633-Pos Board B433
Kinetics and Thermodynamics of the Rate Limiting Conformational Change in the Myosin V Mechanochemical Cycle
Donald J. Jacobs, Darshan Trivedi, Charles C. David,
Christopher M. Yengo.
We have used FRET to examine the kinetics and thermodynamics of the structural changes associated with ADP release in myosin V, which is thought to be a strain sensitive step in many muscle and non-muscle myosins. We also use essential dynamics using FIRST/FRODA starting with three different myosin V X-ray crystal structures to examine the intrinsic flexibility and correlated motions. Our kinetic and steady-state FRET results demonstrate that the nucleotide binding pocket goes from a closed to an open conformation prior to the release of ADP while the actin binding cleft remains closed. Thermodynamic analysis of ADP binding to actomyosin V suggests the collision complex formation is driven by a large enthalpy change and a small change in entropy. The transition from the open to closed pocket actomyosin.ADP state is associated with a large unfavorable decrease in entropy, which suggests the closed pocket conformation is more rigid than the open pocket conformation. Although no crystal structure is available of the closed pocket myosin V.ADP state, our FRET analysis reveals that this conformation may be similar to the myosin V.ATP state. FIRST/FRODA analysis is consistent with these conclusions as the myosin V.ADP structure is more flexible than the Apo structure, while the myosin V.ATP structure is more rigid than myosin V.ADP. Principal component analysis demonstrates that opening and closing of the nucleotide binding pocket correlates with the motions of loop 1 and the transducer region in all three crystal structures. Interestingly, we find that the temperature dependence of the maximum actin-activated myosin V ATPase rate correlates with the pocket opening step, suggesting this is the rate limiting step in the ATPase cycle. Our results provide insight into the structural mechanism of strain-dependent ADP release in myosins.

634-Pos Board B434
The Switch II Region is Critical for the Formation of the Open Cleft Weak Binding Conformation in Myosin V
Darshan V. Trivedi, Charles David, Jörg Rösgen, Donald J. Jacobs,
Christopher M. Yengo.
The impact of two switch II mutations, G440A and E442A, on the conformation of the nucleotide binding pocket and actin binding cleft were examined with temperature dependent FRET analysis of FlAsH labeled myosin V (MV FlAsH). E442A MV FlAsH, which abrogates the salt bridge between switch I and switch II, remains in a closed nucleotide binding pocket state at all temperatures between 4 and 35°C in the presence of ATP indicating a highly stable closed pocket similar to wild-type MV FlAsH. The G440A MV mutant prevents the formation of a highly conserved hydrogen bond to the gamma-phosphate of ATP, and is able to form a closed pocket conformation at 25°C similar to E442A and WT MV. In the presence of ATP,G440A MV FlAsH populates a closed cleft conformation while E442A MV FlAsH forms an open cleft conformation. Our results suggest the switch II region is not critical for formation of the closed nucleotide binding pocket conformation while it is critical for communicating the conformational changes from the nucleotide binding region to the actin binding cleft. These results are supported by essential dynamic analyses using FIRST/FRODA applied to the myosin V crystal structures. To compare our FRET analysis to the thermal unfolding profile of myosin V we examined alpha-helical content by circular dichroism (CD) spectrometry as a function of temperature. We observed a broad transition at lower temperatures and a steep transition at higher temperatures in WT MV FlAsH. Comparing our FRET results with CD will allow us to determine if the conformational changes are associated with changes in secondary structure. Our results are interpreted in the context of identifying communication pathways essential to the energy transduction pathway of myosin motors.

635-Pos Board B435
The HCM Loop Plays a Role in Actin-Activated Product Release in Myosin V
William C. Unrath, Pallavi Penumetcha, Darshan Trivedi,
Christopher M. Yengo.
We examined the functional role of the upper 50 kDa hypertrophic cardiomyopathy (HCM) loop in myosin V. Hypertrophic cardiomyopathy is caused by missense mutations in highly conserved regions of myo2β and one deadly mutation occurs in the HCM loop (R403Q). Since the R403Q mutation has been shown to enhance or decrease the ATPase activity and *in vitro* motility of myosin II, it may be expected that the HCM loop plays a role in actin-activated product release. In our previous work we correlated the conformational change associated with ADP release and maximum ATPase rate in myosin V using FRET analysis. We engineered the R403Q mutation at an analogous site in myosin V 1IQ (R378Q) so that we would be able to investigate the impact of the mutation on actin-activated product release, maximum ATPase rate, *in vitro* motility, and FRET in myosin V. The R378Q mutation reduces the maximum ATPase rate two-fold while it slightly enhances sliding velocity compared to wild-type MV 1IQ. Our results suggest the duty ratio may be reduced as a result of the R378Q mutation. We will directly examine both ADP-release and phosphate-release to evaluate this possibility. To examine the impact of the point mutation on structural dynamics we will determine if conformational changes in the nucleotide-binding pocket and actin-binding cleft are disrupted using our established FRET probes. Our studies further establish a strategy for examining the mechanism of product release in myosin using the three assays: FRET, ATPase, and motility.

636-Pos Board B436
Visualizing and Clarifying the Mechanical Properties of the Myosin V Brownian Search with Optical Tweezers
Keisuke Fujita, Mitsuhiro Iwaki, Atsuko Iwane, Toshio Yanagida.
Myosin V moves processively along actin filaments by taking 36-nm steps in a hand-over-hand manner. These steps involve the rear head quickly releasing from the actin filament and then taking a 72-nm forward movement before rebinding. The 72-nm movement consists of two processes: a conformational change, called the ''lever arm swing'', and a diffusional process of the head, called the ''Brownian search''. The Brownian search is a short-lived state, only recently observed by gold nano particle imaging with high time resolution. It is thought that the Brownian search is important for the released head to find its next actin binding site and for the high energy efficiency of force generation. However, little is known about its mechanical properties. In particular, there are only limited reports describing the interaction between the head and actin during the Brownian search.
Here, we constructed a new optical tweezers system that incorporates a DNA linker to the myosin V based on a previous report (Guydosh et al, Nature 2009). In this experimental setup, the optical tweezers apply an external force to the Brownian search via the DNA linker. By using this measurement system, we succeeded to observe the Brownian search under a controlled load, finding that its behavior changes according to load. These results suggest that our unique system is capable of clarifying the mechanical properties of the Brownian search. At the meeting, we will discuss these mechanical properties and their implications on the acto-myosin V interaction.

637-Pos Board B437
Stepping Mechanism of Myosin V: Insights from the Mutations in the Converter
Sergey V. Mikhailenko, Takashi Ohki, Jun-ichi Yoshimoto, Hiroaki Kubota,
Yusuke Oguchi, Shin'ichi Ishiwata.
Dimeric molecular motor myosin V transports cargoes towards the barbed end of actin filaments in cells, coordinating ATPase cycles in its two head domains to ensure the efficient unidirectional motility. Directional loads modulate the kinetics of nucleotide binding to myosin V, suggesting that the head-head communication may be achieved via intramolecular load, generated when both heads are bound to actin. Here, using point mutations in the converter domain, we directly tested the effect of the intramolecular load on the processive stepping of myosin V. The converter is a compact structure, which transmits tiny conformational changes, induced at the nucleotide-binding site in the process of ATP hydrolysis, to the lever arm. To disturb the transmission mechanism, we replaced with alanines, one at a time, two phenylalanine residues that form a hydrophobic cluster with the C-terminus of the relay helix. We found that the F749A mutation, which is inferred to reduce intramolecular load but does not affect the nucleotide binding or actin affinity, significantly increases the proportion of backward steps, providing strong experimental evidence that the efficient unidirectional processive stepping of myosin V and, possibly, other dimeric processive motors, is ensured by the head-head communication based on the intramolecular load, which coordinates ATPase cycles in two motor domains.

638-Pos Board B438
Inchworm-Like Stepping of Full Length Processive MyoVa
Jessica M. Armstrong, Shane R. Nelson, Elena B. Krementsova,
Kathleen M. Trybus, David M. Warshaw.
Full length MyoVa (FL-MyoVa) adopts a folded, inhibited conformation at low salt, stabilized by electrostatic head-tail interactions, and a fully extended, active conformation at high salt. TIRF microscopy was used to determine the effects of this equilibrium on processivity (i.e. velocity, run length, and stepping dynamics) of single Quantum dot (Qdot)-labeled FL-MyoVa motors.