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Characterizing the Effects of Membrane Fluidity and Lipid Chain Length on the Antimicrobial Activity of Protegrin-1
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Antimicrobial peptides are naturally occurring short amphipathic proteins, innate to the immune system and shown to induce selective lytic activity towards microbial pathogens. Protegrin-1 is an 18-residue, cationic, β-sheet antimicrobial peptide stabilized by two disulfide bonds. Concentration-dependent structural transformations of supported lipid bilayer patches as a result of peptide-membrane interactions have been visualized through the use of atomic force microscopy. A three-stage concentration-dependent transformation has been characterized, which begins with edge instability, followed by pore formation and worm-like micelle formation. This suggests that protegrin-1 acts to lower the line-energy at the edge of the bilayer. Membrane and lipid characteristics, including fluidity, charge and acyl chain length, can alter the activity of antimicrobial peptides. To identify the importance of both acyl-chain length and fluidity on the activity of protegrin-1, these two variables were decoupled. When the bilayers are examined at the same relative fluidity levels, they demonstrate the three-stage transformation observed on a fluid control bilayer, in contrast to the structural transformations that were observed in the gel phase bilayers. This suggests that fluidity exhibits a large influence on the transformations that occur as a result of protegrin-1. To examine the importance of acyl-chain length, the activity of antimicrobial peptides was studied using unsaturated bilayers. Our results indicate that the longer chain bilayers are less susceptible to disruption. This could be due to the hydrophobic mismatch between protegrin-1 and the thicker hydrophobic portion of longer chain lipid bilayers . These results highlight the importance of subtle membrane characteristics in the activity of antimicrobial peptides towards bacterial cells. Lipid bilayers with cholesterol are more accurate eukaryotic cell mimics and will allow examination of the selective preference of antimicrobial peptide activity.

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A Systematic Approach Towards Elucidation of the Mode of Action of a Bacterial Thermosensor
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The membrane sensor and signalling protein DesK is involved in detecting temperature changes in the bacterium Bacillus subtillis. At low temperatures it triggers expression of a desaturase, which introduces double bonds into preexisting phospholipids, thereby regulating membrane fluidity. Recently it was discovered [1] that both sensing and transmission of DesK, which has five transmembrane segments, can be captured into one single chimerical transmembrane segment, the so-called 'minimal sensor'. It was hypothesized that a group of hydrophilic amino acids flanking this transmembrane segment represents the molecular switch responsible for turning on and off the kinase state of DesK. This switch would be regulated by the extent of exposure of this group to the aqueous phase, which in turn would depend on membrane thickness. Here we tested this hypothesis by employing different biophysical approaches, using synthetic peptides corresponding to functional and non-functional mutants of the minimal sensor in artificial model membranes of phosphatidylcholines of varying thickness and at different temperatures. The results of these studies will be reported.
[1] Cybulski et al., manuscript submitted.

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Engineering a Thermosensor To Dissect a Transmembrane Signaling System
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The DesK-DesR two-component system regulates the order of membrane lipids in the bacterium Bacillus subtilis by controlling the expression of the des gene coding for the delta 5-acyl-lipid desaturase. In this work, we address the process by which DesK transmembrane segments (TMS) transmit temperature signals across the membrane by engineering the 5 TMS domain of the DesK into a single-TMS chimeric sensor. This so-called Minimal Sensor (MS) fully retains *in vivo* and *in vitro* the sensing input and transmission output of the parental system. Progressive deletions of TM segments revealed that only the first TM segment (TM1) is essential to regulate the kinase activity. Therefore, our engineered MS combines the N-terminal 17-residue portion of TM1 with the C-terminal 14-residue portion of TM5 which is naturally fused to the cytosolic catalytic domain. The MS N-terminus contains three hydrophilic aminoacids near the lipid-water interface creating an instability hot spot. This boundary-sensitive motif controls the sensing and transmission activity. Accordingly, we hypothesize that membrane thickness is the temperature agent that determines the signaling state of the cold sensor by dictating the hydration level of the meta-stable hydrophilic spot. This hypothesis is supported through the study of the signaling behavior of MS variants purposely constructed.

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Membrane-Associated Folding and Unfolding
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We are studying the molecular events that occur when a peptide inserts across a membrane or exits from it. Using pH jumps to trigger insertion/exit of the pHLIP (pH Low Insertion Peptide) to enable kinetic analysis, we show that insertion occurs in several steps, with rapid (0.1 sec) interfacial helix formation followed by a much slower (100 sec) insertion pathway to form a transmembrane helix. The reverse process of unfolding and peptide exit from the bilayer core, which can be induced by a rapid pH jump from acidic to basic, proceeds much faster than folding/insertion and through different intermediate states. In the exit pathway, the helix-coil transition is initiated while the polypeptide is still inside the membrane. We also designed two pHLIP-variants where Asp and Glu residues were removed from the C-terminus, which inserts across the membrane. The variants preserve the same pH-dependent properties of pHLIP peptide interaction with the membrane, but insertion occurs 10-30 times faster than in the case of the parent pHLIP peptide. A kinetic model of peptide-membrane insertion/folding and exit/unfolding will be discussed. The work was in part supported by grant from the National Institutes of Health, National Cancer Institute RO1 133890 to OAA, DME, YRK.

471-Pos
Enhanced Uptake of Integral Membrane Proteins by Cubic Nanoparticles
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Self-assembled lipidic cubic phases are attracting increasing interest as biocompatible carriers of large biomolecules including proteins, peptides, DNA and drugs.[1] Their unique structure of interpenetrating but unconnected water channels divided by a lipid bilayer can accommodate hydrophobic, hydrophilic and amphiphilic moieties without significant structural perturbation. This has led to their use in a diverse range of applications including the delivery of drugs and other active agents, as biomimetic crystallization media for membrane proteins, as the basis of biofuel cells, and as biosensors.[2]
Lipidic cubic phases can be emulsified into diluted non-viscous aqueous dispersions consisting of cubic nanoparticles, offering significant advantages for many of the applications listed above.[3] Here we have incorporated an integral membrane protein and important neurological drug target within cubic nanoparticles. We have characterised the structural effect on the micro- and meso-scale properties of the nanoparticles. In addition we have shown that protein loading can be significantly enhanced by doping the cubic nanoparticles with a second amphiphile which chemically binds to the integral membrane protein.
[1]Yaghmur A, Glatter O. Advances in Colloid and Interface Science 2009; 147-148; 333-342.
[2]Nazaruk E, Bilewicz R, Lindblom G, Lindholm-Sethson B. Anal Bioanal Chem 2008; 391; 1569-1578.
[3]Spicer PT. Current Opinion in Colloid & Interface Science 2005; 10; 274 - 279.

472-Pos
Real-Time Detection of Apolipoprotein A-I's Lipidation State by Fluorescence Resonance Energy Transfer
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Apolipoprotein A-I (apoA-I), the main protein component of high density lipoprotein (HDL), is the principal facilitator of cholesterol efflux from cholesterol-laden macrophages. Lipid-free apoA-I is the preferred substrate over lipid-associated apoA-I for cholesterol mobilization by the membrane transporter ATP binding cassette A1, which is responsible for more than 60% of cellular cholesterol efflux from cholesterol-laden macrophages. However, more

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.

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.

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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