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).

694-Pos Board B573

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.

696-Pos Board B575

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.

697-Pos Board B576

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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2) Dietrich KA, Sindelar CV, Brewer P, Downing KH, Cremo CR, Rice SE. The kineisn-1 motor protein is regulated by a direct interaction of its head and tail. Proc. Nat. Ac. Sci. 105(26), 8938-8943 (2008).

698-Pos Board B577

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

experiments indicate that no additional ADP is released and this favors states L1 and L2. These conformations can also be distinguished by oxygen isotopic methods because kinesin in L2 should continue to catalyze rapid medium Pi=water exchange, while conformation D2 should not catalyze this exchange reaction because none of the ADP containing heads are in contact with the MT.
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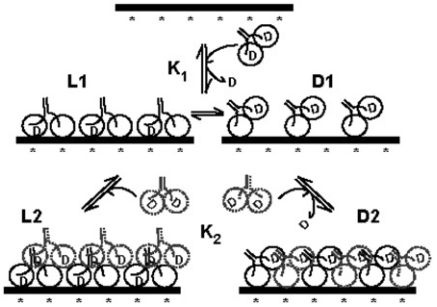

699-Pos Board B578
How Occasional Backstepping Can Speed Up A Processive Motor Protein
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The motor protein kinesin literally walks on two legs along the biopolymer microtubule as it hydrolyzes ATP for its fuel supply. The fraction of accidental backsteps that kinesin takes appears to be about seven orders of magnitude larger than what one would expect given the amount of free energy that ATP hydrolysis makes available. This is puzzling as more than a billion years of natural selection should have optimized the motor protein for its speed and efficiency.
I will point out how the stepping kinesin is a realization of Szilard's information-driven heat engine operating in reverse. A higher backstepping probability allows for more randomness in the walk and, consequently, for the production of more entropy. The production of entropy makes free energy available. With that free energy, the catalytic cycle of the kinesin can be speeded up. I will show quantitatively how the actually measured backstepping rate represents an optimum at which maximal net forward speed is achieved. This result suggests that kinesin uses backstepping as a source of energy and that natural selection has manipulated the backstepping rate to optimize kinesin's speed.

700-Pos Board B579
How Does Kinesin Walk And Coordinate Its Heads?
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Kinesin is a microtubule-associated motor protein which converts chemical energy (stored in ATP molecules) into mechanical work (by transporting cargo). The protein is a dimer and is believed to use its two identical motor domains (heads) alternatively to move along microtubules (MTs), reminiscent of "walking". Although over the past decade much has been learned about the structure and kinetics of the individual kinesin heads, how two of such heads can coordinate their motion during walking is still poorly understood. The most plausible hypothesis is that the heads communicate through a mechanical force mediated by the neck linkers (short peptide chains stretching between the heads and the dimeric coiled-coil tail). Indeed, during a catalytic cycle each neck linker can dock to and undock from its own head domain, indicating that the relative frequencies of these conformations and the rates of the corresponding transitions are strongly dependent on the position of the other head, providing a key to coordination.
By considering the two neck linkers as entropic springs and incorporating the most relevant kinetic and structural properties of the individual heads, we have constructed the first detailed, thermodynamically consistent model of dimeric kinesin that can (i) explain the cooperative motion of the heads during walking and (ii) reproduce much of the available experimental data (speed, dwell time distribution, randomness, processivity, hydrolysis rate, etc.) under a wide range of conditions (nucleotide concentrations, loading force, neck linker length and composition, etc.) simultaneously. Apart from revealing the mechanism by which kinesin operates, our model also allows us to look into the experimentally inaccessible details of the mechanochemical cycle and predict how certain changes in the protein would affect its motion.

701-Pos Board B580
Molecular Simulation Study of Kinesin: Coupling between ATPase Domain Conformational Change and Mechanical Stepping
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Conventional two-headed kinesin is a motor protein that moves unidirectionally by stepping in hand-over-hand manner (akin to human walking) along microtubule (MT) driven by ATP hydrolysis free energy. In the absence of MT, X-ray crystallography revealed primarily two conformations of the head, ATPase domain; ''T'' structure preferred with a bound ATP and ''D'' structure preferred with a bound ADP.
However, relations among the ATPase conformations, stepping motion, and type of bound nucleotide are still rather unclear. Here, we investigated the coupling mechanism between the enzyme structure and mechanical stepping. For this purpose, we performed molecular dynamics simulations with coarse-grained structure-based models. In particular, to investigate structural preference between T and D in front and rear heads bound on MT, we applied multiple-basin energy landscape model (Okazaki et al., 2006).
Through simulations, we found the followings. (1) Enzyme structure can regulate its affinity to MT by the difference in the contact surface area: ''T'' structure has higher affinity to MT than ''D'', which is consistent with experiments. (2) The internal-strain between two heads can regulate the ATPase structural preference: The rear head with forward-directed neck-linker prefer T structure, while the front head with backward-directed neck-linker prefer D structure.

702-Pos Board B581
The Kinesin-1 Tail Conformationally Restricts the Nucleotide Pocket
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The kinesin-1 motor protein transports intracellular cargo to the plus ends of microtubules. In cells, the majority of kinesin-1 exists in a regulated state that binds ADP tightly and has weak microtubule affinity. Regulation involves a direct interaction between the enzymatically active head domains and the regulatory tail domains. This interaction has been shown to inhibit both intrinsic and microtubule-stimulated ADP release, although the mechanism of inhibition is unknown. Here, we use electron paramagnetic resonance and fluorescence spectroscopy to study the kinesin-1 head-tail interaction. We show that the probe mobility of two different spin-labeled nucleotide analogs (2′3′-SLADP and SSL-NANDP) in the kinesin-1 nucleotide pocket is restricted upon addition of exogenous tail domains to truncated kinesin-1 heads. This tail-induced conformational restriction is distinct from the ''closing'' of Switch I that is observed when kinesin-1 binds microtubules. Unlike myosin V, the head-tail interaction is not nucleotide-dependent, and our data demonstrate that the interaction can occur in the absence of an intact gamma-phosphate sensor. Additionally, we find that the head-tail interaction does not stabilize Mg^{2+} in the nucleotide pocket. The conformational restriction also occurs when a tail construct containing a K922A point mutation is used. This mutation eliminates the tail's ability to inhibit ADP release, indicating that the tail does not inhibit nucleotide ejection from the pocket by simple steric hindrance. Our combined data support a mechanism in which the tail forms interactions around the nucleotide pocket and acts as a structural support, positioning the critical K922 residue to exert its inhibitory effect. By ruling out various other mechanisms, we propose that K922 may inhibit ADP release by interacting with the nucleotide alpha/beta-phosphates in a manner analogous to the arginine finger regulators of some G-proteins.

703-Pos Board B582
Kinesin Velocity Increases with the Number of Motors in Gliding Assays against a simple Viscoelastic Load
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In a classic paper, Howard, Hudspeth, and Vale[1] showed that the number of kinesin motors does not affect the velocity of gliding microtubules during motility assays. However, evidence is accumulating from our lab and others that the velocity of vesicle transport *in vivo* increases substantially if 2, 3, or 4 motors pull a single load. How can that be? To resolve this conflict, we performed upside-down kinesin motility assays with full-length Drosophila kinesin heavy chains working against viscous drag comparable to that experienced by moving vesicles in live cells. To do this, the viscosity of the medium was increased to approximately 1 Pa·s by adding 2 mg/mL of a stiff, high-MW polysaccharide here dubbed ''PolymerX''[2]. Also, a single polystyrene bead (d = 2 μm) was attached to the +end of the microtubule. In PolymerX, with a bead attached, 3 μm MTs moved at 150 nm/s, 10 μm MTs moved at 400 nm/s, and greater than 30 μm MTs moved at 700 nm/s, the control velocity. However, without a bead, the velocity of all MTs in PolymerX increased to 700 nm/s. Apparently, a bead-free MT can easily slither end-on through the mesh of PolymerX fibers, but the attached bead cannot. The observed increase in velocity with MT length most likely arises because the number of attached motors is directly proportional to MT length in gliding assays.