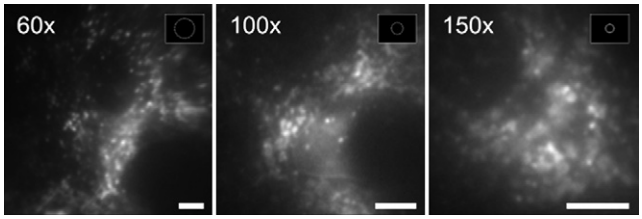
and experiments using objectives with different magnification (and hence different back pupils). We illustrate the system performance by demonstrating ultra-low background TIRF imaging of 200 Hz Qdot blinking, vinculin-EGFP labeled cellular adhesion sites and lysosomal dynamics in cortical astrocytes.



Platform H: Membrane Physical Chemistry I

90-Plat
Charges in phospholipid layers
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The interfacial properties of a membrane are determinant for interaction among bio-membranes / lipid bilayers, or for establishing contact among layer surfaces and substrates approaching from the bulk. The access to the bilayer and its local structural modifications upon interaction with an adsorbing guest molecule are influenced significantly by the presence of charges, and local changes in surface charge density. Results are being presented on model mono- and bilayers prepared from zwitterionic POPC (DPPC) (1-palmitoyl-2-oleoyl-sn-glycero-3-ethylphosphocholine) and its cationic sibling lipids E-POPC (E-DPPC) (1-palmitoyl-2-oleoyl-sn-glycero-3-ethyl-phosphocholine/ di-1,2-palmitoyl-sn-glycero-3-ethyl-phosphocholine) that served to inoculate charge densities at different mol percentages. Monolayer compression isotherms obtained for the mixtures are compared with isotherms of pure POPC as a reference system. The presence of layer charges is manifested in an earlier onset of interaction, the range of interaction is increased. POPC bilayers with the same charge densities as the monolayers studied were then investigated by single molecule tracking using the fluorophore DiI-C18 for diffusion tracing. Initial results indicate a linear decrease of the lateral diffusion coefficient with increasing charge density. In the course of the study indications for domain formation in pure POPC layers were observed as novel peculiarities; these will be presented and discussed. Preliminary results about the adsorption of partially charged phospholipid layers onto hydrogel polyelectrolyte cushions on solid supports will be presented.

91-Plat
Domain/Raft Exploration in Lipid Mono- & Bilayer by Freeze-fracture Electron Microscopy on Nano-Resolution Scale
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Lateral chemical and physical inhomogeneities of biological membranes such as domains/rafts seem to play an important role in signal transduction, membrane traffic, and diseases.
Freeze-fracture electron microscopy (ff-em) as a cryofixation TEM technique is a powerful to explore such small and highly dynamic domains in a probe-free mode. Since the resolution of this technique is 2 nm we are able to study lipid-, protein-, toxin-, as well as drug domains on a nano-resolution scale. Since replica, resistant to beam damage, can be produced from large, micro-meter size objects, ff-em allows us to study nano-scale events in micro-scale biological as well as artificial assemblies. The fact that the fracture plane follows the area of weakest forces, allows insides into the hydrophobic center of lipid bilayer [1-3] as well as into the lipid/gas interface of lipid monolayer stabilizing gas bubbles [4].
Examples will be given for domains in liposomal bilayer made of drugs [5], proteins, and toxin. Lipid-induced modulation of 2-D crystals of bacteriorhodopsin in liposomal bilayer will be shown as an extreme example for domain formation of intrinsic proteins [6-8]. Additionally, liquid ordered (Lo) domains will be shown recently detected in lipid monolayer, stabilizing hydrophobic gas bubbles.
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92-Plat
Sterol Uptake From Liposomes By MβCD Is Influenced By The Extent Of Sterol Superlattice In The Membrane
Berenice Venegas, **Parkson L.-G. Chong** .
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Cholesterol transfer regulates the intracellular distribution and the metabolism of cholesterol, thus having a direct impact on cholesterol homeostasis in cells. The present work investigates the effect of lipid lateral organization (i.e., the extent of sterol superlattice) on sterol transfer from liposomes to methyl-Beta-cyclodextrin (MβCD), a water-soluble macrocyclic compound able to pick up sterols from the membranes. Several sample sets of large unilamellar vesicles (LUVs) composed of POPC, dehydroergosterol (DHE) and Dansyl-PE were examined. Each sample set contained ~15 samples centered at one of critical sterol mole fractions (C_r) theoretically predicted for maximal sterol superlattice formation (e.g., 20.0, 22.2, 25.0, 33.3, 40.0 and 50.0 mol%). Within the same sample set, the DHE content in the sample was varied using 0.4 mol% increments. The molar ratio of DHE to Dansyl-PE was kept constant (15:1) in all samples. The rate of sterol transfer was monitored in real time based on the resonance energy transfer between DHE (donor) and Dansyl-PE (acceptor). The fluorescence intensity of Dansyl-PE versus time was monitored at 500 nm upon addition of MβCD. When DHE is transferred from LUVs to MβCD, the energy transfer efficiency is decreased and, consequently, the fluorescence intensity of Dansyl-PE is decreased over time. The initial rate of DHE transfer was determined by a linear fit of the data collected in the first few seconds of the transfer process. The initial rate of the DHE transfer was found to vary with DHE content in a biphasic manner at C_r. This result demonstrates that the rate of DHE transfer from LUVs to MβCD is governed by the extent of sterol superlattice in the liposomal membrane. (Supported by AHA, NSF and PDOH)

93-Plat
Lipid Diffusion In Domain-forming Bilayers Studied By Pfg-nmr
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The pulsed field gradient (pfg)-NMR method for measurements of translational diffusion of molecules in aligned lipid bilayers is presented. Lateral phase separation of lipids has been successfully studied as well as their dynamics within the bilayer organization. Support was obtained for that the lateral diffusion depends on lipid packing and acyl chain ordering. Therefore, investigations of order parameters of perdeuterated acyl chains, using 2H NMR quadrupole splittings, were useful complements. Here, some of our recent achievements on lipid membranes will be summarized. In particular, bilayers exhibiting two-phase coexistence of liquid disordered (ld) and liquid ordered (lo) phases are considered in detail. Among our major results are that the lateral diffusion is the same for all components, independent of the molecular structure (including cholesterol (CHOL)), if they reside in the same domain in the membrane. Furthermore, quite unexpectedly CHOL seems to partition into the ld and lo phases to roughly the same extent, indicating that CHOL has no strong preference for any of these phases. We propose that the lateral phase separation in bilayers containing one high Tm and one low Tm lipid together with CHOL is driven by the increasing difficulty of incorporating an unsaturated or prenyl lipid into the highly ordered bilayer formed by a saturated lipid and CHOL, i.e. the phase transition is entropy driven to keep the disorder of the hydrocarbon chains of the unsaturated lipid.

94-Plat
Lipid Sorting In Membranes Nanotubes
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Several studies have shown that lipids are sorted at every step of intracellular trafficking [1], for example in [2] it has been shown that COPI-coated vesicles have a different lipid composition than the Golgi apparatus they originate from. But general principles governing lipid sorting are not fully understood yet. In particular, general physical principles in sorting must be investigated closely. As transport intermediates are highly curved, the role of membrane curvature must be considered. As a driving force for sorting we propose, that composition

in the tube should adjust in order to minimize the bending free energy in the curved structures like tubes or small vesicles. Here we have systematically studied lipid sorting in membrane nanotubes of controlled diameter.
We designed an assay where nanotubes are pulled out of Giant Unilamelar vesicles made of Sphingomyelin (BSM), Cholesterol (Chol) and DOPC using optical tweezers. The tube radius is set via micropipette aspiration. The composition in the tube's membrane is measured by recording the fluorescence intensity of labeled lipids under a confocal microscope; simultaneously the force necessary to hold the tube is measured with optical tweezers.
We will show that curvature induced lipid sorting can occur, but only near a phase transition of the ternary system BSM:Chol:DOPC. We will show that the physical origin of sorting by curvature in pure lipid system is a reduction of the free energy of curvature of the membrane in the tube. We will present theoretical considerations supporting these observations.
Finally we will show that protein binding a specific lipid in the membrane can enhance sorting.
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95-Plat
Stoichiometries and Energetics of Cationic Nanoparticle-Membrane Complexes
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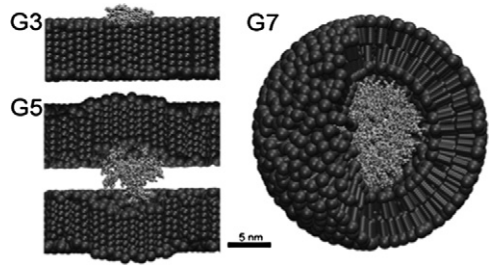
The nanoparticle-membrane interaction is essential to nanotheraputic design and nanotoxicity concerns. The equilibrium structure was determined for phospholipid membranes interacting with one type of nanoparticle, poly(amidoamine) dendrimers, at the atomistic and molecular scale via both experimental and theoretical approaches. The resulting dendrimer-phospholipid complex depends on both the number of primary amines per dendrimer and the dendrimer size. Large dendrimers (> 7 nm diameter) induce vesicle-encased dendrimers and significant membrane disruption. In contrast, small dendrimers (< 5 nm diameter) bind to the membrane surface without individually inducing significant membrane disruption. Techniques such as isothermal titration calorimetry (ITC), molecular dynamics (MD), and differential scanning calorimetry (DSC) were used for examination of the equilibrium structures and identifying the mechanisms of nanoparticle-induced membrane disruption.
Third-, fifth-, and seventh-generation poly(amidoamine) dendrimers (G3, G5, and G7, respectively) are shown here in complexes with phospholipids. The stoichiometries and dimensions of the dendrimer-lipid complexes indicate small dendrimers (G3) saturate with lipids on a planar membrane, medium-sized dendrimers (G5) induces local membrane curvature and/or binds to multiple bilayer surfaces, and each larger dendrimer (G7) becomes encased by a lipid vesicle. These understandings will guide nanoparticle design in both medical and industrial applications.



96-Plat
The Delivery of Lipidic Compounds to Model Membrane Interfaces by Non-lamellar Liquid Crystalline Nano-particles
Pauline Vandoolaeghe[1], Adrian R. Rennie[2], Richard A. Campbell[3], Robert K. Thomas[4], Fredrik Höök[5], Giovanna Fragneto[3], Justas Barauskas[6], Fredrik Tiberg[7], **Tommy Nylander[1]**.
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There is an increasing demand for methods to study processes at the lipid-aqueous solution interface, due to the importance of lipids and lipid self-assembly structures as regulators both for biological activity and for drug delivery vehicles. The biological membrane is one of the most important interfaces that the drug delivery vehicles encounter. The potential use of non-lamellar lipid structures delivery systems in pharmaceutical, food and cosmetic applications has invoked a number of studies of the assembly and interactions of cubic phases of these materials. One such system is a colloidal dispersion of the cubic liquid crystalline phase of glycerol monooleate. We will discuss some aspects of what happens when these liquid crystalline lipid nanoparticle encounters a lipid bilayer, consisting dioleoylphosphaticylcholine, either as supported bilayer or as a vesicle. Null ellipsometry and QCM-D provides kinetic information about the adsorption and triggerable release of the nanoparticles. Using contrast matching of the supported lipid bilayer, neutron reflectivity makes it possible to assess the exchange of material from one ordered lipid phase to another. Synchrotron Small Angle X-Ray Diffraction allowed us to in detail study the phase transition when non-lamellar glycerol monooleate based nanoparticle interact with phospholipid vesicles. Together the four techniques provide insight into the interaction mechanism and shows that the release of the particles are likely to be caused by phase transition of the lipid self-assembled structures.

97-Plat
Construction Of A Tethered-bilayer Lipid Membrane By Physiosorption Of Glycolipid GM_1 To A Hydrophilically Modified Gold Surface
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Interactions of membrane-bound molecules with their environment are difficult to study due to the complex and dynamic nature of biological membranes. The development of model membranes can provide insight into the function of membrane proteins in their natural environment. A phospholipid bilayer deposited at a gold surface offers a unique opportunity to investigate the mechanism of voltage-gated ion channel formation induced by surface-active proteins such as Colicin E1. Studies of voltage-gated phenomena involving transmembrane proteins require a model membrane that is supported at a gold electrode and has a water layer on either side of the bilayer. This can be achieved by creating a tethered lipid bilayer membrane (tBLM) with a hydrophilic spacer region separating the gold surface from the bilayer. We describe here the construction of a lipid bilayer membrane which is tethered from the gold surface using glycolipid, GM_1. The bilayer is composed of 1,2-dimyristoyl-sn-glycerol-3-phosphocholine (DMPC) and cholesterol. GM_1 is physiosorbed to gold by modifying the gold surface with a hydrophilic thiol, 1-thio-D-glucose. Due to the amphiphilic nature of GM_1 this is performed at the air/water interface using the Langmuir-Blodgett technique. The outer leaflet of the bilayer is deposited using the Langmuir-Schafer method. The quality of the bilayer formed at the gold surface was characterized using electrochemical methods, in which the capacitance of the tether bilayer is measured on a single crystal gold electrode (Au(111)). The tBLM was further characterized using Atomic Force Microscopy (AFM). The tBLM was depsited on a substrate composed of Au(111) terraces, force-distance curves were measured using AFM, the thickness of the bilayer was than extracted from these curves. These results will be presented for tBLMs constructed with varying GM_1 content (10, 20, and 30 mole percent).

Platform I: DNA, RNA Structure & Conformation

98-Plat
Computational and Experimental Determination of the tRNA-like Structure in the 3′UTR of the Turnip Crinkle Virus (TCV)
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Turnip crinkle virus (TCV) is a plant virus, which is not capped or polyadenylated. Being one of the smallest plus strand viruses makes it a useful system for studying translation and transcription. Its 3′ proximal region, together with the 5′ UTR, enhances translation. We have employed our massively parallel genetic algorithm, MPGAfold, to predict the secondary structure of the 3′ terminal 195 nt region. Compensatory mutagenesis analyses in vivo and in-line structure probing confirmed the existence of the key predicted features (stem-loop motifs an one H-type pseudoknot) and added another pseudoknot to the model. Based on this information, we employed our 3D molecular modeling software, RNA2D3D, to predict the 3D structure of the core three hairpins and two pseudoknots. Our model structurally resembled a tRNA. Experimental