when the neck linker is constrained in a backward extended conformation. To further investigate the effect of crosslinking on the ATP hydrolysis kinetics, we measured mant-ADP release after rapid mixing with microtubule and subsequent mant-ATP binding rates of the monomers. Stopped flow measurements showed that ADP release and ATP binding rates did not change after constraining in the backward extended conformation, suggesting that either ATP hydrolysis or Pi release step may be the rate-limiting. When the neck linker is constrained in a forward extended conformation, ATP binding rate did not changed but the ADP release rate dramatically decreased. These results suggest that ATP hydrolysis cycle of the motor domain can be differently regulated depending on the direction of the neck linker tension, explaining the alternate catalysis in the dimer.

3946-Pos Board B674
Kinesin Motility on Microtubule Bundles
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Microtubules carry out numerous functions in the cell, one of which is to provide tracks along which microtubule motor proteins walk. In the cell, these filaments often form complex architectures, such as microtubule bundles. Distinct types of bundles can form depending on the orientation of microtubules within the bundle. Bundles that consist of parallel microtubules are found in axons, while bundles comprised of randomly oriented microtubules are present in dendrites. Additionally, antiparallel microtubule arrays are known to exist at the spindle midzone. How do motors navigate these complex microtubule architectures? Here, we study the motility of single kinesin-1 motors on different types of microtubule bundles in vitro. We prepared three types of microtubule bundles: bundles with tightly packed, randomly oriented microtubules formed by depletion forces; spaced, antiparallel bundles formed by the microtubule crosslinking protein, MAP65; and endogenous parallel microtubule bundles derived from neuronal-like processes of CAD cells. We observe that kinesin processivity and velocity are reduced on tightly packed microtubule bundles. This reduction in motility is likely due to staggered, overlapping microtubules within the bundle that act as obstacles for motors. We do not observe reduced motility on microtubule bundles with crosslinking MAPs. We suggest that spacing between microtubules within a bundle created by MAPs organizes the bundle architecture to prevent staggered, overlapping microtubules from acting as obstacles for kinesin motors. Interestingly, we observe single kinesin motors to switch to adjacent, oppositely oriented microtubules within bundles that contain antiparallel microtubules. We show that motors that switch to adjacent tracks during a run exhibit an enhanced processivity. This suggests that the ability of single motors to switch to adjacent tracks could be a mechanism used by kinesin to circumvent obstacles. This study provides new insights into how kinesin motors navigate complex tracks present in the cell.

3947-Pos Board B675
The Mechanism of Determining the Directionality of NCD
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Kinesins are the unidirectional motor proteins, moving on microtubules and being involved in various cellular functions. A motor domain of kinesin comprises the motor core: the ATPase catalytic site and MT-binding site, and the neck region: adjacent to the motor core, and the N or C-terminal region: protruding from the motor core. It is known that neck region and N or C-terminal regions are essential for kinesin's motor activity. Among the kinesin superfamily, kinesin-1 moves toward the plus-end of the microtubule while Ncd is the minus-end directed motor. This difference of directionality has long been studied. A series of experiments using chimera motors whose components are exchanged between kinesin-1 and Ncd demonstrated that the neck region was the determinant of the directionality. However the chimera kinesin-1, whose neck was exchanged with Ncd, also exchanged its C-terminal region with Ncd. Thus, it is uncertain whether the C-terminal region of Ncd is the determinant of the minus-ended directionality. To test this, we engineered other chimeras. A chimera kinesin-1 having only the neck of Ncd did not change the directionality. And another chimera kinesin-1 having both the neck and the C-terminal region of Ncd changed into the minus-ended motor. These results indicate that not only the neck but also the C-terminal region of Ncd is indispensable to produce the minus-end directed motility. We then investigated whether Ncd motor core itself has directionality. We engineered a mutant Ncd whose neck was replaced with an artificial peptide, which is thought not to interact with the motor core. This mutant showed the plus-ended directionality. We conclude that Ncd motor core itself has 'default' plus-end directionality and an appropriate interaction among the neck, the C-terminal region, and the motor core of Ncd makes Ncd the minus-ended motor.

3948-Pos Board B676
Automated, Long-Distance Microtubule Tracking in Gliding Assays
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Microtubules are microscopic tubular structures involved in a number of biological functions, such as cell division, intracellular transport, and mechanosensation. For particular functions, such as mechanosensation in C. elegans, microtubule diameter, or equivalently protofilament number, is tightly regulated. It is tempting to link microtubule diameter to microtubule bending rigidity, and hence to mechanosensation. However, even the link between microtubule diameter and rigidity remains unclear. Two particular challenges in measuring microtubule flexural rigidity as a function of diameter are the intrinsic heterogeneity in microtubule structures in in vitro preparations, and the intrinsic heterogeneity in microtubule rigidities measured for identically prepared microtubules.
In order to address these heterogeneities, we simultaneously determine microtubule diameter and rigidity for single microtubules using statistical properties gathered from a microtubule gliding assay. In this poster, we report a technique we have developed to improve the precision of these measurements. The technique combines a microscope, integrated piezo-elements to control the sample stage, and software to automatically track a single microtubule and reposition the stage with nanometer precision to keep the microtubule centered within the field of view. We have been able to effectively double the field-of-view in each direction (from 30 micrometers to 60 micrometers), resulting in substantially longer microtubule gliding trajectories while maintaining a tracking precision on the order of 10 nm over the entire microtubule trajectory.

3949-Pos Board B677
Cooperative Effects in Transport Systems Driven by Diffusively Anchored Motors
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Intracellular trafficking of membrane-bound organelles is a fundamental process essential to many cellular functions including cell growth and signaling. A variety of such organelles are transported by motor proteins such as kinesins, dyneins and myosins, walking on cellular tracks made of microtubules and actin filaments. Since studying the mechanical and biochemical functioning of these transport systems inside the cell is extremely challenging, transport is often mimicked in-vitro, for example by gliding motility assays. There, cytoskeletal filaments are propelled by motors that are conventionally immobilized on a rigid substrate. In contrast, when transporting membrane-bound organelles inside a cell the motors are diffusively anchored - either directly or via adaptor molecules - to lipid bilayers. Such resulting 'loose' coupling may induce motor co-ordination and is likely to change the collective motor dynamics.
In this study, we investigate the collective behavior of motor proteins anchored to lipid bilayers. Using truncated kinesin-1 motors with a streptavidin-binding-peptide tag we performed gliding motility assays on streptavidin-loaded biotinylated supported lipid bilayers. Our results suggest a dependence of the microtubule gliding velocity on both, the motor density as well as the microtubule length. Based on measurements of the diffusion constants and the velocity of motors and microtubules, a theoretical model is developed to determine (i) the number of microtubule-attached motors and (ii) the force produced by an ensemble of motors.

3950-Pos Board B678
Single Motor Random Walks on Microtubule Bundles
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Intracellular cargo transport uses a combination of dynein and kinesin motors to traverse along microtubule filaments in cells. It has been demonstrated that the motion of cargo within a complex network of microtubules in live cells can be viewed as a hindered random walk with correlation in the step size and dwell times of successive steps (Tabei 2012). A mean zero random walk is surprising considering that these processes are used for directed, unidirectional transport in cells. Further, the individual microtubules within the network are dynamic and the network is constantly changing. Although recent enlightening models have revealed that the motion in cells can be well-described by a subordinated random walk, the combination of fundamental components for the molecular mechanism are complex and still unknown. In order to better inform these models about the activity of single motors within the network, we perform well-defined systematic experiments of processive motors on anti-parallel or randomly-oriented filament bundles. Antiparallel bundles are created using MAP65 to crosslink microtubules. Randomly-oriented bundles are created using depletion forces from inert poly-ethylene glycol molecules in solution. We find that, kinesin