of the monolayer film. Alterations in these properties in the absence of surface active lung surfactant proteins were also observed indicating that interactions between the monolayer at the interface, the ''multilayer reservoir'' and the subphase are essential for the proper functioning of the lung surfactant.

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The effect of proteins and cholesterol on viscoelastic properties and morphologies of Lung Surfactant system

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One of the essential features of healthy lung surfactant (LS) is to reduce the surface tension as well as increases the surface viscosity at the alveolus air-water interface to prevent the collapse. Currently, there is no simple theory explaining physicochemical properties of a monolayer with respect to surface tension or surface viscosity in the dynamic process of breathing. Monitoring the viscoelastic properties of interfacial films allows to predict how such a property depends on lipid and protein composition, packing properties and other variables at different states of the breathing process. Cholesterol and proteins are believed to have drastic effects on lung surfactant system by changing surface shear viscosities but systematic studies regarding this have not been done yet and the compositional effects remained still unkown. Here we focus on the influence of (1) cholesterol and (2) LS proteins such as SP-B_{Mini} and SP-C_{ff} on the surface viscosity and surface tension of pure DPPC monolayer as well as the lung surfactant replacement Survanta.

In order to study the compositional effects on the surface viscosity and surface tension, we have used custom-made Langmuir trough as a viscometer. Our viscometer consists of a Langmuir trough equipped with Helmholz coils which generate a controlled magnetic force to move a magnetic needle floating on the monolayer. The viscometer enables to examine the surface viscosity and surface tension as well as morphological changes in a LS monolayer as varying the amount of cholesterol and LS proteins. Atomic force microscopy has been also used to further investigate the morphological changes of a LS monolayer in nanometre regime after prepared by Langmuir Blodgett technique.

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Interaction of Lung Surfactant Protein A with Phosphatidylcholine Vesicles

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Surfactant protein A (SP-A) , a member of the collectin family found in the lung, binds to dipalmitoylphosphatidylcholine (DPPC), and plays roles in the formation of tubular myelin and the regulation of uptake and secretion of surfactant lipids by alveolar type II cells. The calcium dependent binding of SP-A to both small (SUV) and large (LUV) unilamellar vesicles of phosphatidylcholines (PC) has a dramatic effect on the PC dynamics as measured by ^{1}H linewidths of the acyl chains. For fluid bilayers (dipalmitoyl-PC at 42°C, dimyristoyl-PC and 1-palmitoyl-2-oleoyl-PC at 37°C), ω-CH_3 as well as bulk (CH_2)n and $N(CH_3)_3$ resonances are broadened with the addition of SP-A above a molar ratio of 0.02 SP-A/PC. This binding does not cause SUV or LUV fusion as monitored by negative staining TEM. However, the SP-A binding to PC vesicles exhibits two phases, one where the chain resonances are constrained and a second, requiring increased Ca^{2+} or the presence of 10 mol% cholesterol, characterized by a new downfield $(CH_2)_n$ resonance. The addition of EDTA can partially reverse the second phase of SP-A binding. The changes in PC acyl chain behavior provide insight into how SP-A interacts with multilamellar bodies and how it may aid in insertion of dipalmitoyl-PC into the air-water interface of the lung.

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Studying the Effects of Protein and Lipid Composition on Lung Surfactant Adsorption Through Confocal Microscopy

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Lung surfactant (LS) is a mixture of lipids and proteins that lines the air-water interface of the alveolar walls. It modulates the surface tension in the lungs which greatly reduces the mechanical work of breathing and also prevents the collapse of the alveoli upon expiration. Although lipids are the major constituent of LS, the hydrophobic surfactant proteins SP-B and SP-C play an integral role in proper adsorption of LS to the air-water interface. Understanding the role of these proteins will allow for better design of exogenous LS therapies for the treatment of respiratory distress syndrome. Confocal microscopy's abilities to optically section a sample and simultaneously image multiple dyes provide an ideal tool to study LS adsorption *in vitro*. Combining three-dimensional, multi-component imaging with surface tension measurements allows us to determine whether a particular surfactant mixture can successfully transition from bilayer aggregates in the bulk to a functional monolayer on the interface. SP-B and SP-C are believed to play an integral role in both the transport of aggregates to the interface and the unfolding of surfactant bilayers into a monolayer. Confocal microscopy has allowed us to study the importance of lipid and protein composition on the transport and unfolding of LS.

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Membrane Partitioning of Mechanosensitive Channel Inhibitor GsMTx4: Characterization by Depth-Dependent Fluorescence Quenching and Molecular Dynamics Simulations

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Recently we have demonstrated that membrane partitioning is a property of some (but not all) Inhibitor Cysteine Knot ion channel blockers [Biophysical J. 2007, 93:L20]. GsMTx4 is the only one among the studied blockers that interacts with anionic and zwitterionic lipids with nearly equal affinity. To gain insight into the determinants of its bilayer interactions we have examined several of its mutants and found to our surprise that none of the mutations had significant effects on membrane partitioning. Another surprising feature of GsMTx4 is the almost complete absence of changes in intrinsic fluorescence during membrane insertion. Penetration of GsMTx4 into lipid bilayer was determined using Distribution Analysis of the depth-dependent fluorescence quenching [Biophysical J. 1999, 76:946]. This analysis indicates that GsMTx4 and its W6A mutant penetrate into the bilayer deeper than Melittin. To interpret the fluorescence data and to elucidate peptide-lipid interactions involved in binding of GsMTx4 to PC membranes we performed MD simulations, which showed that anomalous fluorescence behavior of GsMTx4 on membrane partitioning is caused by water penetration into the lipid-peptide interface. The MD simulations also demonstrated high lipid perturbation and preferential interactions of cationic side chains of the peptide with lipid phosphate groups. Acknowledgments: I am grateful to F.Sachs for the gift of GsMTx4, to D.J.Tobias for performing MD simulations and to A.S.Ladokhin for helpful discussions. This research was supported by NIH Grant GM-069783 (A.S.Ladokhin) and KUMC Biomedical Research Training Program Fellowship (Y.O.Posokhov).

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Phase Transition Behaviors And Interactions And Adhesion Of Myelin Lipid Membranes Modulated By Myelin Basic Protein

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Myelin is a stacked membrane structure that allows for fast, efficient conduction of nerve impulses. It has 8 kinds of lipid molecules on two alternating bilayers and proteins such as myelin basic protein (MBP) which has an important role in maintaining myelin structure. The compact bilayer organization of healthy myelin is believed to require a well-defined range of lipid and protein composition, and lipid-protein interaction. Even though we know that multiple sclerosis (MS) is a morphological transformation involving loss of adhesion between myelin lamellae and sometimes formation of myelin vesicle, its mechanism and causes for demyelination are still under investigation.

We have used fluorescence microscopy, Langmuir isotherm, and Langmuir-Blodgett (LB) techniques to investigate how lipid composition of myelin lipid system affects the phase transition behaviors of myelin monolayers and bilayers depending on lateral pressure, temperature, and pH conditions. Model membranes with the composition of the cytoplasmic side of experimental allergic encephalomyelitis (EAE) myelin were also constructed on mica surfaces by LB deposition and the forces between the surfaces measured using the Surface Forces Apparatus (SFA) after exposure to various solution concentrations of MBP.

Our findings clearly demonstrate EAE monolayer remains phase-separated under physiological conditions. If the myelin sheath were to form two phases in vivo there are a variety of effects that could result. The line tension between two segregated domains and a local repulsive force could cause the membrane to bulge leading to vesiculation of the membrane. Force-distance measurements between supported myelin bilayers mimicking the cytoplasmic surface of myelin at various surface coverages of MBP indicate that maximum adhesion and minimum cytoplasmic spacing occurs when each

negative lipid in the membrane can bind to a positive arginine or lysine group on MBP.

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H/D Exchange Provides Insights Into The Orientation Of Bacillus Thuringiensis Pi-phospholipase C Binding To Mixed Component Vesicles

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Determining the orientation and conformation of peripheral membrane proteins when they are docked to target membranes is difficult. In many cases, flexible regions of the proteins provide the major contacts with the membranes. *B. thuringiensis* PI-PLC has two discrete binding sites for phospholipids - the active site for binding PI (or substrate competitors such as PMe or PG), and an activator site that is specific for phosphatidylcholine molecules. We have examined H/D exchange of the protein by Mass Spectrometry for the protein binding to 1:1 and 1:9 PG/PC small unilamellar vesicles (SUVs), conditions chosen for tight binding of the protein. In the absence of SUVs most of the protein amide groups are easily exchanged with D_2O. However, with SUVs are present, the regions helix B and helix C show a reduced exchange rate consistent with protection by the membrane. This is consistent with how this protein is thought to bind to membranes based on mutagenesis studies. Other interesting observations are that the beta strand E exchanges more slowly after binding to membrane while helix E exhibits faster exchange. To further explore how this enzyme is interacting with lipids, the H/D exchange rates of a constructed covalent dimer (disulfide-linked W242C), which is thought to mimic the membrane-induced dimer structure of w.t. PI-PLC in solution, are also examined with or without lipids present. Further study of a mutant protein, W47A/W242A, with very low affinity for vesicles and which is thought to exist as a dimer in solution, also provides some insights into self association of PI-PLC monomers. Clearly, for peripheral membrane protein dynamics, protein/protein, and protein/membrane interactions, H/D exchange experiments coupled with MS can provide information on structural changes not accessible by other structural methods.

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Molecular Dynamics Simulation of the ENTH Domain on Lipid Bilayer

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Clathrin-mediated endocytosis is necessary for a number of cellular phenomena, and the adaptor protein, epsin, has been indicated to play a role during the pathways of these processes. The amino terminal region of epsin is characterized by a highly evolutionary conserved region known as the ENTH (epsin NH_2-terminal homology) domain, which is critical for membrane deformation, although the exact mechanism of this process is still elusive. In order to unravel the behavior of this domain when it interacts with lipid bilayer, atomistic molecular dynamics simulations have been performed for both the wild type and mutants on lipid bilayer. The stability of these ENTH domains and their possible role in membrane deformation are reported.

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FCS of Mutated Phosphatidylinositol-specific Phospholipase C Enzymes Monitors the Interplay of Substrate and Activator Lipid Binding

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Phosphatidylinositol-specific phospholipase C (PI-PLC) enzymes are activated on the surface of vesicles by nonsubstrate phospholipids with the extent of activation tuned by the lipid composition. *Bacillus thuringiensis* PI-PLC can be activated by a small amount of phosphatidylcholine (PC) towards its substrate PI. Fluorescence correlation spectroscopy (FCS) has been used to study fluorescently labeled PI-PLC and a series of mutants (P42G, K44A, K44E, Y88A, and Y246/247/248S) binding to small vesicles of a substrate analogue (phosphatidylglycerol) and PC as a function of the vesicle composition. For PI-PLC and most of the mutant proteins there is a synergistic effect of the two types of phospholipids in anchoring the enzyme to a vesicle. If a mutation alters the affinity of substrate at the active site, binding should vary with X_{PC} and be enhanced in PC-rich region; on the other hand, if a mutation alters the affinity of activator, K_d will increase with increased X_{PC}. In this way, careful determination of the apparent K_d helps to sort out effects of enzyme mutations on activator and substrate sites. FCS is particularly useful in exploring the contribution of K_d to PI cleavage by mutant PI-PLC enzymes. For a fixed X_{PC}, if the bulk concentration of vesicles is above K_d, enzymatic activities should be similar, as long as vesicle binding is the critical step. However, it should drop dramatically when the bulk concentration is below K_d measured by FCS (a long as the substrate analogue is a good mimic for substrate). The results provide a direct analysis of vesicle binding and catalytic activity and shed light into how occupation of the activator site enhances activity. Currently, we are extending the analysis of this system with tethered small unilamellar vesicles and single-molecule methods.

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X-ray reflectivity Structural Study of PKCα-C2 Domain Binding to SOPC/SOPS Lipid Monolayers

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An understanding of signal transduction mechanisms is vital to investigate the causes of diseases. The C2 domain is a conserved protein signaling motif and membrane-targeting domain widely found in signaling proteins. In this work, we study the interaction of the C2 domain of protein kinase Cα (PKCα) with a lipid monolayer of a mixture of SOPC (1-stearoyl-2-oleyl-sn-glycero-3-phosphocholine) and SOPS (1-stearoyl-2-oleoyl-sn-glycero-3-phosphoserine). Recent results from crystallography and EPR studies indicate that PKCα-C2 is likely to orient parallel to the membrane. In this work, we use x-ray reflectivity to directly determine that the PKCα-C2 domain is perpendicular to the membrane. Our new analysis method allows us to test all orientations and demonstrates that our data is inconsistent with the parallel orientation. To carry out this experiment, the PKCα-C2 was injected into the subphase under an SOPC/SOPS (7:3) mixture supported on a buffered aqueous solution. X-ray reflectivity was used to determine the orientation and penetration depth of PKCα-C2 bound to the SOPC/SOPS monolayer. The reflectivity is analyzed in terms of the known crystallographic structure of PKCα-C2 and a slab model that represents the lipid layer, yielding an electron density profile of the lipid layer and bound C2 domain. The orientation of PC/PS-bound PKCα-C2 is described by two angles, $\theta = 35^0$ and $\varphi = 210^0$, and the domain penetrates 7.6 Å into the lipid layer. The structure that we determined is consistent with many observations from mutational studies. The perpendicular model further suggests how PKCα-C2 interacts with other lipid components such as phosphatidylinositol, other domains within PKC such as the C1 domain, and the receptor for activated C-kinase.

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Influence of Lipid Modifications of NRas on the Interaction with different Model Biomembranes and their Orientation at the Lipid Interface

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The membrane associated protein NRas is a member of the Ras-superfamily of GTPases and one of the main regulators of the MAP signal cascade, being responsible, among others, for cell growth and differentiation. Its capability of binding to the membrane is enabled by posttranslational modification with farnesyl and palmitoyl residues. To determine the influence of the nature of these modifications and to investigate the difference between active (GTP) and inactive (GDP) NRas, five different NRas constructs with different lipid anchors and nucleotides (Far/Far (GDP), Hd/Far (GDP), Hd/Hd (GDP), Stbut/Far (GDP) and Hd/Far (GPPNHP)) have been synthesized. By using surface plasmon resonance spectroscopy, we were able to follow the insertion and dissociation process of the lipidated proteins into and out of the membrane. We show that the binding kinetics of the different NRas proteins are markedly influenced by the lipid composition of the membrane, by the nature of the lipid anchors as well as by the nucleotide loading of the protein. Furthermore, we studied the correlation of the insertion process with the orientation of the protein at the lipid interface, using infrared reflection absorption spectroscopy. The results show that the properties of the lipid anchors have a major influence not only on the insertion process, but also on the orientation of the protein at the lipid interface.