ABDM. And cis-trans isomerization of the crosslinked ABDM induced disordered α-helix in the SH1-SH2 region resulting in global conformational change of myosin head.

In this study, we prepared kinesin mutant K380 cys light which has truncated minimum stalk to form dimer. Subsequently, Lys360 and Ala361 in the coiled-coil region of the mutant K380 were substituted by cysteine. The kinesin mutant K380(K350C,A361C) was modified with ABDM. The intermolecular and intramolecular crosslinking were analyzed SDS-PAGE. The optimal condition to crosslinking was 10 times excess molar ABDM for kinesin mutant and modification for 160 min at room temp. The conformational change of the ABDM crosslinked kinesin induced photoisomerization was studied with CD spectroscopy. And the photo-reversible dimerization of ABDM crosslinked kinesin was examined.

685-Pos Board B465 Investigating Cin8 Bi-Directionality as a Molecular Force Sensor Todd Fallesen, Thomas Surrey.

Microtubule Cytoskeleton, Cancer Research UK, London, United Kingdom. Molecular motors in eukaryotic cells are essential for many cellular functions. A diverse population of motors operate upon filaments inside the cell. The kinesin family of motors operates on microtubule (MT) filaments in tasks such as vesicle and organelle transport, mitotic spindle assembly, and chromosome separation in mitosis. A subset of kinesin motors, the kinesin-5 family, can cross-link microtubules. These cross-linking motors organize microtubules in space, which is essential for mitotic spindle assembly. In vitro experiments have demonstrated that the kinesin-5 motor Cin8, from S. cerevisiae, can switch its direction of travel on a MT. This is a remarkable property not observed in other MT motors before. It has been observed through fluorescence microscopy that a single Cin8 motor on a MT moves in an ATP dependent (-) end directed manner, yet when multiple Cin8 motors crosslink two anti-parallel MTs they switch direction. This novel collective effect, essentially results from Cin8 operating not only as a force generator, but also as a novel force sensor. Using a novel binding configuration and optical force spectroscopy, we investigated the biophysical properties of Cin8 motion on microtubules as compared to the well characterized behavior of the Kinesin-1 motor. We test whether a force opposing the motion of Cin8 motors, in gliding assays, can change the direction that the Cin8 motors are collectively moving. Further, using single molecule bead assays, we investigate the behavior of single motors close to their stall force.


1University of Minnesota, Minneapolis, MN, USA, 2Cleveland Clinic, Cleveland, OH, USA. Microtubule motors control a diverse collection of physiologies, including cell division, organelle traffic, and microtubule dynamics. Kinesins use ATP hydrolysis to power a chemical cycle that performs mechanical work. Recent, high-resolution crystallographic and cryo-EM reconstructions, single molecule mechanics, and solution kinetics studies, led to a general mechanochanical scheme for kinesin motors (Clancy, Nature Str. Mol. Biol., 18, 1020-7, (2011)). Nucleotide binding engages key structural elements, including switch I, switch II, and the P loop. Yet, how conformational changes in these switch elements lead in turn to corresponding changes in the microtubule binding domains and the motor mechanical elements (the neck linker and cover strand) remains enigmatic. Likewise, it remains unclear how the thermodynamics and kinetics of these structural transitions differ between two kinesins with very different physiologic roles. We tested two predictions of the model proposed by Clancy et al: the neck-linker gates nucleotide binding; and, coordination between the neck-linker and switch-1 fine tunes the enzymology of specific kinesin motors. We have used a recently developed technique transient time-resolved fluorescence resonance energy transfer, which enables us to detect transitions between multiple structural states, measured with time-resolved FRET during a biochemical transient. We engineered probe-pairs to report the structure, kinetics, and equilibrium constants for nucleotide-driven structural transitions in the neck-linker and switch-1. We then compared these transitions in kinesin-1 to those in Eg5 (kinesin-5). Our results show that: 1. neck-linker docking gates nucleotide binding, as predicted by Clancy et al., and in both classes of kinesins, 2. The state of the neck-linker correlates the information of switch-1, and 3. differences in the equilibrium constants for neck-linker docking during the microtubule bound, ATP stimulated working-stroke, explain the unique force dependence of Kinesin 1 and Eg5.

687-Pos Board B467 Processivity of Kinesin-2 Results from Rear-Head Gating and Not Front-Head Gating Geng-Yuan Chen, David Arginteanu, William O. Hancock.

Department of Bioengineering, University Park, PA, USA. The kinesin-2 family motor KIF3A/B coordinates with dynein to bidirectionally transport intraflagellar particles, melanosome, and neuronal vesicles. Compared to kinesin-1, kinesin-2 is less processive and its processivity is more sensitive to load, suggesting that the gating mechanisms that their control processivity may differ. To understand the motor roles that front-head gating and rear-head gating, we carried out stopped flow kinetics experiments using mann nucleotides, steady state assays, and single-molecule investigations to characterize the entire kinetic cycle a functional mouse KIF3A homodimer that exhibits similar motility to full-length KIF3A/B. Upon first encounter with the microtubule lattice, the motor exchanges mADP with an on-rate of 18 μM-1 s-1 and an off-rate of 27 s-1. When AMPNP was used to entrap the motor in a two-headed bound state, exchange kinetics were unchanged, indicating that rearward strain in the two-headed bound state does not alter nucleotide binding to the front head. Similar lack of front-head gating was found with mATP and when the neck linker domain was shortened from 17 to 14 residues to enhance intramolecular strain. In contrast, microtubule pelleting and single-molecule microscopy assays found that in ADP the motor dissociates with an off-rate of 2.1 s-1 and a KD of 0.5 μM, similar to its behavior in ATP. Hence, kinesin-2 processivity results from rear-head gating and not front-head gating. Based on the kinetics measurements, finally we propose a complete model of the kinesin-2 hydrolysis cycle that accounts for all of the kinetics and motility data. This study provides the direct evidence that rear-head gating does and not front-head gating does not play a role in kinesin-2 processivity and suggests that kinesin-2 mechanochemistry is specifically adapted for bidirectional transport.

688-Pos Board B468 High Speed Microscopy for Observing the Stepping Behavior of Kinesin-1 Motors at Saturating ATP Keith J. Mickolajczyk1, Joanna Arendrea2, Jaime Ortega-Arroyo2, Philipp Kukura2, William O. Hancock1.

1Biomedical Engineering, Penn State University, University Park, PA, USA, 2Chemistry, Oxford University, Oxford, United Kingdom. Kinesin-1 is the most well studied member of the kinesin superfamily of molecular motors and is responsible for the trafficking of vesicles and organelles towards terminal branches of axons. This motor hydrolyzes ATP in a tightly coupled fashion in order to take directed 8 nm steps along a single protofilament of a microtubule with high processivity. However, the comprehensive mechanochanical cycle of kinesin-1 remains elusive, due largely to the technical limitations of ensemble experiments and fluorescence microscopy approaches at the single-molecule level. Questions remain in identifying the rate limiting step in the hydrolysis cycle, as well as the time spent in the bound state. To probe the mechanochanical cycle, we employ a fluorescence-free imaging technique, interferometrically scanning microscopy (iSCAT), to track kinesin-1 at saturating ATP in a reconstituted system with greatly improved spatiotemporal resolution. With point spread function fitting, 8 nm and 16 nm steps are measured for C-terminus and N-terminus labeled motors, respectively. Using novel differences of means and Gaussian mixture model algorithms for the identification of step and sub-step regimes, we investigate the information present in both on-axis and off-axis positional data. Overall, these results point towards new insights into mechanochanical cycle of kinesin-1. The optical system and data analysis tools used present a new platform for investigating the stepping cycles of diverse molecular motors.

689-Pos Board B469 Impact of Structural and Dynamical Complexity on Kinesin Kinetics Bruna D. Jacobson1, Kasra Manavi2, Susan R. Atlas1, Lydia Tapia2.

1Dept. of Physics and Astronomy, University of New Mexico, Albuquerque, NM, USA, 2Dept. of Computer Science, University of New Mexico, Albuquerque, NM, USA. One hypothesis for the onset of Alzheimer’s disease associates the aggregation of excess tau-protein on microtubules with the hindrance of cargo transport by molecular motors. This hypothesis has motivated experimental and modeling studies of kinesin procession in the presence of such obstacles. Very recently, it has been shown that kinesin’s neck linker length is closely related to its ability to bypass obstacles and avoid early detachment from the microtubule, by sidestepping to adjacent microtubule tracks. Here we present results from kinetic models that explicitly account for such sidestepping by analyzing published experimental single-molecule data on the processivity of kinesin-1 and kinesin-2, both with and without obstacles. The mechanochemistry is