View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

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.
Acknowledgements: This work was supported by Bourns College of Engineering and the Bioengineering Center at University of California, Riverside, and grants provided by NIH (2R01-DC02775) and NSF (BES-0522862).

3486-Pos
The Progression of a Novel Liposome-Based Delivery Vehicle Toward in vivo Drug Delivery
Benjamin Wong, Jason Schmidt, Joseph Zasadzinski.
University of California, Santa Barbara, Santa Barbara, CA, USA.
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
Hector H. Huang[1], Joseph B. Pesavento[2], Sharon Rozovsky[3], Kenneth H. Downing[2], Jay T. Groves[1].
[1]University of Berkeley, Berkeley, CA, USA, [2]Lawrence Berkeley National Labs, Berkeley, CA, USA, [3]University of Delaware, Newark, DE, USA.
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
Mariam Ghochani[1], Avinoam Rabinovitch[2], Terry Frey[1], Jim Nulton[1], Peter Salamon[1], Arlette R. Baljon[1].
[1]San Diego State University, San Diego, CA, USA,
[2]Ben Gurion University of the Negev, Beersheba, Israel.
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 obtained. 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 radiuses 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
Nicoletta Kahya, Dick Hoekstra.
University Medical Center, Groningen, Netherlands.
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 galactosyceramides, 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
Marta K. Domanska, Volker Kiessling, Lukas K. Tamm.
University of Virginia, Charlottesville, VA, USA.
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 zippering 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-basedsingle 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 Ca^{2+} 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