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.

2223-Pos Board B242
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.

2225-Pos Board B244
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