

specialized for motion toward either the (+) or (-) end of actin filaments. Previous work has shown that directionality of recombinant myosins may be altered via the genetic insertion [1] or removal [2,3,4] of structural motifs that redirect the lever arm. We have challenged our understanding of myosin structure and function by constructing novel myosin motors that can reversibly switch their direction of motion in response to an external signal. Our general strategy relies on controlling the effective length of lever arms by triggering helix-coil transitions. In one successful design using [Ca<sup>++</sup>] as the control signal, we have built myosin VI variants with chimeric lever arms composed of an alpha-actinin fragment [5] fused to two or more calmodulin-binding IQ repeats. In vitro motility assays show that the engineered motors reverse directionality in response to physiological levels of [Ca<sup>++</sup>], as expected.

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## Platform AB: Membrane Physical Chemistry I

### 1195-Plat

#### Quantification of the Nanomechanical Stability of Multicomponent Lipid Bilayers

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Quantification of the mechanical stability of lipid bilayers is important in establishing the composition-structure-property relations, and shed light on understanding functions of biological membranes. We report a direct correlation of the self-organized structures exhibited in phase-segregated supported lipid bilayers consisting of dioleoylphosphatidylcholine/egg sphingomyelin/cholesterol (DEC) in the absence and presence of ceramide (DEC-Ceramide) with their nanomechanical properties using AFM imaging and high-resolution force mapping. Direct incorporation of ceramide into phase-segregated supported lipid bilayers formed ceramide-enriched domains, where the height topography was found to be imaging setpoint dependent. In contrast, liquid ordered domains in both DEC and DEC-Ceramide presented similar heights regardless of AFM imaging settings. Owing to its capability for simultaneous determination of the topology and interaction forces, AFM-based force mapping was used in our study to directly correlate the structures and mechanical responses of different coexisting phases. We also designed an experiment to directly probe and quantify the nanomechanical stability and rigidity of the ceramide-enriched platforms that play a distinctive role in a variety of cellular processes. Our force mapping results have demonstrated that the ceramide-enriched domains require both methyl  $\beta$ -cyclodextrin (MbCD) and chloroform treatments to weaken their highly ordered organization, suggesting a lipid packing different from typical gel states. Our results also show the expulsion of cholesterol from the sphingolipid/cholesterol-enriched domains as a result of ceramide incorporation. This work provides quantitative information on the nanomechanical stability and rigidity of coexisting phase-segregated lipid bilayers with the presence of ceramide-enriched platforms, indicating that generation of ceramide in cells drastically alters the structural organization and the mechanical property of biological membranes.

### 1196-Plat

#### Simulations of Lipid Bilayer Domain Formation: Effects of Steroid Structure and Asymmetry

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There is a growing amount of evidence that laterally segregated domains of lipids are an integral part of biological membrane structure and function. Using Coarse Grain and Atomistic Molecular Dynamics Simulations we investigate the role of steroid structure and asymmetry in domain formation. Cholesterol, an essential component of animal membranes, appears to be well suited for ordering neighboring lipid chains and promoting domain formation. We demonstrate that alterations to the steroid headgroup hydrophobicity trigger a conversion from domain promoting to domain inhibiting. Those steroids which inhibit domain formation are observed to be less stable in the typical, upright orientation, and instead insert into the bilayer hydrophobic core and reside in

an orientation perpendicular to the bilayer normal axis. A second set of simulations are used to address the role of bilayer asymmetry in domain formation. Cellular membranes are thought to be asymmetric, containing different lipid compositions on opposing leaflets, with the outer leaflet capable of domain formation and the inner leaflet uniformly disordered. These simulations suggest how domain formation is affected by an opposing uniformly disordered leaflet.

### 1197-Plat

#### Probing Structure and Dynamics of Lipid Microdomains with Tagged Proteins and Lipids: A Hybrid Particle-Continuum Simulation Approach

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Lipid rafts are functional microdomains in the cell membrane. They have been implicated in many important cellular processes such as signal transduction, protein sorting and viral entry. At this point, our understanding of the collective dynamics of lipids and lipid clusters in vivo is rather limited. To this end, by employing a hybrid particle-continuum approach, we simulate the coupled dynamics of diffusing probe particles (both proteins and lipids) and the evolving membrane composition. Importantly, we demonstrate that the structure and dynamics of lipid microdomains can be extracted from the fluctuating dynamics of the probe particles. These results suggest novel experimental ways of exploring raft dynamics.

### 1198-Plat

#### Cholesterol-Rich Fluid Membranes Solubilize Ceramide Gel Domains. Implications for the Organization of Mammalian Membranes

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A uniquely sensitive method for ceramide-domain detection allowed us to study in detail cholesterol-ceramide interactions in lipid bilayers with low (physiological) ceramide (Cer) concentrations, and ranging from low or no cholesterol (Chol) (a situation similar to intracellular membranes, such as endoplasmic reticulum) to high Chol, (similar to mammalian plasma membrane). Fluorescence spectroscopy and microscopy experiments were conducted showing that for low Chol amounts Cer segregates into gel domains that disappear upon increasing Chol levels. This was observed in raft (sphingomyelin/Chol-containing) and non-raft (sphingomyelin-absent) membranes, *i.e.* mimicking different types of cell membranes. Chol-Cer interactions have been described mainly as raft sphingomyelin-dependent. In this work, sphingomyelin independence is demonstrated. Moreover, we show that Cer-rich domains re-appear when either Chol is converted by cholesterol oxidase to cholestenone, or temperature is decreased. The inability of cholestenone-rich membranes to dissolve Cer-gel domains shows that the cholesterol ordering and packing properties are fundamental to the mixing process. Cer solubility is dependent on the average gel-fluid transition temperature of the remaining membrane lipids, and is higher in Chol-rich fluid membranes than in Chol-poor ones. We also show that the solubility of Chol in Cer domains is low. The results are rationalized by a ternary phospholipid/ ceramide/ cholesterol phase diagram, providing the framework for a better understanding of biochemical phenomena modulated by Chol-Cer interactions such as cholesterol oxidase activity, lipoprotein metabolism and lipid targeting in cancer therapy. It also suggests that the lipid compositions of different organelles are such that ceramide gel domains are not formed, unless a stress or pathological situation occurs.

Further details in Castro, BM, Silva, LC, Fedorov, A, de Almeida, RFM, and Prieto, M (2009). *J. Biol. Chem.* **284** (5), 22978-22987.

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### 1199-Plat

#### Texture of Membrane Gel Domains

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In this work<sup>1</sup> we investigate the texture of gel (g) domains in supported binary lipid membranes. Lateral organization of lipid bilayer membranes is a topic of fundamental and biological importance. Whereas questions related to the size and composition of fluid domains are well studied, the possibility of texture in condensed solid/gel domains has received limited attention. Gel domains are expected to be prominent in skin membranes and in ceramide domains