3473-Pos
Correlating Obstructed Diffusion with Obstacle Morphology using Single Molecule Tracking and AFM in Supported Lipid Bilayers
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Biophysical research has shown that membrane phospholipids and proteins diffuse not only under normal Brownian diffusion, but with confined and anomalous behavior. We are motivated to understand this anomalous diffusion because it may be involved in many cell functions. We have used single molecule tracking on phase separated, supported lipid bilayers to investigate the origin of this unusual diffusion. DSPC forms ~250 nm, gel phase domains that act as obstacles to diffusion in the DOPC continuous liquid phase. Incorporated into the fluid phase at very low concentration is DMPE labeled with Alexa Fluor 647. When combined with the low background of a single supported bilayer on a quartz substrate, this high quantum yield dye yields a signal to noise ratio greater than 10. By controlling the gel domain morphology and characterizing it with atomic force microscopy, we can correlate the observed single molecule diffusion with the obstacle characteristics. We also compare our results to simulated Brownian diffusion in the presence of experimentally determined obstacle fields. An understanding of this correlation will aid studies that cannot directly characterize the obstacles to diffusion.

3474-Pos
Novel Probes for Sensing Lateral Stress in Membranes
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An understanding of the link between lipid composition and biomechanical properties of the bilayer (e.g. the lateral pressure and bending rigidity) is key to understanding the mechanisms that underpin lipid-protein interactions. Whilst it is now possible to determine parameters such as the spontaneous curvature and bending rigidity of lipid bilayers, evaluation of the lateral pressures within membrane systems remains elusive. Here, we present a novel platform based upon fluorescent probes that are able to sense the stored stresses within lipid bilayers and show how these correlate with the make-up of the membrane. In particular, we have used this system to study the effect of phosphatidylinositol lipids upon model membrane systems.

3475-Pos
Lipid Diffusion in Tethered Bilayer Lipid Membranes (tBLMs)
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The complexity of cells makes molecular-scale characterizations of structure and interactions of biomembranes *in vivo* extremely difficult, thus driving the development of synthetic membrane models. tBLMs are resilient biomimetic systems stabilized by the proximity of an inorganic interface.[1,2] We characterize their in-plane structure, dynamics and dielectric properties using fluorescence microscopy, fluorescence correlation spectroscopy (FCS) and electrochemical impedance spectroscopy (EIS). The in-plane dynamics of tBLMs depend on structural details of the anchor lipid and its lateral density in the bilayer leaflet proximal to the substrate.[3] In tBLMs with homogeneous lateral label distributions, the fluidity of the distal leaflet is comparable to that in vesicle membranes (2D diffusion constant, $D \sim 7\ \mu m^2/s$) while that in the proximal leaflet is moderately reduced ($D \sim 2\text{-}3\ \mu m^2/s$). tBLMs completed with phytanoyl lipids (DPhyPC) show lower label diffusivity than those completed with unsaturated chains (DOPC). In laterally heterogeneous bilayers, the label diffusivity varies only slightly, indicating that distinct regions in the bilayers do not correspond to distinct phases. Concurrently, we investigate the effect of charged lipids (DOPS) and cholesterol on lipid diffusivity. This aims at a characterization of changes in membrane dynamics as amyloid-β (**Aβ**) oligomers associate with tBLMs where they have been shown to affect the resistance of the bilayer to ion conduction.[4,5]
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3476-Pos
Monitoring Submicron and Micron-Size Membrane Compartments using Quantum Dots Monovalently Conjugated to Tracer Molecules
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The current paper describes the application of highly-photostable quantum dot (QD)-conjugated lipids and membrane proteins to explore membrane compartmentalization in model and plasma membranes over a wide range of length and time scales. A rigorous screening protocol is described that assures the bioinertness of QD coatings and the monovalent binding of QDs to tracer molecules. Here, the quality of several bioinert surface coatings is tested by determining their impact on the colloidal stability of CdSe/ZnS QDs in aqueous solution using confocal fluorescence correlation spectroscopy. The monovalent binding of QDs to tracer molecules is verified using a sensitive single molecule tracking assay, which is based on QD-conjugated lipids in a solid-supported lipid bilayer. Three different examples are discussed, in which QD-tracking probes are successfully employed to elucidate the membrane organization in model and plasma membranes. Tracking experiments on compartmentalized polymer-tethered lipid bilayers illustrate that, unlike organic fluorescence dyes, photostable QD-based membrane probes are well-suited to detect micron-size compartments with partially permeable diffusion barriers. Results from wide-field single molecule fluorescence microscopy experiments with frame rates of up to 1000 fps on several cell lines show that QD-conjugated tracer molecules are well-suited for fluorescence-based, long-term, high-speed tracking experiments in plasma membranes. Finally, we discuss changes in membrane compartmentalization induced by insulin as shown through tracking results from QD-conjugated transferrin receptors in healthy and insulin-resistant adipocytes and the impact of chromium picolinate on receptor mobility.

Membrane Structure III

3477-Pos
Phase Behavior and Molecular Interactions of Membranes Containing Phosphatidylcholines and Sterol: A Deuterium NMR Study
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We study the phase behavior and properties of model membranes containing DPPC and POPC with or without sterol using ^{2}H-NMR. The *sn-1* chains of POPC and DPPC are deuterium-labeled in turn, such that information regarding each lipid component can be obtained. NMR spectra were taken as a function of temperature. The chain order of DPPC is greater than that of POPC in all mixtures studied. In DPPC/POPC binary mixtures, coexistence of solid-ordered (so) and liquid-disordered (ld) phases is observed in a wide temperature range. The results for ternary mixtures show that the addition of sterol promotes the formation of the liquid-ordered (lo) phase. Furthermore, the influence of ergosterol on the lipid-lipid interactions is not as robust as that of cholesterol. Cholesterol enhances the DPPC-POPC interaction significantly. The phase behaviors of ternary mixtures will be discussed.

3478-Pos
Evidence of Coexisting Phases in Binary Mixtures of POPC/ceramide3
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Stratum corneum, the uppermost layer of skin epidermis exhibits an unusual lipid composition consisting mostly of long-chain asymmetric ceramides with different head groups, both saturated and unsaturated. Small-angle X-ray scattering (SAXS) was used to investigate the impact of one of the aforementioned ceramides, Ceramide3 (Cer3), on the structure of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) host membranes. Results on the composite model system for three mole ratios Cer3:POPC (5:95; 10:90; 15:85) are presented and compared to properties of the pure systems. All methods applied so far (SAXS, DSC, confocal microscopy) reveal non-ideal miscibility of the two components with macroscopic separation of coexisting phases of different rigidity, indicating a coexistence of two lamellar POPC-rich phases with crystalline Cer3 in all the binary mixtures. An especially developed 4G hybrid approach based on a modified Caillé theory was used to model the POPC-rich phases in the temperature range of [0°C, 90°C]. Average structural parameters such as bilayer thickness, lamellar repeat distance and Caillé parameter were extracted from the model together with the electron density profile for the coexisting POPC-rich phases. For the crystalline structure of pure Cer3 a distorted orthogonal unit cell was found by indexing. In summary, binary mixtures of Cer3:POPC exhibit a complex arrangement within the lipid matrix that we try to file into a simple model to account for the results.

3479-Pos
Physical Properties of the Lipid Diphytanoyl Phosphatidylcholine (DPhPc) used for Ion Channel Measurements
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The synthetic lipid diphytanoyl phosphatidylcholine (DPhPC) has been commonly used in measurements of ion-channel activities and in studies of