3763-Pos
Steered Molecular Dynamics Simulation of Unfolding of Myosin VI Proximal Tail Domain
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Myosin VI is the only member of the myosin motor protein family that moves towards the minus end of actin filaments. Upon dimerization, myosin VI is capable of processive movement with large step size. Why myosin VI can take such large step size is controversial as there are only two CaM-binding sites on the short lever arms. Based on experimental evidence, we had proposed a model in which myosin VI dimerization triggers an unfolding of the proximal tail domain, a three-helix bundle; such extension could account for the large step size. Here we test the model through molecular dynamics simulation. Steered molecular dynamics simulations proved the feasibility of the proximal tail domain unfolding; the domain was seen to readily unfold with its three helices intact; the extended conformation was found to be stable over time. The simulations, furthermore, revealed interactions between apo-CaM and the proximal tail domain that were not seen in available crystal structures, which stabilize a kink at the beginning of the proximal tail domain.

3764-Pos
Dimerization is Essential for the Large Step Size of Myosin VI
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Myosin VI is an unconventional actin-based motor protein that challenges the classical lever arm hypothesis with its minus-end directed processive movement and unusually large step size, in spite of having a short lever arm with a single IQ domain. The presence of a unique insert (Insert 2) and rearrangement in the converter subdomain can account for its reverse directionality and large powerstroke. However, these adaptations are not sufficient in explaining the 30-36nm step sizes of myosin VI which is typically characteristic of motor proteins with longer lever arms, viz. myosin V with 6 IQ domains. Though it is possible that myosin VI can function either as a monomer or a dimer in cells, based on our studies on the functional properties of the protein, it is likely that a dimeric protein will be a more efficient actin anchor and a processive transporter. We have shown that dimerization of full-length myosin VI can be triggered by cargo binding and the cargo-bound motors walk processively on actin filaments with the expected step size. Our recent studies demonstrate that the region immediately distal to the lever arm of the myosin VI motor (the proximal tail) exists as a three-helix bundle and unfolds upon dimerization of two myosin VI monomers, therefore serving as a lever arm extension in myosin VI. Additionally, our studies show that the medial tail domain is necessary for dimerization since specific mutations in this region create constitutive monomers which are non-processive with no gating properties, indicating that dimerization is necessary for the proper functioning of myosin VI.

3765-Pos
Cargo Binding Proteins Trigger Myosin VI Dimerization
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Among the myosin family, while other myosins move toward the barbed(+) end, myosin VI moves toward the pointed (-) end of the actin filament. This unique feature allows myosin VI to fulfill multiple cellular processes such as clathrin dependant endocytosis when bound to Dab2 or clathrin independant when linked to GiPC, vesicules transport to the Golgi and the perinuclear region, vesicules transition from early endosome to late endosome, AMPA receptor trafficking when bound to SAP-97.
Our working model for how myosin VI function in a cell is that the full-length myosin VI molecule primarily exists as a monomer, and folded to form intramolecular interactions involving the cargo-binding domain that block potential dimerization sites. This is consistent with small-angle X-ray scattering data obtained by Spink et al.. Binding to monomeric cargo adaptors leads to an unfolding of the monomers, exposing potential dimerization sites. The unfolded monomers can then be held in close proximity, either via tethering by the adaptor protein and/or via as yet unidentified cargo-binding domain interactions. Alternatively, binding to a dimeric cargo adaptor protein leads to simultaneous unfolding and close opposition of the cargo-binding domains. This distal tethering of two cargo-binding domains allows internal dimerization (likely via coiled coil) to occur at the proximal end of the medial tail, and may include part of the last helix of the three-helix bundle. This internal dimerization causes the three-helix bundle, formerly known as the proximal tail, to unfold, forming an extension of the myosin VI lever arm.Although it has been a controversy whether myosin VI exists as a monomer or dimer, our data and others support the model in which myosin VI exists as monomer in cell but upon binding to cargo binding proteins allows its dimerization and ultimately fulfills its function in cell.

3766-Pos
Single-Molecule and Molecular Dynamics Study of the Dimerization of Myosin VI Medial Tail Domain
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Myosin VI is an actin-based molecular motor with a large step size despite its short lever arm. We recently showed that the medial tail domain of myosin VI, likely a ~70-residue long alpha-helix, is a dimerization region when two monomers of myosin VI are brought into close proximity. This proximity mimics the effect of cargo binding onto myosin VI monomers.
The medial tail domain has peculiar characteristics. Although there are a few hydrophobic residues in the lower amino acid number region, it has a distinct motif alternating between four positively charged residues and four negatively charged residues.
Single-molecule experiments show that a mutant construct of the myosin VI medial tail domain, designed to disrupt the hydrophobic interactions of myosin VI, can still dimerize, therefore ruling out the hypothesis that hydrophobic interaction is the only dimerization mechanism of the medial tail domain. Additionally, it was observed that increasing ionic strength reduces the percentage of myosin VI dimerization. To unveil the molecular mechanisms involved in the dimerization of the medial tail domain, we employed three different molecular dynamics (MD) methodologies, namely: 1) coarse-grained MD facilitating microsecond timescale sampling; 2) the flexible fitting method enabled reversion of coarse-grained to all-atom descriptions; 3) all-atom equilibrium MD. The coarse-grained MD simulation showed the medial-tail domain dimerized, and the all-atom flexible-fitting method identified interactions between two medial-tail domain helices. We used the all-atom equilibrium MD simulation to make sure that the interactions involved in dimerization were stable over time. This set of simulations suggests that an array of salt bridges between positive and negative residues participates in the dimerization process. These results could have an important implication on myosin VI dimerization.

3767-Pos
Mouse Myosinviia is a Monomeric, "slow" Motor with an Intermediate Duty Ratio
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Myosin VIIa is an unconventional myosin which is important in visual and hearing processes. We examined the kinetic and association properties of the baculovirus expressed, truncated mouse myosin VIIa construct containing all 5IQ motifs and the SAH domain (myosinVIIa-S1-SAH). The construct is single-headed with a molecular weight of ~ 130 kDA determined by analytical ultracentrifugation experiments, and only single headed molecules were detected by atomic force microscopy. The relatively high basal steady-state rate of 0.18 ± 0.05 s^{-1} is 3-fold activated by actin with a V_{max} of 0.6 ± 0.02 s^{-1} and a K_{ATPase} of 11.5 ± 2.9 μM. The maximal rate of phosphate dissociation from actomyosinVIIA-ADP-Pi complex measured by the fluorescently labeled phosphate-binding protein could not be obtained because of the weak binding of the myosinVIIA-ADP-Pi complex to actin. A rate of $2.6s^{-1}$ was measured in the presence of 45uM actin. Double mixing stopped-flow experiments measure two rates 4.0 s^{-1} and 0.9 s^{-1} of ADP dissociation from the actomyosin-ADP complex and a rate of 2.0 s^{-1} from myosin-ADP. ATP binds to myosinVIIa with a rate constant of 3.2 $uM^{-1}s^{-1}$. ATP hydrolysis measured by quenched flow gave a rate of 10 s^{-1}, which correlated well with the maximal rate of 13 s^{-1} measured by tryptophan fluorescence. The equilibrium constant of the hydrolysis (K_H) is ~ 1. These data show that mouse myosin VIIa-S1-SAH is a "slow" monomeric, molecular motor with an intermediate duty ratio of >0.4. Therefore for myosin VIIa to reach a high effective duty ratio several

myosin molecules are brought together and localized to the actin bundles in the stereocilia participating in the lateral connections along with the interacting proteins such as vezatin and cadherin.
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3768-Pos
A Comparison of Mechanical Properties of Drosophila and Mouse Myosin 7a
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Myosin 7a is an unconventional myosin present in a range of organisms, and is essential in the function of sensory cells. In *Drosophila*, myosin 7a (D-M7a) is required to maintain bristle structure in Johnston's organ (the auditory center in *Drosophila*). Equivalently, in mice the absence of myosin 7a (M-M7a) disrupts stereocillia structure which adversely effects vestibular function. D-M7a and M-M7a share good sequence homology. Both have a 5-IQ lever-arm, followed by a single α-helix (SAH) domain, and an SH3 domain separating two MyTH4-FERM domains. Here we use the three-bead optical trap assay to compare the kinetics and mechanics of myosin 7a from insect (*Drosophila*) and vertebrate (mouse) species. We use a truncated D-M7a construct (D-M7aTD1), cropped after the SH3 domain to prevent auto-regulation. Due to difficulties with M-M7a expression, a shorter construct truncated after the SAH was used (M-M7aSAH). Data were taken at 50mM ionic strength with 10μM ATP. Step sizes of 10 and 18nm were observed for the D-M7aTD1 and M-M7aSAH, respectively. Variations in light chain binding, and geometric hindrances resulting from the shorter tail in M-M7aSAH, may contribute to this difference. The dwell time data from D-M7aTD1 were fitted well by a single exponential, giving an actin detachment rate (K_{det}) of $1.1s^{-1}$. This compares favourably with the biochemically determined ADP release step. Interpretation of the dwell time data from M-M7aSAH is less straight forward. The data is poorly fit by a single exponential. A double exponential gives fast ($9.5s^{-1}$) and slow ($0.8s^{-1}$) K_{det} rates. Comparison to biochemical data suggests the fast rate is related to ADP release, whereas the slow rate may represent ATPase cycling. We conclude *Drosophila* and mouse myosin 7a exhibit generally similar mechanical properties, though appear differently tuned, perhaps for their species specific function.

3769-Pos
Kinetic Analysis Reveals Differences in the Binding Mechanism of Calmodulin and Calmodulin-Like Protein to the 3 IQ Motifs in Myosin-10
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Myosin-10 (Myo10) is an unconventional myosin associated with dynamic actin remodeling implicated in a multitude of cellular functions. Like most unconventional myosins, Myo10 binds calmodulin (CaM) as the principal light chain. In epithelial cells Myo10 also binds calmodulin-like protein (CLP) as a tissue-specific light chain, resulting in increased Myo10 levels and Myo10-dependent cell motility. This raises questions as to how CaM and CLP compete for the individual IQ sites on Myo10. Indeed, there is little information on the kinetics of light chain binding to any unconventional myosin. Moreover, how Ca^{2+} affects the binding of CaM and CLP to the IQ motifs in Myo10 is unknown. We performed equilibrium and fast-kinetic experiments to elucidate the mechanism of binding of both CaM and CLP to each of the three IQ motifs in Myo10. Our results show that while CaM and CLP bind with moderate affinity to the isolated IQ2 domain in the absence of Ca^{2+}, both light chains display dramatically increased affinity for each of the three IQ domains in the presence of Ca^{2+}. The studies further indicate different binding mechanisms for CLP and CaM to IQ3, suggesting structural differences between the CaM-IQ3 and CLP-IQ3 complexes and supporting differential effects of the two light chains on Myo10 regulation and stability.

3770-Pos
The Molecular Adaptations of Myosin X for Bundled Actin Filament Tracks
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To properly self-organize, cells must be able to direct cargo to specific locations. Although many molecular motors are known to drive cargo transport, the address system that these motors use to move to the proper destination is poorly understood. Recently, we showed that myosin X has a preference for bundled actin filaments. This preference for bundles allows myosin X to identify filopodia, a limited population of actin filaments within the cell. To clarify the bundle selection mechanism, we performed single-molecule mechanical measurements to determine the stepping pattern on bundles. Our observed ~18 nm stepsize is consistent with a filament-straddling mechanism, where each head of myosin X binds to a unique filament in the bundle. A dissection of the domains required for bundle selection reveals a surprising role for the myosin X tail, a region which is likely far from the bundle itself. We find that targeted insertion of a glycine-rich flexible linker within the tail abolishes bundle selectivity, suggesting that the tail adopts a rigid structure that is essential for identifying bundles.

3771-Pos
A Structured Domain is Responsible for Bundle Selection of Myosin X
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Cells organize their contents and regulate cell shape and mechanics through molecular motors functioning on cytoskeletal filaments. Presented with many apparently similar tracks within the cortex, how myosins identify the few actin filaments that lead to their correct cellular destinations is largely unknown. Myosin X, an actin-based motor that concentrates at the distal tips of filopodia, selects the fascin-actin bundle at the filopodial core for motility. While poorly processive on single actin filaments, it takes processive runs on actin bundled by fascin. Such a bundle is the precise structure to which myosin X localizes in vivo. Using single molecule optical trapping experiments we determined the step size to be 17 nm, half of the 36 nm pseudo-helical actin repeat essential for motors to be processive on single actin filaments. This suggested that straddling two filaments within a bundle stimulates this motor's processivity. Using combinatorial chimeric constructs of myosin V and myosin X, we show that the post-IQ region, not the short lever arm (three IQ repeats) or the motor domain, is the main contributor to myosin X's selectivity. This region contains two structures of interest: a charged single alpha-helix (SAH), which may impart unique mechanical or affinity properties to the motor, and a coiled-coil dimerization motif. The structural character of this region was perturbed by insertion of free swivels either before or after the SAH domain. The post-SAH swivel mutant showed no preference for bundled actin for motility, thus providing support to a selectivity model where the search-space of the forward head for the next binding site is constrained to neighboring filaments in a bundle. This result provides insight into the ability of nature to fine-tune myosin motors to serve their specific functions in the cell.

3772-Pos
A Receptor Mediated Delivery System for Single Molecule Imaging in Live Cells
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The high complexity of the eukaryotic cytoskeleton arises from many proteins displaying multiple functions. Live, intact cells are an indispensable system for investigating motility of molecular motors, which depend on their intracellular environments. Here we present a novel system of delivering myosin motors into the cell to investigate their motility by single molecule imaging on the native cytoskeleton. This technology utilizes receptor mediated delivery (RMD) of fluorescently labeled motors. A conjugate of the desired myosin motor and substance P (SP) is internalized upon interaction with the neurokinin-1 receptor (NK-1), allowing the myosin motors access the cell without compromising the integrity of the membrane. RMD has no harmful impact on the cell, leaving the cell membrane intact and the sensitive structures preserved.
We previously demonstrated that myosin X selects the fascin-actin bundle at the filopodial core for motility. Here we show that this motor is successfully delivered with RMD, undergoes endosomal escape and finds its way to its native working environment simply by its functional preference for these unique structures. This is significant as it not only confirms that myosin X recognizes the local structural arrangement of filaments, but also further indicates that the details of cellular actin organization do impact the activity of unconventional myosins. Information on myosin motor motility obtained by RMD allows for the construction of a road map of the actin structures and enables a comparison between various cell types. These paths reveal both the spatial arrangement of the actin filaments (reflecting the complexity and density of cytoskeletal meshwork) as well as the individual motility preferences of myosin motors across cell types.

3773-Pos
The Dynamics for Myosin-X Induced Filopodia Protrusion
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Filopodia are actin-rich finger-like cytoplasmic projections extending from the leading edge of cells. Unconventional myosin-X is involved in the protrusion of