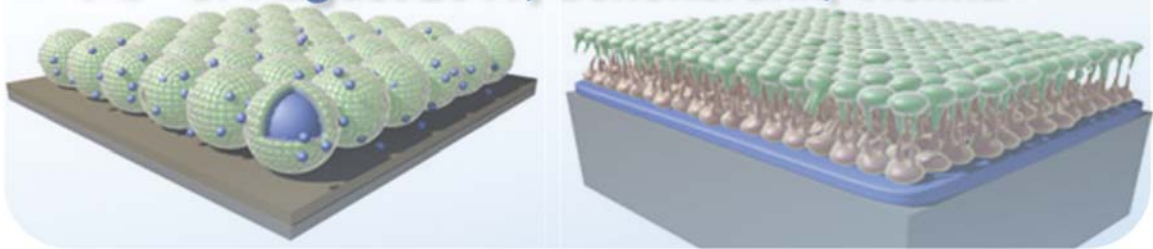


TethMem 2017

7-9th of August 2017, Schönbrunn, Vienna



Abstract booklet

Invited speakers

Cell-Surface Mimics to Study Virus-Membrane Interactions

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Viruses are small pathogenic particles that rely on hijacking a cellular host to multiply and spread. Throughout their life cycle, viruses will therefore on several occasions, come into contact with cellular membranes. Understanding the mechanisms by which virus particles recognize and cross these lipid membranes is of central importance to the development of new antiviral therapies, new drug delivery vectors and new diagnostic tools.

In our work, we take advantage of artificial lipid bilayers to mimic *in vitro* the basic properties of the cell membrane. Using these minimal models we gain insights into the mechanisms by which viral pathogens interact with the cell's barriers and cross them.

In a first example, we focus on the interaction between norovirus virus-like particle and glycolipid-containing membranes and investigate the role of ligands mobility and ligand clustering in modulating the affinity of the virus particle to the membrane. In a second example, we concentrate on the role of Influenza's matrix protein in virus budding and search for mechanisms by which the protein can induce membrane deformations using giant unilamellar vesicles. In a last example we use model membranes carrying glycosaminoglycans, to probe glycoprotein (gC)-mediated attachment and release of the herpes simplex virus. We focus on the potential role of the viral protein glycosylation in modulating the interaction of the virus particles with the cell surface.

Taken together, these examples illustrate the potential of artificial cell membrane mimics in the study of processes occurring at the surface of a cell and demonstrate how such biophysical data can complement more classical cell-biology experiments.

Generating Homogeneous Semisynthetic Glycoproteins

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At least 50% of all human proteins are predicted to experience one or more posttranslational modification (PTM) during their life cycle.¹ These PTMs can result from enzymatic or non-enzymatic reactions and enzymatic PTMs are well-known for being involved in regulating many cellular events such as gene expression, intracellular and extracellular signal transduction, protein-protein as well as cell-cell interactions.^{2,3} To study a major class of enzymatic PTMs, we recently established a strategy for the preparation of homogeneously glycosylated peptides. The tumor marker MUC1, a membrane-attached protein abundantly *O*-glycosylated in its extracellular domain, was chosen as the initial synthetic target. Chemically synthesized mucin peptides are conjugated to a photocleavable ligation auxiliary,^{4,5} obtained via multistep synthesis, that supports NCL and carries a PEG polymer. This facilitates effective enzymatic glycosylation and recovery of the resulting glycopeptides without the need for chromatographic steps.⁵ Modified peptides are linked via auxiliary-mediated NCL and the ligated products are recovered as non-protected glycopeptides after UV irradiation.

We currently expand our approach to sequential ligation reactions and to other protein targets to broaden the applicability of this methodology.

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Mobility-Based Probing of Virus-Lipid and Lipid-Lipid Interactions

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Life depends on complex networks of precisely fine-tuned interactions between different types of biological molecules. Many of these processes are orchestrated by a thin lipid bilayer, 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. Membrane functions often depend on the local, nm-scaled bilayer assembly, which is, since being far below the diffraction limit of optical microscopy, challenging to characterize under physiological conditions. This talk presents two examples, in which a supported lipid bilayer (SLB) is locally modified by formation of lipid complexes (formed either by interaction with virus proteins or with divalent calcium ions), whose properties are probed based on measuring their mobility within the SLB.

The first example focusses on multivalent virus-lipid interactions, which are typically weak in the sense that a single virus has to bind to multiple lipid receptors in parallel to ensure firm bilayer attachment. Quantification of the interaction valency (*i.e.*, the number of receptors involved in the multivalent virus attachment) with single-virus resolution is demanding, which strongly limits our knowledge about virus entry and egress due to the strong dependence of valency on the virus off-rate. Motivated by our previous work on SLB-tethered liposomes,^{1,2} we estimate here the valency of single influenza A/X31 viruses (interacting with the ganglioside GD1a incorporated in the SLB) by measuring virus mobility using total internal reflection fluorescence microscopy (TIRF) and single particle tracking (SPT), allowing the complex off-rate distribution to be deconvoluted from valency effects. Application of this approach to quantify changes of multivalent virus-lipid interactions caused by addition of virus inhibitors will be discussed.

A second example focusses on salt-mediated formation of lipid complexes, a process that is well established from molecular simulations, but has not yet been experimentally studied with single-complex resolution. By combining fluorescence correlation spectroscopy (FCS) with nanoplasmonic antennas that

¹ Block, S. et al. "Quantification of Multivalent Interactions by Tracking Single Biological Nanoparticle Mobility on a Lipid Membrane." (2016) **Nano Letters** DOI: 10.1021/acs.nanolett.6b01511.

² Block, S. et al. "Two-Dimensional Flow Nanometry of Biological Nanoparticles for Accurate Determination of Their Size and Emission Intensity" (2016) **Nature Communications**; DOI: 10.1038/ncomms12956

shrink the FCS probe volume down to the ~20 nm length-scale, we probe the mobility of single dye-conjugated lipids on the nm-scale with high temporal and spatial resolution and show that these lipids diffuse either as single entities or as complexes in the presence of calcium ions. Removal of Ca²⁺ almost completely removes the complex contribution, in agreement with previous theoretical predications on the role of Ca²⁺ in mediating interactions between zwitterionic lipids. Further application of antenna-enhanced FCS to stimuli-induced lipid clustering (*e.g.*, by salts, virus proteins) will be discussed.

Tethered Membranes, Vesicles and Viruses

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We have prepared DNA-lipid conjugates that can be used to create a variety of more complex membrane architectures such as tethered vesicles and tethered bilayer patches that hold the membrane away from the solid support. These architectures and conjugates can also be used to simulate membrane fusion, a central process in cells. This strategy has recently been extended to enveloped viruses that bind to a receptor on the host membrane to initiate infection and then fuse in endosomes. We demonstrate this method by binding influenza virus to target vesicles using DNA-lipid hybridization and then measuring the rates of individual viral fusion events using fluorescence microscopy – single virus fusion kinetics are found to be independent of receptor binding [1]. This approach is now being used to study viruses where the receptor is unknown or challenging to reconstitute, preventing single-virus fusion experiments (e.g. HIV, Ebola and Zika). Many challenges were overcome to implement this strategy for the Zika virus but we now have reliable and interesting results for the pH dependence of the single virus fusion kinetics and efficiency of fusion. This work is a collaboration with Peter Kasson's group at the University of Virginia.

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Emerging Approaches to Fabricate Supported Lipid Bilayers:

Moving Beyond Vesicles

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Controlled self-assembly of model lipid membranes at solid-liquid interfaces opens the door to a wide range of applications across membrane biophysics, biotechnology and medicine. Recently, we developed the solvent-assisted lipid bilayer (SALB) method to form supported lipid bilayers at interfaces. A key feature of the SALB method is that the supported bilayers form in an energetically favored scenario, enabling bilayer fabrication on formerly intractable surfaces like gold. Moreover, the process does not require pre-formed precursor vesicles allowing for arbitrary compositions. Aided by lipid-substrate interactions, surface-adsorbed lipids in organic solvent are rapidly converted into lamellar phase, supported bilayer islands upon addition of aqueous buffer solution. Lipid species in the aqueous solution may attach to the bilayer islands and subsequently rupture to form a contiguous, supported lipid bilayer. Owing to the technically minimal requirements of solvent-assisted lipid self-assembly, we have also developed on-chip lipid microfluidics that take advantage of the SALB method to form miniaturized biomembranes with a rich complexity of components reminiscent of natural cell membranes and that can be utilized on a variety of substrates with different atomic compositions and nanostructure morphologies. In addition to the SALB method, additional innovations have enabled streamlined fabrication of supported lipid bilayers in fully aqueous conditions by utilizing mixtures of phospholipids with minimal preparation requirements. In turn, all these capabilities should further enable academic investigations related to membrane biophysics and pharmaceutical drug development efforts towards high-throughput lipid membrane functional assays.

Molecularly Complete Supported Lipid Bilayers Bring Bioanalytical Devices Closer to *in vivo* Architectures

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The cell membrane is a material with many important biological functions. At first glance, it is a material that defines the interior of the cell from its exterior surroundings. Looking deeper, it is a barrier that protects the cell from pathogens and disrupting agents, but also must allow information to pass in and out for cellular communication. Going further inside the cell, we find that membranes are also used to compartmentalize operations of the cell essential for its health. Integral to all these important duties are the lipids and proteins that make up the membrane and define its physico-chemical properties. Because of the importance of cellular processes to human health, many fundamental studies are aimed at understanding these roles and features of the membrane. And because of the advances of biomolecular engineering, many biotechnology applications are directed towards creating novel devices that capture Mother Nature's engineered designs for biosensing, protein engineering of biological materials, and bioanalytical devices. Thus it is necessary to find ways to interrogate cell membranes and use them in devices that are both compatible with abiotic surfaces and analytical tools, and maintain enough complexity in the system so that the results reflect the *in vivo* situation yet are reasonably simple to decouple phenomena to interpret those results.

Many surface-analytical tools require a planar format, *i.e.*, microscopy, surface plasmon resonance, quartz crystal microbalance, to name a few. Supported lipid bilayers (SLBs) are materials that generally integrate well with many surface characterization techniques and have been used extensively to study membrane processes. However, SLB have suffered from a number of drawbacks like the loss of fluidity of protein components and the loss of native lipid interactions during reconstitution. Both of these features are important for applications that the Daniel laboratory has significant interest: host-pathogen interactions and viral infection. Our goal was to create planar supported bilayers that capture as many features of the host cell as possible in order to use these materials to carry out studies of host-pathogen interactions. We developed a method for the delivery of proteins to SLBs via cell blebs. Cell blebs are sections of the cell membrane that bud off into a proteoliposome during local detachment of the membrane from the cytoskeleton. Native membrane travels with the proteins to the SLB, protecting the

protein, so that it retains its activity and function in the planar platform. In this talk, I will describe our process to generate several different types of proteinaceous planar membranes and characterization of their properties. Next, I will describe the use of these materials in the study of virus-host membrane fusion and delivery of viral genomes across the host membrane. Finally, I will describe work we have done to understand anti-bacterial peptide interaction with planar *E. coli* membranes.

AFM in Supported Bilayer Lipid Membranes

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This presentation will describe our recent studies all the application of atomic force microscopy for the study of: i) membrane domains, ii) the role of micro-domains in ABP localisation and iii) peptide membrane interactions.

Lipid phase heterogeneity and dynamics in cellular membranes, in the form of micro-domains or 'rafts' is fundamental to many cellular processes, yet a full description of the driving forces at play remains unknown. A mechanism proposed to regulate such heterogeneities is critical behavior, whereby, at a critical composition and temperature, the coexistence of lipid phases is highly dynamic and sensitive to perturbation. Whilst previously the dynamics of critical phase fluctuations had to be extrapolated down over several orders of magnitude to that of the expected physiological domain sizes and lifetimes, here we provide direct observation and full characterization of short-lived nanodomain morphology and dynamics at relevant spatial and temporal scales.

Interactions between the actin cytoskeleton, transmembrane proteins and lipids in the cell membrane have been suggested to govern to the behavior of lipid nanodomains in cellular membranes. Here we create model systems composed of phase separated supported lipid bilayers (DOPC-Sphingomyelin-Cholesterol) and the actin binding transmembrane protein ponticulín from *Dictyostelium discoideum*. Atomic force microscopy studies reveal ponticulín preferentially locates to the liquid disordered phase at interface between the liquid ordered and liquid disordered domains via a glycosylphosphatidylinositol (GPI) anchor. This localization increases the area of liquid ordered domain and reduces the line tension between the two phases. Further we show the binding of G-actin monomers over time to individual ponticulín proteins with the greatest binding at domain boundaries. This localization is important biologically as it provides a potential direct route to lipid organization through the polymerization of actin via a single membrane protein, an important step in the understanding of mechanisms behind lipid rafts.

Understanding the mechanism of action of antimicrobial peptides (AMP) is beneficial for the development of this class of antimicrobials. Recently, high resolution techniques such as atomic force microscopy (AFM) have been utilised to determine AMP mechanism of action on planar lipid bilayers and live bacteria. Here we present the biophysical characterisation of Smp43, an AMP with a helical-hinge-

helical topology isolated from the venom of the North African scorpion *Scorpio maurus palmatus* and previously shown to have a favourable therapeutic index. Utilising high speed AFM we detail the attack of an AMP on both prototypical prokaryotic (DOPC/DOPG) and eukaryotic (DOPC/DOPE) planar lipid bilayers in real time. Supported by 2D diffusion models and further experimental data from liposome leakage assays and quartz crystal microbalance-dissipation (QCM-D) analysis, we propose that Smp43 disrupts these membranes via a common mechanism which we have termed 'diffusion limited disruption' that encompasses elements of both the carpet model and the expanding pore mechanism.

Structural Characterization of Biomembranes: the Role and Impact of Composition

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The structural characterization of lipid bilayers presents fundamental interest both in physics, for the study of thin fluctuating soft layers, and in biology, for the understanding of the function of biological membranes. This represents still a challenge: performing measurements on few nanometer thick, soft, visco-elastic and dynamic systems is close to the limits of the available tools and methods.

Neutron scattering techniques are rapidly developing for these studies. Since many biological processes occur at interfaces, the possibility of using neutron reflection to study structural and kinetic aspects of model as well as real biological systems is of considerable interest.

The talk will review some recent progress in the field and provide perspectives for future developments. It will aim at highlighting neutron reflectometry as a versatile method to tackle questions dealing with the understanding and function of biomembranes and their components. The impact of composition on the structure will be highlighted.

The most effective use of neutron reflection involves extensive deuterium substitution and this is becoming more and more an available option in biological systems in general and lipid bilayers in particular [1]. The use of deuterated lipid extracts presents relevant differences both with the hydrogenated counterpart and with synthetic systems [2,3].

The talk will review some progress made in the last few years by using neutron scattering at the ILL in the structural characterization of biomembrane systems, efforts to build and characterize more and more complex systems [2-4], the impact in health related studies [4-6], and will provide perspectives for future developments [7].

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Optofluidics Characterization of Nanoparticle Interaction with Supported Lipid Membranes

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Biological nanoparticles such as extracellular vesicles and exosomes are generating a rapidly growing interest due to the key roles they play in various biological processes and because of their potential use as biomarkers in clinical diagnostics and as efficient carriers in drug-delivery and gene-therapy applications. A large set of tools with single-nanoparticle sensitivity is now available, to which we recently contributed a concept that enables simultaneous fluorescent and scattering-based label-free imaging of surface-bound biological nanoparticles [1]. Examples will be shown that illustrates the use of scattering microscopy to i) investigate supported lipid bilayer formation, ii) label-free measurements of protein binding to individual liposomes and iii) characterize DLVO-controlled non-specific interactions at cell-membrane mimics.[2] Further, with this setup, the fluorescence and scattering intensity of surface-bound nanoparticles can be very precisely determined, but their individual size remains unknown. The size of individual biological nanoparticles can instead be determined by tracking their 3D motion in a bulk solution, using e.g. nanoparticle tracking analysis (NTA). However, due to the random motion of nanoparticles through the illumination volume, NTA does not offer reliable information about their individual scattering and fluorescence intensity. Hence, either the size or emission intensity of nanoparticles can be determined, not both. Since the combination of size and content is decisive for many functions tailored into nanoparticles, this is a severe analytical limitation. By replacing water as the mobile phase, as used in NTA, for a two dimensional fluid supported lipid bilayer, to which biological nanoparticles are directly anchored and imaged, we have developed a new means to simultaneously determine both nanoparticle size and fluorescence / scattering intensity,[3] which may potentially offer flow-cytometry-like sorting based on distinct features of individual nanoparticles. This 2D flow nanometry concept will be discussed in the context of improved characterization of individual nanoparticles of diagnostic and therapeutic significance.

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Membranes in Confined Geometry - How External Forces and Constraints Reveal the Mechanism of Biological Processes

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Small scales permit to answer questions raised by biology that are otherwise not addressable. Among them ultraweak noncovalent intermolecular interactions are of particular interest since they play a pivotal role in many biological processes such as cell adhesion or immunology, where the overall binding strength is controlled through bond association and dissociation dynamics as well as the cooperative action of many parallel bonds. In this context, carbohydrate-carbohydrate interactions are probably the most enigmatic ones allowing individual cells to reversibly enter the multicellular state and to tell apart self and nonself cells. Using colloidal probe microscopy, we investigated the kinetics and thermodynamics of small homomeric Lewis X-Lewis X ensembles formed in the contact zone of a membrane-coated colloidal probe and a solid supported membrane ensuring minimal nonspecific background interactions. Applying a contact model, we could estimate the free binding energy of the formed adhesion cluster as a function of dwell time and thereby determine the precise size of the contact zone, the number of participating bonds, and the intrinsic rates of association and dissociation in the presence of calcium ions.

Apart from mere interaction between membranes, fusion of lipid bilayers is one of the most intricate processes membranes are involved in. Membrane merging is usually prevented by large energy barriers arising from removal of the hydration shell, formation of highly curved structures and eventually fusion pore widening. Here, we measured the force-dependent lifetime of fusion intermediates by means of membrane coated silica spheres serving as force probes in contact with supported lipid bilayers. Analysis of time traces obtained from force clamp experiments allowed us to unequivocally assign different membrane states during the SNARE (soluble N-ethylmaleimide-sensitive-factor attachment receptor)-mediated fusion process to steps in deflection of the cantilever carrying the membrane-coated bead. We identified the removal of the hydration shell and the initial interaction of opposing lipids to be the main energy barrier towards fusion in the presence of SNAREs. Since external normal forces are absent in native systems we also addressed the impact of orthogonal forces, i.e., stretching of the bilayer. For this purpose, isolated patches of planar bilayers containing syntaxin 1 and preassembled SNAP 25 (Δ N-complex) were formed from giant unilamellar vesicles and deposited on a dilatable PDMS sheet, which is part of a

millifluidic chamber allowing to adjust lateral tension in bilayer patches. Docking, hemifusion and fusion of large unilamellar vesicles (LUVs) functionalized with synaptobrevin were followed by fluorescence microscopy. We found that fusion efficiency increases considerably with lateral tension and might indeed be responsible for the gap in time scales between fusion in synapses and model systems.

From Solid Supported Lipid Bilayers to Infection Responsive Wound Dressings: a Journey in Using and Understanding Lipid Membranes

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The team at Bath have developed a sensor for bacterial virulence factors which are indicative of wound infection. The system is a dispersion of phospholipid vesicles dispersed in a hydrogel matrix. Bacterial secretion toxins diffuse into the agarose and lyse the vesicles, releasing self-quenched fluorescent dye which becomes diluted and 'switched on' by dilution in the hydrogel. The system is at an early prototype stage and data relating to its ex-vivo performance in a four-hospital clinical study will be presented. The rest of the talk will discuss our journey to understand which bacterial virulence factors were actually responsible for vesicle lysis, including early work on tethered planar bilayer membranes and suspended lipid vesicles, including some of the various mistakes we made along the way.

Using hydrodynamic trapping to study how membrane-anchored molecules interact

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The lateral interaction between membrane-associated molecules is an important property to better understand the behavior of many biological processes, such as the formation of protein aggregates and receptor signaling. Hydrodynamic trapping is a recently-developed technique that can be used to study these interactions in more detail [1]. The hydrodynamic trap uses the liquid flow through a micropipette to locally move and accumulate molecules coupled to a supported lipid bilayer (SLB). The liquid flow field outside the pipette is well defined and can be converted into a hydrodynamic force per molecule on the SLB [2], which can be related to the accumulation of proteins at different positions in the trap by fluorescence microscopy.

I will here show some examples of how this method can be used to manipulate molecules on SLBs and to obtain physicochemical data of the interaction between membrane-anchored molecules. I will in particular present how the hydrodynamic trap can be used to estimate the intermolecular forces between membrane-anchored molecules at different surface coverage as well as how the molecules orient on the lipid bilayer.

Multiresponsive Behavior of Membranes and Vesicles

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Vesicles formed by lipid bilayers are able to respond to different environments by changing both their morphology and their local molecular composition. Prominent examples for morphological transformations are budding and spontaneous tubulation [1], membrane engulfment of nanoparticles [2], as well as wetting transitions of droplet-vesicle systems [3], all of which can be coupled to the formation of intramembrane domains. This multiresponsive behavior arises from the interplay of curvature elasticity, membrane adhesion, and phase separations. Recently, new experimental methods have been developed to produce giant vesicles with an increased robustness against mechanical perturbation [4,5]. Combining these experimental methods with our quantitative understanding of the membrane behavior will enable us to construct useful modules for synthetic biology.

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Tethered Membranes and Neutron Reflection: Filling a 'Blind Spot' in Structural Biology

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Surface-sensitive scattering, a.k.a reflectometry, of x-rays or neutrons has long been used to characterize the molecular-scale properties of lipid monolayers and bilayers at aqueous surfaces and interfaces,^{1,2} but has not been in the standard toolbox for structural biology due to several limitations. Most seriously, the intrinsic resolution of reflectometry in its application to biological membranes is far less than that of more established methods such as x-ray crystallography or NMR spectroscopy.³ Yet, integrative modeling strategies that combine multiple sources of information have leveled this playground, thus unleashing the intrinsic strengths of reflectometry to provide structures of protein complexes on fluid bilayers that closely resemble biomembranes.

We have developed a comprehensive infrastructure for biological reflectometry that includes sample environment, optimized measurement capabilities, data modeling and a rigorous assessment of the significance of results.⁴ In combination with integrative modeling, these capabilities make neutron reflectometry remarkably sensitive, and new instrumentation is soon expected to shorten data acquisition times by orders of magnitude.⁵ We demonstrated that protein complexes with biomimetic membranes can be structurally assessed at the single-residue level if the structure of a protein is known from other sources.^{6,7} In addition, the protein orientation on the bilayer can be determined with high precision.^{8,9} Because radiation damage is not an issue in neutron scattering, conformational changes of membrane-associated proteins can be triggered by external cues, and structurally characterized.¹⁰ Finally, protein deuteration is a powerful means to distinguish proteins in multi-protein complexes on the bilayer.¹¹ These capabilities provide unique, and otherwise unattainable, insights into membrane protein function. Examples from current work on relevant problems in biology and biomedicine will be discussed.

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Characterization of Single Proteins Using a Lipid-Coated Nanopore

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This talk describes the use of electrolyte-filled nanopores with self-assembled lipid membrane coatings to determine, simultaneously and in real time, the shape, volume, charge, rotational diffusion coefficient, and dipole moment of individual proteins and protein complexes in solution. The talk introduces the main concepts for a quantitative understanding and analysis of modulations in ionic current that arise from rotational dynamics of single proteins as they move through the electric field inside a nanopore. The resulting multi-parametric information raises the possibility to characterize, identify, and count individual proteins and protein complexes in a mixture with implications for protein folding studies, biomarker detection, and routine protein analysis.

Micropatterned Model Membrane to Elucidate the Functional Roles of Lipid Micro-Domains in the Signal Transduction Cascade

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Lipid rafts in the cell membrane are believed to affect various membrane functions, including the signaling by G protein coupled receptors (GPCRs). However, the regulatory roles of lipid rafts in membrane functions are still poorly understood, partially owing to the lack of methods to quantitatively evaluate the affinity of membrane proteins to lipid raft (raftophilicity). We report on a new methodology to gauge the raftophilicity of membrane proteins by using a patterned model membrane. We generated a substrate supported planar lipid bilayer having patterned regions of liquid ordered (L_o) and liquid disordered (L_d) membrane domains. A representative GPCR in vertebrate photoreceptor, i.e. rhodopsin (Rh), was reconstituted into the patterned membrane and its distribution in L_o/L_d regions was evaluated. Rh had a heightened partitioning in L_o domains upon photoactivation followed with the complexation with G protein (transducin), implying that the signal transduction by Rh is modulated by lipid rafts. We determined the raftophilicities of some major membrane proteins involved in the phototransduction. The results are discussed in the light of biochemical, *in vivo*, and *in silico* studies, which demonstrates the utility of quantitative raftophilicity data obtained from the *in vitro* platform of patterned model membrane. We also report the recently developed techniques to combine patterned model membranes with hydrophilic polymer brushes and polydimethylsiloxan (PDMS) microstructures, which should realize a robust and scalable platform for systematically and quantitatively studying the functional roles of the supramolecular architectures in biological membranes.

Functional membranes BASED ON Protein-polymer membranes

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New concepts that combine multifunctional compounds with stable, safe carriers or membranes are on focus in a variety of domains, such as medicine, catalysis, environmental science, and technology. Suitable amphiphilic block copolymers are ideal candidates for applications because they can self-assemble into supramolecular assemblies, such as compartments, or planar membranes with a superior stability, and robustness compared to the lipid based membranes.¹ By combining these polymeric membranes with suitable biological entities, e.g., by incorporating integral membrane proteins or by enzyme encapsulation in polymer compartments it is possible to provide well-defined functions, such as molecular recognition, cooperation, and catalytic activity.¹

Here, we present distinct spaces for desired reactions at the nanometer scale based on protein-polymer assemblies as compartments with triggered activity or as bilayers on solid support.^{2,3} Biopores/channel proteins inserted into the polymer membrane selectively control the exchange of substrates and products with the environment of compartments, resulting in development of stimuli-responsive compartments, which preserve their architecture, while allowing specific *in situ* reactions. Channel proteins/biopores inserted in solid supported membranes serve for a controlled transport of ions or molecules through the synthetic membrane. Protein-decorated synthetic membranes represent smart hybrid systems, which open new avenues in various domains, as for example protein therapy or biosensing approaches.

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Mechanisms of Broad-Spectrum Antiviral Potential of Membrane-Targeting Compounds

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The rapid rise in the number of emerging and re-emerging infectious diseases due to viral pathogens underscores the need for therapeutic approaches that target large classes of viruses using single drugs. To be effective, such “one for many” broad-spectrum antivirals must either interfere with the shared steps of biophysical or biochemical processes of viral entry (i.e., fusion inhibiting mechanisms) or disable disparate virions by targeting their common structural components (i.e., virocidal mechanisms). An obvious target, in this regard, is the lipid bilayer component of the viral envelope. Although derived from the host cell, the viral membrane differs from cellular membranes in many physical-chemical properties including chemical composition, curvature, and lateral fluidity as well as the absence of biogenic reparative capacity. These attributes are shared across a variety of different viruses rendering the viral membrane a discrete and susceptible target for developing broad-spectrum antivirals.

This talk presents broad-spectrum antiviral potential of two distinctly different classes of membrane-active antiviral drug candidates. *First*, using model membranes we show how a peptide derived from the hepatitis C virus nonstructural protein NS5A, acquires a broad-spectrum virocidal activity by disrupting model viral membranes in a size- and composition-dependent manner. *Second*, we demonstrate the ability of a class of synthetic membrane-active amphiphiles in stabilizing positive curvatures, which act to inhibit viral entry.

The Protein Translocation Channel at Work

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Many proteins need to be transported across the membrane of the endoplasmic reticulum (ER) or across the bacterial plasma membrane during or after their biosynthesis. A protein complex that is conserved throughout all kingdoms of life –the Sec61p complex in eukaryotes and the SecYEG complex in bacteria and archaea – represents a major transporting pathway. It acts as a transmembrane pore for secretory proteins, and allows hydrophobic segments of membrane proteins to pass through its lateral gates into the lipid phase. This complex cooperates either with ribosomes or motor proteins, as well as with a variety of other proteins that are required for different steps in the translocation process. We have now obtained mechanistic insight into the molecular details of the protein transport process by having designed minimalistic systems for protein translocation with purified and reconstituted SecYEC complexes at their core (1). We found that the assembly of the translocation machinery with the motor protein SecA occurs in three steps: (i) SecA binds to the lipid bilayer and migrates along its surface as visualized by fluorescence correlation spectroscopy; (ii) It anchors at SecYEG's negatively charged annular lipids as revealed by luminescence resonance energy transfer (LRET), and (iii) SecA binds to SecYEG as visualized by high speed atomic force microscopy of single complexes in suspended membranes (2). We also investigated how the translocon maintains the barrier to small molecules during the translocation of large proteins. We were able to rule out two hypotheses that (i) intrinsic anion selectivity or (ii) spontaneous engulfment of the translocating peptide by a hydrophobic ring of amino acids is sufficient to prevent ion leakage (3). Instead, our electrophysiological experiments revealed the importance of SecYEG's voltage driven conformational changes for barrier preservation (4).

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Mechanosensitive Protein Assemblies

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"Membrane-associated proteins reorganise and assemble 'on demand' in response to stimuli from the cell environment. A remarkable example of such a behaviour are mechanosensitive channels (MSCs): oligomeric transmembrane proteins that regulate the osmotic pressure acting on bacterial cellular walls. Although the crystal structures of several single MSCs have been thoroughly investigated, both in experiments and in computer simulations, the physical mechanisms of gating of a collection of MSCs on biologically relevant time- and length-scales are not well understood. Mesoscopic coarse-grained simulations can be of great help here, enabling us to make experimentally testable predictions. Here we investigate the minimal design requirements of mechanosensitive channels needed for efficient gating, as well as collective gating properties of a population of MSCs. Our results will be of importance for understanding the mechanisms of mechanical sensing in living organisms, and for rational design of synthetic mechanical nanosensors."

The Enigmatic Role of Lipids in Membrane Protein Interactions

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The organization and dynamics of proteins and lipids in the plasma membrane, and their role in membrane functionality, have been subject of a long-lasting debate. Specifically, it is unclear to what extent membrane proteins are affected by their immediate lipid and protein environment and vice versa. To study protein interactions directly in the live cell plasma membrane we developed a method for in vivo micropatterning. Cells transfected with a fluorescent fusion protein (“prey”) are grown on micropatterned surfaces functionalized with specific antibodies to the extracellular domain of a membrane protein (“bait”); the fluorescence copatterning is used as readout for the bait-prey interaction. Specifically, we used the micropatterning approach in combination with single molecule tracking to quantify the influence of a glycosylphosphatidylinositol- (GPI-) anchored protein – a typical marker of liquid ordered phases – on its molecular environment directly in the live cell plasma membrane (1). We found that the captured proteins merely acted as bulky obstacles to the diffusion of other membrane constituents, but did not influence their membrane environment over distances past their actual physical size. Our results imply that the outer leaflet of the plasma membrane is in a homogenous single phase regime under physiological conditions, ruling out the presence of “raft” phases associated with lipid-anchored proteins.

Next, we applied single molecule tracking combined with brightness analysis to study the oligomerization of membrane proteins. For a GPI-anchored GFP we observed cholesterol-dependent homodimerization (2). Similar lipid-dependent oligomerization was observed for the Serotonin transporter (SERT) (3). Surprisingly, oligomerization kinetics were completely different at the plasma membrane versus the ER membrane, indicating that oligomerization is equilibrated at subcellular membranes, but kinetically trapped at the plasma membrane.

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When Structures Relate to Function - Bioarchitectures in the Context of Cells

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Structural – mediated communication between synthetic materials and living cells is still one of the magic boxes in sciences. One major player has been identified: The cellular membrane is involved in this ‚communication‘ at very many levels. At transcriptional and translational level, we have dealt with two very interesting examples of cellular regulation based on presence/absence of structural components in the context of the living unit of a cell. We like to present our findings in the context of odorant perception – as an example, where the membrane context becomes communicative.

Pore-Spanning Membranes: a Tool to Resolve

SNARE-Mediated Single Vesicle Fusion Events

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In vitro single vesicle fusion assays are important tools to analyze the details of SNARE-mediated fusion processes. In this talk, I will discuss the merits and drawbacks of continuous planar pore-spanning membranes (PSMs) to investigate the fusion of large unilamellar vesicles (LUVs) with PSMs, mediated by a minimal neuronal SNARE machinery. PSMs are prepared on functionalized porous silicon substrates with pore diameters in the micrometer range. They harbor the t-SNARE Δ N49-complex to investigate the dynamics and fusogenicity of single LUVs doped with the v-SNARE synaptobrevin 2 by means of confocal fluorescence microscopy.

Fluorescence correlation spectroscopy revealed mobility of both lipids and SNAREs in the PSMs, which is prerequisite for the formation of active fusion complexes. However, t-SNARE doped vesicles docked to the v-SNARE containing supported PSM parts (s-PSMs) were fully immobile, while those docked to the freestanding PSMs (f-PSMs) were mobile with a mean diffusion coefficient of $0.43 \mu\text{m}^2/\text{s}$. Despite the different dynamics of the vesicles on the two membrane types, similar fusion kinetics could be observed giving rise to a common fusion mechanism. Moreover, on s-PSMs, semi stable post fusion structures were resolved. First experiments further demonstrate that the established lipid mixing assay can be extended to a content release assay allowing to monitor fusion pore formation.