the ezrin-actin binding affinity to investigate the influence of ezrin on minimal actin cortices

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The Aim of my project is to investigate the influence of the ezrin-actin binding affinity on minimal actin cortices (MACs) in a bottom-up approach on solid supported membranes (SSMs). Ezrin is part of the ERM protein family and works as an anchor for the actin cortex to the plasma membrane. Ezrin has a N-terminal domain called FERM domain, which contains a phosphatidylinositol (4,5) bisphosphate (PIP_2) binding motif that is needed to be recruited to the plasma membrane. The C-terminal domain of ezrin holds a filamentous actin binding domain. Therefore, when ezrin is activated within a cell, it binds to the plasma membrane *via* its PIP_2-binding motif and connects it to the actin filaments which are part of the actin cortex.

In this project we will use MACs with ezrin as a linker between a PIP_2 doped SSM and an actin network. We mutated the actin-binding site of ezrin to decrease the actin binding ability. Those mutants are known in literature and it was qualitatively shown, that they have a decreased actin binding ability[1]. To measure the ezrin-actin binding affinity quantitatively we established a new approach based on reflectometric interference spectroscopy. After the binding affinity measurements, MACs will be created with the different ezrin mutants as linkers and analyzed. The analysis will be done with high-resolution fluorescent imaging for parameters like mesh size and node density and with video particle tracking for the viscoelastic parameters.

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Polyphilic interactions of the small GTPase Arf1 with membranes

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The small GTPase Arf1 plays an important role in vesicular trafficking at the Golgi membrane. Most prominently, assembly of transport vesicles by the coat protein complex COPI, which proceeds via local deformation of the lipid bilayer, is initiated by this small GTPase in a nucleotide-dependent manner [1]. Moreover, in the yeast *Saccharomyces cerevisiae*, Arf1 was also recognized to participate in the formation of cargo carriers at the Golgi by a complex called exomer [2]. Upon GTP binding, Arf1 undergoes a conformational change and exposes a myristoylated N-terminal amphipathic helix (myrAH), which serves as a membrane binding anchor [3, 4]. The role of GTP hydrolysis in the fission of membrane carriers is still debated. Here, we try to break down the polyphilic nature, i.e. the contributions of the core domain, the amphipathic helix and the lipid anchor, to the membrane binding and membrane remodelling activity of this protein. To this end, lipid membrane binding of different Arf1 variants is studied on a Langmuir film balance and on artificial, unilamellar liposomes. We are currently extending these studies to supported lipid bilayers.

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Functional reconstitution of the insect odorant receptor coreceptor in a novel model membrane architecture

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Insect olfaction is known to be unrivaled in terms of not only sensitivity but also the rapidity of recognition of volatile compounds. A unique transmembrane setup comprised of odorant receptors (ORs) as well as the odorant receptor co-receptor (Orco) allows them to accomplish swift membrane depolarization.[1]

The odorant receptor co-receptor is central to the olfaction sensing process of insects, providing a mechanism facilitating membrane depolarization upon binding of an odorant molecule which is highly conserved across different species. Few mechanistic details of the interaction between Orco, odorant receptors, OBPs (odorant binding proteins) and their ligands are known, as this process takes place in the highly complex environment of the cellular membrane.[2]

So far, biophysical characterization has largely been carried out *via* patch-clamping experiments.[3,4] While this technique can be used to characterize the protein in a native membrane environment, experimental conditions and the composition of the membrane environment cannot be precisely controlled. Model membrane systems in which the olfactory sensing process can be replicated under more controlled experimental conditions could contribute significantly to a better understanding of insect olfactory signalling. We present here for the first time a robust model membrane system, based on a sparsely tethered bilayer setup in which the Orco-subunit can be functionally reconstituted, responding as expected to the synthetic ligand VUAA1.

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Cell-free synthesis of fully assembled and functional transmembrane proteins into supported lipid bilayers

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Supported lipid bilayers (SLBs) hold tremendous promise as cellular-mimetic structures that can be readily interfaced with analytical and screening tools. The incorporation of transmembrane proteins, a key component in biological membranes, is a significant challenge that has limited the use of SLBs for a variety of biotechnological applications. Towards developing this biomimetic interface, we describe an approach using a cell-free expression system for the co-translational insertion of membrane proteins into hybrid supported lipid bilayers (HSLBs) containing phospholipids and diblock copolymers. We use cell free expression techniques and a model transmembrane protein, the large conductance mechanosensitive channel (MscL), to demonstrate two routes to integrate a channel protein into a HSLB. We show that HSLBs can be assembled with integrated membrane proteins by either co-translational integration of protein into hybrid vesicles, followed by fusion of these proteoliposomes to form a HSLB, or preformation of a HSLB followed by the cell-free synthesis of the protein directly into the HSLB. Both approaches lead to the assembly of HSLBs with oriented proteins. Notably, using single-particle tracking, we find that the presence of diblock copolymers facilitates membrane protein mobility in the HSLBs, a critical feature that has been difficult to achieve in pure lipid SLBs. We then use conduct electrical impedance spectroscopy with a SLB supported on a bioelectronic interface to measure the activation of expressed MscL after treatment with a chemical trigger. The approach presented here to integrate membrane proteins directly into preformed HSLBs using cell-free co-translational insertion is an important step toward enabling many biotechnology applications, including biosensing, drug screening, and materials platforms requiring cell membrane-like interfaces that bring together the abiotic and biotic worlds and rely on transmembrane proteins as transduction elements.

A comprehensive comparison of the photophysics of membrane-based fluorophores using “in-membrane electrophoresis” and patterned supported lipid bilayers

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Fluorophores are often incorporated into model lipid membranes to report on the structure, dynamics and physicochemical environment of the membrane. The organization of these fluorophores with respect to each other and the surrounding environment has a significant effect on their photophysical properties. Many lipid-linked fluorophores have been shown to self-quench (i.e., exhibit reduced fluorescence intensity/lifetime) as a function of concentration.[1,2] This self-quenching is often ignored in the lipid bilayer community, however, it can drastically affect the results of fluorescence measurements. Self-quenching is also observed for chlorophyll-containing membrane proteins found in plants which are essential for light absorption in photosynthesis.[3] Therefore, investigating the self-quenching of multiple fluorophores may provide insight into both natural bioenergetics and photophysics in model membranes.

We present a novel platform to assess the photophysics of membrane-based fluorophores and utilize it to perform the first side-by-side comparison of self-quenching of a variety of lipid-linked probes (Texas Red, BODIPY, NBD) and photosynthetic membrane proteins. Using electric fields to direct the migration of fluorophores in patterned lipid membranes and fluorescence lifetime imaging microscopy (FLIM) to assess the photophysical state, quenching was quantified for a continuum of fluorophore concentrations. Analysis of FLIM images allow us to plot graphs of fluorescence quenching against molecular concentration and calculate the critical distance at which multiple fluorophores form traps, with <1 nm resolution. We conclude that the self-quenching of lipid-linked probes is a combination of exciton energy migration and the formation of non-fluorescent trap states. Finally, we show that plant membrane proteins have a higher propensity for self-quenching than lipid-linked fluorophores and may have a different quenching mechanism.

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Structure and mechanics of strained membrane-bound vimentin filaments

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In eukaryotic cells, the shape and mechanical properties are determined by the cytoskeleton, which is composed of actin filaments, microtubules and intermediate filaments.[1] Importantly, intermediate filaments are considered to be the main determinants of cell stiffness and strength.[2] The reason for this is that they can withstand much larger deformations. Thus, they are believed to dominate the mechanical response of cells at higher strains.[1,2,3] Therefore, the organization of intermediate filaments at the plasma membrane and their influence on the mechanical properties of the cells under a variety of strains are of great interest.

Hence, we aim at the development of an *in vitro* model system to mimic the composition of the plasma membrane under strain.

Biotin-labeled vimentin filaments are attached to a biotin-decorated lipid bilayer *via* biotin- affine neutravidin. Since the composite system needs to be laterally stretchable by a motor-driven stretching device, the lipid bilayer is prepared on oxidized, elastic polydimethylsiloxane (PDMS) by vesicle fusion and spreading. Fluorescent beads are embedded in the PDMS to accurately calculate the expansion of the system under strain. We find that the strain speed is crucial in order to observe strain of the vimentin filaments, since the membrane-coupled system can only be stretched when the PDMS stretching is faster than the diffusion of the anchor lipid molecules. Currently, we are able to stretch the membrane-bound vimentin filaments up to 30 %.

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Quantification of the nanoparticle size contribution to the diffusivity when tethered to a lipid bilayer

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When interpreting the diffusivity of nanoparticles bound to a lipid bilayer by ligand-receptor pairs, the particle contribution is often neglected, in comparison to that of the tethers. Nevertheless, in the limit of single or few tethers in highly mobile lipid bilayers this approximation eventually breaks down. When this occurs, the exact influence of particle size to the particle-tether diffusivity is not yet fully understood, due to confinement effects, imprecise measurements of the hydrodynamic boundary conditions, and lack of experimental methods capable of deconvoluting the particle and tether contributions.

By tracking the motion of nanoparticles tethered to a supported lipid bilayer (SLB) under shear-induced flow, which enables simultaneous size, velocity, and diffusivity measurements on the single particle level [1], the contributions of the particle and tethers to the diffusivity were successfully separated in a single measurement [2]. When compared to theoretical expressions, the obtained diffusivity-size relations for both synthetic and extracellular lipid vesicles were not well-described by the conventional no-slip boundary condition, suggesting the occurrence of partial slip. The results also demonstrate that the particle diffusivity can act as a sensitive probe of measuring the distance to the SLB.

This is, to our knowledge, the first direct partial slip measurement on biological nanoparticles, thus extending the understanding about diffusion of biological nanoparticles near lipid bilayers. Furthermore, since the method does not require a known relation between signal intensity and particle size, the approach is generally applicable to nanoparticles bound to an SLB. This latter aspect allows for accurate compensation of the particle contribution to the diffusivity when using nanoparticles to track the motion of cell-membrane-residing molecules.

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Investigating the role of membrane composition in lipid bilayer fluidity and transmembrane protein expression

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Transmembrane proteins play a critical role in a wide variety of cellular processes but are difficult to study due to their embedding in the lipid membrane. Therefore, there exists a need to develop a membrane platform that mimics the environment of a biological cell and has the capability to integrate and express functional, transmembrane proteins. One important design consideration in this system is membrane fluidity since changes in membrane fluidity can affect both membrane protein mobility and activity. To study membrane fluidity, we used a supported lipid bilayer (SLB) platform which allowed for control of the composition of the membrane environment while having many analytical and screening tool options. The first step was to understand how membrane composition affects SLB fluidity without proteins. I performed fluorescence recovery after photobleaching (FRAP) analysis on SLBs comprised of increasing mole fractions of cholesterol in artificial phospholipids of varying saturation. Then, cell-free expression techniques were used to integrate a transmembrane protein, the large-conductance mechanosensitive channel (MscL), into vesicles of the same varying cholesterol mol fractions and lipid saturation as the SLBs to analyze the effects of membrane composition on protein expression by means of fluorescence detection. So far, we have observed that increasing the mol fraction of cholesterol decreases SLB fluidity. Furthermore, increasing the degree of unsaturation, or increasing the amount of double bonds in the lipid tails, increases the fluidity in SLBs. For the protein studies, the data suggests that protein expression decreases with increasing mole fractions of cholesterol; however, increasing the degree of unsaturation increases expression. These results provide vital information for design applications in fields such as cell-mimetic biosensing and artificial cells.

Cell-Membrane Derived Supported Lipid Bilayers

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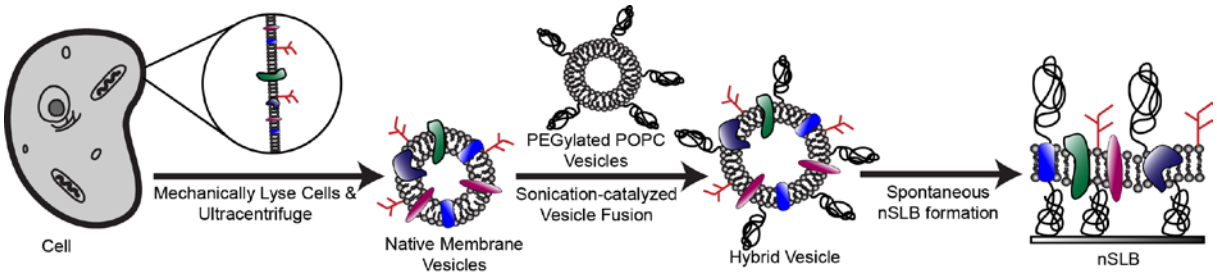
Supported lipid bilayers (SLBs) have a track record spanning more than two decades as model systems for the study of cellular membrane structure and function. While historically SLBs have been composed of simplified lipid mixtures, there has been a recent surge in compositionally complexity in order to better mimic the natural composition of the cellular membrane. To this end, a new class of SLBs derived directly from isolated native membrane preparations, here forth referred to as native-SLBs (nSLBs), have emerged. This new cell-free platform combines the accessibility and control of traditional model membrane systems with the complex natural composition of a chosen donor membrane, along with freedom from metabolic feedback loops and the need to keep the cells healthy.

I will present the current state-of-the-art in nSLB production strategies and how this platform can be applied to investigate a broad spectrum of biological questions ranging from host-pathogen interactions to protein-protein interactions occurring on the membrane surface.



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Direct measurement of current transduction across energy converting cell membranes using a novel membrane-on-a-chip system

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The light-driven reactions of photosynthesis as well as the mitochondrial electron transport chain are hosted within specialized lipid membranes containing a high fraction of redox-active lipids.[1] It is currently believed that diffusion of redox lipids and protein movement are responsible for in-plane charge transfer along these membranes.[2]
Using an electrolyte-gated field-effect-transistor (EG-FET) setup, we show that lipid bilayers containing redox-active lipids can conduct and sustain surprisingly high (mA) in-plane currents similar to organic semiconductors.
Our data suggest that charge transfer within cell walls hosting electron-transfer-chains is driven by self-assembling molecular redox-wires that effectively couple redox-proteins by simultaneous electron and proton hopping within a membrane.
Furthermore, we show a pressure dependence of the current by measuring in-plane conductivity along a monolayer of lipids in a Langmuir-Blodgett Trough.
This alters our understanding of the role of lipid membranes with far-reaching implications, suggesting for example that conducting membranes may be the first step in evolving the complex redox-machineries of life and that electrochemical membrane deterioration may play an important role in mitochondrial aging.
Furthermore, these self-assembling organic 2D-conductors could provide a basis to design more advanced self-assembling and adaptive bio-electronics.

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Preparation of pore-spanning membranes based on porous alumina for the investigation of the antimicrobial peptide lugdunin

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Among numerous other areas of research, model membranes have been extensively used in order to study the activity of antimicrobial peptides in bottom-up approaches. Here, we describe the use of lipid model membranes intending to study the mechanism of action of the novel cyclic antimicrobial peptide lugdunin. It was recently isolated from *Staphylococcus lugdunensis* and displayed promising antimicrobial activity against gram-positive pathogens.[1] Lugdunin is able to induce a proton translocation across lipid model and bacterial cell membranes resulting in the disruption of the membrane potential and subsequently in cell death.[2] However, its exact mechanism of action is still elusive.

We analyzed the structure and orientation of lugdunin in lipid multibilayers by means of ATR-FTIR spectroscopy. Using the amide I band as an indicator of secondary structural elements, we propose a preliminary model of its structure which features peptide nanotubes formed through a self-assembling process and stabilized by a tight hydrogen bonding network between individual peptide molecules. These tubes could act as membrane channels allowing transport of small molecules across lipid bilayers. In order to study the channel activity of lugdunin, we use an artificial system based on pore-spanning lipid bilayers to combine the merits of both solid-supported and black lipid membranes. These membranes exhibit long-term stability provided by the solid support but also allow the monitoring of ion transport across the freestanding membrane parts. Pore-spanning membranes are prepared on planar porous alumina chips fabricated in a two-step anodization process.[3] The resulting surfaces exhibit a hexagonally ordered array of pores with a size of around 60 nm and are primed for the formation of lipid membranes. Both the characterization of the porous substrate as well as the membrane formation process are monitored by electrochemical impedance spectroscopy.

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Looking closer at Ebola and Marburg Glycoprotein Pseudotypes

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Ebola and Marburg Virus are amongst the deadliest human pathogens and have caused several severe outbreaks in the past years. Both are classified BSL4 viruses and to study their binding behavior, pseudotypes which incorporate the glycoprotein (GP) can be used. Pseudotypes allow the investigation of virus-cell interactions in a BSL2 setting. Even though pseudotypes are commonly used, most groups employ them in infection assays looking at virus entry. Fluorescent pseudotypes have been developed and used for microscopy applications but are much less established. Within my project, I plan a detailed characterization of the physico-chemical characteristics of pseudotypes on a single particle level to gain insight about their heterogeneity. Specifically, I aim at addressing following questions: do all pseudotypes show the same binding behavior, do they carry the same amount of GPs, does the fluorescent tag interfere with the interactions we would like to study with these pseudotypes?
Observing these characteristics more closely can lead to improved protocols for pseudotype production and purification. This in turn will allow for improved experiments with more homogenous samples and an improved understanding of pseudotype production will equip us better for thorough interpretations of experimental results.

Modelling cell-free membrane protein expression

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Genes coding for membrane proteins constitute 30% of the genome and the majority of drugs target membrane-bound receptors. Thus, reconstitution of membrane proteins into biomimetic membranes is crucial to advance both knowledge about cell biology, and design of therapeutics. Traditional strategies for recombinant protein expression, purification, and integration into synthetic lipid membranes often do not provide satisfactory yields of functional reconstituted proteins. Here, cell-free membrane protein expression promises a standardized and engineerable alternative. However, currently only limited biophysical models of cell-free membrane protein expression exist, and the field still relies on empirical optimization of process parameters, with little predictability for a given protein sequence. In this work we developed a coarse-grained description of cell-free transcription and co-translational membrane protein folding activity. Our approach, consisting of experimental and computational analysis uncovers key parameters that determine the yield of folded membrane protein in cell-free systems, including a previously underappreciated role of the N-terminal domain for tethering of co-translational ribosome activity to the membrane surface.

Characterization of giant plasma membrane vesicles: Towards a native-like in vitro system

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Since decades, artificial membranes such as supported bilayers, have been applied to investigate the organization and function of individual components of biological membranes. However, if a high compositional complexity is required or membrane components of interest are not easily reconstituted, supported membranes derived from spreading synthetic vesicles fail to mimic the natural situation. An alternative are giant plasma membrane vesicles (GPMVs) derived from the plasma membrane of living cells.

Here, we show that GPMVs can be derived from HEK-293 cells by different vesiculation agents. We analyze the phase-behavior of GPMVs. We furthermore show that these GPMVs can be spread onto solid and porous support to produce pore-spanning plasma membranes.

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Supported Membranes for Phenotyping the Influence of Clinical Agents on Stem Cell Dynamics: Connecting Experiments and Theory

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As human hematopoietic stem cells are sustained in bone marrow, the mobilization of hHSC from bone marrow is a critical step for the treatment of leukemia. At present, many mobilization agents have been developed to disrupt or interfere cytoadhesion mediated via the heterophilic CXCR4-SDF1α axis or the homophilic N-cadherin axis, and induce migration to the peripheral blood stream. However, the mode of action for each agent on marrow/hHSC interface is still largely unknown. Especially, from the clinical viewpoint, it is very important to know whether the inhibition/perturbation of one molecule acts as a pure inhibitor or causes other side effects.

Different from widely used phenotypes obtained from immunofluorescence imaging of “fixed” hHSC, we have proposed to utilize characteristic quantities extracted from the dynamic behavior of live cells, called “dynamic phenotyping”. For this purpose, we utilize supported membranes functionalized with N-cadherin as the model of bone marrow microenvironment. In this study, we shed light on the effect of a clinical agent ADH-1, which is a cyclic penta-peptide aiming to block the homophilic N-cadherin axis, on the dynamic deformation and migration of hHSC. To translate the function of clinical agents into numerical indices, a simple but quantitative theoretical model of “crawling cells” is applied.

Our experimental data shows that ADH-1 significantly reduces the adhesion strength of hHSCs on the surface displaying N-cadherin, confirming the successful disruption of homophilic N-cadherin axis. We also found that dynamic phenotypes, such as mobility, diffusion constant, and persistence time of migration trajectory, are significantly modulated by ADH-1. Intriguingly, numerical simulations show that ADH-1 does not inflate the nonlinear coupling between deformation and motion. This indicates that ADH-1 acts as an antagonist reducing only the number of effective N-cadherin bonds, which is clearly different from other agents targeting CXCR4-SDF1α axis.

The combination of the label-free, quantitative in vitro experiments of primary hHSC on planar lipid model surfaces and the simple theoretical model opens a large potential to numerically identify the differential effects of clinical drugs on dynamic phenotypes of primary cells from leukemia patients.

Binding kinetics of human noroviruses to histo-blood group antigens determined using plasma membrane mimics

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Human norovirus (hNoV) infections are a leading cause of gastroenteritis worldwide. However, the study of hNoVs has long been hampered in lack of adequate in-vitro culture systems, and most knowledge limited to binding studies revealing that histo-blood group-antigens (HBGAs), glycosphingolipids found in intestinal cell membranes, are involved in the infectious cycle. Recently, the development of a culture system based on human intestinal enteroids (HIEs) has facilitated the study of norovirus infections ex-vivo. This culture system allows us to test how membrane HBGA expression correlates to infectious behavior. We characterized lipid extracts from seven HIE cultures with varying HBGA expression. We then used these extracts to make model membranes to study the binding kinetics of norovirus virus-like particles to these membranes using total internal reflection fluorescence microscopy. This allows us to study the dynamics of virus-membrane interactions to gain knowledge on the attachment and detachment behavior of the virus[1]. We see that the virus has higher affinity for membranes of susceptible cells, but even membranes from non-susceptible cells show intermediate binding. We also observe that dissociation correlates better with susceptibility to infection than association[2]. This project demonstrates the importance of host-membrane interactions in norovirus infections and the potential of membrane mimics in studying hNoVs.

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Effects of arginine derivatives and oligopeptides on negatively charged model membranes

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Cell-penetrating peptides (CPPs) are often rich in arginine. They are able to cross cell membranes and carry other molecules into cells without being cytotoxic. The mechanisms by which they accomplish this are still controversial, although endocytotic pathways or spontaneous entry mechanisms have both been proposed. This work takes a bottom-up approach to the role of arginine in CPPs, by comparing an arginine side chain mimic with arginine oligopeptides of different length, with respect to their influence on membrane model systems of various lipid compositions. Simulations as well as breakthrough force spectroscopy experiments, as well as microscopic observation, showed that the arginine sidechain mimic increased likelihood of pore formation and caused membrane dissolution in negatively charged membranes. This destabilisation of membranes was not found for longer oligo-arginines, for any lipid composition or peptide concentration, neither in breakthrough force spectroscopy nor by liposome leakage assays. However, hexa-arginine and longer peptides were able to induce membrane stack formation on negatively charged solid-supported lipid bilayers at low peptide concentration. It was also found that arginine peptides of any length were able to cause stack formation in these bilayer compositions, provided that peptide concentration was high enough. We also demonstrated that behaviour at the membrane stack interface is dependent on peptide length, lipid headgroup size and lipid chain saturation. Our findings combined with literature mention of lamellarisation of model membranes and cell membranes caused by arginine-rich peptides suggest that membrane stack formation and lamellarisation is possibly an important aspect of oligoarginine CPPs' mode of action. However, longer arginine peptides did not show signs of overall membrane destabilization, neither experimentally, nor in simulations, even though they did stabilize pores under specific conditions.

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SARS-CoV-2 surrogates for binding and inhibition studies

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Most of the recent studies on the binding activity of the SARS-CoV-2 Spike protein to ACE-2 are performed on the receptor binding domain only. But other parts of the Spike protein can be involved in intra- and intermolecular interactions with inhibitors and other attachment factors as well. To be able to characterize interactions between different kinds of molecules that potentially interfere with the virus-cell binding, we incorporate the full length transmembrane form of the Spike protein into liposomes. We demonstrated successful incorporation as well as binding function using recombinant proteins and antibodies. To address different mutations, we establish an expression and purification protocol for different full-length spike variants. S-protein containing proteoliposomes can serve as a surrogate virus with defined composition for interaction studies on living cells or (hybrid-) supported lipid bilayers containing native cell membrane components. In a TIRF-based equilibrium binding assay, we can track single surrogate viruses binding to ACE-2 transfected HEK293 cell membrane preparations and probe the binding and inhibition. When prepared as a planar lipid bilayer, we can perform force spectroscopy on the full length Spike protein using an ACE-2 functionalized AFM cantilever.

Supported Membranes for Discriminating Cancer Progression of Human Gastric Cells

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The structure of multicellular tissues becomes disordered according to the cancer progression. Cells in cancerous tissues show wider variety in size and shape (pleomorphism). Although these static, phenomenological features are utilized for cancer staging in the field of pathology, little is known about the mechanism how the pleomorphism and ordering of cells in tissues are correlated with dynamics of single cells. In this research we describe human gastric cells at different cancer grades as self-propelled deformable particles and aim to reveal the relation between their active deformation, adhesion (frictional coupling to the environment), and migratory motion. To model the interaction between gastric cells and extracellular environments, we utilized the supported membranes on the glass substrate and functionalized the surface with laminin E8 fragment. We adjusted the molar fraction of lipid composition to control the average distance of laminin E8 fragment. Human gastric cells from four different cancer stages were seeded, and the active deformation of the adhesion zone was recorded with label-free, reflection interference contrast microscopy (RICM). We found that well differentiated cancer cells hardly migrate nor deform, while poorly differentiated cancer cells actively deform and migrate in a directional manner. We also performed the western blot and found that the expression level of phosphorylated Rac1 of poorly differentiated cancer cells are significantly higher than that of well differentiated cancer cells. Our approach to quantify the deformation and migration of cancer cells on finely defined supported membrane, as “dynamic phenotyping”, could be applicable for cancer staging as a compliment of conventional pathological methods using “fixed” images of immunostained tissues.

References

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