peptide to membranes that also contain the channel-forming peptide gramicidin A allowed us to observe the membrane adsorption of A1AT. The protein interacts with the channel and produces transient current interruptions. The on-rate of these events depends non-linearly on the A1AT concentration and scales with the mole percentage of the charged lipid in the membrane. The measured off-rate is surface-charge independent. Thus, our results suggest that the membrane lipid composition plays an important regulatory role in the physiological activity of the A1AT and its interaction with the membrane.

1831-Pos Board B741
Effect of Hydrophobic Surfactant Proteins SP-B and SP-C on the Permeability of Phospholipid Membranes
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Pulmonary surfactant is a complex mixture of lipids and proteins whose main function is to reduce surface tension at the alveolar air-liquid interface in order to avoid alveolar collapse at the end of expiration and facilitate the work of breathing. It is composed by around 90% lipids and 8-10% specific proteins, including the hydrophobic SP-B and SP-C. In this study, we have analyzed the effect of hydrophobic surfactant proteins on the permeability of phospholipid membranes by two different approaches: fluorescence microscopy of giant vesicles (GV) and electroconduction in planar lipid membranes.
The effect of surfactant proteins on the permeability of GV membranes was assessed under the microscope using the fluorescent water-soluble probes FM®1-43 and calcein. Membrane-sensitive FM®1-43 only labels the external leaflet of membranes, and calcein emits green fluorescence in aqueous media. Neither can permeate through pure lipid membranes. In the presence of physiological amounts of SP-B and SP-C, giant oligolamellar POPC vesicles incorporated FM®1-43 in every single membrane when added to the external medium and were also permeable to calcein. These results suggest the existence of direct connections between aqueous compartments of GV in the presence of these proteins.
On the other hand, planar lipid membranes (PLM) have been widely used to study ionic permeation through phospholipid bilayers mediated by membrane proteins. Permeability of bilayers incorporating small amounts of hydrophobic surfactant proteins has been analyzed in PLMs prepared by the dual monolayer technique. Conductance and selectivity experiments as well as noise analysis of the signals were performed. All our measurements indicate the formation of channel-like structures with defined properties in terms of ionic conductance and selectivity. Possible implications of membrane-permeabilizing structures in the biological context of the pulmonary surfactant system will be discussed.

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The Pivotal Plane of Phosphatidylethanolamine is Unaffected by the Hydrophobic Surfactant Proteins
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Available evidence suggests that the hydrophobic surfactant proteins (SP), SP-B and SP-C, promote adsorption of the surfactant lipids to the alveolar air/water interface by facilitating formation of a rate-limiting negatively-curved structure. In support of this model, the proteins induce several phosphatidylethanolamines to form inverse bicontinuous cubic phases, in which each leaflet has the same saddle-shaped curvature as the hypothetical intermediate. Proteins could promote formation of the cubic phases by altering the spontaneous curvature (c_0) of the phospholipids, which would change the dimensions of the inverse hexagonal (H_{II}) phase. With 1,2-dioleoyl phosphatidylethanolamine (DOPE) in excess water, the SP had no effect on the H_{II} phase, suggesting a constant c_0. The SP, however, might still change c_0 by shifting the location of the pivotal plane at which c_0 is calculated. To determine if the proteins shift the pivotal plane, we measured X-ray diffraction from DOPE with 0 - 1% SP at 22 ^{o}C with different hydrations. Behavior at the different SP concentrations was indistinguishable. With increasing hydration the hexagonal lattice expanded until reaching the same limiting size at the same limiting hydration, indicating that the location of the Luzatti plane, which defines the boundary between the aqueous core and lipid, was unaffected. Detailed structural analysis of the lattice-dimensions at lower hydrations confirmed the existence of a pivotal plane at all levels of protein, and the derived location of the pivotal plane relative to the Luzatti plane (V_p/V_l) showed no response to protein concentration. Since neither V_p/V_l, nor the location of the Luzatti plane change with SP, the location of the pivotal plane remains constant. Unaffected position of the pivotal plane confirms that the SP induce cubic phases without changing the spontaneous curvature. (Studies conducted at the Stanford Synchrotron Radiation Lightsource).

Membrane Structure I

1833-Pos Board B743
Extending Techniques to Prepare Asymmetric Vesicles to Additional Lipid Compositions: Lipid Structure Affects the Ability to Maintain Lipid Asymmetry
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We recently developed a method in which methyl-beta-cyclodextrin (MβCD)-induced lipid exchange was used to prepare model membrane vesicles with a stable asymmetry and which, like plasma membranes, have an outer leaflet rich in sphingomyelin (SM) and have an inner leaflet rich in ordinary glycerophospholipids, such as phosphatidylcholine (PC), phosphatidylethanolamine, and phosphatidylserine. Experiments were carried out to determine how the phospholipid head group and acyl chain structure affected the ability to form stable asymmetric vesicles with SM-rich outer leaflets. In addition to the examples given above, stable asymmetric vesicles could be formed with inner leaflets rich in phosphatidylglycerol or cardiolipin. When PC with different acyl chain structures were compared, asymmetric vesicles with inner leaflets rich in PC could be formed when PC had one saturated acyl chain and one mono or polyunsaturated acyl chain, two monounsaturated acyl chains of various lengths, or when the PC had phytanoyl acyl chains, but not when the PC had two polyunsaturated acyl chains, although SM could be exchanged into vesicles in every case. The lack of asymmetry when vesicles had lipids with two polyunsaturated acyl chains was due to fast flip-flop. Based on these studies, and because lipids with two polyunsaturated acyl chains are of very low abundance in nature, we conclude that, at least in the absence of proteins, natural membranes of various compositions can maintain stable lipid asymmetry. These studies show that asymmetric vesicles with a wide variety of inner leaflet lipids can be prepared.

1834-Pos Board B744
Triton X −100 and TM Helices Increase Ordered Domain (lipid Raft) Size
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It has been postulated that the formation of co-existing liquid ordered (Lo) domains rich in sphingolipids and cholesterol and liquid disordered (Ld) domains rich in unsaturated lipids, has an important role in cell membrane structure and function. Detergent TX-100 favorably partitions into and dissolves the Ld phase. It is used to isolate insoluble Lo-like membranes known as detergent resistant membrane (DRM) from cells. The relationship between DRM and pre-existing Lo domains in cells is not clear. We carried out experiments to find out how TX-100, and TM helices (which are cell membranes components), change membrane properties by investigating their effect on liposomes composed of brain sphingomyelin/1-palmitoyl-2-oleoyl-phosphatidylcholine/cholesterol. As measured by anisotropy and tempo quenching, methods that detect local environment at the nearest neighbor level, neither TX-100 nor TM helices affected the thermal stability of membrane order. In contrast, FRET, which detects proximity at longer length scales, detected that ordered domains disappeared at a lower temperature than that estimated from anisotropy or quenching, both with and without TX-100 and TM helices. FRET detected domains at higher temperatures in the presence of TX-100 or TM peptides than in their absence. The amount of ordered domains and their thermal stability appeared to increase as the interaction distance (Ro) of the FRET pair decreased. These differences are most easily explained by a difference in the size-sensitivity of the detection techniques, such that FRET is unable to detect Lo domains smaller than Ro in size, and by the conclusion that TX-100 increases ordered domain size. However, in presence of TM peptides, this increase in domain size is less evident, as the TM peptide by itself already increases domain size. Thus, in natural membranes the effect of TX-100 upon domain formation may not be significant.

1835-Pos Board B745
X-Ray Phase Contrast Imaging of Freestanding Lipid Model Membranes
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Membranes are considered as the most important interfaces in biology, and can be visualized under physiological conditions by optical techniques such as phase contrast and fluorescence light microscopy. While the contour lines and large lateral domains of biological membranes can be imaged, the density profile of the membrane and associated changes cannot be resolved by visible light. We report on hard x-ray phase contrast imaging of black lipid