Membrane Physical Chemistry II

1414-Pos

Spatially-Resolved Fluorescence Spectra of Patterned Lipid Bilayers

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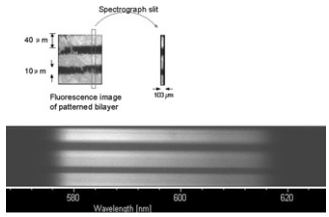
Planar supported lipid bilayers can be micropatterned such that the lipid composition of localized regions differ from that of the surrounding region. These micropatterned bilayers can serve as model systems to study the dynamics of microdomains in lipid bilayers. We have obtained spatially-resolved 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

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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

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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

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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

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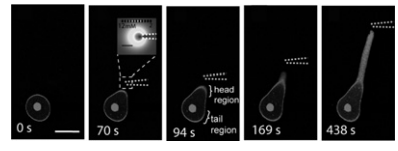
Local signaling, cell polarization, and protrusive growth are key steps in directed migration of biological cells guided by chemical gradients. Here we present a minimal system which captures several key features of cellular migration from signaling-to-motion. The model system consists of flat, negatively 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

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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

hydrated supporting cushion for membrane deposition was chosen for investigation. Polyelectrolyte multilayered films were prepared using layer-by-layer physi-sorption with either terminating positively (CHIT) or negatively (HEP) charged PE layers. Thereafter the vesicle fusion technique was applied to deposit model membranes onto the support. In this study giant unilamellar vesicles (GUV) and small unilamellar vesicles (SUV) composed of a mixture of zwitterionic POPC and its cationic derivative E-POPC were used, the latter providing a positive surface charge density in the lipid bilayer. The topology and integrity of the lipid layer on its PEM-support were investigated by a combination of confocal fluorescence microscopy and atomic force microscopy.

1420-Pos
Mechanics of POPC Bilayers in Presence of Alkali Salts
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Membranes mechanical properties are affected by solvent properties, i.e. the salt content. In this study, we use POPC GUVs (Giant Unilamellar Vesicles) as model membranes and we measure the membrane mechanical moduli by flickering analysis and micropipette technique for a series of alkali salt solutions. Salt concentration effects and ion specificity are investigated in these measurements. Membrane mechanical moduli are shown to display a complex dependence on the salt solution composition.

1421-Pos
Reproduction of Fatty Acid Vesicles
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Compartmentalization is an essential step for the origin of life to allow for the formation of more complex biotic building blocks. Possibly in early life preliminary compartments were formed out of prebiotic molecules, such as fatty acids. These molecules organize in bilayers at neutral pH and they show intriguing behavior with respect to self-reproduction of vesicles [1], in that fast addition of fatty acid at high pH to preexisting seed vesicles at neutral pH results in a fast formation of new vesicles with a size distribution that is closely related to that of the seed vesicles. The mechanism behind this so-called matrix effect is still a puzzle. One possibility would be that the fatty acids insert in the outer monolayer of the vesicle and that an excess of material in the outer leaflet with respect to the inner leaflet is then the driving force for budding and subsequent fission. In order to test this possible mechanism, we varied the rate of addition (insertion) of the new material. Slow addition would give the system time for movement of fatty acids from the outer leaflet to the inner leaflet and thus to remove this imbalance. Our results show that such a decrease of the addition rate indeed leads to growth of the vesicles instead of division, supporting our hypothesis. We also found, by including a fluorescent dye which is self-quenching at high concentrations, that during the fission process the content of the vesicles does not leak to the exterior. These observations agree well with results and predictions from coarse-grained molecular dynamics [2] and provide a plausible mechanism for the matrix effect [1].
[1] Luisi et al. J. Phys. Chem. B 2008, 112, 14655-14664 [2] Markvoort et al., in preparation

1422-Pos
Effect of Solubilization on Rhodopsin Thermal Denaturation in Rod Outer Segment Disk Membranes
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The photoreceptor, rhodopsin is a GPCR in rod outer segment disk membranes. Activation by light converts the dark-adapted form, rhodopsin to the bleached form, opsin. Differential scanning calorimetry (DSC) studies showed that rhodopsin and opsin each exhibit an irreversible scan rate dependent endothermic transition (Tm) at approximately 72°C and 55°C respectively as well as a scan rate dependent exothermic transition. Solubilization was used to examine the contribution of the bilayer. Disk membranes were subjected to sub-solubilizing and solubilizing concentrations of octylglucoside (OG) until rhodopsin was completely delipidated. DSC experiments were performed using a MicroCal VP-DSC microcalorimeter. Samples were scanned at 15, 30, 60 and 90°/hr. Because the protein transitions are irreversible, a second scan was used to determine the baseline. As the OG partitioned into the bilayer the endothermic Tm and EACT (activation energy of denaturation) rapidly decreased. Both then remained constant following rhodopsin solubilization. At low detergent concentration the exothermic Tm increased rapidly then remained constant after solubilization. Unlike the endothermic EACT, the degree of solubilization had little effect on the exothermic transition EACT. Digitonin was also used to examine the effect of solubilization. Unlike OG, this detergent binds to cholesterol which is constitutes approximately 10% of the disk lipids (average). The endothermic transition was less affected by digitonin than by OG. The EACT was also determined by thermal bleaching and was in agreement with the DSC data. These results indicate an endothermic transition is observed due to a weakening of the tertiary structure interactions as rhodopsin is heated. This may be accompanied by changes in the packing of the trans-membrane helices as well as changes in protein-lipid interactions. It is likely the exothermic transition results from aggregation.

1423-Pos
A Coarse Grained Molecular Dynamics Study of Self-Reproduction of Fatty Acid Vesicles
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The formation of self-reproducing fatty acid vesicles is believed to be a quintessential building block required for (the origin of) life. In literature a number of vesicle reproduction experiments have been reported [1]. Of particular interest is the so called matrix effect where the final size distribution of vesicles formed upon addition of new surfactants is strongly biased toward the size distribution of pre-existing vesicles. However, the mechanism behind this is poorly understood. Here, we regard this mechanism using coarse grained molecular dynamics simulations with a parameter set based on a previously published force field [2]. These simulations show fission of a vesicle on continuous addition of new fatty acid molecules to the vesicle exterior. The new molecules are incorporated in the outer leaflet of the vesicle's membrane. As the rate of fatty acid redistribution among the leaflets by means of flip-flop is lower than the rate of fatty acid uptake in the outer leaflet, an excess of molecules in the outer leaflet compared to the inner leaflet is formed. This results in spontaneous membrane curvature, causing the vesicle to deform into twin-vesicles. Eventually, the built-up spontaneous curvature induces the formation of a narrow neck, that finally breaks, resulting in full fission. Importantly, no leakage from the interior of the vesicles was observed. The reproduction pathway shown in these simulations agrees with published experimental data, as well as with new data from our group (see abstract Nicole Pfleger). Our coarse grained molecular dynamics simulations offer further insight at the molecular level of the self-reproduction pathway of fatty acid vesicles.
[1] Luisi et al. (2008) J. Phys. Chem. B 112, 14655-14664.
[2] Markvoort et al. (2005) J. Phys. Chem. B 109, 22649-22654.

1424-Pos
On the Miscibility of Cardiolipin with the Major Lipid Components of the Bacterial Inner Membrane
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The thermotropic phase behavior and organization of model membranes derived from binary mixtures of tetramyristoylcardiolipin (TMCL) with dimyristoyl phosphatidylethanolamine (DMPE) and dimyristoyl phosphatidylglycerol (DMPG) were studied by differential scanning calorimetry (DSC) and fourier transform infrared (FTIR) spectroscopy. Cardiolipin-containing DMPE and DMPG model membranes all exhibit complex multi-component hydrocarbon chain-melting (L_β/L_α) phase transitions upon heating and cooling. This suggests that TMCL is poorly miscible with both DMPE and DMPG and that the domains formed prior to the onset of the L_β/L_α phase transitions are probably retained in the liquid-crystalline state. For all mixtures, the temperatures of the L_β/L_α phase transitions are generally higher than those predicted by an ideal mixing of the components, and the enthalpies of these phase transitions seem to be better correlated with the component mol fraction of hydrocarbon chains than with the molar composition of the mixture *per se*. Finally, when cooled to low temperatures, cardiolipin-rich (i.e., $>$ 30 mol % TMCL) membranes tend to form lamellar-crystalline phases which exhibit spectroscopic characteristics comparable to those exhibited by the lamellar crystalline phases of pure cardiolipin. Together, our experimental observations indicate that cardiolipin is poorly miscible with major lipid components of one of the biological membrane systems (Escherichia coli) in which it occurs naturally. The possibility that it may also be poorly miscible in the biologically relevant liquid-crystalline phase also has interesting structural and functional implications.
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