1 ms. This improved time resolution reveals three populations of acto-myosin attachments with lifetimes that differ by more than 10-fold, likely representing three different biochemical and/or mechanical states. The slowest of these rates is ATP dependent at low MgATP concentrations and corresponds well with the expected rate of ATP-induced dissociation. The populations with shorter lifetimes likely represent dissociation from actomyosin states that precede ATP-induced dissociation. The force dependence of these lifetimes and the relative amplitude of each phase reveal information about mechano-chemical transitions in cardiac myosin that have not previously been probed in the single molecule regime. In addition, ensemble averages of individual attachment events reveal the amplitude and timing of both sub-steps of β-cardiac myosin's power stroke. Through these ensemble averages, we can separately resolve the pre-powerstroke/actin-bound state and the post-powerstroke state, representing the first single-molecule observation of the initial actin displacement by cardiac myosin under load. Supported by NIH grant P015GM087253.

1510-Pos Board B461

Converter Mutation Disrupts Lever arm Rotation in Myosin V

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Myosins are proposed to utilize a conserved structural mechanism to generate force in which small conformational changes in the active site result in a large swing of the lever arm or light chain binding region. The converter domain is a flexible region that provides a link between the catalytic domain and lever arm and is proposed to play a critical role in the allosteric communication between these two domains. We introduced the R712G mutation in the converter domain and examined the impact of this mutation on the structural and functional properties of myosin V. The mutation resulted in a 16% reduction in the maximum actin-activated ATPase rate and no change in the actin concentration at which the ATPase activity is one-half maximal (*K*ATPase). The sliding velocities examined in the in vitro motility assay were very similar between WT and R712G MV. We have developed a novel FRET system in myosin V (MV) that allows examination of the dynamics of lever arm motion. We labeled MV 11IQ containing an N-terminal (NT) tetracysteine motif with the bisarsenical dye FlAsH (MV.NT.FlAsH). The first IQ motif of MV.NT.FlAsH was exchanged with QSY-9 labeled CaM, a non-fluorescent acceptor. We followed the motion of the lever-arm during the ATP binding (recovery stroke) and actin-activated product release (power stroke) steps using stopped-flow FRET. The R712G mutation reduced the rate of the recovery stroke by 23% while having little impact on the fast power stroke that occurs prior to phosphate release. Thus, a mutation in the converter domain can specifically impact the recovery stroke without altering the power stroke demonstrating different allosteric mechanisms are responsible for these two key structural transitions.

1511-Pos Board B462

Myosin Steps Symmetrically along Actin

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Myosin 5a is a homodimer belonging to the family of processive molecular motors that transform chemical energy into directional motion along the cytoskeleton network. Despite a well understood kinetic cycle, the type of hand-over-hand motion describing each step remains unknown due to the intrinsic short lifetime of the unbound head state. Here, we differentially label the two motor domains of myosin 5a, one with a fluorescent quantum dot and the other with a small gold nanoparticle and simultaneously trace the three dimensional motion of each domain by combining single molecule fluorescence with interferometric scattering microscopy (iSCAT).
Within individual trajectories, we observed an intermediate state for each labelled unbound head that is located to one and the same side of the actin filament from which myosin repeatedly sampled the desired actin site until strong binding occurred. Analysis over several hundred molecules showed no preference in the relative position of the transient state with respect to actin. The observation that the transient state orientation can change for the same molecule by binding to another actin filament shows that the directionality of the transient is not an intrinsic property of the motor. Instead the decision is made upon binding to actin, with both cases equally probable. Our work provides critical insight on the highly efficient mechano-chemical cycle of myosin 5a and provides strong evidence for a symmetric hand-over-hand model in which the trailing head swings by the leading head on the same side repeatedly.

1512-Pos Board B463

Myosin-5 and Myosin-6 Differentially Detect Actin Filament Age

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Unlike a static and immobile skeleton, the actin cytoskeleton is a highly dynamic network of filamentous actin (F-actin) polymers that continuously turn over. In addition to generating mechanical forces and sensing mechanical deformation, dynamic F-actin networks serve as cellular tracks for myosin motor traffic. Unfortunately, much of our understanding of processive myosins comes from in vitro studies where motility was studied on pre-assembled and artificially stabilized, static F-actin tracks. In this work, we examine the role of actin dynamics in single-molecule myosin motility using assembling F-actin. We chose the two highly processive motors, myosin-5 and myosin-6. On dynamic F-actin, the barbed-end directed myosin-5 is 1.5-fold more processive, whereas its pointed-end directed counterpart myosin-6 is 1.7-fold less processive (both relative to static F-actin). Moreover, while myosin-5 takes longer runs on ADP/Pi-rich (young) portions of the growing filament, myosin-6 takes longer runs along ADP-rich (old) F-actin. These results suggest that actin changes conformation upon Pi release, and that these two myosins respond to this change in opposite ways. Taken together, these experiments define a new mechanism of how myosin traffic may sort on different F-actin networks.

1513-Pos Board B464

Adapter Proteins Activate Myosin-Va during Cargo Transport

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Myosin-Va (myoVa), one of the best characterized actin-based molecular motors, transports a variety of intracellular cargos. In order to bind a specific cargo, myoVa forms a tri-partite complex with a Rab effector protein (i.e. adapter) and a Rab GTPase protein (e.g. Rab27a) that is inserted in the granule membrane. MyoVa delivers insulin granules to the plasma membrane in pancreatic beta-cells. Interestingly, there are four known adapter proteins expressed in beta-cells, i.e. Granuphilin-A/B, Rabphilin and MyRIP, all of which bind myoVa. The role of these adapter proteins in cargo transport is poorly understood.
Using TIRF microscopy, we measured the speed, run-length and stepping behavior of myoVa in presence of Qdot-labeled adapter proteins. At 25 mM KCl, the adapter proteins do not show appreciable activation of the inhibited myoVa motor. However, at physiological salt concentration, the adapter proteins significantly increase the run-length and the run-frequency of myoVa on actin filaments. Specifically, in the presence of Granuphilin A, the myoVa run-frequency increases ~6-fold, with an ~3.5-fold run-length enhancement as the motor steps (72nm) normally, but at half the speed. By labeling Granuphilin-A and MyRIP with a Qdot, we observed binding of these adapters directly to actin filaments, suggesting that they enhance the motor's run-length and slow speed by a tethering mechanism, similar to Melanophilin (Sckolnick, et al., 2013). In contrast, Granuphilin-B and Rabphilin have little binding affinity for actin. Nonetheless, they bind to and activate myoVa, because the full-length myoVa step size becomes regular like the constitutively active, truncated myoVa-HMM. A common feature of these adapter proteins is that they ensure that the motor remains active while attached to the cargo. However, only some adapter proteins have actin-tethering capacity, which may enhance the long-range vesicle transport. These functional differences may play synergistic roles in the cell.

1514-Pos Board B465

Activation of Drosophila Melanogaster Myosin-5 Motor Function by Calcium and Cargo-Binding Protein

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In *Drosophila melanogaster* compound eye, myosin-5 (DmM5) plays two distinct roles in response to light stimulation: to transport pigment granules from cytosol to the rhabdomere base to decrease light exposure and to transport rhodopsin-bearing vesicles to the rhabdomere base to compensate for the rhodopsin loss during light exposure. The association of DmM5 with pigment granule and rhodopsin-bearing vesicle are mediated by two cargo-binding proteins, i.e., by dRab11 and Ltd, respectively. However, little is known how these two cargo-binding proteins affect the motor function of DmM5. Here we succeeded in expressing the recombinant DmM5 and studied its regulation by calcium and cargo-binding proteins. The actin-activated ATPase activity of DmM5 is significantly lower than that of the truncated DmM5 without the C-terminal globular tail domain (GTD), indicating that the GTD is the