actin filament sliding assay and a dual-beam, optical tweezers apparatus to perform single molecule mechanical studies. For these studies we used a two-headed, heavy meromyosin-like (HMM) construct of myosin-10 that contained a leucine-zipper at the C-terminal end to force dimerization. The actin filament gliding assay showed that myosin-10-HMM moves filaments at a velocity of ~125 nm $s^{-1}$. This is similar to the speed of intact, GFP-tagged, myosin-10 moving within filopodia of live mammalian cells (~140 nm $s^{-1}$) measured by TIRF microscopy. Optical trapping results showed that the average power-stroke size was ~10 nm, with the rate of ATP binding of ~1.3 $\mu M^{-1}$ $s^{-1}$. In most of the raw data traces, we observed displacements of unitary size, however at low ATP concentrations (2 μM) we also observed a number of interactions that exhibited multiple, staircase-like movements consisting of up to 3 steps per binding interaction. This behaviour is characteristic of a *processive* molecular motor. We will discuss these measurements in the context of mechano-chemical coupling and the functional significance in the living cell.

**721-Pos Board B600**

**Bayesian Estimation for Hidden Information of a Single Molecular Motor**

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In single-molecule experiments of molecular motors, it is a widely adopted strategy to visualize the motion of a molecule by attaching a large probe particle. In such a situation, only the motion of the probe can be monitored while the motion of the molecule is hidden. Therefore, in order to study a stepwise motion of the motor in detail, one has to estimate the system parameters and the hidden trajectory of the motor molecule at the same time on the basis of the observed trajectory of the probe. In this presentation, we will present a novel approach for this problem, which is based on the statistical mechanics and empirical Bayesian estimation. The method consists of several steps. First, we temporarily fix the parameters of the system. Then, we can utilize the Bayes theorem to estimate the trajectory of the motor from the trajectory of the probe. On the basis of the estimated trajectory, the marginal likelihood can be calculated using the WKB method. Finally, by maximizing the marginal likelihood, we can estimate the system parameters along with the trajectory of the motor. Although the above mentioned tasks appear straightforward, it is necessary to adopt appropriate approximation schemes in order to reduce the computational cost as well as artifacts resulting from the finite time resolution of the measurements. It is found that our method gives a reasonable solution to this problem. We will discuss the effectiveness of the method presented here by using a simple model that consists of two Brownian particles (a molecule and a probe) connected by a spring.

**722-Pos Board B601**

**ADP Affinity of Myosin VI is Regulated by Off-Axis Load**

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Individual dimeric molecules of unconventional myosin VI move processively along an actin filament, taking multiple steps in a hand-over-hand fashion. Recently we showed experimentally that the efficient processive movement is achieved via asymmetric rates of ADP binding under the forward and the backward loads, that is, to the trailing and the leading heads, respectively, such that ADP dissociates more readily from the trailing head, though the difference in the ADP affinity between the heads is less pronounced than in case of myosin V-6IQ construct. Several lines of evidence, obtained by other groups, suggest that during the processive stepping both the azimuthal angle of myosin's VI lever arm and the path of the motor along an actin filament are very variable, which implies the existence of an off-axis load during the double-headed binding of myosin VI to actin. To reveal the effect of an off-axis load on the efficiency of the processive stepping, we directly measured the ADP affinity of individual single-headed myosin VI molecules bound to actin, under loads applied at certain angles to the long axis of actin filament, towards both barbed and pointed ends. The obtained results indicate that the off-axis load significantly affects ADP affinity of myosin VI motor domain and is an important factor regulating the efficient processive movement of the dimeric molecules of myosin VI.

**723-Pos Board B602**

**The Tail Binds To The Head-Neck Domain To Form A Folded-Back Conformation That Inhibits The Actin-Activated ATPase Activity Of Drosophila Myosin VIIA**

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Myosin VIIA is an unconventional myosin, responsible for human Usher Syndrome type 1B, which causes hearing and visual loss. We have analyzed the molecular mechanism of regulation of myosin VIIA, which is not yet understood. While it was originally thought that myosin VIIA is a dimeric myosin, our electron microscopic (EM) observations revealed that full-length Drosophila myosin VIIA (DM7A) is monomeric. Interestingly, the tail domain markedly inhibits the actin-activated ATPase activity of tail-less DM7A in the absence of Ca2+ but not below pCa 6. By examining various deletion constructs, we found that deletion of the distal IQ domain, the C-terminal domain of the tail, or the N-terminal domain of the tail abolishes the tail-induced inhibition of ATPase activity.
EM study of full-length DM7A suggests that the tail domain folds back on to the head, such that the tail and the head/neck domain contact each other. Single particle analysis of full-length DM7A and of tail-less molecules to which exogenous tail has been added reveals that the tail contacts the head/neck domain at two sites, the motor core domain and the neck domain. These observations, together with the functional results, suggest that DM7A is inactivated by forming an inhibited conformation, in which the tail interacts with the motor and lever-arm domains. This is the first report to identify the regulatory mechanism of a single-headed unconventional myosin.

**724-Pos Board B603**

**The Medial-tail Domain of Myosin-VI as a Dimerization Region**

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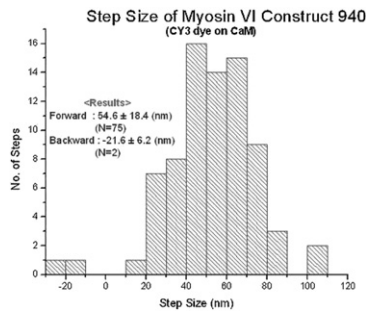
Myosin-VI has been one of the least-understood unconventional molecular motors due to its peculiar characteristics. For instance, each myosin-VI monomer has a short lever arm (~10nm) which contains only two Calmodulins while taking large steps (~36nm center-of-mass movements). Recently, the Spudich group proposed, based on bacterial expressed fragments, that the medial-tail domain of myosin-VI is a single alpha helix (~10nm), which can account for its large step sizes if dimerization occurs after the medial-tail domain. They also suggested that dimerization is formed via the cargo-binding domain. However, these results are contrary to our previous paper that showed that a myosin-VI construct without the cargo-binding domain can dimerize and walk processively. To solve this paradox, we prepared a myosin-VI construct truncated in the middle of the medial-tail domain (940-construct). A FIONA assay showed that this construct does dimerize either via antibody induction or actin-saturation method, and it walks processively with the same step size as the full-length myosin-VI construct. These results suggest that the medial-tail domain of myosin-VI is involved in dimerization and imply that the proximal-tail domain must be the major contributor to the unexpectedly large step size.




**725-Pos Board B604**

**Processivity of Myosin V and X on two-dimensional (2D) paracrystalline actin array**

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Myosin movement in vivo takes place on a wide variety of F-actin structures, including single filaments and 2D/3D bundled networks. Using in vitro single molecule motility techniqes, we have investigated the processivity and stepping characteristics of myosin V HMM and myosin X HMM with a leucine zipper on single actin filaments and 2D actin bundles. To answer how myosin V and myosin X step on actin bundles, we observed single molecule motility of fluorescently labeled myosin V and X using total internal reflection fluorescent microscopy, and analyzed the step-size, run length, speed, and direction of the movements on actin-bundles. Actin was polymerized and cross-linked on a charged lipid monolayer in Teflon wells to create regular 2D actin arrays. Two cross-linking proteins were used: alpha-actinin, which produces non-polarized bundles with 40 nm filament spacing, and fascin

which produces polarized actin bundles with 13 nm filament spacing. We applied a modified particle tracking program, which allowed us to analyze thousands of simultaneous myosin tracks and determine the run lengths and velocities typical of processive movement on the bundled networks. Myosin V moved processively on all types of in vitro actin structures. Myosin X moved well on polarized fascin cross-linked bundles, but movement was impaired or nonexistent on non-polarized alpha-actinin bundles. We hypothesize that forward runs of myosin X on alpha-actinin cross-linked bundles are inhibited because myosin X might makes ''sidesteps" to a neighboring filament, which stalls the run. The presence of an SAH domain in the lever arm of myosin X could increase the working stroke or flexibility of the lever arm allowing it to more easily sidestep across the larger alpha-actinin filament spacing.

**726-Pos Board B605**

**Cargo-mediated dimerization of Myosin VI**

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Myosin VI is one of 18 known classes of the molecular motor superfamily called myosin (1,2). All myosins rapidly bind and hydrolyze ATP in the presence or absence of actin. Until recently it was thought that all myosins moved toward the barbed (+) end of the actin filament. Myosin VI is the exception to that rule and may be unique among the myosin family members in that it moves toward the pointed (-) end of the actin filament (3).

Our working model for myosin VI in a cell is that the full-length protein exists as a monomer if not bound to cargo. Binding of myosin VI monomers to cargo alters the conformation of the molecule, possibly exposing the high probability coiled-coil region (dimerization domain). Once dimerized, the myosin VI can move a vesicle processively toward the minus-end of an actin filament. GiPC and optineurin, two of the known myosin VI binding partners can dimerize, and thus potentially can initiate the dimerization of myosin VI when it binds. Both GiPC and optineurin has been expressed in insect Sf9 cells. Surface plasmon resonance (SPR) analysis showed that both GiPC and optineurin interact with full-length myosin VI within the nanomolar range. Both GiPC and optineurin when incubated with full-length myosin VI initiated its dimerization showed by ATPase assays, EM and TIRF microscopy.

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**727-Pos Board B606**

**Characterization of drosophila myosin 7a mechanics**

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Myosin 7a is an unconventional myosin which participates in the sensory cell functions of numerous organisms, including humans, zebra fish, and flies. In drosophila, myosin 7a (DmM7a) appears responsible for bristle morphology, including the antennae involved in auditory transduction. The composition of motifs within the molecule is as follows: a motor head, containing sub-domains broadly typical of the myosin super-family, which connects to 5 IQ's, followed by the tail region. Within the tail are a putative coiled-coil followed by two tandem MyTH4-FERM domains separated by an SH3 domain. Here, data obtained using the optical trap three bead assay - the practice of using photon force to manipulate micrometer-scale beads to observe single molecule events - are presented for DmM7a. A truncated DmM7a construct (DmM7aTD1), cropped after the tail SH3 domain, was observed to interact with an actin filament at low ionic strength (50 mM KCl). Under the same conditions no interactions were seen with the full length version (DmM7aFL), however, at high ionic strength (200 mM) DmM7aFL became active. These findings are in agreement with recent studies demonstrating that the tail performs an internal regulatory function which is electrostatic in nature. The actin detachment rates (Kdet), calculated from dwell times, were similar for DmM7aTD1 and DmM7aFL at 10 μM ATP, approximately 0.2 $s^{-1}$. The Kdet for DmM7aFL was dependent on ATP concentration, and was increased at 1 mM ATP. These data support previous studies showing M7a to be a high duty motor with slow ATPase activity. Attempts to dimerise DmM7a on actin were unsuccessful based on the absence of ''stepping'' events which are a hallmark of processivity. This supports the case for DmM7a having a role in tension maintenance.

**728-Pos Board B607**

**Prefoldin 4 (PFD4): A putative new partner of myosin Va (MyoVa) in melanosome transport**

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Recruitment of MyoVa and the proper transport of melanosomes during pigment dispersion requires the central region of melanophilin (Mlph, 90 kDa) to bind MyoVa and the N-terminal region bound to the melanosome membrane via Rab27a. The interaction among these proteins is the key to melanosome transport. Previously, we identified and mapped for the first time the interaction between PFD4 (~14kDa) and Mlph using the yeast 2-hybrid system (in vivo) and a biochemical assay (in vitro). PFD4 is a subunit of prefoldin (PFD, ~87kDa), a chaperone that delivers unfolded proteins to a chaperonin for correct folding. Our in-vivo results suggest that PFD4 interacts with Mlph at the same MyoVa binding site. Here we confirm that interaction using pull-down assays and fluorescence spectroscopy; PFD4 competes with MyoVa for the Mlph binding site and residues 400-590 (putative coiled coil) of Mlph are crucial for PFD4 binding. In-vitro fluorescence anisotropy reveals interaction of fluorescein-labeled full-length Mlph with MyoVa tail or PFD4, by an increase in anisotropy and polarization values. Neither mutated A453P full-length Mlph nor the 400-590 segment caused a significant change in anisotropy when incubated with MyoVa; thus these constructs do not bind Mlph. Full-length Mlph also did not bind muscle myosin II. The MyoVa binding domain for Mlph and fragments 150-400, 300-433, 400-590, and Mlph A453P seems to be intrinsically unstructured. When we pre-incubated Mlph with PFD4 or MyoVa the circular dichroism spectrum showed that binding Mlph 150-400 and 150-590 with PFD4 and MyoVa tail possibly causes an increase in α-helix content. Support: CNPq, FAPERJ, PRONEX, CAPES (Brazil); Wellcome Trust (UK)

**729-Pos Board B608**

**The Mechanism of Filament Rotation in Gliding Assays with Non-Processive Myosin Motors**

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We present a model study of gliding assays in which actin filaments are moved by non-processive myosin motors. We show that even if the power stroke of the motor protein has no lateral asymmetry, the filaments will move in a helical, rather than straight fashion. Notably, the handedness of this twirling motion is the opposite from that of the actin filaments. It stems from the fact that the gliding actin filament has "target zones" where its subunits are oriented towards the surface and are therefore more accessible for myosin heads. Because each myosin head has a higher binding probability before it reaches the center of the target zone than afterwards, this results in a left-handed helical motion of the actin filament. We present a stochastic simulation and an approximative analytical solution to study this effect. We show that the pitch of the helix depends on the filament velocity, which in turn depends on the ATP concentration. It reaches about 400nm for slow gliding and increases with higher speeds. These values are in good agreement with recent experiments.

**730-Pos Board B609**

**Non-muscle Myosin IIb Is A Processive Actin-based Motor**

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Proper tension maintainence in the cytoskeleton is essential for regulated cell polarity, cell motility and division. Non-muscle myosin IIB (NmIIB) generates tension in the actin cortex of non-muscle cells. Recent biochemical studies show that both heads of a NmIIB dimer can interact with a single actin filament and that this conformation demonstrates load dependent release of ADP. Using a three bead optical trapping assay we recorded NmIIB interactions with actin filaments to determine if a NmIIB dimer cycles along an actin filament in a processive manner. Our results show for the first time that NmIIB is the first myosin II to exhibit evidence of processive stepping behavior. Analysis of this data reveals a forward displacement of ~5 nm. Surprisingly, NmIIB can and does take frequent backward steps of ~5 nm. The short step size of NmIIB suggests that this motor twists actin. Actin twisting could facilitate the removal of actin crosslinking proteins from the cytoskeleton. Our data supports a model in which NmIIB takes processive forward steps to generate additional tension and also takes backwards steps to relieve tension in the actin cytoskeleton, suggesting that NmIIb is a general regulator of cytoskeleton tension.

**731-Pos Board B610**

**Lever Arm Length Determines The Azimuthal But Not The Axial Orientation Of Myosin V During Processive Motility**

**John H. Lewis**, John F. Beausang, H.L. Sweeney, Yale E. Goldman.