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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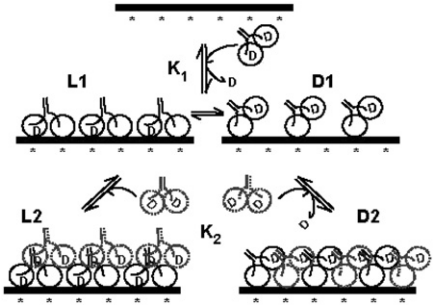

699-Pos Board B578
How Occasional Backstepping Can Speed Up A Processive Motor Protein
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The motor protein kinesin literally walks on two legs along the biopolymer microtubule as it hydrolyzes ATP for its fuel supply. The fraction of accidental backsteps 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.