In situ Monitoring of Structural Changes in Model Membranes upon Cholesterol Depletion via X-ray Diffraction

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compared to the pure lipid was seen at low G_{M1} concentrations. In binary mixtures containing positively charged lipids, a similar magnitude of condensation occurred at all G_{M1} ratios. For less fluid lipid nears their triple point temperature, the addition of G_{M1} caused minimal condensation suggesting the effect is specific to lipids that can be easily ordered.

3381-Pos Board B486

Disaccharides and Monosaccharides Exert Contrasting Effects on the Lamellar-Hexagonal Phase Transition

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We have investigated how several disaccharides and monosaccharides affect the lamellar-hexagonal transition of the lipid SOPE (1-stearoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine). The disaccharides sucrose and trehalose have similar effects, each lowering the lamellar-hexagonal phase transition temperature by about 9° C per molarity. Likewise, the monosaccharides fructose and glucose each affect the lamellar-hexagonal phase transition in a similar way to each other, but strikingly different than the dissacharides. The monosaccharides raise the phase transition temperature for concentrations up to about 0.5 molar, at which point increasing the concentration lowers the phase transition temperature.

3382-Pos Board B487

Sterol Affinity for Glycosphingolipid Containing Bilayer Membranes - effect of Sphingolipid Structure

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Glycosphingolipids are major constituents of plasma membranes where they participate in the formation of ordered microdomains. These sphingolipid enriched domains are suggested to be involved in e.g. cellular signaling and toxin and viral entry. The membrane rafts are one type of ordered domains specifically enriched in cholesterol, whereas glycosphingolipids also may form sterol poor domains so called glycosynapses. The aim of this study was to investigate how the glycosphingolipid structure influences sterol partitioning into glycosphingolipid containing membranes. To assess this we analyzed sterol partitioning between methyl-β-cyclodextrin and large unilamellar vesicles of different composition. Sterol incorporation in the vesicles was determined by measuring fluorescence anisotropy of the cholesterol analog cholestatrienol. The sphingolipids studied include palmitoyl galactosylceramide and palmitoyl glucosylceramide, differing only in the stereochemistry of the sugar head group, and the corresponding glycosphingolipids containing 2-hydroxylated acyl chains. Preliminary results confirm our previous results that the stereochemistry of the sugar head group affects sterol affinity for the glycosphingolipids, being slightly higher for glucosylceramide than galactosylceramide. The ability of the different glycosphingolipids to form ordered, possibly sterol enriched, domains in multicomponent membranes was additionally analyzed with a fluorescence quenching approach.

3383-Pos Board B488

Investigating the Molecular Order of Mixures of Polyunsaturated Fatty Acids with Cholesterol

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Cholesterol influences the fluidity of the membrane as well as other vital functions. The amount of cholesterol in a membrane is critical to ensure that the membrane works properly. Studies have shown that there are areas within the membrane bilayer where there is a higher concentration of cholesterol. These are known as rafts and may be important for the proper function of membrane proteins [Simons *et al*]. Despite this, we still do not fully understand how cholesterol circulates within the cells, and how it alters the molecular order of the membrane. We are investigating the molecular order of mixtures of 1,2-dimyristoyl (d_{54})-sn-glycero-3-phosphocholine (DMPC-d_{54}) and several polyunsaturated fatty acids with varying degrees of hydrocarbon chain unsaturation with and without cholesterol. Introducing cholesterol to the mixtures allows us to determine how it influences the membrane's molecular order and lets us probe the orientation of cholesterol within the bilayer. The experiments have been performed using solid state deuterium NMR techniques.

Simons, K., and E. Ikonen, 1997. Functional rafts in cell membranes. *Nature* 387:569-572

3384-Pos Board B489

In situ Monitoring of Structural Changes in Model Membranes upon Cholesterol Depletion via X-ray Diffraction

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The importance of cholesterol in the molecular structure and organization of cell membranes is a topic of great research interest. It has been hypothesized that the lateral heterogeneity of cell membranes arises from the dynamic self-assembly of cholesterol enriched nanodomains. In order to elucidate the fundamental molecular interactions involved in the assembly of these nanodomains, binary lipid monolayers of dimyristoylphosphatidylethanolamine (DMPE) and dihydrocholesterol (DChol) were studied as model systems and probed using grazing incidence x-ray diffraction (GIXD). Mixed DMPE/DChol systems were shown to exhibit short-ranged lateral ordering consistent with previous data for a lipidic alloy of egg sphingomyelin and DChol that obeys Vegard's law [Phys. Rev. Lett 2009, 103, 028103]. In the presence of β-cyclodextrin (CD), DChol was selectively removed from the membrane. GIXD was used to monitor the changes of lipid ordering during CD mediated desorption of DChol to the subphase. The chemical of amount of CD to DChol was greater than a factor of 1000 and complete DChol depletion was expected. However, it was observed that a significant amount of DChol remains in the membrane during the experimental time frame of a couple of hours and this resistance to CD transfer could be due to the stability of condensed complexes formed between DMPE and DChol.

3385-Pos Board B490

The Maximum Solubility of Cholesterol in POPC/POPE Lipid Mixtures

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Cholesterol is a major constituent of cell membranes and has many important cell functions. The maximum solubility of cholesterol in a lipid bilayer is the highest mole fraction of cholesterol that can be incorporated into a lipid bilayer before cholesterol crystals precipitate. The maximum solubility can provide valuable information about cholesterol-phospholipid interaction. In this study, the maximum solubility of cholesterol in mixtures of POPE/POPC lipid bilayer has been investigated systematically using a cholesterol oxidase (COD) reaction rate assay. The maximum solubility of cholesterol was determined to be 67 mol % in POPC bilayers and 50 mol % in POPE bilayers. In mixtures of POPE/POPC, the maximum solubility of cholesterol increases linearly as a function of the ratio POPC/(POPE+POPC). The data indicates that cholesterol prefers the large headgroup lipid (POPC) over the small headgroup lipid (POPE) and the maximum solubility increases with the population of large headgroup lipid (POPC), which are consistent with the Umbrella Model. Previously, it has been suggested that cholesterol may form a ''hexagonal'' regular distribution pattern at the maximum solubility limit in POPE bilayers and a ''maze'' pattern at the maximum solubility in POPC bilayers. Whether such domains also exist at the maximum solubility limit in POPE/POPC mixtures is investigated using AFM.

3386-Pos Board B491

Orientation of Tie-Lines in the Phase Diagram of DOPC:DPPC: Cholesterol Molel Biomembranes

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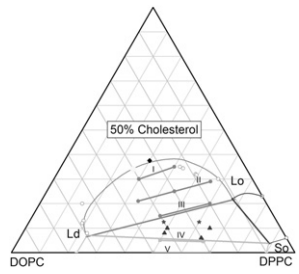
We report the direction of tie-lines of coexisting phases in a ternary diagram of DOPC:DPPC:Cholesterol lipid bilayers, which has been a system of interest in the discussion of biological rafts. For coexisting Ld and Lo phases we find that the orientation angle α of the tie-lines increases as the cholesterol concentration increases and it also increases as temperature increases from T=15°C to T=30°C. Results at lower cholesterol concentrations support the existence of a different 2-phase coexistence region of Ld and So phases and the existence of a 3-phase region separating the two 2-phase regions. Our method uses the X-ray lamellar D-spacings observed in oriented bilayers as a function of varying hydration. Although this method does not obtain the ends of the tie-lines, it gives precise values ($\pm 1°$) of their angles α in the ternary phase diagram.



3387-Pos Board B492

Lipid Areas Obtained from the Simultaneous Analysis of Neutron and X-ray Scattering

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Despite their importance to biophysical research, published lipid areas have been relatively scarce and for the most part, inconsistent. Noteworthy are the discrepancies between lipid areas as determined by standalone X-ray and neutron scattering experiments - arguably two of the most commonly used techniques in structural biology. Although they each have their advantages and disadvantages, when used in combination their advantages can be maximized. In particular, the large scattering contrast in the case of neutrons best resolves the overall bilayers thickness that is directly related to lipid lateral area. On the other hand, high resolution X-ray experiments yield detailed intra molecular structural information [Kucerka et al., Biophys. J. 95, 2356 (2008)].