

known as the hysteresis. Using an optical technique, we have examined the hysteresis for two lipids, SOPE (1-Stearoyl-2-Oleoyl-sn-Glycero-3-Phosphoethanolamine) and DSPE (1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine). SOPE contains a saturated tail and an unsaturated tail, while in DSPE both tails are saturated. We find that the hysteresis exhibits a power law dependence on the temperature ramping rate and that the hysteresis is markedly reduced for the completely saturated lipid DSPE as compared to the mono-unsaturated lipid SOPE. In turn, the hysteresis of SOPE is markedly reduced compared to that of DOPE, a lipid with two mono-unsaturated tails.

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Preferential Interaction of α -tocopherol with PUFA-containing Lipids Characterized by Isothermal Titration Calorimetry

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It is becoming generally accepted that membranes laterally segregate into patches (domains) of different lipid composition to provide the environment necessary for the function of a resident protein. Liquid ordered (l_o) lipid rafts enriched in saturated sphingolipids and cholesterol are the best known example. Much less studied are liquid disordered (l_d) domains rich in polyunsaturated phospholipids and depleted in cholesterol, the antithesis of rafts. They are the focus of our research. We hypothesize that α -tocopherol (vitamin E), a lipid-soluble antioxidant found in low concentration in plasma membranes, has preferential affinity for polyunsaturated fatty acid (PUFA)-containing phospholipids in these l_d non-raft regions. In this manner protection of the lipid species most vulnerable to peroxidation due to their multiple double carbon bonds, would be optimized. To test this hypothesis we utilize isothermal titration calorimetry (ITC) to assay the partitioning of α -tocopherol between large unilamellar vesicles (LUV) and methyl- β -cyclodextrin (cyd), a water-soluble molecule with a hydrophobic cavity that binds small hydrophobic molecules. The approach emulates one that has successfully been applied to measure the binding of cholesterol and the results of preliminary experiments have shown that α -tocopherol can be bound by cyd. Partition coefficients K_X measured for α -tocopherol as a function of phospholipid unsaturation are presented and compared with values measured for cholesterol that, in contrast to our proposal for α -tocopherol, has poor affinity for PUFA.

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The Effect of *Trans* Unsaturation on Molecular Organization in a Phospholipid Membrane

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Despite recognition that the ingestion of *trans* fatty acids (TFA) formed during the partial hydrogenation of vegetable oils may unfavorably affect biochemical function, the impact on the conformation of the molecules into which they incorporate is unknown. We synthesized analogs of 1-elaidoyl-2-stearoylphosphatidylcholine (*t*18:1-18:0PC) and 1-oleoyl-2-stearoylphosphatidylcholine (*c*18:1-18:0PC) with a perdeuterated 18:0 *sn*-2 chain and employed solid state ²H NMR, complemented by computer simulations, to compare molecular organization in a model membrane containing a single "manmade" *trans* or "natural" *cis* double bond. Moment analysis of the ²H NMR spectra recorded as a function of temperature showed that the chain melting temperature for the *trans* isomer (31.5 °C) is depressed compared to the *cis* isomer (7 °C), reflecting an ability to pack more favorably in the gel state, an interpretation supported by molecular modeling. The calculated intra-molecular van der Waals' attraction between acyl chains is greater for *t*18:1 than *c*18:1 acid because the *trans* chain adopts a *t's's'Δs't* conformation, as opposed to *t's'Δs'g'* in a *cis* chain, around the double bond. The average order parameters evaluated for the perdeuterated *sn*-2 chain of *t*18:1-18:0PC and *c*18:1-18:0PC in the liquid crystalline phase coincide within <5%, a result that was reproduced in molecular dynamics (MD) simulations. The values for the average order parameter are 20% below the equivalent saturated PC (18:0-18:0 PC), which is attributed to the increased disorder in the hydrophobic core arising from differences in chain packing. We now have synthesized analogs with a perdeuterated *t*18:1 and *c*18:1 *sn*-1 chain to directly probe the conformational organization of *trans* vs. *cis* chain. (Supported by ACS, PRF 43281-AC7.)

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Scaffolded Vesicles as a Model Membrane System

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Biological cell membranes are complex systems that consist primarily of a phospholipid bilayer, into which cholesterol, proteins etc. may be integrated.

Due to the complexity of biological cell membranes, it is desirable to develop model membrane systems that can be more easily studied. Scaffolded vesicles are an example of such a system. A scaffolded vesicle model membrane system offers a number of advantages with respect to other model systems. By using a porous material as the scaffold, one can achieve an aqueous environment on both sides of the model membrane. This allows for the study of membrane transport processes. The scaffold's porosity may also allow one to more easily integrate transmembrane proteins into the bilayer. Importantly, such a system would remain accessible to both electrochemical and surface analytical techniques. Using FTIR-ATR spectroscopy, the orientation of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) coated on the porous scaffold (as a 70:30 DMPC:cholesterol bilayer) will be determined. Proteins may then be incorporated into the bilayer of the scaffolded model membrane system for study.

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Mechanical Effects of Peripherally Binding Proteins on Membrane Tethers

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Our understanding of cell membrane remodeling by proteins has been informed largely by observations of membrane tubulation by proteins *in vitro* and *in vivo*. Structural and spectroscopic studies have revealed some important details of the interactions between these proteins and lipids. However, a quantitative description of membrane curvature sensing and generation by proteins, which would guide assessment of the roles of specific proteins and evaluation of hypothesized mechanisms of action, is currently lacking.

We are studying membrane curvature sensing and generation by purified proteins using a fluorescence microscopy-based biomimetic curvature gradient manipulation system, the properties of which are described by membrane elasticity theory. Using tethers of controllable curvature pulled from giant vesicles, we monitor protein partitioning between vesicle and tether, and the effects of proteins on tether properties. These measurements direct our assessment and development of statistical mechanical models that clarify the parameters responsible for protein sensing and control of membrane curvature. Our quantitative framework is used with varying membrane composition and solution conditions to reveal subtle differences between various proteins in their membrane restructuring and curvature propensities.

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Modeling Morphogenesis of Outer Segments of Vertebrate Photoreceptor Cells

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The vertebrate eye contains two main types of photoreceptor cells: rods and cones. The outer segment of the rod cells are cylindrical in shape and contain 500-1000 pancake-shaped structures stacked on top of each other, ensheathed within a plasma membrane. Each individual pancake can be thought of as an individual sac with an enclosing membrane, a structure known as a vesicle. The shape of these vesicles is very important since misshaping of the vesicles can lead to loss of eyesight. We will discuss how the quasi-equilibrium shapes of these vesicles could be determined by membrane energetics, and will introduce a Metropolis algorithm to obtain thermodynamically stable vesicle shapes.

Interfacial Protein-Lipid Interactions II

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Pulmonary Surfactant Protein C Reduces the Size of Liquid Ordered Domains in a Ternary Membrane Model System

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Surfactant protein C (SP-C) is the smallest pulmonary surfactant protein and is required for the formation and stability of surface-active films at the air-liquid interface in the lung. The protein consists of a hydrophobic transmembrane α -helix and a cationic N-terminal segment, which contains two palmitoylated cysteines. In the present work, we compared the effect of native palmitoylated and recombinant non-palmitoylated versions of full length SP-C on the liquid ordered (l_o)/liquid disordered (l_d) phase coexistence in a ternary membrane model system consisting of DPPC, DOPC and cholesterol. This model has