motors that either speed up, slow down, or switch directions in response to illumination. These genetically encoded motors should be directly deployable inside living cells, and may also be useful for controlling directed transport outside of cellular contexts.

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**2890-Pos Board B660**

**Stepping of Myosin V with Point Mutations in the Converter**

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Myosin V is a dimeric molecular motor, which transports organelles toward the barbed end of actin filaments in cells. Its highly efficient unidirectional motility requires the coordination of ATPase cycles in two head domains, to ensure that the rate-limiting ADP release almost exclusively occurs in the trailing head and the motor steps forward. Single-molecule measurements revealed that the directional loads modulate the kinetics of nucleotide binding to myosin V, suggesting that the head-head communication may be based on intramolecular load, generated when both heads are bound to actin. Here we directly tested the effect of the intramolecular load on the processive stepping of myosin V, using point mutations in the converter domain, which are inferred to reduce intramolecular load but do not affect the nucleotide binding or actin affinity. 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. The effects of the mutations on the myosin's V motility were tested by multiple kinetic and single-molecule assays. We found that the F749A mutation significantly increases the proportion of backward steps, whereas the F697A mutation completely abolishes the processive stepping of myosin V. These results provide strong experimental evidence that the efficient unidirectional processive stepping of myosin V is ensured by the head-head communication based on the intramolecular load, which coordinates ATPase cycles in two motor domains.

**2891-Pos Board B661**

**Conservation Analysis of Myosin Families**

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Myosins are involved in many cellular tasks, including organelle trafficking, cytokinesis, maintenance of cell shape, and muscle contraction. Different myosins perform different tasks with distinct mechanical and chemical characteristics. Although their core structures are similar, their subtle differences in sequence can cause drastic differences in these characteristics. This study used sequence analysis to identify conserved and unique structures of several myosin families including myosin II, myosin V, and myosin VI. Using myosin VI as an example, we identified several myosin VI-specific residues where all myosin VI proteins have an identical amino acid but no other myosin has the same amino acid in the same aligned column. P444 may contributes to the weaker binding to actin in myosin VI comparing to myosin II and V. M701 is located at the tip of SH1 helix which is the linkage between motor domain and converter. F763 is located in the converter which strongly interacts with the motor domain to position the converter. This method can also be applied to other myosin families to gain mechanistic understanding of myosin functions.

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**The Adjacent Binding State Enables Myosin VI Dual Function**

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Myosin VI is an ATP driven molecular motor that functions as both a vesicle transporter and a cytoskeletal anchor. Recently, we reported that myosin VI generates three types of steps by taking either a distant binding or adjacent binding state. The adjacent binding state is unique to myosin VI and therefore may help explain some of myosin VI's distinct features including its dual functions. To better understand this state, we performed simultaneous observations of the head and tail domains and of the two head domains during motility. We found that the lever arms tilt forward in the adjacent binding state and that the nucleotide binding affinities are similar to that of the rear head domain in the distant binding state. From these results, we propose a model for how the adjacent binding state leads to myosin VI dual function.

**2893-Pos Board B663**

**Myosin VI Comes Together - Structure and Motor Regulation by Binding Partners**

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Myosin VI is an unconventional myosin motor that moves towards the minus end of actin filaments. The motor is known to interact with various binding partners and function as an anchor and a vesicle carrier in many diverse functions, including endocytosis and exocytosis. The multiple functions are likely to be regulated through the binding partners. The functional units of the motor (monomers/dimers) and the effects of binding partners remain unclear.

Here we present a quantitative FRET based assay to measure the association of binding partners and determine the effect upon the oligomeric state of myosin VI. We have shown that myosin VI tail forms relatively tight interactions with the binding partners NDP52 (Kd 1 μM) and Dab2 (Kd 5 μM). We also found that these binding partners oligomerize myosin VI, increasing the affinity of oligomerization from >80 μM to 4 μM. We purpose this is achieved by the binding partners relieving auto-inhibition of the cargo-binding domain. Furthermore, we have characterized the interactions of binding partners with full length myosin VI using size-exclusion chromatography and sucrose density gradients. Single molecule photobleaching assays have been performed to determine the stoichiometry of the complexes. Functional measurements have been carried out using ATPase and single molecule motility assays in the presence of the binding partners to determine effects upon the motor activity.

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**Examination of the Kinetics of Myosin VI during Endocytosis**

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The molecular motor myosin VI has been implicated in endocytosis, a trafficking pathway mediating intracellular uptake of items such as membrane receptors and nutrients. Previous studies demonstrate the localization of the large insert isoform of myosin VI to clathrin-coated vesicles and the no insert isoform of myosin VI to early endosomes. The kinetics of myosin VI functionality on these endocytic structures has not yet been examined, however. This study uses fluorescence recovery after photobleaching (FRAP) to examine the turnover kinetics of the no insert and large insert isoforms of myosin VI during endocytosis. The results demonstrate that myosin VI turns over dynamically on endocytic structures and that different isoforms on different intracellular compartments have distinct turnover rates. The FRAP assay system is then implemented with a novel live cell expression of an artificial dimer of myosin VI to demonstrate the dimeric functionality of myosin VI on endocytic structures in vivo. Further studies of the turnover rates of the myosin VI binding partner Dab2 on clathrin-coated structures demonstrate that Dab2 turns over more rapidly than full length myosin VI, a novel indication of the relative turnover rates of a motor protein versus its binding partner on a given cellular compartment. The turnover kinetics of specific myosin VI motor domain mutants on endocytic structures are also examined, e.g. the D179Y mutant implicated in progressive hearing loss, a study which offers insight into the functional source of myosin VI-related deafness. In addition to providing insight into the endocytic functionality of myosin VI, these FRAP studies offer general insight into the dynamics of myosin motor proteins on intracellular structures, the functional differences between isoforms of given myosin proteins, and the comparative turnover rates of myosin proteins and their binding partners.

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**The Shaker-1 Mouse Myosin VIIa has a Drastically Compromised ATP Hydrolysis Mechanism**

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The shaker-1 mice carry a missense mutation in the myosin VIIa gene (R502P) and exhibit deafness, circling behavior and mild retinal degeneration.