2880-Pos Board B650
Actin Straightens the Myosin Relay Helix during the Powerstroke
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We have used transient time-resolved FRET, $(TR)^2$FRET, to resolve the structural kinetics of the myosin relay helix during the actin-activated power stroke. The relay helix plays a critical role in force generation in all myosin isoforms coupling structural changes in the ATPase site with rotation of the light chain binding domain and force generation. Previous work demonstrated that the relay helix of the myosin II motor domain from *Dictyostelium Discoideum* exists in dynamic equilibrium between a bent pre-powerstroke state stabilized by ATP, and a straight post-powerstroke state which predominates in the presence of ADP or when the nucleotide binding site is empty. We predict that actin binding to the myosin.ADP.Pi complex will reverse helix bending. This transition should parallel the myosin powerstroke. To test this hypothesis, a Cys-lite *Dictyostelium* myosin motor domain was labeled at two introduced Cys residues shown to detect relay helix bending using DEER, TR-FRET and $(TR)^2$FRET spectroscopy. We preformed a $(TR)^2$FRET experiment measuring the structural distribution of the relay helix every millisecond after rapid mixing of myosin.ADP/Pi with actin. This experiment showed that actin binding straightens the bent helix. We characterized the actin dependence of helix straightening and then correlated this dependence with the kinetics of actin binding and phosphate dissociation from the myosin active site. Numerical simulations were used to model a system containing pre and post-powerstroke structural states, actin binding, hydrolysis and phosphate dissociation. We globally fit this model to the $(TR)^2$FRET, actin binding, and phosphate dissociation transients. From this analysis, we are able to determine the timing of helix straightening with respect to actin binding and the critical step of phosphate dissociation which acts as the thermodynamic driving force for myosin's force generating powerstroke.

2881-Pos Board B651
Differential Impact of Temperature and Magnesium on Myosin V and Myosin II
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We examined the impact of temperature and free magnesium concentration on monomeric FlAsH labeled myosin V (MV FlAsH), dimeric myosin V (MV HMM), and dimeric fast skeletal muscle myosin II (SK HMM) using ATPase and motility assays. Our results indicate that MV HMM and SK HMM both have a linear dependence on temperature that is similar in both ATPase and motility assays. However, MV FlAsH contains a different temperature dependence in ATPase and motility assays suggesting its short lever arm may impart a high strain dependence in the motility assay. MV HMM and MV FlAsH are inhibited by high concentrations of magnesium in both ATPase and motility assays. The rate-limiting step in myosin V is known to be ADP release, which we demonstrate correlates well with the magnesium and temperature dependence of ATPase and motility assays. Interestingly, SK HMM exhibits magnesium inhibition in ATPase assays, but only a slight decrease is observed in the motility assay. In SK HMM the rate-limiting step in ATPase assays is thought to be attachment to actin or phosphate release, while in motility assays it is controversial. Our results indicate that SK HMM is better described by an attachment limited model in the motility assay. Magnesium may reduce the duty ratio of SK HMM which alters ATPase activity but not velocity in the motility assay. Future experiments will determine if magnesium alters the actin binding and/or the product release steps in myosin V and skeletal muscle myosin. Myosin V contains a tyrosine (residue 439) in the switch II region, which is an alanine at the corresponding position in myosin II, suggesting this residue may play a key role in differentially altering magnesium coordination in the active site of myosins.

2882-Pos Board B652
Magnesium Regulates Myosin V Motor Activity by Altering Key Conformational Changes in the Nucleotide Binding Pocket
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We investigated how magnesium impacts key conformational changes in the nucleotide binding pocket of myosin V and how these alterations impact the mechanochemical cycle. The conformation of the nucleotide binding pocket was examined using our established FRET system in which myosin V labeled with FlAsH in the upper 50 kDa domain participates in energy transfer with mant labeled nucleotides. Our previous work demonstrated the rate limiting conformation change in the actomyosin V ATPase cycle is opening of the nucleotide binding pocket which precedes ADP release from the open state. We examined the maximum actin-activated ATPase activity of MV FlAsH at a range of free magnesium concentrations (0-10 mM) and find that the highest activity occurs at 0.5 mM magnesium, while there is a 50-60% reduction in activity above 4 mM magnesium. We also demonstrate that the motor activity assayed by in vitro motility is similarly dependent on magnesium concentrations. Transient kinetic studies of mantADP binding/release with actomyosin V FlAsH demonstrate the equilibrium between the open and closed nucleotide binding pocket conformations is dependent on magnesium with the closed state stabilized by magnesium. We find that the kinetics of the nucleotide binding pocket opening step correlates well with the ATPase and motility results over a wide range of magnesium concentrations. In the absence of magnesium (presence of 4 mM EDTA) the nucleotide binding pocket populates a single conformation that is dramatically open at higher temperatures. In addition, magnesium significantly slows the rate of ADP release from the open state. Our results shed light on the structural mechanism of ADP release in myosin V and allow us to speculate about the conserved conformational pathways involved in strain sensitive ADP release in myosins.

2883-Pos Board B653
The Substep of Myosin Va is Associated with an Increase in Stiffness of the Myosin Va Head Domain
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Vertebrate myosin Va transports cargo by processive movement that is optimized by coordination of the two heads via intramolecular strain (Veigel et al Nat Cell Biol 2002). Such coordination requires substantial stiffness of the head domains. Otherwise one head cannot ''sense'' changes in the other head. Substantial stiffness, however, generates substantial restoring forces impeding diffusive movement of the free head to its next binding site about 10nm beyond the throw generated by the stroke of the myosin Va. Such conflicting constraints raise the question whether stiffness of the myosin Va head domain may change during the ATPase cycle.
We measured myosin Va head stiffness with a modified three-bead trapping assay. One bead of the dumbbell was attached end-on to the actin filament to reduce compliance of this link. To determine myosin Va stiffness we applied ramp-shaped movements to the stage and followed the position of the end-on attached bead. When a myosin Va head bound to the dumbbell, the dumbbell started to follow the stage movement, however, with reduced amplitude. The reduced amplitude we attributed to elastic distortion of the myosin Va head, elastic extension of the actin-bead link, and compliance between the myosin Va molecule and the nitrocellulose coated third bead. Compliances of the actin-bead links were determined according to Smith et al. (Biophys J 2001) and by force-extension curves.
Coinciding with the substep in myosin Va binding events bead movement suddenly followed the imposed stage movement more closely than before the substep, indicating suddenly reduced elastic extension of myosin Va. Quantitative analysis suggests a stiffness of the chicken myosin Va head domain of about 0.2pN/nm before the substep and a 2.5 to 3-fold increase in stiffness with the substep.

2884-Pos Board B654
Single Molecule Measurement of the Myosin V Energy Transduction Process
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Myosin V is an ATPase molecular motor that takes 36 nm processive steps along actin filaments in a hand-over-hand fashion. Experiments using gold nano particles have revealed that this movement consists of two processes: a deterministic lever arm swing and diffusional Brownian search. Although both are thought to contribute to the ATP chemo-mechanical energy transduction, details of their contributions remain poorly understood.
Here, we constructed a new optical tweezers system that incorporates a DNA linker to the myosin V to investigate this matter. The optical tweezers exert an external force onto one of the motor domains via the DNA linker, allowing us to observe conformational changes by the lever arm during gait motion. Furthermore, by analyzing the load dependency of the conformational change, we