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

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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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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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Board B213

We employ all-atom and coarse-grained simulation approaches to investigate the thermodynamics of two ionizable and aromatic protein side chains known to play important roles in membrane protein structure and function. We compute free energy profiles for arginine and tryptophan side chains, isolated or attached to transmembrane alpha-helical peptides, across lipid bilayers. We observed that arginine remains almost exclusively protonated and experiences a large, 17 kcal/mol barrier to cross the lipid membrane. This finding is in opposition to a recent model of voltage gated ion channel activity and translocon-based experiments that have been interpreted to suggest that there could be much smaller free energy penalties. Using the same coarse grained model recently employed in simulations of voltage sensor domains, we obtained barriers that are 3–4 times smaller than values obtained from our all-atom free energy profiles. The comparison of solvation free energies and interactions energies for protein side chain analogs with corresponding experimental and quantum mechanical values demonstrated that results from all-atom simulations are accurate to the order of 1 kcal/mol, whereas the coarse grained model failed to reproduce all relevant energetics by an order of magnitude. These findings reinforce the utility of fully atomistic free energy simulations for uncovering the microscopic mechanisms of protein-lipid interactions and highlight the need for all empirical force fields to be carefully tied into available experimental and quantum mechanical results.

2099-Pos Membrane Permeabilization By Human High Density Lipoprotein

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Board B214

A subset of human high density lipoprotein known as trypanosome lytic factor (TLF) confers innate immune resistance to a variety of trypanosome species, most notably Trypanosoma brucei brucei, the causative agent of wasting disease in African cattle. Trypanosome lytic factor is distinguished from other HDLs by the presence of two proteins, apolipoprotein L-1 (apoL-1) and haptoglobin-related protein (Hpr). It has been shown that purified apoL-1 or Hpr are sufficient for trypanosome killing. Trypanosome killing in normal human serum requires endocytosis of the TLF particle and trafficking to the lysosome. The acidic environment facilitates lysosomal membrane disruption and subsequent trypanosome lysis. In order to understand the mechanism of membrane permeabilization by TLF and its components we have investigated their interaction with model liposomes. Here we show that TLF particles bind and efficiently permeabillize liposomes at acidic but not neutral pH. Binding is ionic strength insensitive and appears to involve the entire particle as opposed to individual components. Additionally we show that apoL-1 or Hpr alone are sufficient for membrane permeabilization and exhibit similar pH dependence.

2100-Pos Evaluating Side-Chain Hydrophobicity using Bilayer-Spanning Channels: Serine-containing Gramicidin Channels

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We have used gramicidin channels as a framework to investigate the consequences of burying polar, hydrogen-bonding hydroxyl groups among the hydrocarbon chains near the center of lipid bilayer membranes. Serine was introduced instead of alanine at position 3 or 5 in the sequence formyl-VG^{2}A^{3}LA^{5}VVVWLWLWLW-ethanolamide (D-residues underlined). In head-to-head, dimeric, membrane-spanning gramicidin channels, these sequence positions are located near the lipid bilayer center and subunit interface. Positions 3 and 5 were tested within the context of either Gly or D-Ala at position 2, because D-Ala causes the channel lifetimes to increase significantly (Mattice *et al.* 1995. *Biochemistry* 34:6827.) Size-exclusion chromatograms and circular dichroism spectra indicate that the serine incorporation is well tolerated and has little effect on channel structure. Each of the gramicidins forms well-defined channels in planar bilayers. As expected, the polar Ser residues decrease the analogues' channel-forming potencies, as well as the average channel lifetimes, as compared to the corresponding Ala-containing channels. The Na^+ currents through heterodimeric channels exhibit rectification, attributable to asymmetric placement of a single Ser hydroxyl group with respect to the bilayer center. For the serine-3 analogues, the lifetimes of heterodimers are *less* than the geometric mean of the corresponding homodimer lifetimes, suggesting probable Ser/Ser side chain hydrogen bonding in the homodimer. The properties of the symmetric channels allow us to estimate the energetic penalty for burying serine hydroxyl groups in the bilayer core. The properties of the hybrid channels allow us to estimate the energy of the hydrogen bonding interaction in the hydrocarbon chain environment.

2101-Pos The Energetics And Protonation State Of An Arginine Side Chain In A Lipid Bilayer

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Board B216

Recent cell biology experiments and a model for voltage gated ion channel gating have suggested small free energy penalties for arginine side chains to cross membranes, challenging a long hold view in membrane biophysics. We have studied side chain analogs as well as transmembrane helix models to elucidate the underlying thermodynamics of arginine translocation and ionization in a membrane environment. Both charged and neutral species were found to

face significant free energy barriers, but with very different underlying mechanisms: the energetics of charged species involving strong interactions with water molecules and lipid head groups throughout the bilayer while the neutral species experiences simple dehydration. We found that local membrane deformations preferentially stabilize the charged form leading to a significant pKa shift only at the bilayer center. However, we conclude that the energetics for arginine movement in membranes is governed almost exclusively by the protonated form. In contrast, analog molecules experience much greater shifts due to exaggerated variations in energetics across the bilayer, emphasizing the important role played by the host protein. The large free energy barrier experienced by arginine suggests that these side chains would not be exposed to the lipid hydrocarbon region of a bilayer, with implications for models of voltage gated ion channel activity.

2102-Pos Effects of Cholesterol and Phosphoethanolamine on Thermodynamics for Self-Association of an Inert Transmembrane

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Board B217

Physico-chemical properties of bilayers, such as hydrophobic thickness, membrane rigidity, and lateral pressure should affect self-association of transmembrane helices, which is a crucial thermodynamic step for membrane protein folding, by changing the balance between helix-helix, helix-lipid, and lipid-lipid interactions. We have measured the complete set of thermodynamic parameters (ΔG, ΔH, ΔS, and ΔC_p) at 5–55°C for antiparallel dimer formation of the inert transmembrane helix X-$(AALALAA)_3$-Y (X = NBD and Y = NH_2 (**I**) or X = Ac and Y = DABMI(**II**)) by fluorescence resonance energy transfer from **I** to **II** in PC vesicles (Biochemistry **2006**, *45*, 3370). Here, we examine the effects of cholesterol and PE, which modulate the rigidity and lateral pressure of bilayers, respectively. It was confirmed that the peptide assumes a transmembrane helical structure in POPC bilayers containing cholesterol (30 mol%) or POPE (50 mol%), by polarized ATR-FTIR spectroscopy. Stronger dimerization was observed in POPC/cholesterol (7/3) vesicles ($\Delta G = -20.9$ kJ mol^{-1}, $\Delta H = -65.3$ kJ mol^{-1}, $-T\Delta S = 44.4$ kJ mol^{-1}, and $\Delta C_p = 1.3$ kJ K^{-1}mol^{-1}) than in POPC vesicles ($\Delta G = -12.8$ kJ mol^{-1}, $\Delta H = -27.9$ kJ mol^{-1} and $-T\Delta S = 15.1$ kJ mol^{-1}) at 35°C. The effect of POPE on thermodynamics for the formation of the antiparallel dimer will also be reported.

2103-Pos Properties of WALP23 in Mixtures of Oriented Lipids Investigated by Solid-State ^{2}H and ^{31}P NMR Spectroscopy

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Biological membranes represent complex mixtures of lipids of different types with varying hydrocarbon chain lengths. In this project the behavior of model WALP23 peptides (Killian *et al*. 1996. *Biochemistry 35*:1037; Strandberg *et al.* 2004. *Biophys J 86*:3709) in lipid mixtures was investigated by using ^{31}P as a probe for the lipid head groups and ^{2}H-alanine as a probe for the peptide. The bilayer normal was aligned either parallel (β=0°) or perpendicular (β=90°) to the applied magnetic field. WALP23 (acetyl-GWW$(LA)_8$LWWA-ethanolamide) has a hydrophobic length approximately corresponding to the hydrophobic portion of DOPC (di-C18:1Δ9c) bilayers. In shorter DLPC (di-C12:0), the peptide "kinks" at position Leu^{12} (Daily *et al. Biophys J* in press), while in longer DEuPC (di-C22:1Δ13c) it induces an isotropic lipid phase. Therefore, it was of interest to study WALP23 behavior in lipid mixtures composed of differing proportions of DLPC, DOPC and DEuPC. It was found that the bilayer structure is generally preserved in lipid mixtures, as shown by ^{31}P NMR, although some isotropic phase may be induced depending upon the peptide/lipid ratio and the DEuPC/(total lipid) ratio. WALP23 incorporates into the lipid bilayers, judging by the two-fold reductions of quadrupolar splitting magnitudes at β=90° compared to β=0°. The influence of bilayer lipid composition upon the geometry and average orientation of WALP23 will be examined in detail.

Intracellular Channels

2104-Pos Wetting, Ionic Conductance, and Structural Stability of the Phospholamban Pentamer

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Board B219

Phospholamban (PLN) is a 6-kDa membrane protein that forms a stable pentamer in a lipid bilayer membrane. As a monomer, PLN directly inhibits the Calcium ATPase, thereby regulating cardiac muscle contraction. Earlier reports have suggested that a PLN oligomer can also regulate calcium flux across the sarcoplasmic reticulum membrane by acting as a calcium ion channel. The recent NMR structure of a PLN pentamer revealed a 7.2-A-diameter, entirely hydrophobic pore. In this study, we used molecular dynamics to investigate the stability of the NMR model and determine whether the PLN pentamer can transport ions. A microscopic model of the PLN pentamer was constructed by merging the NMR structure with a patch of a solvated lipid bilayer membrane and physiological solution. To explore the structural dynamics of the pentamer, the system was equilibrated for over 30 ns. Within less than one nanosecond of the onset of equilibration, a chain of water that was initially placed in the pentamer's pore disappeared. After several nanoseconds, the pentamer underwent structural rearrangements resulting in partial closure of the pore at the cytoplasmic side.