than 95% of apoA-I is lipid-bound and associated with plasma mature HDL. To study the mechanisms that promote the production of lipid-free/lipid-poor (cholesterol-efflux capable) apoA-I in the arterial walls, we developed an apoA-I variant capable of reporting the lipidation-state of apoA-I in real-time.

We employed fluorescence resonance energy transfer (FRET) to generate an apoA-I reporter with lipidation-state specific fluorescence. ApoA-I's four endogenous tryptophans (Trp) were substituted with phenylalanines and a single Trp was substituted in at position 19, as the FRET donor. A cysteine residue substituted in at position 136 was labeled with the fluorophore AEDANS, as the FRET acceptor. The resultant apoA-I variant, apoA-I:W19-AED136, was lipidated to varying degrees producing rHDL of different sizes. The fluorescence emission spectrum of lipid free apoA-I:W19-AED136 and each of the rHDL particles was collected. Structural differences in the conformation of lipid-free apoA-I and apoA-I associated with different rHDL sizes altered the relative positions of the FRET donor-acceptor pair, leading to lipidation state specific fluorescence ''fingerprints''. Lipid-free apoA-I:W19-AED136 showed the highest degree of energy transfer (E=0.571), and apoA-I exhibited decreasing levels of energy transfer with increasing rHDL particle size (7.8 nm (E=0.387), 8.4 nm (E=0.0780), and 9.6 nm (E=0.0334)).

ApoA-I:W19-AED136 was successfully used to measure the transition rate of apoA-I between lipid-associated and lipid-free states, potentially, the rate limiting step of macrophage cholesterol efflux in the atherosclerotic plaque.

473-Pos

FVIIIa Binding to Phosphatidylserine-Membranes and Its Influence by Annexin V

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Binding of Factor VIII (FVIII) to Phosphatidylserine (PS)-expressing platelets is a key process in the intravascular pathway of the blood coagulation cascade. Deficiency of FVIII leads to a severe disease, hemophilia. In the human blood system binding of FVIII to platelets is influenced by many cofactors. One important cofactor is Annexin V, a protein that binds to PS-containing membranes in a Calcium-dependent manner.

Annexin is known to inhibit binding of activated Factor VIII to membranes while it does not interfere with binding of inactivated FVIII to membranes in the absence of other cofactors. We investigate the binding behaviour of FVIII, activated FVIII and Annexin to PS/PC model membranes using Fluorescence Correlation Spectroscopy. Based on the understanding of the binding mechanism of each protein, we analyse their mutual inhibition behaviour. Finally, we perform the binding experiments [1] in blood plasma to measure in a more natural environment compared to buffer solution.

[1] Engelke, H., Dorn, I., Rädler, J.O., Soft Matter, in press.

474-Pos

Effect of Hydrophobic Surfactant Proteins SP-B and SP-C on the Phase and Morphology of Protein Deficient Native Surfactant Films

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We present alterations in the phase, morphology and mechanical properties of a native replacement surfactant film induced either by the presence or absence of surfactant specific proteins SP-B and SP-C. Using Langmuir isotherms and fluorescence microscopy, the individual lipid-protein interactions in a complicated native surfactant system are explored. The surface tension lowering property of Survanta™, a native surfactant, is significantly compromised in the absence of the proteins, as is the ability of the film to undergo reversible collapse. A lack of proteins also causes the characteristic shoulder, prevalent at ~ 40 mN/m in most lung surfactant mixtures, to disappear. A lack of this characteristic shoulder illustrates the inability of the film to undergo reversible squeeze out by forming ''surface associated surfactant reservoirs''. Addition of SP-B causes an increase in the amount of surfactant material adsorbed from the sub-phase. Further it increases the monolayer stability and the compressibility modulus of the protein deficient film. SP-B is therefore responsible for helping the film achieve a high enough surface pressure during compression, as well as quick re-absorption of material during expansion. SP-C plays a dominant role in the formation of bilayer patches containing unsaturated lipids. SP-C also changes the mechanisms of monolayer collapse, and the film collapses via the formation of reversible collapse cracks. However, it is only in the presence of both SP-B and SP-C that the monolayer films are able to perform all the biophysical functions necessary for the proper working of the lung surfactant. These observations provide conclusive evidence showing that both SP-B and SP-C have distinct biophysical functions in the lung surfactant system, making them equally necessary for the long term survival of air-breathing mammals.

475-Pos

Anionic Polymers Reverse Serum Inhibition of Pulmonary Surfactant by Promoting Accumulation of Surfactant Near the Air-Liquid Interface

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Acute respiratory distress syndrome (ARDS) is a common pathology, including a spectrum of respiratory diseases associated with lung injury, and exhibiting a high overall mortality and morbidity rate. Inactivation of surfactant by serum and inflammatory components leaked into the alveolar spaces is considered as an important pathogenic factor within ARDS.

The mechanism by which inhibition is taking place depends on the nature of the inhibitory substance and could affect either the ability of surfactant to adsorb into the air-water interface or the ability of surfactant films themselves to reach the lowest surface tensions along the compression-expansion breathing cycles. Up to now, different polymers have proven to be useful to reverse or prevent inactivation of surfactant. We have explored the performance of inhibited surfactant and potential reactivating conditions using a fluorescent high-throughput method that detects and quantitates accumulation of surfactant near the air-liquid interface. This accumulation can be correlated in a first step with the concomitant decrease in surface tension that occurs when surface active lipids are transferred into the air-exposed side. Using this method we have evaluated inhibition of native porcine surfactant and of several clinical surfactants by serum, and the ability of hyaluronic acid (HA) to reverse or prevent this inhibition. A comparison was also made with the effect of other polymers. In general terms, presence of polymers in the subphase increases significantly the amount of surfactant associated with interfacial regions and seems to overcome, at least partially, the barrier to adsorption imposed by serum. Results obtained from a massive number of samples showed a very high reproducibility and a high correlation with data obtained using traditional methods to assess surfactant activity, such as surface balances or the Captive Bubble Surfactometer.

476-Pos

Confocal Microscopy and Competitive Adsorption: A New Look At Polymer-Enhanced Lung Surfactant Adsorption

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Lung surfactant (LS) is a mixture of lipids and proteins that lines the air-liquid interface of the alveolar walls and modulates the surface tension in the lungs. It therefore greatly reduces the mechanical work of breathing as well as prevents alveolar collapse upon expiration. Blood serum leaking into the alveoli as a result of trauma can lead to LS inhibition, which is one characteristic of acute respiratory distress syndrome (ARDS). The competitive adsorption of serum proteins, such as albumin, to the air-liquid interface of the alveoli blocks LS from forming a functional monolayer during ARDS. The addition of hydrophilic polymers, such as polyethylene glycol and chitosan, to the liquid subphase has been shown to enhance interfacial LS adsorption *in vitro*. Optimal amounts of polymer allow LS to form a functional monolayer in the presence of albumin, thus reversing inhibition. Albumin must be displaced from the air-liquid interface in order for a functional monolayer of LS to form. Imaging of the competitive adsorption process with confocal microscopy has allowed us to better understand the mechanisms behind forming an interfacial LS monolayer under inhibitory conditions. We can simultaneously track LS, polymer, and albumin, as well as separately visualize phenomena occurring at the interface from those occurring in the bulk. As a result of these capabilities, we have studied how various parameters affect the transport of LS to the interface and the displacement of albumin in order to form a functional surfactant monolayer.

477-Pos

Surface Rheological and Morphological Studies of Peptoid Mimics of Lung Surfactant Protein C

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Surfactant protein C (SP-C) is a lipoprotein secreted by alveolar type II cells that has been implicated in surface-associated activities thought to facilitate breathing and to prevent alveolar collapse. The *N*-terminal cysteine residues of SP-C are palmitoylated, which is thought to be critical in stabilizing the helical structure and maintaining a surface-associated surfactant reservoir. However, the exact function of the two palmitoyl chains is not yet fully understood. In the current study, poly-*N*-substituted glycines or ''peptoids'', a class of novel bio-inspired foldamers, have been employed to study the effects of *N*-terminal alkylation of a petpoid-based mimic of SP-C. Langmuir isotherms were performed to examine the reversibility of non-alkylated and di-alkylated SP-C mimic-containing lipid films during compression and expansion cycles at the air/liquid interface. Atomic force microscopy (AFM) of Langmuir-Blodgett films revealed extensive multilayer formation at high compression for a lipid

film of dipalmitoylphosphatidylcholine, 1-palmitoyl-2-oleoyl-phosphatidylglycerol and palmitic acid with the presence of di-alkylated SP-C mimics, in contrast to the film containing the non-alkylated SP-C mimics. Interfacial stress rheometor (ISR) measurements show that the lipid system with non-alkylated SP-C mimics displays a sharp increase in loss modulus at a surface pressure of ~42mN/m, corresponding to the plateau region in the isotherms. The dramatic increase in surface viscosity is consistent with the increase in the fraction of solid phase in the AFM images. In contrast, di-alkylated SP-C-containing films are more fluid at high surface pressures with a moderate increase in viscosity, suggesting an important role of the di-alkylated chains to associate with lipid acyl chains and maintain the coexistence of both fluid-like and solid phases at high surface pressures.

478-Pos

Simulation Studies on Interactions of Lung Surfactant Protein SP-B with Lipid Monolayers and Vesicles

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We used molecular dynamics simulations to study the interactions of lung surfactant protein SP-B with lipid aggregates at the air/water interface and in water. This is relevant for understanding the mechanism of function of lung surfactant, a thin film of lipids and proteins lining the gas exchange interface in the lung alveoli. The film reduces the surface tension of the air/water interface to low values, and is absolutely necessary for breathing. Its function is associated with transfer of material between the monolayer at the interface and bilayer reservoirs in the aqueous sub-phase, which is mediated by SP-B and SP-C proteins. While these proteins are crucial for function their exact role remains unclear.

We studied model lipid mixtures with lung surfactant protein SP-B and its fragment mini-B using the MARTINI coarse-grained model. The secondary structure of SP-B was obtained using homology modeling and fitting to the known structure of mini-B. We simulated lipid monolayers at the air/water interface with disconnected lipid bilayer patches in water, in the presence of either SP-B or mini-B. The bilayer patches formed vesicles, which did not require proteins. The proteins inserted into the headgroup/interfacial region of lipid aggregates showing preference for positive curvature. SP-B demonstrated stronger surface and fusogenic activity as compared to mini-B, for which aggregation was necessary. The proteins induced local curvature in monolayers, producing small bilayer folds below the equilibrium tension. Binding of an SP-B monomer or a mini-B dimer to opposing leaflets resulted in a stable monolayer-vesicle connection without mixing the lipid content. Formation of a lipid bridge between the connected monolayer and vesicle was observed. SP-B promoted hemifusion of vesicles by bringing them in close contact; hemifusion progressed into formation and expansion of the fusion pore.

479-Pos

Inhibition of Pulmonary Surfactant by Meconium: Biophysical Properties and Molecular Mechanism

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Pulmonary surfactant is a complex mixture of lipids and proteins lining the alveolar air-water interface. Lowering the surface tension at the respiratory interface, pulmonary surfactant stabilizes the respiratory epithelium against physical forces tending to collapse. In addition to constitutive disorders or immaturity in lungs at birth, some environmental factors and pathological events can perilously impair the surfactant system and consequently lead to pulmonary dysfunctions. In newborn infants, meconium aspiration syndrome (MAS), due to exposure of pulmonary surfactant to meconium, can result in severe respiratory failure. Surfactant inactivation plays a key role in the pathophysiology of MAS, preventing low surface tension to be reached. Currently, mechanisms for meconium-induced inactivation of pulmonary surfactant are not clearly understood, although it has been proposed that dysfunction of pulmonary surfactant complexes could be due to exposure to unsaturated membrane lipids, free fatty acids, bile acids, or cholesterol, all present in meconium in variable amounts. Inactivation of pulmonary surfactant by meconium is accompanied by a profound alteration of the thermotropic properties of its membrane structure, with consequences on several functionally-relevant biophysical properties, such as interfacial adsorption and compression-expansion behaviour. Surfactant membranes become substantially fluidized as a consequence of exposure to meconium and this membrane-perturbing effect can be mimicked by exposure of surfactant to a mixture of bile acids and cholesterol. Thus, we propose that solubilisation of cholesterol by bile acids promote abnormal incorporation of cholesterol into surfactant complexes, perturbing their structure and their interfacial function.

480-Pos

Membrane Occupancy-Dependent Rejuvenation of DnaA Is Associated with Its Conformationally Driven Oligomerization

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DnaA, the initiator of chromosome replication in all known eubacteria species, is activated once per cell division cycle. Its overall activity cycle is driven by nucleotide exchange and ATP hydrolysis. Acidic phospholipids in a fluid membrane were shown to promote the rejuvenating nucleotide exchange on DnaA. We have recently shown that the transition into an active form is strongly cooperative with respect to DnaA membrane occupancy. Only at low membrane occupancy DnaA reactivation is efficiently catalyzed by the acidic phospholipids. The present study is aimed at unraveling the molecular outcome of the occupancy dependent DnaA rejuvenation. The comparison with N-terminal truncated protein, tDnaA, the specific labeling of DnaA by the environmentally sensitive fluorophore 2-(4-maleimidoanilino)naphthalene-6-sulfonic acid (MIANS), the CD examination of its secondary structure as well as the cross-linking at the N-terminal of DnaA revealed that: (i) DnaA N-terminal is indispensable in the cooperative transformation between the high and low occupancy states (I and II, respectively), (ii) the transformation between these states is associated with a conformational change, presumably at the N-terminal domain and (iii) State II of the protein on the membrane corresponds to a trimeric or higher form of DnaA. It is suggested that the DnaA conformation attained at low surface density drives its oligomerization which is presumably a pre-requisit to its interaction with *oriC*.

481-Pos

Interaction of Cytochrome-C with Monoolein Liquid Crystals Mesophases

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Phase behaviour and structural properties of monoacylglycerides in water have been investigated for a long time, due to their extended polymorphism. In particular, monoolein (MO) in water shows several mesophases, characterized by a high disordered conformation of the hydrocarbon chains. At such conditions, an embedded protein can influence the physical properties of the lipid matrix, depending on the protein size and polarity. We take advantage of the structural properties of monoolein and cytochrome-c to extensively study the temperature effects on the cubic transition from Pn3m to Im3m by means of small-angle X-ray scattering technique (SAXS) and electronic absorption spectroscopy (EAS). To do so, we made samples composed of monoolein (50 mg/ml) in the presence of 1, 10 and 50 mg/ml of cytochrome-c. Ours preliminary SAXS results indicate that cyto-c is able to change the monoolein water channels, from cubic Pn3m to Im3m. Moreover, such kinetic behaviour is too slow, taking place within some days. EAS measurements indicate that the incorporation of cyto-c within the Monoolein water channels begins after two or three days (after the sample preparation). Besides, after one week of sample preparation the amount of cyto-c within the Monoolein water channels is equal to 60% and 34% for 1 and 10 mg/ml of cyto-c, respectively. Interestingly, increasing the temperature, the unity cell parameter decreases, indicating that water is going out from the unity cell and the symmetry of the liquid crystal phase can changes to another cubic or to hexagonal, depending on the temperature and the sample composition. We believe that these results could bring more insights on the protein-liquid crystal interaction.

482-Pos

Insertion and Folding of Outer Membrane Proteins Into Lipid Bilayers and the Function of the Periplasmic Chaperone FkpA

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In Gram-negative bacteria, outer membrane proteins (OMPs) are translocated in unfolded form across the periplasm before they insert and fold into the outer membrane. When isolated in unfolded form in 8 M urea, OMPs like OmpA develop their barrel structure after urea dilution in the presence of preformed lipid bilayers or detergent micelles. We have previously shown that a periplasmic chaperone, the seventeen kDa protein (Skp) promotes OmpA folding and insertion into lipid bilayers, but only when these bilayers contain negatively charged phosphatidylglycerol [1]. Here we demonstrate that another periplasmic chaperone, FkpA, also facilitates folding and insertion of OMPs like OmpA. Both faster folding kinetics and higher yields in lipid bilayers were observed for OmpA when FkpA (32 kDa) was present. We previously reported that the Skp trimer forms 1:1 complexes with OMPs [2]. Our present fluorescence experiments indicate that the FkpA dimer may form complexes with OMPs at