

3757-Pos**Anomalous Diffusive Behavior in Intracellular Transport Mediated by Molecular Motors**Luciana Bruno^{1,2}, Valeria Levi^{1,2}, Maia Brunstein³, Marcelo Despósito^{1,2}.¹Facultad de Ciencias Exactas y Naturales. Universidad de Buenos Aires, Buenos Aires, Argentina, ²CONICET, Buenos Aires, Argentina, ³Laboratoire de Photonique et Nanostructure, Paris, France.

Intracellular transport of large cargoes, such as organelles, vesicles, or large proteins, is a complex dynamical process that involves the interplay of ATP-consuming molecular motors, cytoskeleton filaments, and the viscoelastic cytoplasm. In this work we investigate the motion of pigment organelles (melanosomes) driven by molecular motors in *Xenopus laevis* melanocytes using a high-spatio-temporal resolution tracking technique.

By analyzing the obtained trajectories, we show that the melanosomes mean-square displacement undergoes a transition from a subdiffusive to a superdiffusive behavior. A stochastic theoretical model, which explicitly considers the collective action of the molecular motors, is introduced to generalize the interpretation of our data. Starting from a generalized Langevin equation, we derive an analytical expression for the mean square displacement, which also takes into account the experimental noise. By fitting theoretical expressions to experimental data we were able to discriminate the exponents that characterize the passive and active contributions to the dynamics. Then, our model gives a quantitative description of active transport in living cells with a reduced number of parameters.

Unconventional Myosins II**3758-Pos****Electron Microscopy of Myosin 6 Molecules**Chun Feng Song¹, Howard D. White², Folma Buss³, John Kendrick-Jones⁴, John Trinick¹.¹Leeds University, Leeds, United Kingdom, ²Eastern Virginia Medical School, Norfolk, VA, USA, ³Cambridge University, Cambridge, United Kingdom, ⁴MRC Laboratory of Molecular Biology, Cambridge, United Kingdom.

Myosin 6 has important roles in cellular processes including endocytosis, secretion and cell migration, and is greatly over-expressed in prostate and ovarian cancer cells. The myosin 6 molecule is unique amongst the 40 so far identified myosins in walking in the opposite direction along actin filaments to all the other myosins so far studied. The molecule also takes unusually large steps considering its lever arm contains only 2 light chains. We have studied expressed intact and truncated chicken brush border myosin 6 molecules by electron microscopy under different nucleotide conditions using both negative staining and rotary metal shadowing, followed by single particle image averaging. In our hands the myosin 6 molecules are almost exclusively monomeric. Image averages after negative staining show predominantly one orientation with a characteristic view of the motor domain through the cleft between the upper and lower 50 kDa sub-domains, and with the light chains angled across the lever arm. In the absence of nucleotide the shape of subfragment 1 (S1) is very similar to the 2 nm reduced resolution apo S1 crystal structure, with the lever curving back along the motor domain towards the nucleotide binding pocket. Image averages of the whole molecule under the same conditions appear to show the tail crossing the motor domain. In nucleotides (ATP, ADP, ATP- γ S) the whole molecule is straight, with the lever and tail in line with the long axis of the motor. The measured length of the tail was substantially different depending on whether the shadowing or staining method was used, although the images were much more detailed after staining. By shadowing the tail was 29 nm long, whereas after staining it was 9 nm. This may indicate that part of the tail is collapsed by negative staining.

3759-Pos**Probing Myosin-VI Processivity using Artificial Lever Arms**Mary W. Elting¹, Zev D. Bryant¹, Jung-Chi Liao^{1,2}, James A. Spudich¹.¹Stanford University, Stanford, CA, USA, ²Columbia University, New York, NY, USA.

The lever arm of myosin VI has an unusual composition in which two different calmodulin-binding domains, a globular three-helix bundle, and an extended single alpha-helix domain may all contribute structural roles. We wish to understand which properties of this lever arm are important for mediating intra-head communication, preventing dissociation from the actin filament, and determining the distribution of stride sizes in a processively stepping dimer. We have replaced parts of the myosin VI lever arm with alpha-actinin repeats in a series of chimeric constructs. In dimers with artificial levers arms, we have found that processivity is surprisingly robust to dramatic changes in the properties of the lever arm, even when the stride size is altered (Liao et al., JMB

2009). In new chimeric constructs, we show that limited processivity is possible even in the absence of both calmodulin-binding regions. We examine the importance of intra-head coordination for processive motion in myosin VI by comparing the predictions of simple kinetic models to measurements of run length distributions for chimeric myosins and for control constructs.

3760-Pos**Coupled Myosin VI Motors Facilitate Unidirectional Movement on an F-actin Network**

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Unconventional myosins interact with the dense cortical actin network during processes such as membrane trafficking, cell migration and mechanotransduction. Our understanding of unconventional myosin function is derived largely from assays that examine the interaction of a single myosin with a single actin filament. In this study we have developed a model system to study the interaction between multiple tethered unconventional myosins and a model F-actin cortex, namely the lamellipodium of a migrating fish epidermal keratocyte. Using myosin VI, which moves towards the pointed-end of actin filaments, we directly determine the polarity of the extracted keratocyte lamellipodium, from the cell periphery to the cell nucleus. We use a combination of experiment and simulation to demonstrate that multiple myosin VI molecules can coordinate to efficiently transport vesicle-size cargo over 10 microns of the dense interlaced actin network. Furthermore, several molecules of monomeric myosin VI, which are non-processive in single molecule assays, can coordinate to transport cargo with similar speeds as dimers.

3761-Pos**A Model for Myosin VI Kinetics Under Load**Peiyong Chuan¹, James A. Spudich¹, Alexander R. Dunn².¹Dept. of Biochemistry, Stanford University School of Medicine, Stanford, CA, USA, ²Dept. of Chemical Engineering, Stanford University, Stanford, CA, USA.

Myosin VI is thought to function both as a transporter and an anchor in vivo. In an earlier study (Altman et al, Cell 2004), inhibition of myosin VI stepping kinetics by load applied using an optical trap was observed at saturating ATP and low ADP concentrations (< 2.5 μ M). A simple mechanism whereby the rate of ADP binding increases exponentially with load was proposed. Here we present myosin VI stepping data taken at a variety of applied loads and ADP concentrations, and show that the Altman model only holds at low ADP concentrations. At higher, physiologically relevant ADP concentrations under load we observe a dwell time that is an order of magnitude smaller than that predicted by the Altman model. We present a modified model in which applied load alters the equilibrium between two myosin VI states with different nucleotide affinities. This new kinetic scheme accurately describes myosin VI behavior at various nucleotide conditions under a large range of loads and provides insight into how myosin VI may function both as a force-generating transporter and as an anchor in vivo.

3762-Pos**Shrec Measurement of Myosin-VI Stepping Motion**

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Myosin-VI is a motor protein that plays an important role in a large variety of cellular events such as vesicle transport and anchoring actin bundles to the plasma membrane. Myosin-VI is thought to move processively as a dimer along an actin filament in a hand-over-hand fashion with large steps similar to myosin-V. However, unlike myosin-V, its step size is largely variable. Recently, we showed using FIONA method that myosin-VI does not have widely distributed step but rather has two step types, a regular large step (72nm) and short step (44nm). (Arimoto et al. Biophysical J. vol 96 p.139a) The large steps were consistent with the hand-over-hand model. The short steps, however, were not explained by canonical stepping model. We also showed that the fraction of short steps largely increases in the presence of ADP, suggesting the short and large steps are regulated in the ADP-dependent manner. In this study, in order to investigate the coordination of two heads during short and large steps, we performed an advanced multi-color FIONA technique called single-molecule high-resolution colocalization (SHREC) which involves labeling the two heads with differently-colored Q-dots. Now we are analyzing what is the condition that myosin-VI switches between two types of stepping manner. Furthermore, to clarify how myosin-VI switches between short and long steps, we are measuring myosin-VI movement under several ADP concentrations.