been previously shown to simulate the phase properties of the lipid components in lung surfactant bilayers and monolayers. Presence of native palmitoylated SP-C reduced the size of lo domains in the DPPC/DOPC/cholesterol membrane model as detected by Förster Resonance Energy Transfer (FRET). Interestingly, very similar effects on the lo/ld equilibrium could be observed in the presence of a recombinant variant of SP-C, in which the two palmitoylcysteines of the native protein had been replaced by phenylalanines. It has been suggested that phenylalanines can act as functional mimics of palmitoylated cysteines in SP-C from some animal species. We therefore propose that the effects of SP-C on domain size could be related to selective interactions of this protein with liquid-ordered membrane regions and that this could be important for SP-C-promoted stabilization of lung surfactant films *in vivo*.

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Molecular Dynamics Simulations of Model Lung Surfactant Monolayers and Surfactant Protein B Fragment

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Surfactant Protein B (SP-B) plays an essential role in the proper functioning of lung surfactant. However, the details of how SP-B interacts with lung surfactant lipids to support lung function are poorly understood. The interactions between an SP-B based peptide and lung surfactant lipid monolayers are investigated using molecular dynamics simulations. Mini-B, a peptide of 34 amino acid residues consisting of the N-terminal and C-terminal alpha helices of full-length SP-B, achieves a similar level of function to full length SP-B in rodent models of respiratory distress. The monolayers probed included pure DPPC, pure POPG, and a mix of 7:3 DPPC:POPG. First the most stable configuration of the peptide-monolayer system is sought by allowing the system to evolve through time from different starting configurations and peptide orientations. It is then determined what effect the counter-ion concentration has on the screening of the electrostatic interaction between the negatively charged headgroups of POPG and the positively charged residues of the peptide. Finally, by placing a bilayer of lipids adjacent to the monolayer, in resemblance to the coexisting lipid reservoirs, the influence Mini-B has on the interaction between the monolayer and bilayer is demonstrated.

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Interactions of SP-B Based Peptide with Lipid and Protein Components of Lung Surfactant

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Lung surfactant (LS) is a mixture of lipids and proteins that reduces the surface tension at the alveolar air-water interface and thus prevents lung collapse and enables normal breathing. Surfactant Protein B (SP-B) is an essential component of LS and is absolutely necessary for survival. SP-B is thought to function by facilitating large-scale rearrangements of lipids and stabilizing the structures at various stages of the breathing cycle. However, neither the structural basis for this ability nor the physiological ramifications of lipid rearrangements are yet understood, in part because a high-resolution structure of SP-B is not yet available. Mini-B is a peptide fragment of SP-B that has been shown in *in vitro* and *in vivo* studies to retain similar activity to the full-length protein. Previously, we determined the structure of Mini-B, first in organic solvent hexafluoroisopropanol (HFIP) and then in detergent micelles composed of sodiumdodecylsulfate (SDS) using solution NMR. In our present work, we have studied the interactions of Mini-B with dodecylphosphocholine (DPC) and SDS micelles. DPC and SDS micelles provide an interfacial environment, with lipid headgroups corresponding to the headgroups of the most abundant lipids in LS, phosphatidylcholine (PC) and phosphatidylglycerol (PG) and thus solution NMR studies of interactions between Mini-B and these micelles can provide insight into LS protein-lipid interactions. We have also investigated the interactions of Mini-B with the most abundant surfactant protein SP-A under similar conditions. These studies further the understanding of the mechanisms of SP-B interactions with other surfactant components in native lung environment.

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Characterization of Transmembrane Peptide-Anchored Lactoferricin in Mixed Lipids

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To investigate the effects of a cationic juxtamembrane sequence on a hydrophobic transmembrane domain, model peptides have been designed with the lactoferricin sequence RRWQWR (LfB) anchored to an α-helical transmembrane peptide (RRWQWR-(spacer)-$(LA)_7$KKA). Spacers to date have included the helix-breaking -GPGG- and the helix-continuing -AA- sequences. The transmembrane domain contains a hydrophobic $(Leu\text{-}Ala)_7$ helical sequence that spans the membrane with two lysine anchors at the C-terminus. Selected alanines, deuterated on the C_α and C_β carbons, were incorporated in the sequences and used for solid-state NMR spectroscopy. Circular dichroism spectra reveal that the presence of the -AA- spacer correlates with a higher helix content in both DMPC and DMPC:DMPG (3:1) bilayer membranes. Solid state ^2H NMR spectra of macroscopically aligned lipid:peptide samples on glass plates reveal in all cases signals from the $C_\beta D_3$ groups and in some cases signals from the individual C_α deuterons. For example, in DMPC and DMPC:DMPG, the $C_\beta D_3$ groups for the peptide with the -GGPG-spacer give very similar quadrupolar splittings, in the range of 7-10 kHz. Interestingly, in DMPC the C_α deuterons, which are often not observed in similar transmembrane peptides, can also be seen for both labeled alanines, at 48 kHz and 70 kHz. In DMPC:DMPG a single $C_\alpha D$ quadrupolar splitting is resolved at 65 kHz. The presence or absence of selected $C_\alpha D$ resonances in NMR spectra of RRWQWR-(spacer)-$(LA)_7$KKA as well as WALP19, each with or without proline, will be considered in oriented samples of different bilayer lipid compositions.

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Interaction of the Protein Retinitis Pigmentosa 2 (RP2) with Langmuir Phospholipid Monolayers

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A severe form of retinitis pigmentosa is linked to mutations of the 350 residues protein RP2 (retinitis pigmentosa 2). This protein contains a α/β C-terminal domain, a highly hydrophobic β-helix and two acylation sites at the N-terminal. It localizes predominantly to the membrane. However, the parameters responsible for the modulation of RP2 binding to membranes are still largely unknown. The objectives of this research work were to characterize the membrane binding properties of RP2 using Langmuir monolayers. The complete sequence of RP2 was expressed and high purity was achieved. RP2 was injected into the subphase underneath phospholipid monolayers bearing different fatty acyl chains (length and unsaturation) and polar headgroups. RP2 binding was monitored by surface pressure measurements. The injection of RP2 underneath phospholipid monolayers led to an increase in surface pressure which indicates its membrane binding. The surface pressure data demonstrate that the adsorption kinetics of RP2 is independent of pH but is strongly affected by the ionic strength of the subphase as well as by the type of phospholipid fatty acyl chain (length and unsaturation) and headgroup. For example, on the basis of its maximum insertion pressure, RP2 shows a preferential binding onto saturated phospholipid monolayers which is consistent with its postulated localization to rafts. This interaction has been further studied by infrared sprectoscopy. In solution, the amide I band is centered at 1630 cm^{-1}, indicating the presence of the β-helix. In contrast, when injected into the subphase in the absence and presence of a phospholipid monolayer, the amide I band is shifted to longer wavenumbers with components at 1640 and 1655 cm^{-1}. These data thus suggest that RP2 has a preferential orientation in monolayers where the α/β C-terminal domain is oriented towards the monolayer.

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Influence of the Lipidation Motif on the Partitioning and Association of N-Ras in Model Membrane Subdomains

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In a combined chemical biological and biophysical approach using time-lapse tapping-mode atomic force microscopy, we studied the partitioning of differently lipidated N-Ras proteins with various membrane-recognition motifs into lipid domains of canonical model raft mixtures. The results provide direct evidence that partitioning of N-Ras occurs preferentially into liquid-disordered lipid domains, independent of the lipid anchor system. N-Ras proteins bearing at least one farnesyl group have a comparable membrane partitioning behavior and show diffusion of the protein into the liquid-disordered/liquid-ordered phase boundary region, thus leading to a decrease of the unfavorable line tension between domains. In addition, except for the monofarnesylated N-Ras, strong intermolecular interactions foster self-association and formation of nanoclusters at the domain boundaries and may serve as an important vehicle

for association processes and nanoclustering, which has also been observed in *in vivo* studies. No significant changes of the localization between GDP- and GTP-loaded N-Ras could be detected. Conversely, the non-biological dual-hexadecylated N-Ras exhibits a time-independent incorporation into the bulk liquid-disordered phase to maintain high conformational entropy of its lipid chains.

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Interactions between POPA and a4b2 nAChR: Insight from MD Simulations

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Extensive experimental studies have validated the necessity of the anionic lipid phosphatidic acid (PA) and/or cholesterol (CHOL) for functional nicotinic acetylcholine receptor (nAChR). At molecular lever, however, it is still unclear how PA and CHOL modulate the functionality of nAChR. We investigated the modulation mechanism through molecular dynamics (MD) simulations of both open- and closed-channel $\alpha4\beta2$ nAChR embedded into a ternary lipid mixture of 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC), 1-palmitoyl-2-oleoyl phosphatidic acid (POPA), and cholesterol with a POPC:POPA:CHOL ratio of 3:1:1. Unique interactions of POPA with the closed- and open-channel nAChR were revealed in MD simulations. We identified several putative POPA binding sites, which were formed by the highly conserved residues at the interfaces of the extracellular and transmembrane domains or the intracellular and transmembrane domains of $\alpha4\beta2$. Our MD simulations also suggested that POPA might stabilize the open-channel structure through better hydrogen bonding and salt-bridging with its residues in the open channel. The total numbers of hydrogen bonds and salt-bridges formed between POPA and nAChR were 3 and 5 times more in the open-channel than in the closed-channel, respectively. The salt-bridges lasted for nanoseconds in the open channel but only ~100 ps in the closed-channel. The POPA molecules that formed salt-bridges with nAChR showed higher order parameters than the POPA in the bulk lipids, while the order parameters for lipids at the lipid-protein interface were generally reduced. These results collectively suggest that the interactions between POPA and nAChR may potentially modulate the channel gating and preferentially enhance receptor function. Supported by NIH (R01GM66358 and R01GM56257) and NCSA through the PSC.

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Membrane Association and Insertion of the C2 Domain to Anionic Lipid Bilayers under Tension

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The rates of enzymatic reactions involved in the blood clotting cascade are enhanced by several orders of magnitude upon binding of coagulation factors to anionic regions of the cell membrane. This key process hinges on two specialized membrane-anchoring domains, the GLA domain and the C2 domain. We have recently reported a membrane-bound model of the GLA domain. Here we report the results of our simulations investigating membrane association and insertion of the C2 domain, which exhibits a completely different behavior from the GLA domain, both in terms of the overall architecture and its Ca^{2+}-independent membrane binding.

Both crystalographically solved, open and closed forms of the C2 domain of factor V were equilibrated over 50 ns in solution and inserted gradually (0.5 Å/ns) into a pre-equilibrated DOPS bilayer. During the insertion, lateral tension of 36 dyn/cm (calibrated based on several independent simulations of pure DOPS bilayers) was applied to the membrane to prevent over-shrinking and to allow its expansion upon C2 binding.

In contrast to the proposed implication of the two states, multiple transitions between the open and closed states were observed in solution. During membrane insertion, however, the open form closed near the surface of the membrane with K23 and R43 residues establishing direct interaction with the membrane. Subsequently, W26 in Loop1 was inserted into the DOPS tail region. The results provide a membrane-bound model of the C2 domain and suggest that, in contrast to the GLA domain, Ca^{2+}-independent, specific interactions between protein side chains and the membrane, associated with backbone conformational changes of the inserted loops, are the primary forces catalyzing membrane binding of the C2 domain.

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Ceramide-1-phosphate Prevents Interaction Of Pten With Phosphatidylinositol-4,5-bisphosphate But Does Not Interact Significantly With The Protein Itself

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PTEN, phosphatase and tensin homologue deleted on chromosome 10, has been identified as one of the most highly mutated or deleted tumor suppressors involved in tumorigenesis, second only to p53. This enzyme works to regulate the PI3K pathway by specifically dephosphorylating $PI(3,4,5)P_3$ at the 3 position of the inositol ring in order to control basal levels of the phosphoinositide, which in turn controls the levels of phosphoAkt within the cell. We have previously shown that PTEN binds specifically to $PI(4,5)P_2$, its product, which causes a conformational change which is thought to allosterically activate the protein. It has been recently discovered that Ceramide-1-Phosphate also plays a role in the PI3K pathway, increasing the levels of phospoAkt within the cell by some unknown mechanism. We have tested the ability of PTEN to interact with model membranes containing Ceramide-1-Phosphate and undergo conformational changes in its presence. Surprisingly, while PTEN does not interact significantly with membranes containing POPC and Ceramide-1-Phosphate, the interaction of PTEN with membranes containing $PI(4,5)P_2$ decreases in the presence of Ceramide-1-Phosphate. Additionally, the conformational changes typically observed upon interaction of PTEN with membranes containing $PI(4,5)P_2$ do not occur when Ceramide-1-Phosphate is added to the membrane. These data suggest that Ceramide-1-Phosphate may affect the PI3K pathway by preventing the interaction and subsequent activation of PTEN by $PI(4,5)P_2$.

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Spectroscopic Studies of Beta-Lactoglobulin with Model Membrane Vesicles

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Bovine beta-lactoglobulin (β-LG) is a lipocalin protein found in mammalian milk. In the native state, its secondary structure is dominated by beta-sheet, though it has the propensity to form α-helices based on secondary structure predictions. We have shown that β-LG can adopt a significant fraction of alpha-helical conformation upon mixing with synthetic phospholipid vesicles. The thermodynamic and kinetic aspects of interaction between β-LG and lipid vesicles have been previously studied. However, the function of β-LG is still not clear. In this work, a leakage experiment has been conducted to analyze the degree of leakage of small molecules through the lipid bilayer as enabled by β-LG. Furthermore, the factor of membrane curvature for the interaction has been investigated by varying the composition of vesicles by changing proportion of PC, PG and PE. Finally, the role of cholesterol for the protein-lipid interaction is studied to illustrate a potential function for β-LG in mammalian species.

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Alterations In Phase And Morphology Of A Lung Surfactant Monolayer in contact with surfactant in the sub-phase induced by cholesterol and native surface active proteins

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Although the presence of cholesterol, the major neutral lipid component, is well known in native surfactants (upto 10 % mass), its role in the surfactant remains uncertain. The most recently FDA approved clinical surfactant contain cholesterol, while two that have been used for 20 years have cholesterol carefully removed. However, they are all successful in treating neonatal respiratory distress syndrome (NRDS) resulting from a lack of surfactant. As a result the optimal concentration of cholesterol, if any at all, remains debated. Here we present indications for an optimal cholesterol concentration by presenting alterations to the phase and morphology of Survanta, a clinically used bovine lung surfactant extract, induced by both physiological and elevated concentrations of cholesterol when the monolayer is in contact with surfactant in the subphase. We find that low cholesterol concentrations (1-2 wt %) help to achieve a lower surface tension by enhancing surfactant material adsorption to the interface. However, increasing the cholesterol concentrations to higher values ($\approx$ 20 wt %) significantly alters the normal surfactant isotherm. Alterations in a typical signature plateau for Survanta at ~ 40 mN/m are noted suggesting a change in the solid phase fraction of the film. Fluorescence microscopic imaging reveals the coexistence of discrete monolayer along with ''multilayer reservoir'' adjacent to the air/water interface. Differences in the collapse structures of the monolayer are also noted indicating an alteration in the mechanical properties