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

stoichiometries of 2:1 or higher. At pH 7, the folding kinetics of OmpA into lipid membanes was well described by double-exponential time courses, both in the presence and in the absence of FkpA. While the presence of FkpA did not affect the rate constants of the faster OmpA folding phase, both the relative contribution of the faster rate to the overall folding kinetics and the rate constants of the slower folding phase were increased when FkpA was present. Our results provide direct evidence that the periplasmic chaperones Skp and FkpA support folding of OMPs into lipid membranes independently of each other.

[1] Patel, G. J., Behrens-Kneip, S., Holst, O., Kleinschmidt, J.H., 2009 Biochemistry, in press.
[2] Qu, J., Behrens, S., Holst, O., Kleinschmidt, J.H., 2007 J. Mol. Biol. 374, 91-105.

483-Pos

Fusion Peptide Effects on Epitope Recognition At Membrane Surfaces by the Broadly Neutralizing Anti-HIV-1 2f5 Monoclonal Antibody

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The HIV-1 glycoprotein fusogenic subunit gp41 is the target for 2F5, a broadly neutralizing monoclonal antibody (MAb2F5) isolated from asymptomatic infected individuals. The 2F5 epitope locates close to the membrane interface within the membrane proximal external region (MPER) that connects the HIV-1 envelope gp41 ectodomain with the transmembrane anchor. Here evidence is presented indicating that the conserved amino-terminal fusion peptide (FP) increases the affinity of this antibody for its membrane-inserted epitope. Structural characterization by circular dichroism together with membrane-disrupting activity measurements suggests the formation of FP-MPER complexes at the surface of lipid bilayer vesicles. MAb2F5 associated more efficiently with lipid vesicles containing FP and MPER peptides as compared to those containing only MPER, or MPER in combination with FPctl, a scrambled version of the FP. Moreover, the N-terminal FP sequence had almost no effect on membrane-inserted epitope binding by MAb4E10, a neutralizing antibody that has been shown to extract its C-terminal MPER epitope from the membrane interface. In combination with recently reported crystallographic data (Julien et al. (2008) J. Mol. Biol., 384, 377-392), these results support a ''catch-and-hold'' mechanism for the process of MAb2F5-epitope binding at membrane surfaces.

484-Pos

Effect of the Conjugation of Peg to the PLL on the Micro- and Mesoscopic Properties of a POPC Bilayer

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Recent studies have demonstrated that the conjugation of poly(ethylene glycol) (PEG) to poly(L-lysine) (PLL) allows these copolymers to be adsorbed to the surfaces of pancreatic islets with minimal toxicity. The molecular mechanism for the effect of the conjugation of PEG to PLL on attenuation of cytotoxicity remains unknown. This knowledge is important for the design of optimized PLL-*g*-PEG copolymers for the design of nanoscale immunoisolation barriers. In this study, we investigated the conformational and electrostatic changes of PLL by the grafting of PEG chains, and the interaction changes of the PLL-*g*-PEG copolymer with a POPC bilayer compared to that of the PLL polymer with molecular dynamics simulations. The results showed that the grafting of PEG chains to PLL resulted in a change in the conformation of PLL from a randomly coiled structure to a structurally more stable globular conformation, and a significant change in electrostatics distribution. The interactions of PLL and PLL-*g*-PEG copolymers with a POPC bilayer showed that conformational and electrostatic changes resultant from the conjugation of PEG to PLL further affected the interaction of the polymer with the lipid bilayer. In addition to resulting in a structural change of the lipid bilayer, the permeability, compressibility, and bending modulus of the lipid bilayer are also affected by PEG grafting to PLL. The results will provide molecular insight for the experimental observation about the effect of the conjugation of PEG to PLL on the reduced cototoxicity.

Keywords: PEG; PLL; conjugation; conformation; electrostatics; lipid bilayer; micro-mesoscopic properties.

485-Pos

Observation of Backbone C_α-Deuteron Signals in Solid-State NMR Spectra of Labeled Alanines in Oriented Transmembrane Peptides

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Solid-state deuterium NMR spectra combined with the Geometric Analysis of Labeled Alanines (GALA) method provide information about the average apparent tilt and dynamics of membrane-spanning peptides in oriented, hydrated lipid bilayer membranes. When Ala-d_4 labels are used, the side-chain methyl (CD_3) signals are readily detected, but the weaker backbone C_α-D resonances are often problematic to observe (van der Wel, Biophys J, 83, 1479) for peptides of the WALP family [GWW$(LA)_n$LWWA]. In spite of this difficulty, we recently have begun to observe surprisingly intense backbone C_α-D resonances in the ^{2}H NMR spectra for some oriented transmembrane peptides, particularly in cases where the $(LA)_n$ core sequence of WALP is interrupted by a ''guest'' residue such as Pro, Lys or Arg. Examples will be discussed. In some cases, the C_α-D signal intensities appear to depend upon the macroscopic orientation of the lipid bilayers with respect to the external magnetic field. The sequence and orientation dependence of the backbone C_α-D signals may therefore reflect the local and global peptide dynamics in ways that are incompletely understood. A selection of spectral examples will be presented and interpreted.

486-Pos

Charged and Aromatic Anchoring Amino Acids Affect the Orientation of Transmembrane Peptides: A Deuterium NMR Study

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In integral proteins, the membrane-spanning segments are often flanked by aromatic and/or charged amino acids, which serve as anchoring residues, promoting protein-lipid interactions. These residues have specific polarity preferences, which are reflected in their typical immersion depths. Namely, tryptophan (W) is localized primarily to the carbonyl region of lipid acyl chains, while charged lysine (K) and arginine (R) prefer more polar regions. Although such patterns are widely recognized, little is known regarding the contributions of these residues for the orientations of transmembrane segments.

Solid-state NMR spectroscopy offers a way to approach this problem. Peptides of the ''WALP'' family, GWW$(LA)_n$LWWA, have served as useful models. Membrane-spanning proteins, nevertheless, typically contain more diverse sets of anchoring amino acids, which may act together to influence molecular geometry and orientation. To address these issues, we have designed the $\underline{X}^{2,22}W^{5,19}$ALP23 peptides (acetyl-G$\underline{X}$ALW^5(LA)$_6$LW^{19}LA$\underline{X}$A-[ethanol] amide) to encompass two different anchor residues. In these peptides the *inner* anchor is kept constant (W), while the *outer* ''anchor'' (separated by a short Leu-Ala spacer) is varied to either W, K, R or the control residue G.

Solid-state ^{2}H NMR spectra were recorded for peptides having Ala CD_3 groups in aligned bilayers of DLPC, DMPC and DOPC. The ''Geometric Analysis of Labeled Alanines'' provided a means to deduce the apparent average peptide orientations. Introduction of charged anchors ($\underline{X}$ = K or R) resulted in a 2-5° increase in the apparent tilt angle compared to $\underline{X}$ = G. Conversely, for $\underline{X}$ = W, the apparent tilt angle decreases 2-9°, accompanied by a significant change in the tilt direction.

487-Pos

Influence of WALP Peptides on Phase Behavior of Cholesterol Containing Ternary Lipid Mixtures

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Partitioning of lipid bilayer membrane components plays a crucial role in biological processes. For example, cellular mechanisms such as signaling, adhesion and transport are often dependent on and regulated by inhomogeneities in the biological membranes. Ternary lipid systems containing cholesterol provide an opportune model for characterizing phase behavior, such as separation between liquid disordered and liquid ordered ("raft") phases. In these model systems one can incorporate (non-perturbing) deuterium labels and use ^{2}H NMR methods to establish the phase behavior of the system as a function of temperature. It is of interest to investigate also the possible influence that membrane-spanning peptide components may have on the segregation of lipids.

In some experiments, we have used the saturated fluid model lipid diphytanoylphosphatidylcholine (DPhPC). We have investigated both DPhPC:DPPC-d_{62}:Cholesterol and DOPC:DPPC-d_{62}:Cholesterol (35:35:30) mixtures, with and without incorporation of WALP peptides of different lengths (acetyl-GWW$(LA)_n$LWWA-ethanolamide).

When WALP peptides are introduced into DPhPC:DPPC-d_{62}: Cholesterol, a peptide length-dependent appearance of non-bilayer phase is observed. The non-bilayer phase is apparent in both ^{2}H and ^{31}P NMR spectra. A prominent isotropic lipid peak has disqualified this system as a model for investigations of longer WALP peptides.

The DOPC:DPPC-d_{62}:Cholesterol model system is better behaved. Upon addition of WALP peptides, no apparent formation of non-bilayer phase is evident. Incorporation of WALP peptides of various lengths results in a slight increase in phase transition temperature, from a range of about 288-293 K with no peptide present, to a range of about 293-298 K when a WALP peptide is present at 1/160 (peptide/total lipid). Results for different length WALP peptides will be discussed.