mutant G220C chimera protein fused with GFP at the neck-linker has been prepared and labeled IAE-DANS. The FRET efficiencies between the fluorescent probe DANS at C220 and GFP in the presence and absence of nucleotides were analyzed.

710-Pos Board B589
Analysis of Conformational Change of Novel Rice Kinesin K16 Using Small Angle X-ray Solution Scattering and EPR
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We have previously revealed that rice kinesin K16 has several unique enzymatic characteristics comparing with conventional kinesin. The most interesting property is that the ADP-free K16 motor domain(MD) is very stable, contrast to conventional kinesin that is very labile in ADP-free state. Recently, we have successfully dissolved the crystal structure of ADP bound K16 motor domain. The overall structure of the K16MD is similar to that of conventional kinesin MD, as expected from the high similarity of amino acid sequence. However, neck-linker region showed an ordered conformation in a position quite different from conventional kinesin. In this study, we designed the K16MD chimera protein fused with GFP at the neck-linker in order to monitor the conformational change of the neck-linker during ATP hydrolysis by small angle X-ray solution scattering and EPR. We determined the Radius gyration (Rg) values of K16-GFP in the presence or absence of nucleotides by X-ray solution scattering. The Rg of nucleotide-free K16-GFP was about 42Å. In the presence of ADP and ATP, the Rg values were 38Å and 39Å, respectively. These results may suggest that the neck-linker of nucleotide free K16 is in the docked conformation, on the other hand, the neck-linker of nucleotide bound state is in the novel conformation observed in crystal structure. We also analyzed conformational change of K16 in the solution by EPR. We constructed K16 mutants which have single cysteine at 331, 335 or 340 and labeled with 4-maleimido-2,2,6,6-tetramethyl-1-piperidinyloxy. But we could not observe notable change of mobility during ATP hydrolysis in the absence of microtubule for the three mutants. Currently, we are analyzing the distance between kinesin core reagion at 47 and neck linker at 328 using the dipolar EPR method.

711-Pos Board B590
The Effect Of Loads on the Collective Behavior of Neurospora Kinesin
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The motor protein kinesin converts the energy from ATP hydrolysis and Brownian motion into directed movement. There is increasing evidence suggesting that several kinesin motors cooperate to transport cargoes. Recent experiments also suggest that the collective behavior of kinesin differs significantly from single-molecule behavior. We study the collective behavior of Neurospora kinesin (Nkin) in vitro. By laser trapping latex beads attached to microtubules through biotin-streptavidin linkages, we are able to apply forces to microtubules being transported by several kinesins attached to the coverslip. The density of motors on the coverslip is related to the average number of motors involved in the transport. We experimentally characterize the transport for a range of loads and motor densities.

Unconventional Myosins

712-Pos Board B591
Photo-Control of Myosin Va using Photoresponsive Calmodulin
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Myosin Va is a processive motor that has a role as an organelle transporter in various cells. Myosin Va consists of motor domain, neck domain, coiled-coil region, and globular tail domain (GTD). The neck domain carries six IQ motifs, which act as the binding site for calmodulin (CaM) or CaM-like light chains. The GTD inhibits the Ca^{2+}/CaM dependent actin-activated ATPase activity of myosin Va. CaM is a physiologically important Ca^{2+}-binding protein that participates in numerous cellular regulatory processes. CaM has a dumbbell-like shape in which two globular domains are connected by a short α-helix. Each of the globular domains has two Ca^{2+}-binding site called as EF-hand. CaM undergoes a conformational change upon binding to calcium, which enables it to bind to specific proteins for a specific response. N- (4-phenylazophenyl) maleimide (PAM) is a photochromic compound that undergoes *cis-trans* isomerization by ultraviolet (UV) - visible (VIS) light irradiation reversibly. Previously we have demonstrated that the binding of the CaM to the target peptide is controlled by the isomerization of PAM. PAM was incorporated into CaM mutants that have a single reactive cysteine residue. The binding of PAM-CaM (N60C), PAM-CaM (D64C) and PAM-CaM (M124C) to M13-YFP were apparently photo-controlled by UV-VIS light irradiation reversibly at the appropriate Ca^{2+} concentration. In the present study, we have tried to photo-control the function of myosin Va using the PAM-CaM by UV-VIS light irradiation reversibly. The part of endogenous CaM of myosin Va heavy meromyosin (M5aHMM) was substituted by exogenous PAM-CaM. The M5aHMM substituted by PAM-CaM (M5aHMM/PAM-CaM) showed normal range of actin-activated ATPase activity. Currently, we are examining to photo-control the actin-activated ATPase activity of M5aHMM/PAM-CaM in the presence of exogenous GTD.

713-Pos Board B592
Modification Of Loop 1 Affects The Nucleotide-Binding Properties Of Myo1c, The Adaptation Motor In The Inner Ear
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Myo1c is a ubiquitously expressed mammalian class I myosin that serves as a component of the hair cell's adaptation-motor complex in the inner ear. We have recently shown that a truncated form of Myo1c consisting of the motor domain and a single IQ domain, $Myo1c^{1IQ}$, has kinetic properties similar to full-length Myo1c (Adamek et al, 2008). We also showed that the ATPase cycle of Myo1c shows a unique response to Ca^{2+}, inhibiting the ATP hydrolysis step 7-fold and accelerating ADP release by 10-fold. Here we probed the role of loop 1, a flexible loop near the nucleotide-binding region, in defining the properties of Myo1c by creating six chimeras. We found that replacement of the charged residues in loop 1 with alanines or the whole loop with a series of alanines did not alter the ATPase, transient kinetics properties and Ca^{2+}-sensitivity of $Myo1c^{1IQ}$. Substitution of loop 1 with that of the corresponding region from tonic smooth muscle myosin II ($Myo1c^{1IQ}$-tonic) or replacement with a single glycine ($Myo1c^{1IQ}$-G) accelerated ADP release 2-3-fold from A.M in Ca^{2+}, whereas substitution with loop 1 from phasic muscle myosin II ($Myo1c^{1IQ}$-phasic) accelerated ADP release 35-fold. $Myo1c^{1IQ}$-tonic translocated actin in vitro twice as fast as wild type and $Myo1c^{1IQ}$-G 3-fold faster. The changes induced in Myo1c showed no resemblance to the behaviour of the loop donor myosins or to the changes observed with similar Myo1b chimeras (Clark et al, 2005).

714-Pos Board B593
Mechanics of myosin V near stall
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Motor proteins of the myosin family are driving many types of cellular motility. Functions are diverse, ranging from muscle contraction to endocytosis, cell locomotion, intracellular transport or signal transduction in hearing. Recent structural, kinetic and single molecule mechanical studies however revealed that the basic mechanisms of chemo-mechanical energy transduction are shared amongst myosin motors. This includes a working stroke in two phases coupled to the release of Pi and ADP and strain dependence of ADP release. Many details of the basic mechanism still remain unclear, including the effect of stall forces on the mechanics of a single motor head. Here we have used a single-headed myosin V construct (6 IQ) to investigate whether the conformational change associated with the working stroke can be reversed at high loads. We used optical tweezers to apply forces near stall for the processively moving dimeric motor (>2pN). We observed backstrokes of ~15nm, consistent with a reversal of the main conformational change of a single myosin V motor head. The dwell times of backstrokes were dependent on load. Implications of these findings for processive movement of the native, dimeric motor are discussed.
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Force Dependence of a Myo1b Truncation Mutant
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Myosin-Is are the single-headed members of the myosin superfamily that associate directly with cell membranes and play roles in regulating membrane dynamics. We previously characterized the force dependence of the widely expressed myosin-I isoform, myo1b, using an optical trap and a novel isometric force clamp. This myo1b isoform, which contains five IQ-motifs, is highly strain sensitive, with forces of < 2 pN decreasing the rate of actin detachment > 75 fold. We estimated the distance parameter (distance to the