compared to those containing C_{16}-ceramide. Work supported by funding from the Sigrid Juselius Foundation (JMH), the Finnish Cultural Foundation (JMH), Evald and Hilda Nissi Foundation (JMH), The Finnish Eye Foundation (JMH), the Academy of Finland (AH, IV, SW), and from the Natural Sciences and Engineering Research Council of Canada (MRM).

843-Pos Board B722

Sterol Solubility in Vesicles (GUV's) Containing the Ternary Lipid Mixture DPPC:DOPC:Sterol by Quantitative NMR

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In the laboratory, giant unilamellar vesicles (GUVs) offer a rich system for studying miscibility of cholesterol and phospholipids in a lipid bilayer. Our laboratory produces vesicles through various methods, but this study will focus on electroformation. Our work and the work of many other laboratories rely on the assumption that the electroformation process creates vesicles with the same lipid composition originally assembled in the electroformation chamber, up to an ultimate sterol solubility limit. A few sterol solubility limits have been previously tested in selected binary systems [1], but are not generally known for ternary systems. It is important for us to test our assumption that the electroformation process does not significantly alter lipid composition, and also to determine the solubility limit of sterols in membranes in order to understand and properly present the results of other GUV studies from our laboratory. Here we describe studies using quantitative NMR to ascertain the solubility of sterols for which convenient chemical assays exist (e.g. cholesterol) and for which chemicals assays are not readily available (e.g. ergosterol).

[1] Huang J., Buboltz J.T., Feigenson G.W., 1999. Maximum Solubility of Cholesterol in Phosphatidylcholine and Phosphatidylethanolamine Bilayers. Biochim. et Biophys. Acta 1417, 89-100.

844-Pos Board B723

Modeling The Temperature Dependence of Membrane Solubilization by Detergents

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It is known that lipid membranes become, typically, less susceptible to solubilization with increasing temperature so that more detergent is needed to start and complete their conversion to mixed micelles. Qualitatively, this can be explained by the fact that thermal chain disordering and headgroup dehydration render the spontaneous curvature of the molecules less positive.

Here we present an alternative model to describe this temperature dependence quantitatively in terms of simple, physically meaningful model parameters. This model relates the onset of solubilization to the detergent concentration when micellization becomes more favorable than membrane insertion. It quantifies the effect of temperature on membrane versus micellar packing effects in terms of the heat capacity changes of partitioning (-0.75 kJ/(mol K)) being more negative than that of micelle formation (-0.52 kJ/(mol K)). We demonstrate the model based on measurements of the CMC, partition coefficient and heat capacity changes for pentaethylene glycol monodecyl ether (C10E5) interacting with membranes of POPC by isothermal titration calorimetry (ITC).

845-Pos Board B724

An Approximate Cooperativity Analysis By Dsc And Uv-vis Phase Of Pseudobinary DXPC-DC8,9PC-Cholesterol Dispersions

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The effect of DC8,9PC on the cooperativity of ordered-fluid phospholipid phase transitions has been investigated by determining the transitions widths in multilayer's dispersions of DXPC - DC8,9PC by DSC. The van' t Hoff values of the main transition enthalpies were calculated using an approximate expression deduced from Zimm and Bragg theory. The DC8,9PC decreases the cooperativity (size of the cooperative unit of synthetic DXPC bilayers). The observation that the membrane lipids in the mixed lipid/DC8,9PC assemblies appear to adopt bilayer structures is important, since it demonstrates that the lipid domains within the colorimetric vesicles exist in the fundamental organizational unit found in cellular membranes. The experiments described in this work shed light upon the effects of external environmental parameters, such as temperature, upon structural and dynamical properties of the organized lipid assemblies. The colorimetric platform also facilitates elucidation of the contribution of distinct membrane components, such as cholesterol, toward shaping membrane properties. This capability could open the way for application of the assay for detailed analyses of the roles played by particular molecules, such as peptides and DNA, in determining membrane functions and properties. Between non polymeric and polymeric species in DXPC: DC8,9PC mixtures the influence of the polymer is from C14 chain-down more cooperative the non-polymeric; from C16 on before DPPC:DC8,9PC is better polymerized than non-polymerized and for DSPC:DC8,9PC there is no difference. Hypothesis is C14 or less non-polymerized are intermixed with no difference but when polymerized, non ideal mixing is formed, polymers containing polymeric units with pockets containing DMPC and/or cholesterol. When chains are C16 or more units of saturated and non saturated mixtures cooperative units are quite similar.

846-Pos Board B725

Energetics of Cholesterol Transfer between Lipid Bilayers

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It is believed that natural biological membranes contain domains of liquid ordered phase enriched in cholesterol and sphingomyelin. Although the existence of these domains, called lipid rafts, is still no firmly established for natural membranes, direct microscopic observations and phase diagrams obtained from the study of three-component mixtures containing saturated phospholipids, unsaturated phospholipids, and cholesterol demonstrate the existence of lipid rafts in synthetic membranes. The presence of the domains or rafts in these membranes is often ascribed to the preferential interactions between cholesterol and saturated phospholipids, for example, between cholesterol and sphingomyelin. We calculated, using molecular dynamics computer simulation technique combined with the umbrella sampling and weighted histogram analysis method (WHAM), the free energy of cholesterol transfer from the bilayer containing unsaturated phosphatidylcholine lipid molecules to the bilayer containing sphingomyelin molecules and find that the affinity of cholesterol to sphingomyelin is higher. By doing the simulations at different temperatures, we calculated the free-energy components, energy and entropy, and show that cholesterol transfer is exothermic and with a loss of entropy. The transfer is promoted by the favorable change in the lipid-lipid interactions near cholesterol and not by the favorable energy of cholesterol-sphingomyelin interaction, as assumed previously.

847-Pos Board B726

Impact Of Ceramide3 On POPC Host Membranes: A Study On Structure And Thermodynamics

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The uppermost layer of skin epidermis exhibits a very peculiar lipid composition consisting mostly of long-chain ceramides of asymmetric chain length. The functional impact of ceramides on 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) membranes was investigated using natural phytosphingosine type ceramide3 (Cer3) as a model system. Results will be presented on structure and thermodynamic phase behavior of the composite model system for three mole ratios Cer3/POPC (5:95; 10:90; 15:85). All methods applied so far (SAXS; DSC; confocal microscopy) reveal non-ideal miscibility of the two compounds with macroscopic separation of coexisting lamellar phases of different rigidity. Two major transition regions were identified: at low temperature(T_{m1}) and at high temperature(T_{m2}). They were attributed to POPC rich and Cer3 rich domains respectively. A slight increase in the d-spacing and as well a shift of T_{m1} towards higher values indicate a solidifying effect of Cer3 on POPC host membranes. At low concentration the Cer3 rich domain (T_{m2}) exhibits normal thermodynamic behavior with freezing point depression due to the presence of POPC. The continuous shift in T_{m1} together with a loss of transition cooperativity for the POPC rich domain hints towards a modification of the bilayer elasticity due to the presence of the Cer3. The overall heat content of the composite system increases with the amount of Cer3 present, signifying a stronger attractive interaction among the lipids. The Cer3 rich phase is almost dehydrated. Giant vesicles (GUVs) exhibit fluid-gel domain coexistence with the typical smooth and flat face facets as signatures of the more fluid and the more rigid membrane regions.

848-Pos Board B727

The Effect Of Glycerol On Membrane Solubilization By Nonionic Surfactants

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Some protocols for solubilizing membrane components by surfactants use glycerol as a co-solute for stabilizing the native state of proteins. We have studied

the effect of glycerol on the surfactant - lipid system in terms of surfactant self-association (critical micelle concentration, CMC), membrane partitioning (partition coefficient K and αHmic), and the onset of membrane solubilization (i.e., bilayer-to-micelle transition, characterized by a specific surfactant-to-lipid mole ratio in the membrane denoted Resat) by isothermal titration calorimetry (ITC). One effect expected for glycerol is its tendency to 'salt out' hydrophobic molecules. This promotes all aggregation phenomena (K increases, CMC decreases) but has little effect on the balance between the aggregates (Resat ~ const.). Furthermore, glycerol gradually dehydrates the polar head group, which renders the effective molecular shape more favorable for a bilayer (K increases) whereas micellization is much less affected (CMC ~ const) so that bilayers are stabilized compared to micelles (Resat increases). Our results indicate that the behavior of the sugar based surfactant octyl glucoside seems governed by the salting out effect, whereas for ethylene oxide surfactant C12EO8, headgroup dehydration seems to be the key effect explaining the effects of glycerol.

849-Pos Board B728

Synthetic and Mycobacterial Trehalose Glycolipids Confer Dehydration Resistance to Supported Phospholipid Monolayers

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We have recently demonstrated that the glycolipid trehalose dimycolate (TDM), a major outer membrane component of dehydration resistant Mycobacterium tuberculosis (MTb), can impart significant dehydration resistance to supported phospholipid membranes. We now report studies of other, related glycolipids both natural and synthetic that exhibit behavior similar to TDM, conferring protection from desiccation to membranes of which they are constituents. We examined solid-supported lipid monolayers, characterizing membrane integrity and two-dimensional fluidity before and after de- and re-hydration with fluorescence imaging and fluorescence recovery after photobleaching (FRAP). As with TDM, the degree of protection is dependent on the fraction of synthetic lipid in the monolayer and there is a distinct minimum fraction needed for protection by each glycolipid. We can control the synthetic lipid fluidity and minimum protecting fraction by designing and synthesizing lipids with particular hydrophobic chain lengths, saturation, and branching, thereby illuminating the role of molecular structure in biophysical function. The advent of these synthetic, protective glycolipids opens the door to the creation of lipid bilayers and liposomes since the relevant hydrophobic and hydrophilic domain sizes can be controlled. These new structures allow investigations of the physical origins of well-known mycobacterial properties beyond dehydration resistance in controlled experimental contexts, such as the inhibition of membrane fusion.

850-Pos Board B729

How Small Polar Molecules Protect Membrane Systems Against Osmotic Stress

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We investigate how small polar molecules, urea and glycerol, can act to protect a phospholipid bilayer system against osmotic stress. The osmotic stress can be caused by a dry environment, freezing, or through exposure to aqueous systems with high osmotic pressure due to solutes like in saline water. A large number of organisms regularly experience osmotic stress and it is a common response to produce small polar molecules intracellularly. We have selected two ternary systems of urea-water-dimyristoylphosphatidylcholine (DMPC) and glycerol-water-DMPC as model systems to investigate the molecular mechanism behind this protective effect, and we put a special emphasis on applications in skin care products. Using solid-state NMR, DSC, X-ray diffraction and sorption-microbalance measurements we study the phase behavior of the lipid system both exposed to an excess of solvent of varying composition and for systems exposed to water at reduced relative humidities. In this we have arrived at a rather detailed thermodynamic characterization. The basic findings are: i) In excess solvent the thermally induced lipid phase transitions are only marginally dependent on the addition of urea(glycerol). ii) For lipid systems with limited access to solvent the phase behavior is basically determined by the amount of solvent irrespective of the urea(glycerol) content. iii) The presence of urea (glycerol) have the effect to retain the lipid in liquid crystalline phase down to low relative humidities (64% for urea, 75% for glycerol at 27°C), whereas the transition to the gel phase occurs already at a relative humidity of 94% in pure water, demonstrating the protective effect of the polar molecules against osmotic stress. iv) In skin care products urea and glycerol are referred to as a moisturizer, which we find slightly misleading as it replaces the water while keeping the physical properties unaltered.

851-Pos Board B730

How Bilayer Curvatures Modulate Molecular Reaction Efficiencies In A Membrane Junction

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Chemical reactions between ligand/receptors taking place in the intermembrane space between opposing lipid bilayers are crucial for maintaining processes vital for eukaryotes and multicellular organisms, e.g. vesicle trafficking and immune responses. The mechanism of chemical reactions taking place in bulk are understood in terms of molecular collective properties such as pressure, temperature and concentration, whereas a reaction taking place between bilayers proceed by more subtle pathways. The properties of the membranes into which the receptors are embedded modulate reaction efficiencies and should be understood in terms of contact area, receptor density and interbilayer forces.

We here present results from a single-vesicle based binding assay showing how the curvature of two lipid bilayers drastically alters binding efficiencies. We studied three biochemical binding reactions (i) trans-SNARE complexation, (ii) streptavidin/biotin recognition and (iii) calcium-mediated lipid chelation all taking place in the confined space of a membrane junction. We measured binding probabilities as a function of membrane curvatures and found that the probability of successfully completing the binding reaction could be changed by up to two orders of magnitude depending on the bilayer shape.

We built a simple model based on receptor membrane density, electrostatic bilayer repulsion and membrane shape to account for the observation. We believe the presented assay and model to have an impact on the understanding of biological recognition reactions taking place across a membrane junction, such as vesicle trafficking and fusion, as well as neuronal and immunological synapse formation.

852-Pos Board B731

Effect of Poloxamer 188 on the Osmotic Response of Cell Membranes

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Poloxamer 188 (P188), an amphiphilic triblock copolymer of poly(ethylene oxide)-block-poly(propylene oxide)-block-poly(ethylene oxide) has been shown to effectively interact with injured plasma membrane and restore its function both *in vitro* and *in vivo*. Elucidation of the mesoscopic and molecular mechanism that mediates the interaction between this triblock copolymer and damaged membranes will help to improve the current approach in development of Poloxamers for therapeutic purposes. Previous work done by our group examined the interaction between P188 with phospholipid monolayers and demonstrated that P188 inserts selectively into damaged portions of the membrane and corrals surrounding lipids. Upon restoration of membrane integrity, the inserted polymer is squeezed out. Here, the effect of P188 on membrane permeability under osmotic stress was investigated by using giant unilamellar phospholipid vesicles, the simplest biomimic membrane which retains the essential curved bilayer structure. Results will be presented detailing how the osmotic gradients and P188 concentration affect P188 in corralling membrane lipids.

853-Pos Board B732

Self-assembly Formation of Multiple Tethered Lipid Bilayers

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Tethered lipid bilayers have been proven powerful as experimental models in studies of membrane-spanning proteins, which are currently the most important targets in drug discovery. Tethering of planar lipid membranes reduces the influence from the solid support on the lateral mobility of the membrane constituents and provides a sufficiently large solvent reservoir underneath the membrane for studying molecular transport events. Inspired by cell-cell junctions, where membrane residing proteins control the separation between two or more membranes without interfering with their integrity, we developed a new self-assembly route for formation of multiple macroscopically homogenous and highly fluid tethered lipid bilayers (lipid diffusivity~5 μm2/s 1) with compartmentalized inter-membrane volumes geometrically confined by membrane-anchored DNA duplexes. The formation of multiple macroscopically homogeneous planar membrane-membrane junctions with sealed inter-membrane liquid reservoirs was accomplished using so called bicelles, which is a versatile class of model membranes generally composed of a mixture of the long-chained dimyristoyl phosphatidylcholine (DMPC) and the short-chained dihexanoyl PC. Quartz crystal microbalance with dissipation (QCM-D) was