Using a combination of all-atom and coarse-grained molecular dynamics simulations to interpret a range of x-ray scattering experiments, we aim to understand the role of membrane deformation in the action of the Parkinson's Disease protein, α-Synuclein. Our simulation results have led to the hypothesis that αS flattens curved membranes by screening the repulsive interactions between negatively charged, acidic headgroups, thereby reducing the effective area per headgroup and relieving the inherent positive curvature of the lipids on the outer leaflet of synaptic vesicles. We hope to address the question of whether αS influences a membrane's mechanical properties as a route to evaluating this hypothesis. Additionally, we aim to understand the role of αS in recruiting sub-domains of positively charged lipids. A second, smaller peptide (the CRAC motif from gp41) is also studied in an effort to build the computational tools necessary for matching the x-ray data that is used for calculating a membrane's material properties.

2528-Pos

Effects of Subphase on Collapse Behavior of Lung Surfactant

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The phase behavior of binary fluids next to interfaces can be complex. If one fluid has a more favored interaction with the interface the fluids can phase separate in some interfacial region extending into the bulk. Using neutron and x-ray reflectivity, we show phase separation of water/glycerol mixtures next to lipid monolayer interfaces. The glycerol forms a thin layer ten angstroms deep underneath the monolayer. This non-equillibrium interfacial phase separation greatly impacts the mechanical properties of the lipid monolayer. Moreover, the thermodynamic driving force for this de-mixing is complex. Usually such de-mixing is observed when two miscible fluids have significantly different surface tensions at a given interface. However glycerol and water are miscible and have nearly identical surface tensions at the air/water interface. Our work probes what surface tension and interfacial free energy mean in the setting of more complex interfaces. The preference partitioning of glycerol to the interface affects the collapse behavior of the lipid film and has implications on the collapse mechanism of lung surfactant which sits atop an alveolar lining fluid enriched in sugar biopolymers.

2529-Pos

Oxygen Diffusion Through Lung Surfactant Layers

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Pulmonary surfactant, a lipid-protein complex covering the air-liquid interface of alveoli, is essential for preventing alveolar collapse at the end of expiration. To do so, surfactant reduces surface tension by forming a surface-active interfacial film, which has to be crossed by oxygen to reach the pulmonary epithelium and the capillary. The effect of the presence of the pulmonary surfactant layer in oxygen diffusion has not been properly evaluated.
Here we have developed a special setup using luminescent Rutenium-containing organo-metallic oxygen sensors to measure oxygen diffusion rates through capillary water layers containing different concentrations of pulmonary surfactant lipid or lipid-protein preparations.
The potential role of surfactant and the structure of surfactant membrane network in terms of facilitating oxygen diffusion through the air-water respiratory interface will be discussed.

2530-Pos

Molecular Organization of the Tear Film Lipid Layer

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Purpose. To describe the molecular organization of the anterior lipid layer of the tear film.
Methods. Artificial tear fluid lipid layers (ATLL) were deposited on the air-water interface and their physico-chemical behavior was compared to egg-yolk phosphatidylcholine (eggPC) monolayers by using Langmuir-film balance techniques, X-ray diffraction, atomic force microscopy, and Brewster angle microscopy. These experimental approaches were complemented by *in silico* molecular level simulations.
Results. In contrast to eggPC monolayers compression isotherms of the ATLL suggested that at higher surface pressures the ATLL films were no longer monolayers. ATLL films had a lower compressibility compared to eggPC lipid films. At π=20 mN/m both samples or part of the samples were in the condensed phase. Brewster angle microscopy suggested that in the case of ATLL a clear phase separation was observed. Atomic force microscopy preformed at π=20 mN/m showed only a smooth surface for eggPC, whereas for ATLL lipoprotein-like particles were protruding from the otherwise smooth lipid film. Computer simulations on eggPC and ATLL yielded a detailed picture of the atomic level organization of eggPC and ATLL residing on the air-water interface and supported the experimental findings.
Conclusions. Here we provide detailed structural analysis of eggPC and ATLL films deposited on the air-water interface. The results are discussed in the context of *in vivo* function of the tear fluid.

2531-Pos

Molecular Scale Texture and Topological Defects in Lipid Membranes: A New Liquid Crystalline Phase

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Lipid membranes are self-organizing structures that define intercellular and intracellular interfaces in biological systems. Grazing incidence x-ray diffraction (GIXD), provides a sensitive probe of the local, molecular structure and packing of lipid molecules within single membranes. For example, diffraction clearly establishes that dipalmitoyl-phosphatidylcholine (DPPC) membrane leaflets are always coupled across the bilayer, and that even when leaflets are deposited independently the membrane rapidly self-organizes so that opposing lipid tails scatter as one entity. Variation in the azimuthal tilt direction of the lipid tails was required to reproduce the diffraction data indicating an orientational texture of lipid molecules and smectic domains formation identical to larger scale textures observed in many 2-D liquid crystalline systems, but at a molecular scale.
A similar phenomenon is also observed when proteins bind to membrane receptors. The interplay between lipids and proteins is complex: lipids can influence the structure and function of membrane proteins and at the same time proteins can impact lipid organization. In this example, lipid monolayers at the air-water interface containing the ganglioside GM1 were studied in the absence and presence of cholera toxin. At low surface pressures, protein binding perturbed the lipid order such that the molecules were no longer close packed, creating topological defects and lipid-protein domains with orientational texture. This new lipid phase may be a mechanism for toxin penetration and potentially has far broader implications in biological signaling.

2532-Pos

Calculation of Interleaflet Domain Coupling in Mixed Lipid Bilayers

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The coupling between the physical states of the two leaflets (monolayers) of a lipid bilayer is a subject of current interest in relation to both the biology of lipid rafts and the physics of model membranes capable of liquid-liquid phase separation. In these model systems there is experimental evidence of a large coupling which maintains micron-scale registry between domains in the two leaflets. Nevertheless, the mechanism of this coupling has been unclear. We have performed a mean-field calculation with molecular detail to evaluate the contribution to this coupling due only to lipid tail interdigitation. By comparing the free energies of symmetric and asymmetric lipid compositions, we obtain a coupling strength of 0.2 kT per square nanometer. This is enough to account for micron-scale domain registry and is in favorable agreement with a recent estimate from a coarse-grained molecular dynamics simulation. Our result supports the hypothesis that lipid interdigitation is the dominant mechanism for interleaflet domain coupling in model membranes capable of liquid-liquid phase separation.

2533-Pos

Assembly of Lipid Bilayers in Large Scaffold Arrays

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In this study we have addressed the assembly of lipid bilayers in arrays and the stability of established membranes in different scaffold geometries.
To establish planar lipid membranes across large scale partition aperture arrays, we created a disposable single-use horizontal chamber design that supports combined optical-electrical measurements. Lipid bilayers could easily and efficiently be established across CO_2 laser micro structured 8×8 aperture partition arrays with average aperture diameters of 301 ± 5 μm.
To demonstrate the functionality of the lipid bilayers established across the 8×8 arrays, controllable reconstitution of the biotechnological and physiological relevant peptides valinomycin and gramicidin A, together with the membrane proteins α-Hemolysin and FomA were carried out. The results showed that the design supports low current (high sensitivity) recordings of membrane peptides and proteins by incorporating gramicidin A, α-Hemolysin and FomA into the established lipid bilayers.Finally, we tested the scalability of the assembly of lipid bilayers by creating rectangular 24×24 and hexagonal 24×27 lipid membrane arrays respectively. The two different geometries of the micro structured aperture arrays seem to support stable and functional membrane arrays, however, with somewhat different electrical properties. We propose that the presented design may be suitable for further developments of sensitive biosensor assays.

2534-Pos
Comparison of the Effects of Cholesterol or 3β-Hydroxy-5-Oxo-5,6-Secocholestan-6-Al on the Thermotropic and Structural Properties of Mixtures of Phosphatidylethanolamine and Phosphatidylcholine
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The oxidation of cholesterol with ozone produces 3β-hydroxy-5-oxo-5,6-secocholestan-6-al. This oxysterol has been implicated in a number of pathological conditions *in vivo* including atherosclerotic plaque formation and amyloidogenesis. We have shown previously that this oxysterol strongly modifies the physical properties of model membranes composed of different phosphatidylethanolamines or phosphatidylserine. In the present work we have extended our studies to ternary mixtures composed of phosphatidylethanolamine and phosphatidylcholine with sterols, either 3β-hydroxy-5-oxo-5,6- secocholestan-6-al or cholesterol. We use differential scanning calorimetry and small angle X-ray diffraction to characterize the phase behavior of mixtures of dipalmitoleoylphosphatidylethanolamine (diPoPE) and dipalmitoleoylphosphatidylcholine (diPoPC) or 1-palmitoyl-2-oleoyl-phosphatidylethanoloamine (POPE) and 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC). We compare the effect of the two sterols on the temperature of the transition of the ternary system from the liquid crystalline to the hexagonal phase (T_H) and on the curvature of the resulting cylindrical micelles. Addition of low concentrations of diPoPC increases T_H while adding cholesterol to this mixture significantly lowers T_H. The effect of 3β-hydroxy-5-oxo-5,6-secocholestan-6-al is much weaker than that of cholesterol. With regard to the curvature of the cylindrical micelles, the addition of diPoPC and 3β-hydroxy-5-oxo-5,6-secocholestan-6-al have opposing effects, while cholesterol does not effect the curvature at all. Low concentrations of POPC in POPE cause an increase in T_H and decrease the curvature of the cylindrical micelles, while cholesterol has the opposite effect.

2535-Pos
Self-Assembly Simulations of Membranes Containing Phospholipid Oxidation Products
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Products of phospholipid oxidation (OXPLs) are involved with the genesis or pathology of several diseases. OXPLs can modify the physical properties of biological membranes, thereby possibly altering several biological processes near membranes including signaling pathways. We have used atomistic and course grained simulations to investigate the properties of OXPL-containing lipid bilayers. We ran self assembly simulations of mixtures of palmitoyl-oleoyl-phosphatidylcholine (POPC) with two different OXPLs: PazePC, which is anionic, and PoxnoPC, which is zwitterionic. The total sampling time exceeds 1 millisecond. Despite having shortened and polar acyl chains, the two OXPLs POPC self assemble into stable lipid bilayers with POPC. The bilayers can accommodate at least 25% OXPL, although such bilayers have a lower area compressibility modulus. As as example of the modification of a membrane-associated biological process, we show that KALP-23 peptides partition differently in POPC-OXPL and POPC bilayers. The peptides adopt a transmembrane orientation more easily when OXPLs are present in the bilayers.

2536-Pos
Study of the Cholesterol Umbrella Effect in DPPC and DOPC Bilayers by Molecular Dynamics Simulation
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The instability of cholesterol clusters in dipalmitoylphosphatidylcholine (DPPC) and dioleoylphosphatidylcholine (DOPC) lipid bilayers was investigated via atomistic Molecular Dynamics (MD) simulation. Cholesterol clusters in phosphatidylcholine (PC) bilayers are found to be very unstable and to readily disperse into cholesterol monomers. The instability may result from the difficulty for the system to prevent water exposure to cholesterol's aggregated hydrophobic bodies in a cluster. The system responds to artificially arranged cholesterol clusters in several interesting manners: (i) Cholesterol clusters quickly form a "frustum" shape to reduce water penetration through cholesterol headgroups; (ii) Many clusters bury themselves deeper into the bilayer interior, causing local bilayer deformation; (iii) Cholesterol fluctuates rapidly, both laterally and vertically to the bilayer plane, in order to escape from clusters. These fluctuations result in the disintegration of clusters, and in one incidence, a highly unusual flip-flop event of a cholesterol across the DOPC bilayer occurs. Our results show that cholesterols have a strong tendency to avoid forming clusters in lipid bilayers and that the fundamental cholesterol-cholesterol interaction is unfavorable. Furthermore, the radial distribution functions of cholesterol hydroxyl oxygen to various headgroup atoms of PC reveal that the PC headgroups surrounding cholesterol have a clear tendency to reorient and extend toward cholesterol. The range of this "Umbrella Effect" can reach up to 2-3 nm, larger than previously reported.

2537-Pos
Flip-Flop Motions of Lipid Molecules in Mixed Bilayer Systems
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The cell membrane is composed of a wide variety of lipid molecules, cholesterols, and membrane proteins. Lipid molecules in the membrane have several time scales of motions ranging from femtosecond to seconds. The flip-flop motion, in which lipid molecules move from one leaflet to the other, is known to be one of the slowest: it typically occurs within several tens of seconds, or much longer. Recently, experimental studies revealed that cholesterols (CHOL), diacylglycerols (DAG), and ceramides (CER) show fast flip-flop motions in some membranes. However, the molecular mechanisms underling the motions remain elusive.
In this work, we performed coarse-grained molecular dynamics simulations, using MALTINI force field parameters. We examined flip-flop motions of CHOL, DAG, and CER in phospholipid bilayer systems, composing of DAPC(di-20:4), SAPC(18:0-20:4), and POPC(16:0-18:1). In the simulations using DAPC membranes, we observed flip-flop motions of CHOL, DAG, and CER within a microsecond. The flip-flop rate of CHOL was the highest, whereas that of DAG was lower than CHOL. CER flipped only once during the simulation. This tendency of flip-flop motions is strongly correlated with the relative positions of the lipids to the bilayer membranes: CHOL stays almost at the center of the membrane, whereas the head group of CER is located at the water/membrane interface and interacted with solvent molecules strongly.
The flip-flop motions of lipids were also affected with the membrane environment. Within 1-microsecond simulations, CHOL flipped 257 times in DAPC, 196 times in SAPC, and 5 times in POPC. Thus, the flip-flop rate is strongly correlated with the number of double bonds in the acyl chains of bilayer phospholipids, suggesting the importance of the membrane fluidity. These simulation results qualitatively agree with existing experimental data and shed light on the molecular mechanisms underlying the dynamics of biomembranes.

2538-Pos
The Effect of Cholesterol on Membrane Chain-Chain Packing
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Membrane structure is very important issue for cell. Membrane is not only the ''wall'' for protecting cell but also the interface for exchanging signal, ions and molecules. Many evidences show that membrane protein will fold to functional structure by associating with suitable membrane structure. Lipid chain-chain packing is one of important structures and will affect membrane thickness, lipid lateral diffusion and membrane domain formation. We will use grazing incident X-ray diffraction to probe lipid chain-chain packing. The 12keV X-ray light source in BL13A beam line of NSRRC and home-made humidity-temperature controlled chamber will be applied in the measurements. Cholesterol will