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