the design of liposomes for fusogenic delivery to OMV targets. There is therapeutic interest in delivery to Gram-negative OMV, e.g. where they exist in biofilm infections, or to elucidate a semi-synthetic approach to modify OMV lipid composition to generate useful delivery vehicles.

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Macrophage Cell Fusion and Crosstalk with Myoblast Fusion

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Cell-to-cell fusion plays a pivotal role in various developmental processes, tissue homeostasis, immune response and, possibly, cancer. One of the key challenges in characterizing these complex and relatively slow membrane fusion events is to uncouple actual fusion stage from the preceding differentiation processes, which prepare the cells for fusion. Here we have focused on two very important and well characterized examples of cell-to-cell fusion, macrophage fusion that leads to osteoclast or giant cells formation and myoblast fusion in muscle development and regeneration. Proteins that mediate fusion stages of these processes remain obscure. Application of RANKL to RAW macrophage-like cells commits the cells to fusion with most fusion events to take place 72-96 hours later. We blocked this robust fusion by applying a reversible hemifusion inhibitor lysophosphatidylcholine (LPC) at 72 hours post-RANKL, and removed LPC at 88 hours. This approach has allowed us to accumulate the ready-to-fuse macrophages and then observe cell fusion events that would normally develop within 16h to develop within 30-90 min. Synchronization of cell fusion using LPC block has also worked for myoblast fusion. Antibodies against annexin V inhibited both macrophage fusion and myoblast fusion suggesting similarities between protein machineries involved in these fusion processes. We also found that co-incubation of fusion-committed RAW cells and primary myoblasts promotes myotube formation and inhibits osteoclast formation suggesting an intriguing cross-talk between these cells in the context of muscle regeneration as well as in inflammation & bone biology.

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An Anionic Phospholipid Enables the Hydrophobic Surfactant Proteins to Alter Spontaneous Curvature

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The cationic, hydrophobic surfactant proteins, SP-B and SP-C (SPs), promote adsorption of vesicles to an air water interface and greatly increase the rate of forming an alveolar film. The available evidence suggests that the SPs may facilitate adsorption by promoting the generation of a negatively curved intermediate. The studies here tested whether the proteins could change the curvature of phospholipid leaflets in the inverse hexagonal (H_{II}) phase. Experiments with the uncharged dioleoyl phosphatidylethanolamine (DOPE) showed no change in the lattice-constant with added SPs. Pulmonary surfactant contains ~10% (mol:mol) anionic phospholipids. We therefore also examined mixtures of DOPE with 10% (mol:mol) of the anionic dioleoyl phosphatidylglycerol (DOPG) to investigate selective interactions with the SPs. Small angle X-ray scattering established the phase diagram (temperature - % protein) and lattice-constants for these lipids combined with the physiological mixture of the SPs. The hexagonal lattice-constant decreased linearly with increasing amounts of protein before reaching a temperature-dependent maximum change of 8-14% at ~1% (w:w) protein. To test whether this effect was strictly electrostatic, NaCl concentrations up to 3M provided electrostatic screening. The salt diminished but did not eliminate the dose-related change in the lattice-constant. Measurements at different hydrations showed that the separation between the pivotal plane and the aqueous core was unaffected by the proteins, indicating that the change in the lattice-constant produced by the proteins reflects a more negative spontaneous curvature. These results provide direct evidence that the hydrophobic surfactant proteins can enhance the negative curvature of lipid leaflets. Studies were conducted at the Stanford Synchrotron Radiation Lightsource.

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Dynamics of the Influenza Hemagglutinin Fusion Peptide: A Comparison of MD and NMR

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Membrane insertion of influenza hemagglutinin (HA) is essential for viral membrane fusion. Previous NMR relaxation studies on bicelles [1, 2] analyzed the 23 conserved N-terminal residues of the HA2 subunit (fusion peptide). It was shown that this peptide, which adopted a helical hairpin structure, experiences wobbling motions relative to the bilayer surface on the ns timescale. Here, the dynamics of the HA fusion peptide hairpin on a DMPC membrane are studied using molecular dynamics (MD) simulations. Simulations were performed with the acidic groups (E11 and D19) protonated and unprotonated. Internal correlation functions of backbone N-H vectors are determined over the 100-ns MD simulations, and are fit to the Lipari-Szabo model free approach [3]. The calculated order parameters and correlation times are similar to those determined experimentally. Starting from an initial orientation parallel to the membrane, during the simulations the hairpin rotated nearly 90° around the axis that is parallel to the two helices, with the N-terminal helix buried more deeply in the lipid tail region.

[1] J. L. Lorieau, J. M. Louis, and A. Bax, Proceedings of the National Academy of Sciences of the United States of America 107 (2010) 11341.
[2] J. L. Lorieau, J. M. Louis, and A. Bax, Journal of the American Chemical Society 133 (2011) 14184.
[3] G. Lipari and A. Szabo, Biophysical Journal 37 (1982) A380.

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Lipid Bilayer Curvature Frustration

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The free energy of bending of surfaces composed of biological lipids is derived, computed, and compared with experiment. Simulations of two distinct systems, one a planar bilayer, the other the inverse hexagonal phase indicate consistent mechanical properties and curvature preferences, with DOPE having a spontaneous curvature, R0 = −26 Angstroms and DOPC preferring to be approximately flat (R0=-65 Angstroms). Additionally, a well-defined pivotal plane, where a DOPE leaflet bends at constant area, has been determined to be near the glycerol region of the lipid, consistent with the experimentally predicted plane. By examining the curvature frustration of both high and low curvature, the transferability of experimentally determined bending constants is supported. How to predict the effects of biologically active molecules on the mechanical properties of lipid bilayers under well-controlled conditions will be examined.

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Mechansim of the Early Stages of the Lamellar/Bicontinuous Cubic Phase Transition

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The transition between lamellar and bicontinuous inverted cubic (Q_{II}) phases is mediated by catenoidal bilayer channels, which are also the structures that are produced by membrane fusion. The Q_{II} phase forms by production of these channels in an array of initially flat bilayers. As the number of channels increase with increasing temperature, they initially form a disordered, metastable array in the lamellar phase, which has been referred to as the ''isotropic phase.'' The isotropic phase accomplishes the topological change between the lamellar and Q_{II} phases. The disordered lattice subsequently transforms into the Q_{II} phase by local bending of the bilayers. There are discordant observations concerning the rate of formation and temperature interval of existence for the ''isotopic phase.'' Here, the expected dimensions of channels are predicted as a function of temperature, as well as the extent of ''isotropic phase'' formation as a function of temperature and sample water content. The predictions are made using the fourth order curvature energy model previously used to rationalize the stability of the Q_{II} phase in phospholipids (Langmuir, 2010, **26**:8673). The extent of channel and ''isotropic phase'' formation is sensitive to the interaction energy of the flat bilayers in the lamellar phase, the lateral dimensions of the lamellar phase bilayers and the sample water volume fraction, as well as the temperature and curvature elastic parameters of the lipid. The theory, as well as the hysteretic nature of the transition process, accounts for the apparent conflict between early observations, made mostly by phosphorus NMR.

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Calculating Minimal Energy Shapes of Fusion Pores

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We have developed a new computational model to calculate the shape of fusion pores that are in a state of minimal elastic energy as a function of pore length and lumen radius. A Helfrich Hamiltonian accounting for splay and lipid tilt was used for calculations. Minimal-energy shapes were obtained by numerically solving steepest descent partial differential equations derived from the Hamiltonian. The energy landscape was calculated by describing the bilayer as a single surface–the midplane between monolayers–or by describing the

bilayer as two abutted monolayers, each with a neutral surface. The constraint imposed by mathematically placing two monolayers in apposition causes minimal energy to be larger than that predicated by (incorrectly) assuming that the elastic properties of a bilayer can be quantitatively captured through a single surface. Independent of pore size, the deformation of tilt did not appreciably affect elastic energies; in other words, membrane splay dominates elastic energies. For small radii, shapes of minimal energy were close to the shape of a catenoid. For large pores, however, deviations of minimal energy shapes from catenoids were large, resulting from the necessity that the membranes be parallel and the separation between them fixed at distances far from the rim of the pore. Energies for minimal shapes were 15-60kT less than the energy of the toroidal shape for pore radii in the range of 2-16 nm and for initially parallel membranes that were separated by 2-4 nm. For the smallest pore possible (i.e., an initial pore), a toroidal geometry overestimated the minimal energy by 30 kT. For pores with radius larger than length, membrane separation near the rim of the pore exceeds the distance between the parallel membranes. These shapes of minimal elastic energy can now be used to calculate fusion pore dynamics.

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Free Energy Landscapes of Vesicle Fusion by Umbrella Sampling MD Simulations

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Despite intensive investigation, the energy landscapes governing membrane fusion in vitro and in vivo remain uncertain. A plethora of factors including small molecules, ions, fusion proteins, and osmotic pressure gradients are known to influence fusion rates, but these perturbations only hint at the underlying molecular mechanisms.

The barriers and metastable structures that characterize fusion free energy landscapes are inherently difficult to resolve atomistically due to the fluid, disordered nature of membranes. These pathways are also difficult to access with molecular resolution simulations, namely molecular dynamics (MD), due to the time scales associated with spontaneous fusion and the lack order parameters capable of driving fusion progress through high energy intermediates.

To address this challenge, we have developed a novel umbrella sampling method paired with an order parameter capable of driving and controlling fusion progress. Our initial results for 20 nm POPC vesicles give a barrier of 43 kBT along a pathway beginning as a metastable stalk, proceeding over a barrier with a hemifused structure and then ending as an opened fusion pore. Though marginally metastable, the hemifusion diaphragm does not expand, likely due to the small vesicle size and the lack of a lipid reservoir, but instead either reverts back to a stalk or proceeds forward to form a fusion pore.

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Local Stresses in Fusing Membranes from Molecular Simulation

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Membrane fusion involves transient and non-uniform stresses on the participating membranes. It is believed that these stresses help drive evolution of lipidic fusion intermediates and determine fusion pathways and outcomes. It has also been shown both via experiments and molecular dynamics simulations that lipid composition can dramatically affect fusion kinetics and efficiency. We have developed a means to measure locally resolved pressure in molecular dynamics simulations and implemented it in the GROMACS software. We use this to measure pressure stresses on highly-curved fusion intermediates. The non-uniform, fluctuating, and spatially curved nature of these intermediates makes measurements challenging; we utilize techniques from computational geometry to assist convergence in our measurements. We interpret our findings in the context of prior fusion theories of lipidic stalk formation, hemifusion interstitial energy, and pore formation. We also examine local membrane pressure changes near fusion peptides.

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A Molecular Mechanism of Lipid Membrane Fusion

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Membrane fusion is an essential molecular event involved in many cellular processes, such as exocytosis, endocytosis, intracellular vesicle trafficking, fertilization, and viral infection to target cells. In spite of extensive studies of membrane fusion, however, the basic molecular mechanisms in biological systems are not well understood. Probably, it is due to the complex nature of biological membranes and the variety of possible molecular pathways for membrane fusion. We have studied the membrane fusion process, particularly ion-induced membrane fusion. Biological membrane fusion seems to occur with either ion-induced or non-ion-induced membrane process, particularly the later case is for virus membrane fusion system. Dr. Chernomordik and his co-workers have studied on non-ion-induced lipid membrane fusion and developed the so-called ''Stalk-intermediate model'' before total membrane fusion. That fusion model has been well received by many membrane fusion investigators, particularly in the virus fusion field. Stalk formation between two lipid membranes may occur due to undulation of lipid molecules or local binding of the lipid bilayers, which results in the formation of a local region of outer monolayer fusion. The stalk hypothesis can be described by macroscopic models treating bilayers and monolayers as homogeneous elastic surfaces. We have also studied non-ion-induced bilayer membrane fusion. Our membrane fusion theory is based on the interaction energies between the two membranes due to alternation of the membrane surface properties, e.g., hydrophilicity and hydrohobicity of interacting membranes, and then lipid membrane close approach and due to membrane curvature. Although the membrane interaction processes are different between the two models, these membrane fusion properties are the same as those of our ion-induced lipid membrane fusion.

Protein-Lipid Interactions I

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Influence of pH and Side-Chain Negative Charge on the Behavior of Designed Transmembrane Peptides in Lipid Bilayers

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GWALP23 (acetyl-GGALW^{5}LALALALALALALW^{19}LAGA-amide) is a favorable model peptide for investigations of single-residue effects on protein-lipid interactions and the properties of membrane-spanning helices (J. Biol. Chem. 285, 31723). GWALP23 has favorable properties in bilayer membranes because the peptide exhibits only limited dynamic averaging of NMR observables such as the ^{2}H quadrupolar splitting or the ^{15}N-^{1}H dipolar coupling (Biophys. J. 101, 2939). To investigate the potential influence of negatively charged side chains upon system properties, we have substituted a single Leu residue with Glu at different positions and incorporated specific ^{2}H-Ala labels in the core of the single-Trp peptide Y^{5}GWALP23 (see Biochemistry 51, 2044). Solid state ^{2}H NMR experiments were used to examine the peptide orientation and dynamics as functions of the lipid bilayer thickness and pH in hydrated lipid bilayer membranes. We observed well defined ^{2}H quadrupolar splittings for Y^{5}GWALP23-E16 in the pH range from 4.0 to 8.2, suggesting that the peptide helix is well oriented in DOPC lipid bilayers. The glutamic acid residue, though protonated, seemed to confer multi-state behavior at pH 2.5, and the resulting populations exhibited slow exchange on the NMR time scale. The deprotonation of E16 at pH 8.2 did not have any effect on the peptide orientation, perhaps suggesting that the close proximity of E16 to W19 (on the next helical turn) could provide stability to the peptide helix. We are also studying the peptide-lipid behavior when Glu is substituted in position 12 and/or 14, individually or together.

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Functional Consequences of Incomplete Hydrophobic Matching at TM1 of the LeuT Transporter

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The Leucine Transporter (LeuT) is the prototype for structure-function studies of mammalian Neurotransmitter: Sodium Symporters such as DAT, SERT and NET, the transporters for dopamine, serotonin, and norepinephrine, respectively. Its functional sensitivity to the environment, i.e., membranes or detergents in various compositions, has engaged much recent research. As the role of the environment in the function and organization of transmembrane proteins has been shown to involve hydrophobic mismatch, we investigated the membrane deformation and extent of hydrophobic matching for LeuT with the recently described hybrid Continuum-Molecular Dynamics (CTMD) method that combines elastic continuum formulations with an atomistic description of the lipid-protein interface from molecular dynamics simulations. The analysis was performed for functionally relevant conformations of LeuT embedded in two different model membranes: a POPC lipid bilayer and a model bacterial bilayer composed of a 3:1 mixture of POPE and POPG lipids. In both bilayers we found significant membrane thinning and water penetration near the membrane-facing Lys288 of TM7, a positively charged residue embedded