EPR spectra recorded with the spin label, cholestane, in terms of a superposition of spectral components from the two environments. We compare our results on 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) to other methods and measure the affinity of cholesterol for phospholipids with increasing levels of unsaturation.

1501-Pos Board B271
Phase Separation in Model Membranes Controlled by Hybrid Lipids
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Biological membranes are currently considered as a heterogeneous and highly dynamical organization of lipids and proteins. Some of these components phase separate in finite-size domains (known as 'rafts') to play a vital role in cellular signaling and transport process. Recent theoretical approaches have suggested that the hybrid lipids (lipids with one saturated and one unsaturated tail) stabilize nano-sized domains by controlling the line tension between 'gel' and 'fluid' phases [1,2]. Our x-ray scattering experiments on highly oriented multilamellar samples prepared on a hydrophilic surface have exhibited a strong effect of such hybrid lipids on the phase separation behavior in mixtures of saturated and unsaturated lipids. The phase transition temperature is found to decrease dramatically with the added mole percent of the hybrid lipids. Further, the bilayer repeat distance and the amount of phases are found to vary systematically as a function of the added hybrid lipids. This experimental study opens up an opportunity to verify the theoretical ideas about the role of hybrid lipids in forming the 'raft' in cellular membranes.
[1] R. Brewster, et al., Biophys. J. (2009), 97, 1087-1094.
[2] T. Yamamoto et al., Euro. Phys. Lett. (2010), 91, 28002.

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Probing the Organization and Dynamics of Lipid Probes in Phase Separated Supported Bilayers using Super-Resolution Fluorescence Localization Microscopy
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Vesicles containing two coexisting liquid phases are widely used to study lateral heterogeneity of membrane components. We recently developed a method to deposit unilamellar supported bilayers on an agarose cushion that conserves the macroscopic phase separation and circular domain morphology usually found in free-floating giant vesicles. We are using these supported bilayers in conjunction with single particle tracking and super-resolution fluorescence localization imaging to probe domain structure, probe mobility, and probe confinement in well defined model membranes. We use the fluorescent lipid analogs DiD and DiIC12 to probe the liquid-disordered phase, while we use Alexa-647 labeled Cholera Toxin B subunit bound to the ganglioside GM1 to label the liquid-ordered phase. By reconstructing images of accumulated localized single probe centers, we observe domain morphology at ~20nm lateral resolution. By calculating mean squared displacements (MSD) from an ensemble of single molecule trajectories, we measure diffusion coefficients for the different probes as a function of membrane composition and temperature. As expected, we observe confinement of probes when they partition strongly into the discontinuous phase, and measured MSD vs. time curves are consistent with confinement within circular domains of the size given by our reconstructed images. We are currently investigating the organization and dynamics of probes in membranes undergoing critical fluctuations, and are applying these same imaging and analysis techniques to quantify the diffusion of these same probes in the plasma membranes of living cells.

1503-Pos Board B273
Dynamics and Size of Crosslinking-Induced Lipid Nanodomains in Model Membranes
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Changes of membrane organization upon crosslinking of its components trigger cell signaling response to various exogenous factors. Crosslinking of raft gangliosides GM1 with cholera toxin (CTxB) was demonstrated to cause microscopic phase separation in model membranes and the CTxB-GM1 complexes forming a minimal lipid raft unit are subject of ongoing cell membrane research. Yet, those subdiffraction sized rafts have never been described in terms of size and dynamics. By means of two-color z-scan fluorescence correlation spectroscopy, we show that the nano-sized domains are formed in model membranes at lower sphingomyelin content than needed for the large scale phase separation and that the CTxB-GM1 complexes are confined in the domains poorly stabilized with sphingomyelin. Fluorescence resonance energy transfer together with Monte Carlo modeling of the donor decay response reveal the domain radius of approximately 8 nm, which increases at higher sphingomyelin content. We observed two types of differently behaving domains, which suggests a dual role of the crosslinker: first, local transient condensation of the GM1 molecules compensating lack of sphingomyelin and second, coalescence of existing nanodomains ending in large scale phase separation.

1504-Pos Board B274
AFM Determination of the Elasticity of Model Biomembranes
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The utility of AFM (Atomic Force Microscopy) to study surface morphologies and molecular level forces is well known. It is also possible to apply well-defined small forces on soft surfaces using the AFM. The latter is being used here to determine the elasticity (Young's modulus) of model membrane lipid bilayers. The mechanical properties of biological membranes are of interest in understanding various biologically related processes like cell hemolysis, viral fusion and pore formation, etc. and are closely related to the cell stability. The method involves determination of the force curves by compressing the lipid bilayer by indenting the AFM tip on the bilayer surface, and the resulting elastic response of the bilayer to the applied force (load) is analysed using theoretical models for the tip geometry. We will discuss the sample preparation technique and the results of our experiments on coexisting fluid-phase domains in typical 'lipid raft' mixtures, as well as the effects of varying the level of unsaturation of the lipid carbon chains.

1505-Pos Board B275
Rafts: The Manifestation of a Curvature-Induced Microemulsion
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To explain the appearance of heterogenieties in the plasma membrane, I propose a hypothesis which begins with the observation that the environment of many different lipids in the membrane's two leaves makes it susceptible to fluctuations of a non-zero curvature. These couple to composition fluctuations at non-zero wave numbers which are readily excited thermally. When this coupling is strong, it is well known that it leads to microphase separation and modulated phases. I note that when the coupling is less strong, the tendency towards modulation remains manifest in a liquid phase that exhibits transient structure of a characteristic size; that is, it is a microemulsion. It is induced by curvature, not by a line-active agent. The structure is manifest in a structure function which displays a peak at non-zero wavenumber, and a correlation function which exhibits exponentially-damped oscillations as a function of distance. The damping occurs over the usual correlation length, and the oscillations occur with a characteristic wavelength. This is found to be on the order of the square root of the ratio of the bending modulus of the bilayer to its surface tension. For the plasma membrane in the presence of a cytoskeleton, this distance is on the order of 100 nm. The theory predicts interesting possibilities for correlations between the two leaves. For example, a region rich in saturated lipids in the outer leaf would be surrounded by an annulus poorer in them, and would be opposite a region in the inner leaf poor in saturated lipids surrounded by an annulus richer in them. Experiments to verify the hypothesis of rafts as a curvature-driven microemulsion are proposed.

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A Statistical Mechanical Model of Cholesterol/Phospholipid Mixtures: Linking Condensed Complexes, Superlattices, and the Phase Diagram
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Despite extensive studies for nearly three decades, lateral distribution of molecules in cholesterol/phospholipid bilayers remains elusive. Here we present a statistical mechanical model of cholesterol/phospholipid mixtures that is able to rationalize almost every critical mole fraction (X_{cr}) value previously reported for sterol superlattice formation as well as the observed biphasic changes in membrane properties at X_{cr}. This model is able to explain how cholesterol superlattices and cholesterol-phospholipid condensed complexes are inter-related. This model gives a more detailed characterization of the *LG_I region* (a broader region than the *liquid disordered - liquid ordered* mixed phase region), which is considered to be a sludge-like mixture of fluid phase and aggregates of rigid clusters. A rigid cluster is formed by a cholesterol molecule and phospholipid molecules that are condensed to the cholesterol. Rigid clusters of similar size tend to form aggregates, in which cholesterol molecules are regularly distributed into superlattices. According to this model, the extent and type of sterol superlattices, thus the lateral distribution of the entire membrane, should vary with cholesterol mole fraction in a delicate, predictable and non-monotonic manner, which should have profound functional implications.