1189-Plat
Calcium Regulation of myo1b Tension Sensing
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We recently demonstrated that the widely expressed myosin-I isoform, myo1b, is exquisitely sensitive to tension (Laakso et al. 2008. Science. 321:133-6), where it transitions from a low duty-ratio to a high duty-ratio motor at very low opposing forces (< 1 pN). These forces are transmitted to the motor through the IQ-motif-containing light-chain-binding-domain (LCBD), which is structurally stabilized by calmodulin molecules. Calcium binding to these calmodulins affects the ATPase and motile properties of myo1b (Coluccio & Geeves. 1999. J. Biol. Chem. 274:21575-80). Using stopped-flow fluorescence, we confirmed that calcium accelerates the biochemical rates of phosphate and ADP release by 2 - 5 fold. We performed single molecule optical-trap experiments in the presence of 25 μM ATP and 0, 1 or 9 μM free calcium. At low forces in the presence of calcium, we found an acceleration of the actin detachment kinetics, which is consistent with stopped-flow measurements. We also found that the average displacement of the myo1b step decreases to ~ 0 nm. The decoupling of the LCBD displacement from the motor-domain kinetics prompted us to test how calcium impacts the force sensitivity of actin detachment kinetics. Using an isometric clamp, the addition of 9 μM calcium resulted in a 5-fold decrease in the distance parameter that describes force sensitivity. Finally, we measured the kinetics of calcium binding to myo1b and determined that it occurs in two steps. The first step is very fast and calcium dependent, while the second step is significantly slower and independent of calcium concentration. These results show clearly that calcium regulates the ability of myo1b to sense and sustain tension.

1190-Plat
Myosin 5A Walking Mechanism: The Structural Basis of Slow ADP Dissociatin from the Lead Head
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Using electron microscopy and image averaging, we have observed myosin 5a walking along actin filaments in the presence of low concentrations of ATP. Most molecules are attached with 13 actin subunits between heads but ~10% are bound with 11 or 15 subunit spacings. Most lead heads are in the pre-powerstroke conformation, but some post-powerstroke lead heads are observed, especially at smaller separations where there is less strain in the myosin. Post-powerstroke lead heads have the converter at the front of the motor domain with its lever bent strongly backwards. Lead heads attached at the 13 subunit spacing are 98 % in pre-powerstroke state, tethered there by the trail head. However, heads spaced by 11 subunits are more evenly distributed (60:40) pre- to post-powerstroke. No post-powerstroke lead heads are seen in heads spaced by 15 actin subunits. These results are consistent with an energy difference of 10 kJ/mole between the pre- and post-power stroke conformations at 11 and 13 actin subunit separation. The post-powerstoke lead head is a new attached state of myosin: the motor domain has completed its powerstroke at the expense of severe lever distortion, but with little cargo movement. The rate of ADP dissociation from lead heads measured by stopped-flow fluorescence is >30 fold slower than from trail heads. The slower rate can be explained by a mechanism in which ADP only dissociates from the post-powerstroke state. ADP dissociation from the lead head is therefore inhibited by an unfavorable equilibrium between the pre-and post-powerstroke conformations. Supported by NIH EB00209.

1191-Plat
Processive Runs of Full Length Myosin VA Are Interrupted by Pauses and Dwells
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Full length myosin Va (FL-MyoVa) forms an inhibited, folded conformation at low salt, stabilized by interactions between the globular tails and the heads. High ionic strength disrupts this interaction, resulting in an extended, active processive motor. In vivo, it has been postulated that cargo binding disrupts the folded conformation and activates the motor. It is possible that splice variations in the tail (-B+D+F, melanocyte; +B-D-F, brain) could modify the ability of myosin Va to form the inhibited state. Two FL-MyoVa splice variants and an HMM-MyoVa, with biotin tags for Qdot labeling, were expressed in Sf9 cells. Sedimentation velocity experiments showed similar transitions from the folded-to-extended conformation for the two splice variants as a function of salt. TIRF microscopy was then used to observe processive runs on actin. The velocities of both FL-MyoVa splice variants were similar, and increased 270% (171-460nm/sec) with increasing KCl concentration (25-200mM). In contrast, the velocity of HMM-MyoVa increased by a more modest 50% (381-586nm/sec). The trajectories of the FL-MyoVa and HMM-MyoVa were also strikingly different. Both FL-MyoVa splice variants underwent processive runs that were interrupted by periods during which the motor dwelled at fixed points on the actin filament, presumably in the folded, inhibited state. At lower KCl concentration, FL-MyoVa dwelled approximately half of the total trajectory duration. Increasing ionic strength decreased duration of the dwells. HMM-MyoVa was fully active and maintained continuous processive movement at all KCl concentrations. The slower overall velocities for the FL-MyoVa splice variants, compared to HMM-MyoVa, results from inclusion of the dwell periods. We propose that during a processive run, a single FL-MyoVa can switch between an active and inhibited state without dissociating from actin, and that this phenomenon is independent of splice variations in the tail domain.

1192-Plat
Simultaneous Observation of Tail and Head Movements of Myosin V During Processive Motion Provides Insight into Its Stepping Dynamics
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Processive stepping of myosin V (myoV) on actin has been studied either by tracking the position of the tail, which follows the motion of the molecule as a whole, or by tracking the position of one or both heads. Here we combine these two approaches, and attach a quantum dot (Qdot) to one of the motor domains, and a bead to the tail. Using optical trapping and total internal reflection microscopy, the position of one head and the tail are simultaneously observed as myoV moves processively on an actin filament against increasing load. Our results show that the head (Qdot) moves continually with 72.9 ± 10.3 nm step size, while the tail (bead) moves with a step size of 34.7 ± 8.6 nm. For every two tail steps, the head moves only one step. One of the tail steps takes place concurrently with the head step. Back steps were occasionally observed. Analysis shows that before taking a back step, the head moves 68 ± 11nm while the tail moves 31.9 ± 9.7nm, which suggests that the leading head lands on the 11th actin subunit instead of its normal 13th actin subunit. Interestingly, during a backstep the tail moves -28.6 ± 13.7nm, while the step size distribution for the head shows multiple peaks. This suggests that the head has multiple binding positions along the actin filaments, while the tail has a more defined conformation. Our observation supports a hand-over-hand model for processive movement of myoV, and reveals the cause of the back stepping behavior of myosin V under physiologically relevant loading forces (<2pN).

1193-Plat
Contribution of the Myosin VI Tail Domain to Processive Stepping and Intramolecular Tension Sensing
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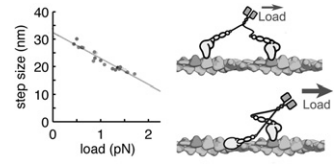
Myosin VI is proposed to act as both a molecular transporter and as a cytoskeletal anchor in vivo. The structural traits and kinetic mechanisms by which myosin VI takes processive, ~36 nm steps along actin are controversial. In particular, the portion of the molecule C-terminal to the canonical lever arm, termed the medial tail (MT), has been hypothesized to act as either a lever arm extension or as a dimerization motif. We created constructs in which the MT is interrupted by glycine-rich molecular swivels in order to test competing models of the MT's contribution to processive stepping. Disruption of the MT results in decreased processive run lengths measured using single-molecule fluorescence microscopy and a decreased step size under applied load as measured in an optical trap (see Figure). We used single-molecule gold nanoparticle tracking and optical trapping to examine the mechanism of coordination between the heads of dimeric myosin VI. We conclude that intramolecular tension prevents ADP release from the lead head. This mechanism likely increases both the motor's processivity and its ability to act as an anchor under physiological conditions.



1194-Plat
Engineering A Controllable Bidirectional Molecular Motor
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Myosin superfamily motors play crucial roles in cellular functions such as motility, cell division and organelle trafficking. Different myosin classes are

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++] 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++], as expected.

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Platform AB: Membrane Physical Chemistry I

1195-Plat
Quantification of the Nanomechanical Stablility 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 β-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 th is 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[1] 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