polarization microscopy to determine the mobility and orientation of the kinesin motor domains. We first investigated conditions mimicking a state when only one head is bound to the microtubule and the other one is tethered. For this we made heterodimeric constructs with impaired microtubule binding in one head. Our results indicate that the tethered head is very mobile. We then investigated the orientation of the head domains in homodimeric constructs moving processively at saturating or limiting [ATP]. At saturating [ATP] both motor domains are well oriented relative to the microtubule but at limiting [ATP] there is an increase in mobility. This result indicates that before ATP-binding one motor domain is mobile.

693-Pos Board B572
Cooperative Movement Of Wild-type Kinesin And Velocity-deficient Mutants
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In the classic sliding filament assay, the molecular motor kinesin exhibits processive movement on microtubules with a velocity that is invariant over a large range of motor concentrations. This indicates that kinesin motors move synchronously at high density, but studies examining the motility of 2–3 kinesin motors have shown a surprising lack of synchronization. These results together led us to believe that kinesin motors under high density conditions can pull one another off the microtubule track, accelerating dissociation. Using a computational model, we can demonstrate that this would enable synchronization of MT movement without complete motor to motor synchronization. To test this experimentally, we combined kinesin dimers containing a mutation in the neck-linker (termed VKN) that elicits a 3-fold reduction in velocity when compared to wild-type motor (5.4μm/min vs. 16.2 μm/min) with wild type motors in the sliding filament assay. No significant amount of microtubule buckling was observed for any mixture of wild-type and mutant motors; even at limiting dilutions, and speckled microtubules moved at the same velocities throughout their length, indicating that the motors behave cooperatively, coordinating their movement through a shared interaction with the microtubule. We plan to examine whether this cooperativity is positive (WT motors accelerating VKN mutant movement) or negative (VKN slowing down WT).

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Kinesin Chimera Protein Fused with Calmodulin as a Molecular Shuttle
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Recently attention is focused on the application of molecular shuttles based on the motor protein kinesin and microtubule to drug delivery system (DDS) and lab-on-a-chip. In vivo, kinesin carries cargoes of biomolecular e.g., organelle which attach to the tail domain of kinesin. However, the molecular mechanism of the attaching and detaching of cargo is still obscure. Therefore, artificial binding systems have to be introduced on the molecular shuttle. Previously biotin-avidin and antigen-antibody reaction system have been used to attach kinesin to target cargoes. Although the systems are highly specific and tight, these are flawed as irreversible binding. In this study, we employed reversible cargo loading system using calmodulin (CaM) and M13 peptide for the molecular shuttle. We have designed kinesin K560 chimera protein fused with CaM at the C-terminal tail region of kinesin (K560-CaM). K560-CaM was successfully expressed by *E. coli*. expression system and purified. And M13 peptide fused with yellow fluorescent protein (M13-YFP) was also prepared as a target cargo. The ATPase activity and the microtubules gliding activity of K560-CaM were almost in the normal range of the kinesin wild type. The Ca^{2+} dependent reversible binding of K560-CaM and M13-YFP was observed with HPLC using size-exclusion column.

695-Pos Board B574
Evidence For Kinesin-1 Passing Obstacles On The Microtubule
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We have performed single molecule imaging with automated particle tracking and extensive statistical analysis of kinesin-1 motility in the presence of obstacles on microtubules in vitro. Minimal GFP-labeled wildtype kinesin predominantly detached immediately from the microtubule track in the presence of either motile or static (kinesin) roadblocks. Moreover, automated analysis allowed us to detect short pauses (<200 ms) within a processive run. For the case of motile obstacles we reasoned that the encountered obstacle unbinds quickly and allows further movement. But, surprisingly, also in the presence of static obstacles short pauses were detected, suggesting that kinesin is indeed able to 'pass' the obstacle. We propose that while processive kinesin passes an obstacle it may change protofilaments.

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Unique Conformation of Kinesin-1′s Neck Linker in the Nucleotide-free State
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Kinesin-1 is a motor protein that moves along microtubule in a hand-over-hand manner. The neck linker-docking model has been proposed to explain processive motility but has been questioned because its free energy change is too small to account for the force production. An alternative model proposes that the tethered head freely diffuses and is captured preferentially at the forward binding site, although the structural basis is not clear. To explain this mechanism, we recently proposed that the neck linker has to adapt a backward-pointing conformation to promote ADP release (Mori et al. Nature 2007). Previous cryo-electron microscopy (cryo-EM) studies (Rice et al. Nature 1999) showed distinct densities from the gold cluster attached to the distal end of the neck linker in the nucleotide-free state, but the direction of the neck linker extension was uncertain. To identify the conformation of the neck linker in the nucleotide-free state, we attached gold cluster to the middle of the neck linker and observed the gold-labeled kinesin motor heads on the microtubule using cryo-EM at <15 Å resolution. The gold-density showed ellipsoidal shape extended along the protofilament and these densities were located rearward to the beginning of the neck linker. This density distribution indicates that the mobility of the neck linker is restricted toward the minus-end of the microtubule presumably due to steric constraints, which is consistent with the recently solved nucleotide-free kinesin crystal structure (Makino et al. this meeting). This conformational preference of the neck linker after ADP release provides structural basis for the preferential binding of the tethered head: ADP release and tight microtubule-binding is prohibited at the trailing position because the neck linker is pulled forward and is permitted only at the leading position.

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Examination Of The Kinesin-1 Tail Interaction With Microtubules
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It is well known that the kinesin-1 tail domain contains a second microtubule binding-site independent of the binding-site located in the head domain (1,2), but the affinity and location of the tail-microtubule interaction on tubulin is not known. We have used fluorescence anisotropy to measure a Kd of kinesin-1 tail for microtubules in the submicromolar range, and we are currently performing experiments to determine the specific tubulin residues involved in forming this interaction. We hypothesize that the tail binding-site will include the extreme C-terminus of tubulin, which we will test by measuring the affinity of tail for tubulin with its C-terminal residues cleaved. Any effects that kinesin head domains or select microtubule-associated proteins may have on the affinity of the tail for microtubules will also be analyzed by fluorescence anisotropy, and the possibility for the tail to inhibit the ATPase activity of the head while bound to microtubules will be tested with an enzyme-coupled ATPase assay. These studies will test the hypothesis that the kinesin tail can fold over and simultaneously contact both the head domain and microtubules, producing a state in which both the ATPase activity of the head domain is inhibited and the kinesin molecule is anchored to the microtubule via its tail domain, as proposed by Dietrich et al (2).
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Location of Tethered Head of Kinesin-1 When Bound to a Microtubule
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A dimer of kinesin-1 motor domains (heads), each with a bound ADP, looses only one of its 2 ADP molecules on binding to a microtubule (MT). The first equivalent of one kinesin dimer per two tubulin heterodimers (one head per tubulin heterodimer) binds tightly (K_1), but additional kinesin binding can occur at higher kinesin concentration (K_2). Whether the heads are arranged as in L1,2 or D1,2 is controversial. These cases can be distinguished by two additional criteria as indicated in the figure. One is that binding of a second outer layer of kinesin on going from L1 to L2 should not result in additional release of ADP, whereas additional ADP will be released on going from D1 to D2. Initial