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