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

Thomas S. Wilhlem, Rachel R. Boerner, **Paul E. Harper**.

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

Y. Jenny E. Isaksson, Max Lönnfors, Pia-Maria Grandell,
Thomas K.M. Nyholm, J. Peter Slotte.

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

Iain M. Braithwaite, James H. Davis.

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

Kathleen D. Cao, Luka Pocivavsek, Niels Holten-Andersen,
Stephanie A. Harmon, Mati Meron, Binhua Lin, Ka Yee, C. Lee.

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

Serkan Balyimez, Soyeun Park, Juyang Huang.

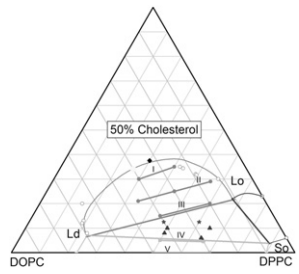
Cholesterol is a major constituent of cell membranes and has many important cell functions. The maximum solubility of cholesterol in a lipid bilayer is the highest mole fraction of cholesterol that can be incorporated into a lipid bilayer before cholesterol crystals precipitate. The maximum solubility 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

Pradeep Uppamoochikkal, Stephanie Tristram-Nagle, **John F. Nagle**.

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

Norbert Kucerka, Mu-Ping Nieh, John Katsaras.

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)].

We have utilized our recently developed method to characterize lipid areas of various phospholipids with varying numbers of carbons and double bonds. In the case of lipids with unsaturated fatty acid chains our results suggest that lipid areas change with increasing hydrocarbon chain length, but not linearly - lateral lipid area is the result of the fine balance between the hydrocarbon chain length and double bond position. Furthermore, we discovered that the most dramatic change in lipid area occurs after the introduction of the first double bond to the lipid's acyl chains.
Besides their importance in biology, lipid areas play a central role in molecular dynamics (MD) simulations, where their inconsistencies have been highlighted by the disparate results arising from MD simulations using different force fields. Since MD force fields are considered to be "well tuned" if they are able to reproduce experimental data, more reliable experimental information is necessary for their future development.

3388-Pos Board B493
Cardiolipin, a Key Component to Mimic the E. coli Bacterial Membrane in Model System Membranes
Sílvia Lopes, Cristina Neves, Peter Eaton, Paula Gameiro.
The phase transition temperatures of several lipidic systems were determined using two different techniques: dynamic light scattering (DLS) and steady-state fluorescence anisotropy, using two fluorescent probes that report different membrane regions (TMA DPH and DPH). Atomic force microscopy (AFM) was used as a complementary technique to characterize different lipid model systems under study. The systems were chosen due to the increased interest in bacterial membrane studies due to the problem of antibiotic drug resistance. The simpler models studied comprised of mixtures of POPE and POPG lipids, which form a commonly used model system for E. coli membranes. Given the important role of cardiolipin (CL) in natural membranes, a ternary model system, POPE/POPG/CL, was then considered. The results obtained in these mimetic systems were compared to those obtained for the natural systems E. coli polar and total lipid extract. DLS and fluorescence anisotropy are not commonly used to study lipid phase transitions, but it was shown that they can give useful information about the thermotropic behaviors of model systems for bacterial membranes. These two techniques provided very similar results, validating their use as methods to measure phase transitions in lipid model systems. The temperature transitions obtained from these two very different techniques and the AFM results clearly show that cardiolipin is a fundamental component to mimic bacteria membranes. The results suggest that the less commonly used ternary system is a considerably better mimic for natural E. coli membranes than binary lipid mixture.

3389-Pos Board B494
Bioenergetics Explains the Structures of Membrane Lipids: Cholesterol, Plant Sterols, Unusual Fatty Acid Chains and Polyisoprenes
Thomas H. Haines.
All living membranes support cation gradients, which they maintain by cation pumps: proton pumps or – for the animal plasma membrane – sodium pumps. This includes the organelle membranes of the eukaryote. The negative side of the gradient faces the cells' cytoplasm. Lipid bilayers leak both H+ and Na+ at rates that are equivalent in vivo (H+ is ~10-5 cm/sec without a membrane potential([H+] is ~10-7) whereas Na+ is ~10-12 cm/sec without a membrane potential ([Na+] is ~10-1). The resident membrane potential increases the rate of leakage. Cation leakage requires the cell to spend ATP energy pumping the cations back out. Resting cells spend 70 to 80% of their ATP on cation pumping. Cholesterol, found in animals, is the only lipid tested that inhibits Na+ leakage across phospholipid bilayers. It decreases leakage to 1/3 membranes w/o it. Meanwhile many membrane lipid structures inhibit H+ leakage by: 1) decreasing water diffusion through bilayers; 2) thickening the bilayer; 3) packing the bilayer cleavage with hydrocarbon.
1) sterols, hopanoids, tetrahymenol decrease membrane water permeability. 2) polyisoprenes, CoQ, squalene, dolichol, vitamin E., and carotenes thicken the membrane bilayer. 3) Iso- and anteiso-fatty acids, branched plant sterols, and chains in extreme acidophiles terminating with cyclohexane or cycloheptane groups.
A unique phospholipid, cardiolipin (CL), displays a high pK2 (~8.0) in bilayers. This appears to facilitate ATP synthesis in membranes that use the F0F¬1ATPase to make ATP. Except for the chloroplast with its CF0CF¬1ATPase, CL always accompanies the F0F¬1ATPase.
In sum, membrane lipid structures are uniquely designed to support membrane bioenergetics. This makes the structures of membrane lipids as biochemically functional as are the structures of amino acids, nucleotides and carbohydrates are for proteins, nucleic acids and CHO polymers.

3390-Pos Board B495
Material Properties of Matrix Lipids Determine Conformation and Intermolecular Reactivity of a Diacetylenic Phosphatidylcholine in the Lipid Bilayer
Anu Puri, Hyunbum Jang, Amichai Yavlovich, Alex Haber,
Athar M. Masood, Timothy D. Veenstra, Ulrich Baxa, Ruth Nussinov,
Robert Blumenthal.
Photopolymerizable phospholipid $DC_{8,9}PC$ (1,2-bis-(tricosa-10,12-diynoyl)-*sn*-glycero-3-phosphocholine) exhibits unique assembly characteristics in the lipid bilayer. Due to the presence of the diacetylene groups, $DC_{8,9}PC$ undergoes polymerization upon UV (254 nm) exposure and assumes chromogenic properties. Photopolymerization in a gel phase lipid matrix (DPPC) monitored by UV-VIS absorption spectroscopy occurred within 2 minutes after UV treatment, whereas no spectral shifts were observed when $DC_{8,9}PC$ was incorporated in a liquid phase matrix (POPC). Calcein release from DPPC/$DC_{8,9}PC$ liposomes was observed after a lag of 10 minutes following UV triggering, whereas no release occurred from POPC/$DC_{8,9}PC$ liposomes. LC-MS analysis showed a decrease in $DC_{8,9}PC$ monomer without any change in DPPC concentration in UV-treated samples. Cryo-electron microscopy revealed fiber-like structures in the UV-treated DPPC/$DC_{8,9}PC$ liposomes with few intact vesicles remaining indicating that the leakage of calcein was due to the disruption of liposomes. Molecular Dynamics (MD) simulations of DPPC/$DC_{8,9}PC$ bilayer indicate that lipid tails in the gel phase are more highly ordered than in the fluid phase of POPC/$DC_{8,9}PC$ bilayer, packing each other into close proximity. We speculate that well-packed fatty acyl chains can increase the probability of light-induced polymerization in $DC_{8,9}PC$. Further, MD simulations suggest that motions of $DC_{8,9}PC$ in the gel phase bilayer are more restricted than in the fluid bilayer. The restricted motional flexibility of $DC_{8,9}PC$ enables the reactive acetylenes in individual molecules to align and undergo the polymerization reaction, whereas the unrestricted motions in the fluid bilayer lead to a dampening of UV-triggered polymerization due to the lack of appropriate alignment of the fatty acyl chains. These studies may have implications for physicochemical effects at the nanoscale that may occur in biological membranes as a result of signaling, transport, and fusion.

3391-Pos Board B496
SANS Investigation of the Response of DMPC-DMPG Lipid Bilayers to Membrane-Active Peptides
Shuo Qian, William T. Heller.
Membrane-active peptides disrupt the integrity of cell membranes and form transmembrane pores in model lipid bilayers. Alamethicin and melittin are two extremely well-characterized examples of membrane-active peptides that are known to undergo a concentration-dependent transition from a surface-adsorbed state to a state in which transmembrane pores are formed, resulting in the death of the target cell. The action of these peptides strongly depends on the composition of the lipid bilayer membrane. In particular, charged lipids and cholesterol are thought to drive the cellular specificity of the cytotoxicity of these membrane active peptides. Further, lipid rafts, enriched domains in multi-component membranes, can concentrate or exclude proteins and peptides associated with lipid bilayers. SANS with contrast variation was used to probe the response of small-unilamellar vesicles (SUVs) composed of mixtures of the neutral lipid DMPC with the charged lipid DMPG to the presence of alamethicin and melittin. SUVs made of chain-deuterated d54-DMPC and DMPG at a molar ratio of 7: 3 were studied in the absence and presence of the two peptides in H2O/D2O mixtures containing 90% D2O solution. The measurements in 90% D2O, which is at the match point of the readily available d54-DMPC, greatly enhances the scattering from the hydrogenated components and ensure maximum signal for any in-bilayer aggregates or an asymmetric distribution between the leaflets of the bilayer of hydrogenated material that may form. The SANS experiments were performed using the BIO-SANS of HFIR at ORNL, which provided excellent signal for the dilute SUVs solutions. We found in both alamethicin and melittin, the asymmetric distribution of two lipids between the two monolayers increases with the peptide concentration. Interestingly, melittin was found to produce a stronger effect than alamethicin.

3392-Pos Board B497
Inclusion of Menaquinone in Lipid Membranes Decreases Susceptibility to Antimicrobial Peptides
Julia Nepper.
Most studies on the interaction of antimicrobial peptides with lipid bilayers have used unsaturated, fluid-state phospholipids to model bacterial membranes. However, unsaturated lipids are rarely found in cell membranes of gram-(+) bacteria, including *Staphylococcus aureus*. To maintain cell membrane