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.

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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.

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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.

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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.

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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.

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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