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

complex, in which LIS1 binds the dynein motor domain in an ADP-VO4 transition state-specific manner. LIS1 enhanced the affinity of dynein for microtubules, but only under transition state conditions. In single molecule bead assays, LIS1 dramatically prolonged the interaction of dynein with microtubules under load, providing the first evidence for a role in dynein force regulation. To measure the resistance of dynein to detachment from microtubules directly, we have now used ''superforce'' analysis, subjecting beads with associated single dyneins to sudden increases in laser trap strength above the dynein stall force. LIS1 alone or in combination with NudE decreased the detachment of dynein from microtubules by up to 5-fold. To test how LIS1 and NudE affect dynein force production under multi-motor conditions, we monitored beads coated with ~2-3 dynein molecules at a laser trap strength of 3.7 pN. LIS1 and NudE induced an increased frequency of multi-motor events and caused a dramatic increase in trap escape. In silico simulations confirm that the prolongation of individual dynein-microtubule interactions should result in enhanced force production by multiple motors. These results appear to explain the need for LIS1 and NudE in dynein-dependent, high-load intracellular movements, where multi-motor activity is critical. Support: GM47434, GM068952, HD40182, GM070676.

3779-Pos
Dynamic Regulation of Bidirectional Vesicle Transport
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The motor proteins dynein and kinesin function collectively to achieve long-range, bidirectional transport along microtubules. Transport in live cells, imaged using phase microscopy, exhibits distinct modes of motility, with fast, unidirectional movement in both anterograde and retrograde directions as well as saltatory, bidirectional movement. To examine transport in a simplified environment, we isolated axonal transport vesicles from transgenic mice expressing GFP-dynamitin. The fluorescent vesicles were imaged with high resolution using total internal reflection fluorescence microscopy. Using automated tracking software, we tracked all vesicles associated with polarity-marked microtubules. The purified vesicles move bidirectionally with 40% of motility in the anterograde direction and 60% in the retrograde direction, similar to the bidirectional population of vesicles observed in live cells. Inhibitory antibodies to dynein modulate the direction of transport. We compared the predictions of a simple tug-of-war model, proposed by Müller et al., [PNAS, 2008] to the observed motility in vitro, and found good agreement when 6-7 dynein motors and 1 kinesin motor are active. This prediction is in striking agreement to quantitation of motor numbers through photobleaching and quantitative western blotting, which estimate approximately 6 dynein motors per vesicle and a ratio of 6.3 ± 0.7 dynein motors to each kinesin-1 motor. Together, the analysis of vesicle transport in live cells, purified vesicles in vitro, and mathematical modeling indicate that vesicles move robustly with a small complement of motors. The results suggest an efficient regulatory scheme where small changes in the number of active motors manifest in large changes in the motility of the cargo.

3780-Pos
Steady States of a tug-of-war Model for Organelle Transport
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Constructing a reasonable model to describe the motion of organelles and vesicles moved by motor proteins in cells is not straightforward but may assist in understanding the mechanism. Recently, Muller, Klumpp and Lipowsky (PNAS 105, 4609-14, 2008) have developed a tug-of-war model to describe this motion. Their model exhibits several qualitatively different motility regimes that depend on the precise value of the single motor parameters. They suggested that parameter variation could be used by a cell to regulate its cargo traffic. We have carried out a detailed theoretical analysis of this tug-of-war model in the limit that the numbers of the two different motor species bound to the cargo becomes large [1]. All the stable, i.e., biophysically observable steady states and their stability domains can then be obtained. Depending on the parameter values, the tug-of-war model may exhibit either uni-, bi- or tristability. The steady state motion of the cargo, transported by two different motor protein species, is determined by the initial numbers of the motors bound to the track. Monte Carlo simulations confirm that our theoretical results are accurate when there are a large number of motors but also remain useful for only a few motors. Theoretical analysis of the small motor-number situation is planned.
[1] Y. Zhang, Phys. Rev. E. 79, 061918 (2009).

3781-Pos
Non-Monotonic Force-Dissociation Rate Relation Improves Ensemble Performance of Multiple Molecular Motors
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Molecular motor-based intracellular transport is important for cell function. There is mounting experimental evidence that multiple motors work together to transport cargos in a cell. However, how these motor assemblies perform collectively is poorly understood and in particular it is still unclear how different mechano-chemical parameters of single motors affect ensemble function. Here we investigate whether using monotonic force-dissociation relation above and below motor stall force is a correct approximation for single motor dynamics and whether it can reproduce experimentally observed in-vitro multiple motor behavior. This is a relevant and important question because recent models of multiple motor based transport use Kramers's theory formula for the force dependent dissociation rates of a single motor employing an assumption that the force-dissociation relation for single motors is monotonic. We find that a detailed analysis of our experimental observations is inconsistent with such an assumption. Instead, we propose a class of single motor models where force dissociation relations need not be monotonic above single motor stall force and which successfully explain the experimental observations.

3782-Pos
The Birefringence and Molecular Fine- Structure of Axonemes
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To shed light on the molecular arrangements and structural changes occurring inside microtubule based structures, we observed single axonemes using a liquid crystal polarized light microscope (LC-PolScope, Oldenbourg et al., Biophys J., 74, 645-54, 1998) to measure their birefringence, a sensitive indicator of structural parameters characterizing ''crystal-like'' biopolymers directly in living cells. The birefringence of rod-like particles such as axonemes is caused by their shape and by the arrangement of molecular bonds inside their structure. The latter is called intrinsic birefringence and can be used to monitor the molecular conformation during dynamic processes such as axoneme beating under physiological conditions. Similar to microtubules, we found that axonemes have an intrinsic birefringence that is about 10% of their total birefringence and that can be used to detect structural changes inside axonemes by measuring their retardance (=birefringence times thickness of sample) with polarized light microscopy. We analyzed individual axoneme retardance and found that the maximum retardance of a single demembranated axoneme was 1.7nm. After extracting the outer dynein arms the retardance decreased to ~1.3nm. Thus, dynein molecular motors contribute to the birefringence of axonemes. We also analyzed the retardance of static axonemes that were bent as a result of axonemal beating frozen due to ATP depletion. The axoneme retardance varied in a systematic manner that seemed to be related to the curvature of the bends. Our combined results lead us to propose that the structure of microtubules and dynein contribute to axoneme birefringence, in addition to its form birefringence. The intrinsic birefringence of bent axonemes is likely caused by structural changes inside axonemes and is affected by the mutual interactions between microtubules and dynein molecular motors.

Cell & Bacterial Mechanics & Motility III

3783-Pos
Physics of Phagocytosis in Self Recognition: Particle Size and Myosin Forces
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Cells make a number of key decisions by actively applying forces to the objects that they 'touch'. How a macrophage decides to adhere to and 'eat'a foreign object (microbes to drug carriers) while leaving 'self'cells alone is a central decision in macrophage function. The molecularly specific adhesion systems that activate (or inhibit) will be shown to function from nanometer to micron length scales and to be made efficient by signaling (or not) to force-generating myosin motors in macrophages. Additional effects of particle shape - relevant to filamentous viruses perhaps - will be discussed. Collectively, the results suggest new means of achieving compatibility and the CD47 findings even appear relevant to engraftment of stem cells.