

Thymidine block that arrests cells at the border between G1 and S phases. Critical temperatures were elevated in GPMVs isolated from cells in cell cycle phases that precede cell division (G2 and M) compared to other stages (G1 and S). In unsynchronized cells, critical temperatures were found to be inversely proportional to cell density, suggesting that contact inhibition and associated arrest of cell growth results in lower plasma membrane critical temperatures. Lower critical temperatures were also observed when growth was arrested through overnight serum starvation, and elevated critical temperatures were restored 24h after the addition of serum containing medium. Transition temperatures are also lowered in GPMVs prepared from cells undergoing apoptosis through the application of TRAIL, and vesicles contain a more rigid liquid-ordered or gel phase at low temperature. These results are in agreement with past studies that have indicated that plasma membrane composition varies within the cell cycle. Since GPMV critical temperatures are hypothesized to reflect on the magnitude of lipid-mediated heterogeneity in intact cells, these results suggest that membrane heterogeneity is greatest in cells undergoing rapid growth and cell division and is suppressed in cells under low growth conditions.

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Molecular and Mechanical Manipulation of Membrane Domains in Planar Supported Bilayers

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Nano-membrane domains are hypothesized to play an integral role in many cell signaling pathways. Their transient nature and biocomplexity underlies a myriad of fundamental questions about lipid-lipid and lipid-protein interactions and their roles in cellular functions. As a result, there is a need for innovative approaches for understanding different biophysical aspects of membrane assemblies and their underlying, multiscale dynamics. Here, we integrate dynamic holographic optical trapping (HOT) and fluorescence imaging with fluorescence correlation spectroscopy (FCS) to characterize membrane domain nucleation in biomimetic planar supported bilayers. The dynamic HOT system allows for the creation of multiple traps from a single light source, each of which can be controlled individually in real time. Silica microspheres are being trapped into arbitrary patterns for system optimization. Receptor-bound microspheres associated with nano-domains in planar supported bilayers act as handles for dynamic HOT manipulation. Our hypothesis is that by trapping multiple microsphere-bound receptors, the associated heterogeneous lipid domains will nucleate a larger domain upon interaction in a manner that depends on the lipid type, cholesterol and protein content. Fluorescence imaging is used to visualize lipid domain formation, and subsequent lateral diffusion of lipid species will be measured with FCS as a function of trap-induced confinement. These results will ultimately lead to new insights into domain formation in membranes.

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Lipopolymer Crowding in Polymer-Tethered Lipid Bilayers Alters Lipid Mixing Behavior and Protein Sequestration in the Presence of Raft-Mimicking Lipid Mixtures

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It is now well established that the cytoplasm and plasma membrane of cells are characterized by high concentrations of proteins. Consequently, macromolecular crowding and confinement effects are believed to play important roles in the organization, dynamics, and function of proteins in cellular environments. However, the functional significance of molecular crowding remains unclear and conclusions are still controversial, due to the complexity of cellular systems. To address this experimental challenge, here we introduce a model membrane system on the basis of a polymer-tethered lipid bilayer, in which lipopolymers act as crowding agents. Our experiments show that changes in lipopolymer concentration have a profound effect on the lipid mixing behavior of raft-mimicking lipid mixtures forming micron-size liquid-ordered (L_o) and liquid-disordered (L_d) phase separations. Complementary fluorescence correlation spectrometry (FCS), confocal fluorescence intensity analysis, and photon counting histogram (PCH) analysis experiments demonstrate that lipopolymer-mediated molecular crowding also influences the lateral diffusion of lipids and membrane proteins, as well as membrane protein sequestration in raft-mimicking lipid mixtures. Interestingly, our experiments confirm that elevated lipopolymer concentrations cause the formation

of buckling structures, which can be seen as a stress relaxation phenomenon. Notably, the extent of membrane buckling is substantially different in L_o and L_d lipid regions.

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Why Cholesterol should be Found Largely in the Cytoplasmic Leaf of the Plasma Membrane

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In the mammalian plasma membrane, cholesterol can translocate rapidly between the exoplasmic and cytoplasmic leaves, and has been found predominantly in the latter. We hypothesize that it is drawn to the inner leaf to reduce the bending free energy of the membrane caused by the presence there of phosphatidylethanolamine (PE). It does this in two ways: first by simply diluting the amount of PE in the inner leaf and second by ordering the tails of the PE so as to reduce its spontaneous curvature. Incorporating this mechanism into a model free energy for the bilayer, we find that between 50 and 60% of the total cholesterol should be in the inner leaf of human erythrocytes.

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Do Gel Phase Lipid Bilayers Behave Like Euler Elastica?

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The elasticity of fluid phase lipid membranes can be characterized using the Helfrich Hamiltonian, which depends only on the square of the total curvature and the Gaussian curvature and the corresponding curvature moduli: the mean bending modulus and the Gaussian curvature modulus. Even at large curvatures approaching the inverse bilayer thickness, the effect of higher order terms of the elastic energy are minimal. Recently, a method has been developed to derive the bending modulus for fluid membranes from the stress-strain relationship of a buckled membrane [1,2]. The method also predicts the shape of the membrane, an *Euler Elastica*, and this serves as a check that the membrane indeed follows quadratic curvature elasticity. Using a coarse-grained lipid model, we analyze the shape of a buckled membrane in a gel phase and show that it does not behave like an *Euler Elastica*, even at low curvatures. The deviation from the theory suggests that higher order terms of the total curvature reduce the energy penalty for high curvatures. We present an extended version of the Helfrich Hamiltonian that captures this effect and show that it describes the shapes that we observe in simulations. We then calculate the bending modulus, as well as the modulus describing higher order corrections.

[1] Noguchi H., "Anisotropic surface tension of buckled fluid membranes", *Phys. Rev. E* **83**, 061919 (2011).

[2] Hu M., Diggins P., and Deserno M., "Determining the bending modulus of a lipid membrane by simulating buckling", *J. Chem. Phys.* **138**, 214110 (2013).

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Revisiting the Link between Lipid Membrane Elasticity and Microscopic Continuum Models

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Fluid lipid membranes can be described by a continuum-elastic Hamiltonian that features two central parameters mean and Gaussian curvature modulus. Of those two, the Gaussian modulus is much less understood, since it affects the membrane energetics only through topology or boundaries and is thus difficult to measure experimentally. Moreover, recent work by Hu et al. [Biophys. J. 102, 1403 (2012)] to determine this modulus from computational studies revealed discrepancies with more microscopic expectations, according to which this modulus should be given by the second moment of the lateral trans-membrane stress profile. Our goal in the present study is to revisit the arguments linking the Gaussian modulus to properties of thin sheets, using thin plate theory as a starting point, but allowing for a number of generalizations. For instance, membranes are more generally described as anisotropic continua with in-plane fluidity and a nontrivial distribution of pre-stresses. It is easy to see that in this case linear elasticity, combined with the additional but common approximation of a linear strain tensor, will predict that the Gaussian curvature modulus vanishes. We therefore need to include the possibility of nonlinear strains (while still using linear constitutive equations), but this in turn leads to some subtle inconsistencies within Monge gauge. Another difficulty is that virtually all existing theories treat Young's modulus and the Poisson ratio as constant throughout the width of the membrane. Starting from fundamental linear elasticity theory, amended by a nonlinear strain tensor, and using consistent geometrical constructions, we

then investigate the elastic properties of membranes, especially the Gaussian curvature modulus.

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Molecular Level Modeling of the Effects of Charge and Sugars on the Phase Equilibrium of Model Lipid Bilayers

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We present a mean-field theoretical molecular model capable of addressing the disparity in results drawn from experiments studying the structural and thermodynamic characteristics within multicomponent anionic model membranes. The experiments were drawn under identical system conditions, differing only in lipid preparation method—electrophoresis, in the presence of sugar solutions, versus a gentle hydration protocol, absent of sugars. The discrepancies in the membrane phase behavior were noted, but left uncharacterized due to constraints on the experimental conditions that limited the yields necessary to evaluate a comparison drawn between the techniques.

Such experimental limitations highlight the utility of our theory, from which we can capture the non-trivial coupling between organizational and physicochemical properties within the system. We use a molecular model to quantify the isolated and net contributions of glucose, sucrose, and counterion distribution to the stability of phase-coexistence regions in ternary anionic membranes comprised of dipalmitoylphosphatidylcholine (DPPC), anionic dioleoylphosphatidylglycerol (DOPG), and cholesterol. The theory is based on a free energy formulation that explicitly accounts for the architecture and charge distribution of all system molecules, while treating the intermolecular interactions within a mean-field approach. A distinguishing attribute of our theory is the rigorous construction of all system components from a molecular foundation, realistically characterizing the system's physics on the scale of the monomer. The size and shape of each monomeric unit are treated explicitly, and all possible conformations, as well as a comprehensive set of translational and rotational states, are included.

As delineated in this work, anionic lipid head-regions present hydroxyl groups capable of forming hydrogen-bond networks. The introduction of sugar molecules motivates strong interactions with these networks, tremendously disrupting the stability of phase coexistence regions. Screening of counterions partially alleviates this effect.

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Edge Structure of through Pore in Lipid Membrane

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Theoretical model of edge structure of through pore in lipid membrane was developed. We assumed that membrane monolayers are not necessary in close contact with each other everywhere; instead, hydrophobic void can appear between monolayers of the membrane near the pore edge. If formed, the void is supposed to be filled by organic solvent or low-polarity impurities, which are usually present in artificial and cell membranes. The monolayer of pore edge was divided to three regions: almost vertical monolayer, almost horizontal monolayer, and horizontal monolayer of bilayer part of the membrane. All three regions were assumed continuously conjugated. Pore line tension was calculated in the framework of theory of elasticity of liquid crystals adapted to lipid membranes, by taking into account splay and tilt deformations. Line tension depends on the value of surface tension at the boundary of lipid tails with the void. For sufficiently high surface tension the void collapses. Calculated values of pore edge line tension are in good agreement with available experimental data both for membranes containing and lacking organic solvent or low-polarity impurities.

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Bolalipid Membranes: Elasticity Theory Approach

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Bolalipids is a new and very intriguing and promising object for the investigations. Bolalipids are the main component of extremophilic bacteria's cell mem-

brane and mostly responsible their exceptional stability and vitality under the high temperature, pressure and environment acidity. As opposed to "conventional" lipids bolalipids are composed of two polar head connected by two hydrocarbonic tails and can either pierce through the whole membrane or form a U-shape configurations. Bolalipids' investigation is at its opening stage. There is lack of experimental data on the model membranes, on the theoretical side the researches are limited by computer simulations data and some microscopical models. However, for the effective progress in the investigation of the bolalipid membranes the elasticity theory should be developed. Similar theory applied to the conventional lipid membranes gives results that are in an excellent agreement with the experimental data. That is why we have chosen the way of construction the effective elasticity theory of bolalipids on the basis of conventional lipid one.

In the present work we constructed the phenomenological elasticity theory of the bolalipid membranes. The set of all feasible types of deformations are found. It have been shown that, unlike to conventional lipids, bolalipid's elasticity theory demands two bending moduli. Possible experiments for the determining of the elastic moduli are suggested. Theory takes into account the presence of the two configurations of bolalipids in the membrane: U-shape and O-shape configurations. The way of the experimental determination of the ratio of these configurations is proposed.

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Physical Properties and Membrane Packing in Hybrid Archaeosomes

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In this study, we have characterized the physical properties of liposomes made of archaeal bipolar tetraether lipids (BTL) and "conventional" monopolar diester lipids (termed as hybrid archaeosomes). We used the polar lipid fraction E (PLFE) isolated from the thermoacidophilic archaeon *Sulfolobus acidocaldarius* as the BTL component of the hybrid archaeosomes. Hybrid archaeosomes were made by extrusion with the particle size ranging from 100-300 nm. Dynamic light scattering and zeta potential measurements showed that the presence of PLFE greatly stabilizes liposomes. The steady-state polarization of DPH fluorescence in PLFE/PC mixtures decreases with increasing temperature (18-56°C) in a molar ratio dependent manner. At low temperatures, hybrid archaeosomes have membrane packing, as inferred from DPH polarization, similar to that in gel state of disaturated PC bilayers. At the high temperature end, membrane packing of the hybrid archaeosomes is significantly tighter than that of PC fluid state. A similar conclusion can be drawn from the studies using Laurdan's generalized polarization. Additionally, due to tight membrane packing, drug release from hybrid archaeosome is much slower than from liposomes composed of only diester lipids. Confocal fluorescence microscopy showed that these hybrid archaeosomes can be taken up by live cells (e.g., MCF-7 breast cancer cells) and deliver entrapped drug molecules to the cell's nucleus, but with a rate significantly lower than that obtained from conventional diester liposomes.

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Temperature-Dependent Lipid Phase Transitions Occurring in Higher Organisms

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Previously published data concerning the temperature dependence of the neural responses of frog, squid and rat have been examined for unusual features occurring at the gestation temperature, T_g , of such organisms. The temperature dependencies of the resting potential and action potential of frog and squid exhibit behaviors that strongly suggest the onset of a membrane state change when $T > T_g$. Anomalies in the flow of current in the rat ganglion lead to a similar conclusion. Membranes formed of whole lipid extracts from various organisms, including extracts from neural tissues, have been observed to exhibit a phase transition at a temperature $T^* = T_g$. Based on approximations to the classic Hodgkin-Huxley equations, analysis of axonal response indicates that observed changes in the resting potential and peak of the action potential reflect chemo-mechanical coupling between voltage-switchable ion channels and temperature-linked, lipid phases of the plasma membrane. The behavior of the resting potential and action potential when $T > T_g$ can be rationalized by postulating the emergence of a different lipid state that alters channel properties. The change in neural properties with increasing temperature for these animals has an activation energy of approximately 26 kcal/mole. Short exposure to temperatures above T_g alters the stability of the channels, whereas elevating the temperature for longer periods appears to result in irreversible degradation of the tissue.