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Membrane Association of the PTEN Tumor Suppressor: Reference Structure of a PIP Phosphatase on a Lipid Bilayer
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The phosphatidylinositolphosphate phosphatase PTEN is the second most frequently mutated protein in human tumors. Its membrane association, allosteric activation and membrane dissociation are poorly understood. We recently reported PTEN binding affinities to membranes of different compositions [1] and a preliminary investigation of the protein-membrane complex with neutron reflectometry (NR). Here we use NR to validate molecular dynamics (MD) simulations of the protein and study conformational differences of the protein in solution and on anionic membranes. NR shows that full-length PTEN binds to such membranes roughly in the conformation and orientation suggested by the crystal structure of a truncated PTEN protein, in contrast with a recently presented model which suggested that membrane binding depends critically on the SUMOylation of the CBR3 loop of PTEN's C2 domain [2]. Our MD simulations confirm that PTEN is peripherally bound to the bilayer surface and show slight differences of the protein structure in solution and in the membrane-bound state, where the protein body flattens against the bilayer surface. PTEN's C2 domain binds phosphatidylserine (PS) tightly through its CBR3 loop, and its phosphatase domain also forms electrostatic interactions with PS. NR and MD results show consistently that PTEN's unstructured, anionic C-terminal tail is repelled from the bilayer surface. In contrast, this tail is tightly tugged against the C2 domain in solution, partially obstructing the membrane-binding interface of the protein. Arresting the C-terminal tail in this conformation by phosphorylation may provide a control mechanism for PTEN's membrane binding and activity.
[1] Shenoy et al., 2012, PLoS ONE 7, e32591.
[2] Huang et al., 2012, Nat. Commun. 3, 911.

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Computational Studies of the Integrin Receptor in Complex Biologically Relevant Lipid Bilayers
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Integrins are large heterodimeric (αβ) cell surface receptors which play a key role in the formation of focal adhesion complexes and are involved in various signal transduction pathways. They are located in the plasma membrane and they activated to a high affinity state by the formation of a complex between the integrin β-subunit tail, the membrane and talin. Despite the existence of structural data for all domains of integrins, the structure of the complete integrin receptor and details of its interactions with bilayers remain unclear. In this study, a model of the complete integrin receptor in complex with the talin F2-F3 domain was constructed using recent structural data. The complex was inserted in biologically relevant bilayers, whose lipid composition resembles that of the plasma membrane, containing phospholipids, cholesterol and sphingolipids to study the dynamics of the integrin receptor and its effect in bilayer structure and dynamic. The results of this study demonstrate the dynamic nature of the integrin receptor and suggest that the presence of the integrin receptor alters the lipid organization between the two leaflets of the bilayer in an asymmetric way, including a slowing-down of lipids in an annulus around the protein due to the interactions of the lipids with the integrin receptor.

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Selective Modification of Lipid Bilayer Dynamics and Fluidity Properties by Kl4 (Mimetic of Surfactant Protein B)
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KL_4, a 21 residue mimetic of surfactant protein B, is effective in the treatment of infant respiratory distress syndrome (RDS) and functions by lowering aveolar surface tension and promoting oxygen exchange. Here we compare the results from previous NMR investigations to those of fluorescence characterization of the effects of KL_4 on the lipid dynamics and organization in liposomes. We utilized a pyrene phospholipid analog to investigate the effect of KL_4 on lipid organization and acyl chain dynamics by monitoring changes in excimer-to-monomer (I_e/I_m) ratio. This experiment probes the environment of the hydrophobic core of DPPC/POPG and POPC/POPG liposomes. An average decrease of ~27-40% and ~0-10% in I_e/I_m was observed in the DPPC/POPG and POPC/POPG LUVs, respectively, with increasing peptide concentration (0.5 to 5 mol%). This decrease is directly proportional to a lowered probability of excimer formation, which is highly dependent on proximal interactions of an excited monomer with a pyrene moiety at ground state.
The ability of the peptide to modify membrane fluidity properties was studied via anisotropy measurements of a rhodamine-labeled phospholipid. A steady increase in the order was observed in the DPPC/POPG liposomes with relatively constant fluorescence intensity, while collisional quenching was observed in the POPC/POPG liposomes. These observations agree with NMR observations and proposed mechanisms of peptide-mediated lipid trafficking. Further studies on the orientation and penetration of the peptide were interrogated using power saturation SDSL EPR.

2220-Pos Board B239
Exploring Membrane-Bound form of the C2 Domain by HMMM Model
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The membrane anchoring of several coagulation factors to the cellular membrane is mediated by the C2 domain, which binds to the anionic regions of the membrane. Upon membrane binding, the rates of enzymatic reactions of the coagulation factors are increased by several orders of magnitude. The mechanism of the rate acceleration is not clear, since the membrane-bound form of the C2 domain, and therefore the complex forms of the C2-containing factors are not yet solved at atomic resolution.
We have constructed membrane-bound forms of the C2 domain of the coagulation factor V (FV-C2) and investigated specific protein-lipid interactions, using all-atom molecular dynamics (MD) simulations and the HMMM model (BJ (2012) 102, 2130). Spontaneous binding of FV-C2 to a PS membrane was observed within 2-25 ns for twelve different trials of 50-ns-long MDs. FV-C2 interacted with the membrane through three loops (termed Spikes 1, 2 and 3), achieving a stable orientation. Multiple trajectories of the successful membrane-bindings provided reliable statistics for analysis of the protein structure and protein-lipid interactions, which may be unattainable without the HMMM model. There are two crystalographically solved structures available for the FV-C2; open and closed forms, based on the distance between Spikes 1 and 3. In the HMMM trajectories, however, neither structure is well populated as membrane-bound form of the FV-C2. In the half of the HMMM trajectories, a PS headgoup is observed to interact with K23, Q48, and S78 located at the inner side of Spikes 1-3 (''PS-specificity pocket''), but the orientation of the headgroup is opposite to previously suggested. Several basic side chains around the Spikes were observed to make initial protein-lipid contacts, changing their orientations as the protein nears the membrane.

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Exploring Protein-Lipid Interactions using Gramicidin a as a Model System
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Gramicidin A (gA) is a 15 amino acid peptide with an alternating L and D sequence that dimerizes to form a membrane-spanning monovalent cation channel. Among gA's key features are four tryptophan (Trp) residues per monomer, which have been implicated as important contributors to the stability and orientation of the channels in bilayers. using molecular dynamics simulations, we have explored the Trp residues' effects on gA channel-lipid interactions. First, a modified gA (Me-gA) was built using 1-methyltryptophan, which disrupts the ability of the indoles to be hydrogen bond donors. These Me-gA dimers were simulated for 100 ns in pure DLPC, DMPC, DOPC, and POPC bilayers, and the results were compared to the previously published results using wild type gA channels. Second, both gA and Me-gA channels were simulated for about 3 μs in bilayer systems with the same average hydrophobic length (equimolar $DC_{16:1}PC$+$DC_{24:1}PC$ mixture, equimolar $DC_{18:1}PC$+$DC_{22:1}PC$ mixture, and pure $DC_{20:1}PC$) to explore the effects of hydrophobic mismatch on lipid distribution adjacent to the protein. In all systems, protein structure and orientation characteristics were calculated. Lipid bilayer properties, such as thickness, surface area per lipid, and lateral compressibility were also quantified as functions of distance from the channel/lipid boundary to elucidate the mutual protein-lipid/protein-bilayer interactions. Preliminary results show that Me-gA systems exhibit a reduced first shell trough (by 1-3 Å) and the bilayers become thicker around Me-gA in all pure bilayer types in comparison to wild type gA. This suggests that the lipids stretch to accommodate the slightly longer hydrophobic thickness of Me-gA. The results from the mixed bilayer simulations indicate lipid redistribution around the channels based

on hydrophobic mismatch. These redistribution events appear to begin about 500 ns after beginning the simulations.

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Pre-Exposure of Pulmonary Surfactant to Hyaluronic Acid Alters its Structure and Interfacial Properties

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Pulmonary surfactant is a complex mixture of lipids and proteins lining the alveolar air-water interface. By lowering the surface tension, pulmonary surfactant stabilizes the respiratory epithelium against physical forces tending to collapse it. Dysfunction of surfactant is associated with respiratory pathologies such as acute respiratory distress syndrome (ARDS) or meconium aspiration syndrome (MAS), where naturally occurring inhibitory agents reach the lung. We have already confirmed the higher resistance to inhibition of preparations combining pulmonary surfactant and polymers such as hyaluronan (HA) and the potential use of these additives to design new therapeutic surfactant preparations. In the present study we have analysed the effect of HA on the structure and surface behaviour of pulmonary surfactant complexes, in order to investigate the possible mechanism for HA-promoted reactivation. We have observed significant effects of HA on structural properties such as aggregation of surfactant membranes, or size, distribution and packing of segregated ordered lipid domains. We have also observed that HA promotes refining of surfactant composition, i.e. enrichment of pelletable large surfactant complexes in saturated phospholipids. Surprisingly, we have detected no apparent direct interaction between surfactant complexes and HA even though we have observed 100% of transfer of surfactant complexes into the interface only in the presence of this polymer. All of these irreversible changes in surfactant structure and activity are observed only upon pre-exposure of surfactant to polymer concentrations at which the polymer forms an entangled meshwork. We propose that the polymer meshwork is responsible for entropy-mediated changes in surfactant structure, which may enhance surfactant function and thus resistance to inactivation.

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Identification of the Regulatory Anionic Lipid Site in Kir Channels

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Inwardly rectifying potassium channels (Kir) are regulated by multiple factors, including multiple lipids. In addition to a specific requirement for phosphatidyl-4,5-bisphosphate (PI(4,5)P2) for channel activity of Kir2.1, analysis of purified proteins reconstituted into liposomes has revealed a secondary requirement for non-specific anionic lipids, which increase PI(4,5)P2 sensitivity by ~100 fold [Cheng et al. 2011, Biophys. J. 100, 620-628]. Recent crystal structures of eukaryotic Kir channels in complex with PI(4,5)P2 reveal a common PI(4,5) P2 binding site [Hansen et al, 2011, Nature. 477, 495-498; Whorton & MacKinnon, 2011, Cell. 147, 199-208], but they have not identified the synergistic anionic lipid site.

We have performed extensive docking simulations to identify potential interaction sites of different phospholipids with Kir2.1 channels. These simulations indicate two distinct binding sites; a high affinity site that corresponds to the crystallographic PI(4,5)P2 binding pocket and a lower affinity site, involving two lysine residues further towards the periphery of the cytoplasmic domain, that may correspond to the secondary anionic lipid site. When the two lysine residues are mutated to cysteine, channel activity is essentially abolished, even in the presence of PIP2. Cysteine modification of these residues by decyl-MTS, which essentially provides a 'lipid tether' to the residue, restores channel activity in the presence of low levels of PIP2. These results point strongly to the identified site as being the site for non-specific anionic lipid interaction, and support a model in which the anionic lipid interaction (or 'lipid tethering') pulls the Kir domain towards the membrane, facilitating PI(4,5)P2-mediated channel opening.

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Interaction at the Membrane Midplane Mediates Interleaflet Coupling

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Transmembrane signaling implies that peripheral protein binding to one leaflet be detected by the opposite leaflet. Even without involvement of raft lipids we showed (Horner et. al. Biophys. J. 2009) that, peripheral binding of charged molecules (poly-lysine, PLL) to planar lipid bilayers is detected at the other side. Addition of PLL to both sides of the membrane sandwiched lipids between two PLL molecules, indicating a formed nanodomain. As the 2dim surface tension between the monolayers is thought to be of comparable size as the line tension in one of the monolayers, interactions at the membrane midplane don't explain the existence of small nano domains. We tested this assumption by measuring the individual lipid mobility's in both leaflets of a free standing planar lipid bilayers and developed a model. The adsorption of a polypeptide decreased lipid mobility of the adjacent (lower) monolayer. This was revealed by fluorescence correlation spectroscopy (FCS). Depending on the size of the polypeptide, lipid diffusion was either slower or faster than that of the polypeptide. Although only one lipid type was used in both monolayers, lipid mobility's were not equal. The polypeptide decelerated lipid movement in the distant monolayer to a much smaller degree than it did affect lipid mobility in the adjacent monolayer. Based on these observations we propose a model which suggests that the coupling between monolayers is due to friction at the membrane midplane. The interlayer friction coefficient seems to be much larger than the in-layer friction coefficient which explains the existence of small nano-domains.

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Molecular Basis of the Interaction between Signaling Lipids and Proteins; the Special Case of Phosphatidic Acid

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Intricate networks of signaling cascades regulate cellular development and response to environmental factors. within these cascades, the lipid second messenger phosphatidic acid (PA) is an important regulator in all eukaryotes. Rapid but transient increase of PA levels regulate growth, acclimatization and survival during development and stress response. Signaling lipids, including PA, function by selectively recruiting proteins to the membrane. This facilitates either the activation or inhibition of these target proteins. However, the exact details of phospholipid-mediated stress response mechanisms are still largely unclear, partially due to the lack of characterized target proteins of PA. Here we report on characterization of the interaction between various regulatory proteins and lipid second messengers by determining their lipid binding specificity and affinity for PA. Furthermore, to assess relative binding affinity in variable structural lipid environments, in vitro liposome-binding assays with liposomes covering a range of lipid compositions are performed. In our binding studies, phosphatidylethanolamine is utilized to study the role of membrane curvature and electrostatics in lipid-binding.

Specific attention is given to the electrostatic/hydrogen bond switch model. Hydrogen bond formation between basic amino acids and the PA phosphomonoester headgroup increases the negative charge of PA and allows for docking of the protein binding domain. The elucidation of the mechanism behind the binding of proteins to lipid second messengers such as PA will further develop our understanding of their function in plant growth and stress responses. This in turn can help in understanding regulation and cell functioning in numerous organisms.

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Cholesterol Stabilizes Ion Channel Function during Sphingomyelinase Hydrolysis

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Sphingomyelinase, a central enzyme of sphingolipid metabolism, hydrolyses sphingomyelin to ceramide, which is a potent modulator of membrane properties. using well-defined mixtures of palmitoyl-oleoyl-phosphatidylcholine (POPC), sphingomyelin (SM), ceramide (Cer), and cholesterol (Chol), we mimicked sphingomyelinase activity by gradually replacing sphingomyelin by ceramide. It is well-known that ceramide induces in such systems a phase coexistence of L_α and L_β domains, where SM/Cer pairs preferentially locate in the gel domains and POPC and Chol enrich in the fluid domains. Our interests were focused on the effects of SM depletion from and Chol enrichment in the fluid domains, respectively, and in particular on the effects on ion channel function. By applying a combination of x-ray diffraction with osmotic stress we were able to determine the bending rigidities for the coexisting domains, as well as estimates for the spontaneous curvatures and Gaussian moduli of curvature. We found a small (2%) changes in bending rigidity, and curvature modulus, but a significant (46%) drop in spontaneous curvature. In the absence of cholesterol this drop is somewhat smaller (38%). However, it is way excelled by the 73% changes in bending rigidity and Gaussian curvature. using a simple