

peptide-lipid interactions. As opposed to most natural lipids, DPhPC is more chemically stable, and therefore, it can provide for a more robust model of lipid membranes. However, despite its frequent use, DPhPC has been less investigated than other PC lipids. To measure the physical properties of DPhPC membranes we employ a combination of complementary methods that include x-ray scattering, Nuclear Magnetic Resonance (NMR), and osmotic stress. By X-ray scattering, we obtain the repeat distances (D-spacings) of multilamellar structures formed by DPhPC in solution. By applying osmotic stress, we are able to modify and control intermembrane spacings and molecular conformations, the latter measured by NMR. These structural measurements together with ion channel activity measurements using the standard gramicidin A channels allow us to compare DPhPC with more common and better studied PC lipids. In this way, we can help rationalize the use of DPhPC as a model membrane for studies of membrane function.

3480-Pos

Protein-Lipid Interaction and Domain Formation in Asymmetric Membranes

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The lateral organization of lipids and receptors in cellular membranes is connected to a multitude of biological and pathological processes. The role of sphingolipid- and cholesterol-rich lipid "rafts" as membrane domains that control protein-protein interaction has recently attracted much attention. While the model membrane approach has yielded fundamental insights into cell membrane structure and function, the majority of these studies did not take into account the very important asymmetry between the composition of the inner and the outer leaflets of cell membranes. Therefore, certain key questions remain still unanswered: for example, how is the partition of membrane components in raft domains affected by the presence of non-raft lipids in the inner leaflet? To answer questions of this type we have developed a new method based upon cyclodextrin-induced lipid exchange to create giant unilamellar vesicles (GUVs) featuring the same type of asymmetry encountered in biological membranes. These vesicles can be produced with a very large yield, while avoiding use of organic solvents. The asymmetry of the bilayer can be confirmed by leaflet-targeted Fluorescence Correlation Spectroscopy (FCS). The use of asymmetric GUVs as novel model bilayers should allow the investigation of the principles underlying communication between raft-like domains in opposite leaflets of the bilayer, as well as investigation of the origin of lipid and membrane protein affinity for rafts.

3481-Pos

Thermal Energies and Invariant Lipid Structures

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Indiana University Purdue University Indianapolis, Indianapolis, IN, USA. Biological membranes pose many interesting problems amenable to a physicist's perspective. A central effort of a physicist is to discover invariant properties as conditions and systems change. As an example, for a lipid bilayer system one can vary the type and length of the hydrocarbon chains, in addition to the specific lipid headgroup. Furthermore, lipid bilayers in solution exhibit a range of molecular motions with amplitudes determined by the available thermal energy. How will membrane properties behave under these changes? The theoretical framework to answer this question is within classical equilibrium thermodynamics. We use a mean-torque potential model [1] to analyze order parameter data from solid-state NMR measurements on lipid bilayers to describe how bilayer properties scale with temperature and acyl chain length. We find an invariant description of acyl chain packing, which allows us to address the correspondence between changes in acyl length and changes in temperature. We present the functional form of this scaling relationship, the conclusions that can be drawn from such a temperature study, and how this invariance is ultimately a step in determining the guiding principles of lipid mixture organization in biological membranes. [1] H. I. Petrache, S. W. Dodd, and M. F. Brown. *Biophys. J.* **2000**, 79:3172-3192.

3482-Pos

Specific Spatial and Orientational Order in Phospholipid Membranes Induced by Cholesterol

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¹Tampere University of Technology, Tampere, Finland, ²The University of Western Ontario, London, ON, Canada, ³Barcelona University, Barcelona, Spain, ⁴Helsinki University of Technology, Helsinki, Finland. Cholesterol plays a major role in formation of laterally ordered membrane structures such as lipid rafts. These domains have been found to be involved in a variety of cellular functions, implying that there is immediate interest to understand the structure as well as the dynamics of rafts, and in particular the role of cholesterol in promoting order in rafts. Nonetheless, due to the molecular scales asso-

ciated with lipid rafts and the soft nature of membrane domains overall, the atomic-level mechanisms responsible for cholesterol's specific ordering capability have remained unresolved. Our atomistic simulations [1] reveal that this ordering and the associated packing effects in membranes largely result from cholesterol's molecular structure, which differentiates cholesterol from other sterols. Cholesterol molecules are found to prefer a specific spatial and orientational molecular in-plane organization, where cholesterol molecules are located in the second coordination shell, avoiding direct cholesterol-cholesterol contacts, and forming a three-fold symmetric arrangement with proximal cholesterol molecules. At larger distances, the lateral three-fold organization is broken by thermal fluctuations. Other sterols having less structural asymmetry are found to lack the three-fold arrangement that is characteristic to cholesterol.

[1] H. Martinez-Seara, T. Róg, M. Karttunen, I. Vattulainen, and R. Reigada. Manuscript under review (2009).

3483-Pos

Near-Field Structural Studies of Lipid Bilayers

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We use a Near-field Scanning Optical Microscope (NSOM) in conjunction with a Photo Elastic Modulator (PEM) to conduct birefringence (ne-no) measurements with a spatial resolution of ~80nm. With our current setup we are able to distinguish changes in retardance S on the order of 10⁻⁴ radians. Simultaneously while gathering information about S we extract information about the samples optical orientation θ referenced to the system's axis, with an accuracy of ~7.24x10⁻⁴ radians. We use our system on 1,2-dipalmitoylphosphatidylcholine (DPPC) bilayers, which at room temperature are in the gel state, (i.e.: their acyl chains have a ~32o degree azimuthal tilt with respect to the membranes normal). Modeling the membrane as a uniaxial crystal we are able determine the position of the acyl chains by measuring the birefringence and optical orientation. By controlling the temperature of our sample we hope to better study the structural changes that occur during phase transitions from gel to liquid states. The investigation of other lipid mixtures and the transformations they undergo during different phases will also be discussed.

3484-Pos

Calgary Lipids: A Lipid Force Field for Molecular Simulations

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Lipid bilayers are fundamental components of biological membranes. The most biologically relevant state of lipid bilayers is the fully hydrated fluid phase (fluid-disordered or liquid-ordered). Due to their fluidity, it is not possible to obtain experimentally atomic level structures of single bilayers. Computer simulations have been widely used to study lipid bilayers, providing detailed structural information that can aid the interpretation of experimental results. A large body of literature is available on simulations of lipid bilayers, but different studies used different simulation conditions and different force fields, which makes it difficult to compare results for different lipids.

We present here a new united-atoms force fields for lipids, compatible with common protein force fields. Partial charges and dihedral angle profiles were derived from quantum mechanics calculations on lipid fragments, while Lennard-Jones parameters were tuned by fitting thermodynamic data for simple molecular building blocks. We built a library of structures of 60 different lipid bilayers, generated using molecular dynamics simulations in identical conditions. The library provides comparable structural data for bilayers with 4 different head groups and 15 pairs of acyl chains, including the most common lipid molecules. Structural parameters (such as the area per lipid and the electron density profiles) as well as dynamic properties (diffusion coefficients, deuterium order parameters) have been calculated for each bilayer, allowing for a systematic analysis of the effect of chain length, chain unsaturation and the chemical nature of the head group.

3485-Pos

Viscoelastic Properties of Plasma Membranes Varies with Cholesterol Level

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Cholesterol is an important lipid component of mammalian cell plasma membranes. It contributes to the biophysical properties of the membrane, and plays an important role in the regulation of the membrane protein function.

In this study, effects of cholesterol on viscoelastic properties of the plasma membrane are investigated. We use optical tweezers to extract nanotubes (tethers) from the plasma membrane of human embryonic kidney (HEK) cells. We obtain time-resolved tether force measurements under cholesterol depleted and cholesterol enriched conditions. Using these data, elastic and viscous parameters of the plasma membrane are quantified and correlated to the changes in the membrane cholesterol level.

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3486-Pos

The Progression of a Novel Liposome-Based Delivery Vehicle Toward in vivo Drug Delivery

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The primary aim of nanoscale drug delivery is to increase the efficacy of drug treatment by both lowering injected dose and reducing drug exposure to healthy tissue. Currently, liposomes are commonly used as carriers in industrial formulations for the treatment of cancer, but still suffer from drug retention and targeting issues. Previous work has established the vesosome, a large lipid bilayer enclosing multiple smaller liposomes, as an alternative to the liposome that offers improved retention and functionality. However, to be able to compare the relative effectiveness of the vesosome to the liposome, it is necessary to advance the vesosome to in vivo testing. We have developed a method to functionalize the vesosome for biocompatibility and examined the in vivo lifetime and biodistribution and initial results are a proof-of-concept that the vesosome can be used as a nanoscale drug carrier. To improve upon the vesosome, the formulation and synthesis have been modified to improve product purity, maintain half-life and introduce active targeting capacity. Continuing work focuses on using the new formulation of the vesosome to quantify its in vivo behavior and compare it to the vesicle.

3487-Pos

A Platform to Study Curvature Effects of Proteins on Membranes

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The topography of a cell membrane is heterogeneous but well regulated. Specific cell processes, such as clathrin-mediated endocytosis, locally induce a large degree of curvature in the cell membrane and require several proteins to bind and shape the membrane. The N-terminal Homology domain of Epsin (ENTH) is a membrane binding domain involved in endocytosis that is believed to induce local curvature in lipid bilayers. Previous investigations, looking at small round vesicles in the presence of the ENTH domain using negative-stained electron microscope images, have shown tubulation of vesicles in the presence of purified ENTH domain. However vesicle dehydration from the staining process makes it difficult to discriminate morphological artifacts from protein-induced curvature.

Cryo-electron microscopy (cryo-EM) avoids dehydration artifacts from heavy stains and preserves the round morphology of lipid vesicles. Using cryo-EM, we explore two methods of vesicle preparation: extrusion of lipids through a porous membrane with 100 nm pores and solubilization of lipids in detergent and slow dialysis of the detergent. We show that the extrusion method introduces a small sample of elongated and non-spherical shapes in the vesicle population, whereas the detergent-dialysis method produces perfectly round vesicles.

Using the detergent-dialysis method to produce round vesicles, we can add putative curvature-inducing proteins to these vesicles and observe the effects of these proteins on vesicle curvature using cryo-EM. This provides an ideal platform to study the effects of curvature-inducing proteins on vesicle morphology.

3488-Pos

Investigation of Mitochondrial Crista Membrane Morphologies in Terms of Minimizing the Free Energy of the Configuration

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Electron tomograms have revealed that in normal mitochondria crista membrane contains both flat lamellar and tubular components that are connected to inner boundary membrane through crista junctions. We propose a free energy model for this configuration in an effort to use observable geometrical features to predict thermodynamic properties of mitochondria such as surface tension and pressure that are not directly observable. To that end, a set of geometric measurements from the structural features of mitochondria were ob-

tained. Structural features were measured from 3D electron tomograms of mitochondria. These tomograms were obtained by collecting tilt series of 300nm sections of mitochondria, aligning the projection images of each tilt series to a common origin, and applying a filtered backprojection algorithm to the tilt series to calculate tomograms of mitochondria in each section. Full tomograms were obtained by joining the reconstructions of up to four serial 300nm sections. From the measured structural features, the input parameters for our model are extrapolated. The free energy model combined with these geometric measurements predicts that tubular structures are stabilized by tensile forces of 10-20 pN, comparable to those typical of motor proteins. It also predicts the pressure differences of 0.01-0.1 atm across crista membrane and surface tensions of less than 0.2 pN/nm, the point at which a membrane ruptures. Geometric measurements predict relations between lamellar radii versus number of tubes describing the constraints within crista morphology of normal mitochondria. We are further interested in investigating if through mitochondrial fusion the observed loose tubular membranes in the mitochondrial matrix can come together to form crista membranes and minimize the free energy. We are investigating the geometric changes in the crista morphology that can minimize the free energy when OPA1 is missing.

3489-Pos

Domain Assembly in Giant Unilamellar Vesicles Prepared from Myelin Lipids

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Myelin is a specialized membrane, which is produced in large amounts by differentiated oligodendrocytes and wraps around the axon of neurons for insulating purposes. Any alteration in the spatio-temporal architecture of myelin leads to severe neurological symptoms in neurodegenerative diseases, i.e. multiple sclerosis. Compared to most plasma membranes, myelin is mostly composed of lipids (~80% of the total weight) and has a rather peculiar lipid composition, as it is highly enriched in galactosylceramides, sulfatides, and glycosphingolipids.

In the present study, we reconstitute myelin lipids in giant unilamellar vesicles and apply confocal optical microscopy to visualize the specific molecular organization and domain assembly. In particular, the distinct effect of sulfatides and/or ceramides on lipid segregation is investigated in giant unilamellar vesicles prepared from various mixtures of sphingolipids, phospholipids and cholesterol. The biochemical dissection of distinct domains and the isolation of specific lipid components, as shown here, will contribute to the understanding of the organization of myelin and the spatio-temporal regulation of its subdomains.

Membrane Fusion

3490-Pos

Fast Single Vesicle SNARE-Mediated Membrane Fusion Assay in Planar Supported Bilayers Reveals Details About Fusion Mechanism

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SNAREs (*soluble N-ethylmaleimide-sensitive factor attachment protein receptors*) mediate membrane fusion in neuronal exocytosis and intracellular membrane trafficking. It is widely accepted that the zipper interaction between syntaxin1a, SNAP25 and synaptobrevin2 brings plasma- and vesicle membrane together and leads to their merger. Membrane fusion has been extensively studied by liposome and single vesicle-planar membrane fusion assays. However, both approaches have been criticized because of slow fusion rates incompatible with cellular rates or lack of physiological specificity, respectively.

Here we present a fluorescence-based single vesicle-planar bilayer fusion assay with millisecond time resolution and an improved reconstitution procedure that preserves the native topology and mobility of the SNAREs. The acceptor-SNARE complex, composed of syntaxin1a (SyxH3), SNAP25 and a short synaptobrevin2 peptide (Syb49-96), was reconstituted into planar bilayers by a combined Langmuir-Blodgett - vesicle fusion technique. Docking and fusion of single Rh-DOPE labeled Syb vesicles to the supported acceptor-SNARE membranes were observed by total internal reflection fluorescence microscopy. No docking or fusion was observed in protein-free control membranes. Vesicle SNARE docking was dependent on the acceptor-SNARE complex density in the membranes. Moreover, docking was SNAP25-dependent, and subsequent fusion did not require Ca²⁺ and was efficient at ambient temperature. A detailed kinetic analysis of >1000 single fusion events revealed that each fusion reaction consists of 6 to 9 activating steps with 8 steps fitting the data best. This could be interpreted by fusion sites consisting of 8 SNARE complexes that each activate in a single rate-limiting step in 8 ms. We find that different lipid compositions of the supported and the vesicle membrane have a relatively minor influence on docking, but modulate the fusion efficiency and fusion kinetics more dramatically. Supported by NIH grant GM072694