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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.

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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.

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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.

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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*.

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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.

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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