myosin molecules are brought together and localized to the actin bundles in the stereocilia participating in the lateral connections along with the interacting proteins such as vezatin and cadherin.
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3768-Pos
A Comparison of Mechanical Properties of Drosophila and Mouse Myosin 7a
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Myosin 7a is an unconventional myosin present in a range of organisms, and is essential in the function of sensory cells. In *Drosophila*, myosin 7a (D-M7a) is required to maintain bristle structure in Johnston's organ (the auditory center in *Drosophila*). Equivalently, in mice the absence of myosin 7a (M-M7a) disrupts stereocillia structure which adversely effects vestibular function. D-M7a and M-M7a share good sequence homology. Both have a 5-IQ lever-arm, followed by a single α-helix (SAH) domain, and an SH3 domain separating two MyTH4-FERM domains. Here we use the three-bead optical trap assay to compare the kinetics and mechanics of myosin 7a from insect (*Drosophila*) and vertebrate (mouse) species. We use a truncated D-M7a construct (D-M7aTD1), cropped after the SH3 domain to prevent auto-regulation. Due to difficulties with M-M7a expression, a shorter construct truncated after the SAH was used (M-M7aSAH). Data were taken at 50mM ionic strength with 10μM ATP. Step sizes of 10 and 18nm were observed for the D-M7aTD1 and M-M7aSAH, respectively. Variations in light chain binding, and geometric hindrances resulting from the shorter tail in M-M7aSAH, may contribute to this difference. The dwell time data from D-M7aTD1 were fitted well by a single exponential, giving an actin detachment rate (K_{det}) of $1.1s^{-1}$. This compares favourably with the biochemically determined ADP release step. Interpretation of the dwell time data from M-M7aSAH is less straight forward. The data is poorly fit by a single exponential. A double exponential gives fast ($9.5s^{-1}$) and slow ($0.8s^{-1}$) K_{det} rates. Comparison to biochemical data suggests the fast rate is related to ADP release, whereas the slow rate may represent ATPase cycling. We conclude *Drosophila* and mouse myosin 7a exhibit generally similar mechanical properties, though appear differently tuned, perhaps for their species specific function.

3769-Pos
Kinetic Analysis Reveals Differences in the Binding Mechanism of Calmodulin and Calmodulin-Like Protein to the 3 IQ Motifs in Myosin-10
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Myosin-10 (Myo10) is an unconventional myosin associated with dynamic actin remodeling implicated in a multitude of cellular functions. Like most unconventional myosins, Myo10 binds calmodulin (CaM) as the principal light chain. In epithelial cells Myo10 also binds calmodulin-like protein (CLP) as a tissue-specific light chain, resulting in increased Myo10 levels and Myo10-dependent cell motility. This raises questions as to how CaM and CLP compete for the individual IQ sites on Myo10. Indeed, there is little information on the kinetics of light chain binding to any unconventional myosin. Moreover, how Ca^{2+} affects the binding of CaM and CLP to the IQ motifs in Myo10 is unknown. We performed equilibrium and fast-kinetic experiments to elucidate the mechanism of binding of both CaM and CLP to each of the three IQ motifs in Myo10. Our results show that while CaM and CLP bind with moderate affinity to the isolated IQ2 domain in the absence of Ca^{2+}, both light chains display dramatically increased affinity for each of the three IQ domains in the presence of Ca^{2+}. The studies further indicate different binding mechanisms for CLP and CaM to IQ3, suggesting structural differences between the CaM-IQ3 and CLP-IQ3 complexes and supporting differential effects of the two light chains on Myo10 regulation and stability.

3770-Pos
The Molecular Adaptations of Myosin X for Bundled Actin Filament Tracks
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To properly self-organize, cells must be able to direct cargo to specific locations. Although many molecular motors are known to drive cargo transport, the address system that these motors use to move to the proper destination is poorly understood. Recently, we showed that myosin X has a preference for bundled actin filaments. This preference for bundles allows myosin X to identify filopodia, a limited population of actin filaments within the cell. To clarify the bundle selection mechanism, we performed single-molecule mechanical measurements to determine the stepping pattern on bundles. Our observed ~18 nm stepsize is consistent with a filament-straddling mechanism, where each head of myosin X binds to a unique filament in the bundle. A dissection of the domains required for bundle selection reveals a surprising role for the myosin X tail, a region which is likely far from the bundle itself. We find that targeted insertion of a glycine-rich flexible linker within the tail abolishes bundle selectivity, suggesting that the tail adopts a rigid structure that is essential for identifying bundles.

3771-Pos
A Structured Domain is Responsible for Bundle Selection of Myosin X
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Cells organize their contents and regulate cell shape and mechanics through molecular motors functioning on cytoskeletal filaments. Presented with many apparently similar tracks within the cortex, how myosins identify the few actin filaments that lead to their correct cellular destinations is largely unknown. Myosin X, an actin-based motor that concentrates at the distal tips of filopodia, selects the fascin-actin bundle at the filopodial core for motility. While poorly processive on single actin filaments, it takes processive runs on actin bundled by fascin. Such a bundle is the precise structure to which myosin X localizes in vivo. Using single molecule optical trapping experiments we determined the step size to be 17 nm, half of the 36 nm pseudo-helical actin repeat essential for motors to be processive on single actin filaments. This suggested that straddling two filaments within a bundle stimulates this motor's processivity. Using combinatorial chimeric constructs of myosin V and myosin X, we show that the post-IQ region, not the short lever arm (three IQ repeats) or the motor domain, is the main contributor to myosin X's selectivity. This region contains two structures of interest: a charged single alpha-helix (SAH), which may impart unique mechanical or affinity properties to the motor, and a coiled-coil dimerization motif. The structural character of this region was perturbed by insertion of free swivels either before or after the SAH domain. The post-SAH swivel mutant showed no preference for bundled actin for motility, thus providing support to a selectivity model where the search-space of the forward head for the next binding site is constrained to neighboring filaments in a bundle. This result provides insight into the ability of nature to fine-tune myosin motors to serve their specific functions in the cell.

3772-Pos
A Receptor Mediated Delivery System for Single Molecule Imaging in Live Cells
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The high complexity of the eukaryotic cytoskeleton arises from many proteins displaying multiple functions. Live, intact cells are an indispensable system for investigating motility of molecular motors, which depend on their intracellular environments. Here we present a novel system of delivering myosin motors into the cell to investigate their motility by single molecule imaging on the native cytoskeleton. This technology utilizes receptor mediated delivery (RMD) of fluorescently labeled motors. A conjugate of the desired myosin motor and substance P (SP) is internalized upon interaction with the neurokinin-1 receptor (NK-1), allowing the myosin motors access the cell without compromising the integrity of the membrane. RMD has no harmful impact on the cell, leaving the cell membrane intact and the sensitive structures preserved.
We previously demonstrated that myosin X selects the fascin-actin bundle at the filopodial core for motility. Here we show that this motor is successfully delivered with RMD, undergoes endosomal escape and finds its way to its native working environment simply by its functional preference for these unique structures. This is significant as it not only confirms that myosin X recognizes the local structural arrangement of filaments, but also further indicates that the details of cellular actin organization do impact the activity of unconventional myosins. Information on myosin motor motility obtained by RMD allows for the construction of a road map of the actin structures and enables a comparison between various cell types. These paths reveal both the spatial arrangement of the actin filaments (reflecting the complexity and density of cytoskeletal meshwork) as well as the individual motility preferences of myosin motors across cell types.

3773-Pos
The Dynamics for Myosin-X Induced Filopodia Protrusion
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Filopodia are actin-rich finger-like cytoplasmic projections extending from the leading edge of cells. Unconventional myosin-X is involved in the protrusion of

filopodia. However, the underlying mechanism of myosin-X induced filopodia formation is obscure. It is critical to directly observe the movements of myosin-X during various stages of filopodia protrusion (initiation, extension and retraction) in order to understand the mechanism underlying the myosin-X induced filopodia formation. we observed real-time movements of myosin-X fused with GFP (Green Fluorescent Protein) in filopodia of living cells using a total internal reflection fluorescent microscope, which enabled us to specifically observe the filopodia attached to a glass surface in living cells and trace the movements of myosin-X at the single-molecule level.
Myosin-X was recruited to the initiation site at the leading edge where it assembles with exponential kinetics before the filopodia extension. The myosin-X induced filopodia showed repeated extension-retraction cycles, with each extension of 2.4 μm, which was critical to produce long filopodia. Myosin-X, lacking FERM domain, could move to the tip like wild type, however, it transported towards the cell body during filopodia retraction and it could not show multiple extension-retraction cycles, thus failed to produce long filopodia. During the filopodia protrusion, myosin-X lacking FERM domain moves within filopodia with a velocity of ~600 nm/s same as wild-type myosin-X, suggesting that the myosin-X transports cargoes most likely integrin-β in filopodia without the interaction with the membrane via FERM domain. Based upon these results, we proposed the model of myosin-X induced filopodia protrusion. However, it is still unclear how myosin-X can initiate filopodia formation and promote the phased extension. It is plausible that myosin-X has a unique feature to generate force to promote the cytoskeletal structural change and membrane extension, but further studies are required to clarify this possibility.

3774-Pos
The Effect of the Interaction Between the Myosin-X Ferm Domain and Integrin on Filopodia Protrusion
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Filopodia are thin actin-rich plasma membrane structures found at the leading edge of migrating cells. Filopodia protrusions are regulated in part by myosin X, an unconventional myosin with a FERM domain that interacts with the adhesion factor integrin-β.We have previously found the long filopodia needed for cell adhesion were produced by having filopodia repeat cycles of short (~3um) extensions and retractions (phased elongation). This phased elongation could be suppressed by deleting the FERM domain. This result indicates that filopodia protrusion mediated by myosin-X is strongly responsible for cell adhesion.
To further investigate the importance of the FERM domain, we have examined the effects of substrate coating on filopodia. Two types of substrate coating, fibronectin and poly-lysine, were used. Fibronectin is an extracellular matrix glycoprotein that binds to integrins (integrin dependent), while poly-lysine is a synthetic molecules used to enhance cell attachment to plastic and glass surfaces (integrin independent). We found that the protrusion velocity of filopodia on fibronectin was less than that on poly-lysine, which is likely due to friction between the FERM domain and integrin.Also, the filopodia length of one elongation (one extension and retraction) on fibronectin was 1.5 fold longer than that on poly-lysine. However, this was compensated for by the number of phased elongation resulting in approximately equal filopodia lengths regardless of the substrate coating.
We are now observing the movement of myosin-X lacking FERM domain using the same substrate coatings. At this meeting, we will compare these results with the above in detail.

Microtubule Motors-Dynein

3775-Pos
Cysteine Mapping of Cytoplasmic Dynein Motor Domain
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Cytoplasmic dynein is a large cytoskeletal protein complex comprised of a heterodimer of heavy chains, intermediate chains, light intermediate chains and light chains. Cytoplasmic dynein is responsible for transporting cargo, other proteins, vesicles and organelles, throughout the cell by movement along microtubules in a retrograde fashion. This activity is mediated by a series of conformational changes to the motor domain induced by ATP binding, hydrolysis and the release of ADP to give a power-stroke motion. There are, however, many unknowns regarding the conformational changes and structure of the motor domain. The extremely large size of the motor domain (380 kDa) makes structural characterization a challenging task. As an initial step towards this goal, cysteine mapping of the motor domain was performed. Preliminary results from fluorescence spectroscopy indicate that 6 out of the 47 cysteines react with ThioGlo(r)1 (methyl 10-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-9-methoxy-3-oxo-3*H*-benzo[*f*]chromene-2-carboxylate) in the motor domain's native state. Under denaturing conditions, an additional 15 cysteines are revealed. The non-reactivity of the remaining 26 cysteines suggests the presence of 13 disulfide bonds in the motor domain. These results are being analyzed with mass spectrometry to confirm and identify the accessible, buried and oxidized cysteines. This information will be instrumental in mapping the location of residues within cytoplasmic dynein's motor domain. In addition to characterizing the structure of the motor domain, gold particle-bearing labels reactive with surface-accessible cysteines are being explored to provide cryoelectron microscopy data on the motor domain.

3776-Pos
Cytoplasmic Dynein is not a Classical Duty Ratio Motor
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Cytoplasmic dynein is not a classical duty ratio motor
The mechanical cross bridge cycle of cytoplasmic dynein has often been compared with that of myosin. Cytoplasmic dynein and myosin 5 are both organelle bound motors responsible for transport of their cargo over a long distance. It has also been demonstrated that myosin 5 is a processive motor and that its processivity is regulated by the duty ratio; i.e., the ratio between bound and free state during the cross bridge cycle. As the binding of ATP leads to the dissociation of the motor filament complex a decrease of the ATP concentration results in an increased duty ratio.
It was the aim of our study to investigate whether the processivity of cytoplasmic dynein is also governed by the duty ratio. With the optical trap single molecule measurements were carried out in a two bead dumbbell approach. At 100 μM ATP consecutive 8 nm steps up to stall force were observed often resulting in repeated 8nm forward and backward steps at stall force. To our surprise however, at low ATP concentrations dynein underwent only single binding events with an apparent working stroke of 8nm.
These results can not be explained by a simple one site model for ATP binding where processivity is governed by the duty ratio. In contrast to myosin, dynein possesses two essential ATP binding sites. At low ATP concentrations we hypothesis that only one of the ATP binding sites is occupied, thereby resulting in a loss of processivity.

3777-Pos
Collective Dynamics of Cytoplasmic Dynein Motors In Vitro
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Molecular motors are necessary for fundamental biological functions such as cell division and intracellular transport. These processes, which can lead to concerted movements in the cell often rely on the interplay of a multitude of motors exerting forces on microtubules. While current insight into the mechano-chemistry of single motor proteins is quite advanced, it is not sufficient for understanding collective motor activity.
Meiotic nuclear oscillations in the fission yeast Schizosaccharomyces pombe represent an easily accessible model process to study intracellular movements driven by a multitude of dynein motors [Vogel et al.]. We are developing a novel in vitro assay to identify the minimal set of components and conditions required to obtain oscillations similar to those in S. pombe. Initially we study the behavior of anti-parallel microtubule doublets [Leduc et al.] gliding on dynein. The parameters to be tested comprise motor density, ATP concentration and eventually the on- and off-rates of the motor proteins. The results of these investigations will provide insight into the collective behavior of motor proteins leading to large-scale movements in living cells.
Vogel et al., PLoS Biol., 7 (2009)
Leduc et al., in preparation

3778-Pos
LIS1 and Nude Permit Multiple Dynein Motors to Cooperate to Transport High Loads
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Cytoplasmic dynein is involved in a wide range of intracellular movements including fast vesicular transport and slow nuclear translocation. How one motor contributes to fast, low load movement as well as slow, high-load movement is unknown. We have found that two dynein regulatory factors, LIS1 and NudE, cooperate to convert dynein to a novel persistent force state under load (MBC 19(suppl.), 1546). We found NudE to recruit LIS1 to dynein to form a triple