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.

3146-Pos Board B193

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.

3147-Pos Board B194

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.

3148-Pos Board B195

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 $PI(4,5)P_2$.

3149-Pos Board B196

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.

3150-Pos Board B197

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

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.

3151-Pos Board B198

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.

3152-Pos Board B199

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.

3153-Pos Board B200

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.

3154-Pos Board B201

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).

3155-Pos Board B202

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