Platform V: Membrane Dynamics & Bilayer Probes

1137-Plat

Curvature Sorting of Lipids and Proteins in the Strong Segregation Limit: Curvature Mediated Domain Nucleation and Steady State Transport in Tubular Membranes with Phase Separation

Michael C. Heinrich, Aiwei Tian, Tom C. Lubensky, Tobias Baumgart.
University of Pennsylvania, Philadelphia, PA, USA.

Intracellular sorting centers, including the trans-Golgi network, the endoplasmic reticulum, and the endocytic recycling compartment, all contain membrane tube elements with cylindrical curvatures. The question of how curvature and sorting are coupled is central to the understanding of the function of organelle homeostasis, membrane trafficking, and intracellular sorting. Of particular interest, but underexplored, are non-equilibrium phenomena fundamentally linked to membrane transport.

Here we present a straightforward and well controlled model system that allows us to characterize lipid transport at steady state: ternary lipid mixture tubular membranes pulled from phase-separated giant unilamellar vesicles (GUVs) by means of optical tweezers. The tubule composition, when initially formed from the liquid-ordered (Lo) phase region of the vesicle, is dependent on the velocity at which it is pulled: fast extraction velocities create tethers of the Lo phase, while slow extraction velocities can generate tethers that are liquid-disordered (Ld) phase. Thus, the speed with which highly curved tubules are formed may possibly serve as a control variable in living cells to adjust tubule composition. Furthermore, in tubules which are initially Lo phase, we find curvature-induced nucleation of Ld domains at the neck between tubule and vesicle. These Ld domains display characteristic, curvature dependent parabolic growth behavior that can be understood via a straightforward analytical mass transfer model that we derived from linear irreversible thermodynamics. We have also developed numerical schemes that capture shape transitions of tubular membranes on the basis of measured biophysical parameters in support of our experimental findings.

1138-Plat

Dynamics of Bicelle Model Membrane Promote Domain Formation

Hyo Soon Cho, **Megan Spence.**
University of Pittsburgh, Pittsburgh, PA, USA.

We have created a magnetically-aligned bicelle model membrane system (containing POPC, DMPC, and cholesterol) that laterally separates to form lipid domains. Lipid domains have been observed in a number of ternary systems (cholesterol, saturated lipid, unsaturated lipid), but rarely in systems containing POPC and never in systems containing POPC and DMPC. Using pulsed-field gradient NMR techniques, we measured the lateral diffusion constant for the lipids as a function of diffusion time. The time dependence of the diffusion constant showed the presence of domains ~1um in size at 295K. The domains increase in size with temperature, until an isotropic membrane structure appears, indicating that the domains are liquid-disordered phase. Variable temperature 1H-MAS NMR of the domains supports this assignment. Bicelles are magnetically-aligned, highly perforated bilayers often used in solid state NMR for membrane protein structure determination. The formation of POPC/DMPC/cholesterol domains may result from the rapid ($\tau c \sim 10\text{-}6$ s) undulations of the bicelle bilayer surface. The domain-forming bicelles are also suitable for NMR structure determination of raft-associated membrane proteins.

1139-Plat

Dynamics of 3D Axisymmetric Multicomponent Vesicles in A Viscous Fluid

Jin Sun Sohn[1], Shuwang Li[2], Xiaofan Li[2], John S. Lowengrub[1].
[1]University of California, Irvine, Irvine, CA, USA, [2]Illinois Institute of Technology, Chicago, IL, USA.

Multicomponent vesicles are hollow, closed biomembranes with a lipid bilayer membrane containing different types of lipids and cholesterol. Recent experiments on giant unilamellar vesicles demonstrate that there exists a variety of behavior of multicomponent vesicles. Under this understanding, we develop and investigate numerically a thermodynamically consistent model of three dimensional axisymmetric multicomponent vesicles in an incompressible viscous fluid. The model is derived using an energy variation approach that accounts for different lipid surface phases, the excess energy (line energy) associated with surface phase domain boundaries, bending energy, spontaneous curvature, Gaussian bending energy, local inextensibility and fluid flow via the Stokes equations. The equations are high-order (fourth order)nonlinear and nonlocal due to incompressibility of the fluid and the local inextensibility of the vesicle membrane. To solve the equations numerically, we develop a nonstiff, pseudo-spectral boundary integral method that relies on an analysis of the equations at small scales. We present simulations of multicomponent vesicles in a quiescent and an extensional flow and investigate the effect of varying the average surface concentration of an initially unstable mixture of lipid phases. The phases then redistribute and alter the morphology of the vesicle and its dynamics. A comparison of results with experimental vesicle morphologies yields good agreement.

1140-Plat

Lipid Phase Specific Organisation of the Transmembrane Anchor of Influenza Hemagglutinin

Joerg Nikolaus, Andreas Herrmann.
Humboldt University Berlin, Berlin, Germany.

Correct transport and localization of membrane proteins to distinct cellular organelles are crucial for cell growth but also for the infection cycle of enveloped viruses. Viral membrane proteins like hemagglutinin of influenza virus, anchored in the membrane via a single transmembrane domain (TMD), are transported to the plasma membrane and - together with other virus components - recruited to the budding site. Cholesterol and glycosphingolipid-enriched membrane microdomains are considered as assembly and budding sites for enveloped viruses such as influenza virus. Besides a most likely location of a sorting signal within the TMD sequence also the width of the bilayer is important, which is, amongst other factors, controlled by the cholesterol content of the membrane.

Using different fluorescent-based approaches we study the impact of membrane hydrophobic thickness and membrane packing properties on the sorting behavior of virus derived TMD peptides containing a Trp residue in its center. The emission λmax depending on the hydrophobicity of the surrounding of the Trp residue and parallax analysis of fluorescence quenching are used to determine the Trp location in the lipid bilayer revealing a transmembrane orientation. Membrane thickness is altered by the use of lipids having different acyl chain length and also by the addition of cholesterol or decane shifting λmax. Incorporation of Rhodamine labeled TMD into giant unilamellar vesicles prepared from lipids with varying length and also from ternary lipid mixtures forming distinct liquid phases allows us to study phase dependent TMD localization by fluorescence microscopy. Typically, in the model system the TMD sorts into the liquid disordered phase in contrary to the raft association of hemagglutinin in cells. To address the latter by a more appropriate system, we used viral lipids for vesicle preparation mimicking the natural environment of the TMD.

1141-Plat

Nanometric Phase Transitions on Phospholipid Membranes Using Plasmonic Heating of Single Gold Nanoparticles

Alexander S. Urban, Michael Fedoruk, Stefan Wimmer,
Fernando D. Stefani, Jochen Feldmann.
LMU Munich, Munich, Germany.

Lately impressive advances have been made revealing the structure and pathways of cellular and subcellular systems by optical techniques with nanometric resolution [1]. Full understanding of the function of these systems requires additional energetic information of the processes involved. In order to obtain such information locally, it is necessary to develop remotely controlled nanoscale heat sources.

We demonstrate the capability of single gold nanoparticles as optically controlled nanoscopic sources of heat. Gold nanoparticles attached to giant unilamellar vesicles in the gel-phase can induce reversible, local phase transitions to the fluid-phase when illuminated at their plasmon resonance [2]. The optically heated nanoparticles melt a nanoscale region of the membrane and exhibit an enhanced diffusion over the membrane. The diffusion is analyzed by single particle tracking for various phospholipids and laser power densities. As a result, we can control the nanoscale phase transition and obtain local information on the dynamics of the membrane.

The results illustrate the use of single gold nanoparticles for local nanoscale thermodynamic investigations on phospholipid membranes. The approach presented here can be easily extended by combining it with other microscopy methods and optical tweezers techniques. This may also open new possibilities to position nanoparticles on cell membranes and thermally manipulate biomolecules or membrane processes.

[1] R. Schmidt, C. A. Wurm, S. Jakobs, J. Engelhardt, A. Egner and S.W. Hell, Nat. Methods (2008) 5, 539
[2] A.S. Urban et al., Nanoletters (2009) 9, 2903-2908