initial interaction of the defensins with the microbial membrane is thought to occur via non-specific interactions based on surfaces charges and amphipathicity. However it is not understood how these peptides are able to selectively target the membranes of the pathogens and ultimately kill them. To date structures of alpha defensins have been reported for human neutrophils, rabbit kidney and mouse intestinal epithelium. We report the first NMR structure of an alpha defensin from myeloid cells of rhesus macaque. It adopts the canonical alpha defensin fold with the core structure made of three-stranded beta sheets stabilized by three intra-molecular disulfide bridges. However there are differences in the defensin electrostatic surface that could manifest a distinct flexibility and mode of action. Results from the studies of interaction of defensins with bicelles (bilayer containing micelles) will be presented and the possible role of each of the residues involved in its interaction with membranes will be discussed.

Interfacial Protein-Lipid Interactions, Surfactants

2090-Pos Palmitoylation Promotes Stabilization of Interdigitated-Like Phospholipid Phases by the N-terminal Segment of Pulmonary Surfactant Protein SP-C

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Board B205

SP-C is the smallest pulmonary surfactant protein and is required for the formation and stability of surface-active films at the air-liquid interface in the lung. The protein consists of a transmembrane hydrophobic α-helix and a cationic N-terminal segment that contains palmitoylated cysteines. In the present work, the lipid/protein interactions of palmitoylated and non-palmitoylated versions of synthetic peptides designed to mimic the N-terminal segment of SP-C have been analyzed and compared by electron spin resonance (ESR) spectroscopy.

Both palmitoylated and non-palmitoylated peptides decrease the mobility of phosphatidylcholine (5- PCSL) and phosphatidylglycerol (5-PGSL) spin probes in dipalmitoylphosphatidylcholine (DPPC) or dipalmitoylphosphatidylglycerol (DPPG) bilayers. Both peptides have a greater effect at temperatures below than above the main gel-to-liquid-crystalline phase transition, although the palmitoylated peptide induced greater lipid immobilisation than does the non-palmitoylated form.

The effect of SP-C N-terminal peptides on the chain flexibility gradient of DPPC and DPPG bilayers at temperatures below the main gel-to-liquid-crystalline phase transition, registered by spin probes at different positions in the *sn*-2 acyl chains, is consistent with the existence of a peptide-promoted interdigitated phase. The palmitoylated peptide, but not the non-palmitoylated version, is able to stably segregate interdigitated and non-interdigitated populations of phospholipids in DPPC bilayers at 37°C. This feature suggests that the palmitoylated N-terminal segment takes part in and stabilizes ordered domains such as those containing interdigitated lipids. We propose that palmitoylation may be important to promote and facilitate association of SP-C and SP-C-containing membranes with ordered lipid structures like those potentially existing in highly compressed states of the interfacial surfactant film.

2091-Pos Polyelectrolyte Mediated Competitive Adsorption Between Lung Surfactant and Serum Proteins

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Board B206

The adsorption of lung surfactant (LS) to an air-liquid interface is strongly inhibited by the competitive adsorption of surface active serum proteins and is likely the explanation of LS inactivation in Acute Respiratory Distress Syndrome (ARDS). Utilizing a cycling Langmuir trough as an in vitro model to approximate LS/serum protein behavior at alveolar interface, we show that LS adsorption to the interface is restored by the addition of cationic polyelectrolytes such as chitosan, suggesting a promising therapy for ARDS. Fluorescence microscopy images show distinct changes in interfacial morphology between serum protein and LS-covered regions offering a visual confirmation of LS adsorption to the interface. Freeze fracture transmission electron microscopy (FFTEM) images of model LS vesicles show untreated vesicles are ~50 nm in diameter and uniformly distributed throughout the solution while treatment with a low concentration of chitosan (0.05 mg/mL) causes aggregation of the vesicles.

This competitive adsorption of serum proteins to the alveolar air-liquid interface can be modeled as an energy barrier to LS adsorption and can be analyzed using a variation of the classical Smolukowski description of colloidal stability. The serum proteins generate both a steric and electrostatic barrier to LS adsorption due to net repulsion between the negatively charged surfactant aggregates and serum proteins. Chitosan binds to and neutralizes the negatively charged LS and serum protein surfaces, lowering the electrostatic barrier to LS adsorption.

2092-Pos Mechanisms of Surfactant Membrane Inactivation by C-Reactive Protein

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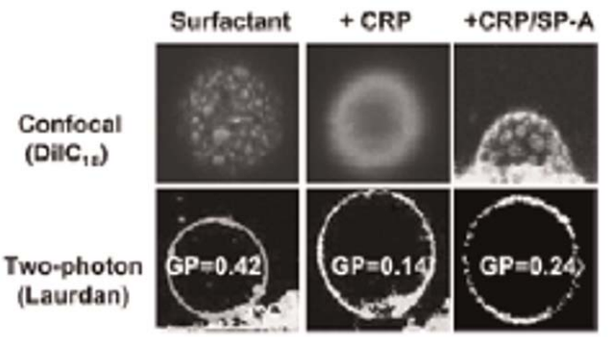
Board B207

C-reactive protein (CRP) is a plasma protein that notably increases in serum and bronchoalveolar lavage following tissue injury, infection, or inflammation. Our objective was to investigate the mechanisms involved in CRP-inhibition of animal-derived pulmonary surfactant (PS), which consists of surfactant membranes containing the hydrophobic surfactant peptides SP-B and SP-C, and to determine whether addition of surfactant protein A (SP-A) reverses this inhibition. The results indicated that CRP inhibited surface adsorption of PS by increasing membrane fluidity as determined by fluorescence anisotropy, DSC, and confocal and two-photon excitation fluorescence microscopy of GUVs formed from PS. CRP caused disappearance of the Lo/Ld phase coexistence characteristic of surfactant membranes. SP-A blocked CRP effects on those membranes and CRP-inhibition of surfactant surface adsorption.



2093-Pos Modification of Film Conformation - a New Mechanism for Surfactant Inhibition by Plasma Proteins

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Board B208

Pulmonary surfactant (PS) dysfunction arising from serum protein leakage into the alveolar space appears to be operative in the acute respiratory distress syndrome (ARDS). Hence, albumin-inhibited PS is a commonly used in vitro model for studying surfactant abnormality in ARDS. However, the mechanism by which PS is inhibited by albumin is still controversial. The present work examined film conformation of albumin-inhibited bovine lipid extract surfactant (BLES) with/without surfactant protein A (SP-A) using atomic force microscopy (AFM). BLES and albumin were co-spread at an air-water interface from aqueous media. The co-spreading minimized the adsorption barrier of phospholipid (PL) vesicles of BLES imposed by pre-adsorbed albumin molecules, i.e., inhibition due to competitive adsorption. Compared to functional BLES films, including albumin compromised the normal PL phase transition and separation in BLES monolayers and inhibited the monolayer-to-multilayer transition. Fluorescence confocal microscopy (FCM) confirmed that albumin remained at the interface at surface pressures considerably higher than its equilibrium spreading pressure (~ 20 mN/m). These inhibitory effects were attributed to a biophysical mechanism in which albumin molecules interact with the PL molecules at the interface and hence significantly increase the film compressibility. Consequently, a much larger area reduction is required to achieve high surface pressures (i.e., low surface tensions). This new mechanism of surfactant inhibition does not necessarily contradict other inhibition mechanisms due to serum proteins, such as competitive adsorption. Rather, it complements the competitive adsorption mechanisms by including an additional deleterious effect of albumin molecules once present in the interface. Addition of SP-A cannot effectively counteract these inhibitory effects, indicating that SP-A reverses surfactant inhibition primarily by enhancing PL adsorption rather than by directly increasing lipid packing at the interface.

2094-Pos Lipid-Specific Interactions of Lung Surfactant Protein D with Model Membranes

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Board B209

Surfactant protein D (SP-D), a member of the collectin family, plays an important role in the innate immune response as well as pulmonary surfactant homeostasis. Among its diverse range of ligands, phosphatidylinositol (PI) is the best-defined endogenous phospholipid ligand. The mechanisms of how SP-D interacts with PI, and how this interaction affects membrane properties, most notably lipid dynamics and permeability of vesicles, have not been extensively studied. To this end, we have designed model membrane systems for NMR study that utilize d_{75}-1,2-dipalmitoylphosphatidylcholine (^{2}H-DPPC) as the "host" lipid for solubilizing a variety of proteo-lipids (e.g. PI) as the "guest" lipid. Vesicles prepared from these lipid mixtures (4:1 DPPC/guest) were used to assess how SP-D affected acyl chain motions by analyzing SP-D-induced ^{1}H line broadening. We have confirmed that the SP-D interaction with these vesicles is specific for PI (other anionic phospholipids do not bind significantly) and calcium-dependent. Once bound to the PI, SP-D also perturbs the DPPC methylene chains that surround PI. ^{13}C-labeled DPPC can also be used as a direct probe of how the SP-D affects that phospholipid in these mixed bilayers. By using a fluorescent dye entrapped in DPPC/PI vesicles we could also show that very low amounts of SP-D (below what leads to significant line broadening) caused dye leakage from the model membrane. These perturbations of lipid packing by SP-D could be linked to its role in the reorganization of surfactant ultrastructure. Similar perturbations might also contribute to SP-D dependent permeabilization of microbial membranes.

2095-Pos How Pulmonary Surfactant Attains Low Surface Tensions - New Evidence from Atomic Force Microscopy

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Board B210

Monolayers of a functional pulmonary surfactant (PS) can attain very low surface tensions well below their equilibrium value. The mechanism by which PS monolayers reach such low surface tensions while maintaining film stability is still unknown. Fluorescence microscopy (FM) studies indicate that phospholipid (PL) phase transition and separation may be important for the normal biophysical properties of PS. PL phase transition/separation in the monolayers of bovine lipid extract surfactant (BLES) was studied using atomic force microscopy (AFM). AFM revealed PL phase separations upon film compression and a monolayer-to-multilayer transition at surface pressure 40–50 mN/m. The tilted-condensed (TC) phase consisted of domains not only in micrometer scale, as previously detected by FM, but also in nanometer scale, below the resolution limits of conventional optical methods. There is a marked tendency for microdomains to dissociate into nanodomains upon film compression, such that the nanodomains account for a significantly larger fraction of the TC phase than the microdomains, especially before the onset of the monolayer-to-multilayer transition. The nanodomains were uniformly embedded within the liquid-expanded (LE) phase, thus forming a 2D alloy-type structure which could impart both flexibility and stability to the film. Upon further compression, such an alloy structure could also facilitate partial collapse of surfactant monolayers from the domain boundaries, i.e., the monolayer-to-multilayer transition at 40–50 mN/m. These resultant multilayer structures would provide additional stability to PS films, thereby allowing the attainment of very low near-zero surface tensions. Addition of surfactant protein A (SP-A) increased generation of the nanodomains and promoted the formation of multilayers. We concluded that the nanodomains may play a predominant role in affecting the biophysical properties of PS films.

2096-Pos Electrical Properties of Supported Bilayers on Polymer Cushions

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Board B211

We have demonstrated the formation of electrically addressable bilayer membranes formed by Langmuir-Blodgett-based deposition on single crystal silicon. We have shown that a polymeric cushion between the bilayer and the silicon substrate is required to allow for lateral mobility of lipid molecules, as well as for the creation of a reservoir below the membrane. This study investigates the effects of polymer concentration, compression pressure, and lipid composition on the electrical properties of this biomimetic bilayer platform. Specifically, we investigate PEG concentrations 0.25, 0.5, two and four times the molar crossover concentration of 5.9% polyethylene glycol (PEG-2000), and utilize impedance spectroscopy to measure the resistance and capacitance of these bilayers. A small increase in capacitance in the lipid bilayer is seen with increasing PEG concentrations. The resistance of the bilayer is maximized at PEG concentrations between 5.9 and 11.8%, or twice the crossover concentration. At concentrations outside the 5.9 to 11.8% concentration range, the bilayer resistance is generally lower and difficult to reproduce. This work identifies experimental conditions under which this biomimetic bilayer platform most closely mimics the electrical properties of cell membranes. This platform will be useful for the study of the assembly and electrical properties of channel forming transmembrane peptides.

2097-Pos Clustering Of Cardiolipin Into Domains By The Sarcomeric Mitochondrial Creatine Kinase And Its Mutant Lacking The Cluster Of Cationic Residues Near The Carboxyl Terminus

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Board B212

There are two isoforms of human mitochondrial creatine kinase, the sarcomeric form (sMtCK) found in striated muscle, and the ubiquitous form (uMtCK) found in most other tissues including brain and kidney. We compared the properties of sMtCK with that of uMtCK to induce the clustering of cardiolipin in model membranes composed of cardiolipin and phosphatidylethanolamine. We find that the sMtCK has lower tendency to induce the clustering of lipids. We relate the relative ability to form lipid clusters to the conformation stability of the protein and the extent of its interaction with membranes. In addition, we explore the role of the cluster of cationic residues near the carboxyl terminus of the protein, the putative membrane binding domain.

Protein-Hydrocarbon Chain Interactions

2098-Pos Exploring the Thermodynamics of Protein Side Chains in Membranes with Fully Atomistic and Coarse Grained Simulations

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