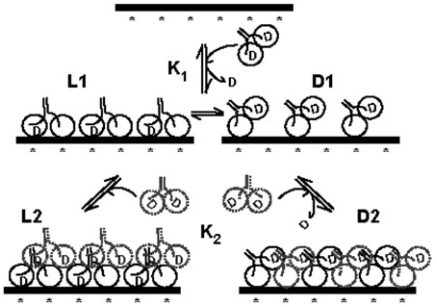
experiments indicate that no additional ADP is released and this favors states L1 and L2. These conformations can also be distinguished by oxygen isotopic methods because kinesin in L2 should continue to catalyze rapid medium Pi=water exchange, while conformation D2 should not catalyze this exchange reaction because none of the ADP containing heads are in contact with the MT.
Supported by NSF grant MCB-0615549.




**699-Pos Board B578**
**How Occasional Backstepping Can Speed Up A Processive Motor Protein**
**Martin Bier**.
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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?**
**Imre Derenyi**, Andras Czovek, Gergely J. Szollosi.
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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**
**Ryo Kanada**, Kei-ichi Okazaki, Shoji Takada.
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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**
**George Holzwarth**, Jason Gagliano, Matthew Walb, Jed C. Macosko.
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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.

Support from the NIH NS053493 and the Dreyfus Foundation is gratefully acknowledged.
[1] Howard, Hudspeth, and Vale, Nature 342, 154–158 (1989).
[2] The Brownian motion of 2 μm beads in dilute PolymerX solutions and of 0.2–2 μm unattached vesicles in live cells, when analyzed by the Generalized Stokes-Einstein method, show similar G′ and G″ in the two environments.

**704-Pos Board B583**
**Quantum-dot Assisted Characterization Of Helical Motor Paths On Microtubules**
**Bert Nitzsche**, Felix Ruhnow, Stefan Diez.
Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.
Advanced techniques in single-molecule optical microscopy have contributed greatly to our current view on the dynamics of motor proteins. While so far most studies have been limited to the 2-D imaging on a CCD-camera chip, a complete understanding of motor protein function requires insight in how motor proteins move in 3-D on the lattice of cytoskeletal filaments.
Here, we report a novel and versatile method to study the interactions of motor proteins with cytoskeletal filaments in 3-D with nanometer accuracy. We sparsely label reconstituted microtubules with quantum dots and use fluorescence microscopy to image their longitudinal and rotational movement over reflective silicon surfaces coated with motor proteins. We determine the 2-D xy-positions of the QDs with sub-pixel accuracy by nanometer tracking and combine this data with simultaneous height measurements based on fluorescence-interference contrast microscopy. We use this technique (i) to investigate the stability of the paths of cooperating processive kinesin-1 motors and (ii) to study the asymmetry in the powerstrokes of non-processive microtubule motors.

**705-Pos Board B584**
**Diffusive Movement Of A Processive Kinesin On Microtubules**
**Hailong Lu**, Yusuf M. Ali, Carol S. Bookwalter, David M. Warshaw, Kathleen M. Trybus.
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Conventional kinesin-1 is a processive motor protein that moves unidirectionally on microtubules. We found that when full-length kinesin containing a HIS tag at its C-terminus is bound to an anti-HIS Quantum dot (Qdot), it shows diffusive movement on microtubules in the presence of either ATP or ADP. Diffusive behavior was first described for the depolymerizing kinesin-13, MCAK (Helenius et al., 2006). When bound to a carboxylated Qdot, the same kinesin construct moves processively in the presence of ATP, but does not interact with microtubules in ADP. Further investigation with a truncated construct lacking the last 75 amino acids (kinesin-ΔC) showed both unidirectional and diffusive movement on microtubules in solutions containing a mixture of ADP and ATP. The diffusion constant depends on the concentration of ADP/ATP. When tested in solutions containing only ADP, kinesin-ΔC shows purely diffusive movement. We interpret these data to imply that kinesin-1 diffuses on microtubules when it is in the inactive, folded conformation, and it moves processively when in its active, extended conformation. We speculate that in the folded state, kinesin with bound ADP retains a relatively high binding affinity for microtubules compared to extended kinesin, thus allowing it to diffuse.

**706-Pos Board B585**
**Alternating Site Mechanism Of Kinesin-1 Characterized By Single-molecule FRET Of Fluorescent ATP Analogues**
**Guenther Woehlke**[1], Sander Verbrugge[2], Bettina Ebbing[1], Erwin Peterman[2].
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Kinesin-1 motor proteins move along microtubules in repetitive steps of 8 nm at the expense of ATP. To determine nucleotide dwell times during these processive runs, we are using here a FRET method at the single-molecule level that detects nucleotide binding to kinesin motor heads. We show that the fluorescent ATP analogue used produces processive motility with kinetic parameters altered less than two and a half-fold compared to normal ATP. Using our confocal fluorescence kinesin motility assay, we obtain fluorescence intensity time traces that are analyzed using autocorrelation techniques, yielding a time resolution of about a millisecond for the intensity fluctuations due to fluorescent ATP binding and release. To compare these experimental autocorrelation curves to kinetic models, we use Monte-Carlo simulations. We find that the experimental data can only be described satisfactory on the basis of models assuming an alternating site mechanism, thus supporting the view that kinesin's two motor domains hydrolyze ATP and step in a sequential way.

**707-Pos Board B586**
**Expression and Characterization of Novel Rice Kinesin E15**
**Masako Tsuchida**[1], Masafumi D. Yamada[2], Kazunori Kondo[2], Shinsaku Maruta[1].
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Kinesin is an ATP driven motor protein that moves along microtubules. Kinesin plays important physiological roles in intracellular transport, mitosis and meiosis, control of microtubule dynamics, and signal transduction. Kinesin species derived from vertebrates have been well characterized. In contrast, only a few kinesis have been characterized in plants. E15 is one of the kinesin encoded on rice genome. E15 has COOH-terminal motor domain and exhibits a high homology with the kinesin-14 family in *Arabidopsis thaliana*. However, this kinesin is not similar to other kinesin -14 family kinesins derived from animal, e.g., DmNcd, ScKar3, and CeKlp. Consequently, kinesin E15 may be plant-specific kinesin. In this study, we expressed the motor domain of a novel rice plant-specific kinesin, E15, in *Escherichia coli*. and studied its enzymatic characteristics and compared with other related kinesins. Molecular weight of the E15 motor domain was 37.6 kD. The MT-dependent ATPase activity was higher and the affinity for MT was weaker than rice kinesin K16 that we have previously reported. The optimum pH was pH 6.0–6.5, which is similar to K16. Interaction of E15 with fluorescent ATP analogues was also studied for the kinetic characterization. E15 showed weaker affinity for nucleotide than other kinesins. Currently, we are preparing E15 dimer for the motility assay.

**708-Pos Board B587**
**Kinetic characterization of the Rice Kinesins using Fluorescent-ATP Analogue**
**Nozomi Umezu**[1], Yuko Kubo[1], Kazunori Kondo[2], Toshiaki Mitsui[3], Shinsaku Maruta[1].
[1]Division of Bioinformatics, Graduate School of Engineering, Soka University, Tokyo, Japan, [2]Depertment of Bioinformatics, Faculty of Engineering, Soka University, Tokyo, Japan, [3]Graduate School of Science and Technology, Niigata University, Niigata, Japan.
Kinesin is an ATP driven motor protein that plays important physiological roles in intracellular transport, mitosis and meiosis, control of microtubule dynamics and signal transduction. Kinesins derived from vertebrate have been well studied on their characterization. However, not so many studies for kinesins of plants have been done yet. Previously, we have expressed the novel rice kinesin K16 by *E.coli*. Biochemical and crystallographic studies of the K16 motor domain demonstrated that K16 has very unique properties and conformation, which may reflect the plant specific physiological role. We have also succeeded to express other several rice kinesins. In this study, we focused on rice specific kinesins D04, L05, N14 and O12. The kinesin motor domains of D04 and L05 are found at the N-terminal. In our preliminary study, D04 and L05 belong to kinesin-4 sub family and kinesin-7 (CENP-E) sub family, respectively. On the other hand, N14 and O12 are the C-terminal motor domain. N14 and O12 belong to kinesin-14 family. Kinetic characterizations of these kinesin motor domains were studied using fluorescent ATP analogue, NBD-ATP. The binding of NBD-ATP to the ATPase site and release from the site were monitored by the change of fluorescence intensity. The kinetic parameters of rice kinesins were compared with other related kinesins. The kinetic parameters of rice kinesins were apparently different from that of conventional kinesin.

**709-Pos Board B588**
**Analysis of Crystal Structure and Solution Structure of the Motor Domain of Rice Kinesin K16**
**Shinsaku Maruta**[1], Keiko Tanaka[1], Yuko Kubo[1], Toshiaki Mitsui[2], Zui Fujimoto[3].
[1]Graduate School of Engineering, Soka University, Hachioji, Tokyo, Japan, [2]Niigata University, Graduate School of Science and Technology, Niigata, Japan, [3]Department of Biochemistry, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan.
The novel rice plant specific kinesin K16 has several unique enzymatic characteristics comparing with conventional kinesin. The most interesting property is that the ADP-free K16 motor domain is very stable, contrast to conventional kinesin that is very labile in ADP-free state. Recently, we have determined crystal structure of the novel rice kinesin K16 motor domain (K16MD) in complex with MgADP at 2.5A resolution. The overall structure of the K16MD is similar to that of conventional kinesin motor domains, as expected from the high similarity of amino acid sequence (43.2 %). However, the neck-linker of the ADP bound K16 motor domain showed an ordered conformation in a position quite different from that observed in conventional kinesin, which may reflect the unique enzymatic characteristics of rice kinesin K16. In the present study, we analyzed the inner structure of the K16 motor domain in detail and compared the structure with Eg5 and other related kinesins. It has been revealed that K16MD does not have interaction of amino acids side chains, which stabilizes the docking conformation of neck-linker. We have also analyzed the conformation of neck-linker in the solution using the K16 by FRET. Motor domain