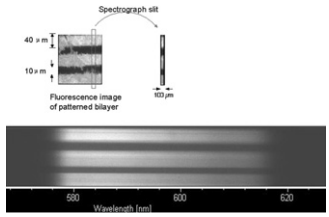
Membrane Physical Chemistry II

1414-Pos

Spatially-Resolved Fluorescence Spectra of Patterned Lipid Bilayers

Teiwei Luo, **Alfred Kwok**.
Pomona College, Claremont, CA, USA.

Planar supported lipid bilayers can be micropatterned such that the lipid composition of localized regions differ from that of the surrounding region. These micropatterned bilayers can serve as model systems to study the dynamics of microdomains in lipid bilayers. We have obtained spatially-resolved fluorescence spectra of bilayers patterned with alternating rows of 1% Rhodamine-DMPE/POPC and lipid voids with epifluorescence and TIRF (total internal reflection fluorescence) excitation. A 60X water immersion objective is used to image a 100-micron slice of the bilayer onto the entrance slit of an imaging spectrograph. A CCD camera at the exit port of the spectrograph records the fluorescence spectra from the bilayer. In conventional fluorescence spectroscopy, the signal from all the pixels of each column of the CCD camera, which corresponds to signal from a specific wavelength, is integrated to produce a single spectrum. In our experiment, such integration is not performed. Since the fluorescence spectra from the alternating rows of Rhodamine-DMPE/POPC and voids are imaged onto different rows of the CCD camera, their spectra can be spatially resolved.



Spatially resolved fluorescence spectrum

1415-Pos

Tethered Lipid Bilayers that Mimic the Composition of Neuronal Membranes

Matteo Broccio[1], Rima Budvytyte[2], Gintaras Valincius[2], Mathias Loesche[1].
[1]Physics Dept, Carnegie Mellon University, Pittsburgh, PA, USA,
[2]Institute of Biochemistry, Vilnius, Lithuania.

For the study of biomolecular interactions with membranes, biomimetic lipid membrane models are a trade-off between robustness and amenability to various characterization techniques on the one hand and limitations in the compositional variety characteristic of biological membranes on the other. We have developed tethered bilayer lipid membranes (tBLMs) as a long-term stable and versatile experimental model in which thiolated lipopolymers span a hydrated layer that separates the membrane from its solid support[1]. Such tBLMs may be prepared either by ''rapid solvent exchange''[2], which leads to highly insulating bilayer but provides limited control over membane composition, or by vesicle fusion, which provides better control over membrane composition but leads to membranes with lower resistance. Here we report on tBLMs that mimic mammalian neuronal membrane lipid compositions by containing various phospholipids, cholesterol, sphingomyelin and cerebrosides. Electrochemical parameters of these neuronal membrane mimics as a function of composition were studied with electrochemical impedance spectroscopy. In tBLMs prepared by rapid solvent exchange, membrane capacitance has a sigmoidal dependence on cholesterol content. These results are compared with those from tBLMs prepared by the fusion of vesicles, whose cholesterol content can be determined with routine biochemical assays. This work aims at establishing complex membrane mimics for studies of Aβ oligomer interactions with bilayers to assess their influence on the lipid component of neuronal membranes in Alzheimer's disease.

Supported by the NIH (1P01AG032131) and the AHAF (A2008-307).

[1]Valincius, G., et al., 2008. Biophys. J. 95:4845-4861.
[2]Cornell, B.A, et al., 1997. Nature 387:580-583.

1416-Pos

Fabrication of a Membrane Interferometer Containing Electrodes

Laura D. Hughes, Prasad V. Ganesan, Steven G. Boxer.
Stanford University, Stanford, CA, USA.

Despite the advantages of supported lipid membranes, one remaining problem has been the incorporation of membrane proteins, as membrane proteins tend to lose their functionality near a surface. To address this limitation but retain the advantages of a nearby surface, we have developed a system where a lipid bilayer is separated a few hundred nanometers from an atomically flat mirror (Ganesan and Boxer, PNAS, 2009, vol. 106, p. 5627). This mirror allows the use of Fluorescence Interference Contrast Microscopy (FLIC) and Variable Incidence Angle-FLIC (VIA-FLIC), two surface characterization techniques that precisely locate the height of fluorescent objects relative to the silicon surface with nanometer resolution. Both FLIC and VIA-FLIC have been used to measure changes in curvature of the bilayer in response to osmotic perturbations of the solution above the bilayer. Current work focuses on changing the architecture of the substrate to allow access to the volume both above and below the bilayer. These changes to the substrate will enable concurrent electrical and optical measurements of voltage-gated membrane proteins, as well as increased control over osmotic balance. Progress towards this goal will be described.

1417-Pos

Conformational Flexibility in Membrane Binding Proteins: Synaptotagmin I C2A

Jacob W. Gauer[1], Jesse Murphy[1], Kristofer Knutson[1], R. Bryan Sutton[2], Greg Gillispie[3], Anne Hinderliter[1].
[1]University of Minnesota Duluth, Duluth, MN, USA, [2]Texas Tech University Health Sciences Center, Lubbock, TX, USA, [3]Fluorescence Innovations, Bozeman, MT, USA.

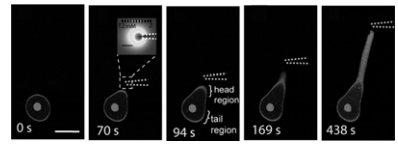
Thermodynamic parameters capture the averaged contribution to a system's energetics. In the case of binding proteins, such as Synaptotagmin I, the first step toward addressing how and where the energy is distributed within that protein is to ascertain the magnitude of the interactions within that protein. Our aim is to understand how binding information is conveyed throughout this protein during the role it plays in regulated exocytosis. While many detailed molecular approaches have identified putative regions where interactions occur, it is their energetics that dictates their response. Here, denaturation studies of the C2A domain of Synaptotagmin I were carried out in conditions that are physiologically relevant to regulated exocytosis where calcium ions and phospholipids were either present or absent. Denaturation data was collected using two techniques: differential scanning calorimetry (DSC) and lifetime fluorescence. A global analysis approach combining these data sets was used where the data was simultaneously fit to models derived from thermodynamic principles. The enthalpy associated with the denaturation of the C2A domain of Synaptotagmin I in the absence of all ligands was found to be quite low when compared to other proteins of the similar molecular weight. This suggests some conformational flexibility in the interactions which hold the protein together. In addition, the denaturation behavior is shown to be different upon binding ligand, suggesting that conformational flexibility is impacted by ligand binding. This material is based in part upon work supported by the National Science Foundation under CAREER - MCB 0747339.

1418-Pos

Protrusive Growth and Periodic Contractile Motion in Surface-Adhered Vesicles Induced by Ca^{2+}-Gradients

Tatsiana Lobovkina[1], **Irep Gözen**[1], Yavuz Erkan[1], Jessica Olofsson[1], Stephen G. Weber[2], Owe Orwar[1].
[1]Chalmers University of Technology, Göteborg, Sweden, [2]University of Pittsburgh, Pittsburgh, PA, USA.

Local signaling, cell polarization, and protrusive growth are key steps in directed migration of biological cells guided by chemical gradients. Here we present a minimal system which captures several key features of cellular migration from signaling-to-motion. The model system consists of flat, negatively charged phospholipid vesicles, a negatively charged surface, and a local, and controllable point-source supply of calcium ions. In the presence of a Ca^{2+} gradient, the surface-adhered vesicles form protrusions in the direction of the gradient. We also observe membrane shape oscillations between expanded (flattened), and spherical states as a function of the Ca^{2+}-concentration. The observed phenomena can be of importance in explaining motile action in prebiotic, primitive, and biomimetic systems, as well as in development of novel soft-matter nano- and microscale mechanical devices.



1419-Pos

Deposition of Model Biomimetic Membranes on a Soft Support

Agnieszka Gorska[1,2], Malgorzata Hermanowska[2], Aleksander Balter[1], Beate Klösgen[2].
[1]Institute of Physics, Nicolaus Copernicus University, Torun, Poland,
[2]Institute of Physics and Chemistry, University of Southern Denmark, Odense, Denmark.

The lipid bilayer is the first site of all cellular interactions with the extracellular environment. The interactions between the membrane and its local surroundings are influenced by the presence of charges, within the membrane itself and as well in the near environment. The investigation of a biomimetic system requires an environment which will not modify the basic properties of the membrane to be probed. In this study a polyelectrolyte multilayer (PEM) consisting of alternating layers of chitosan and heparin (CHIT/HEP) as a soft and highly