microtubules, arranged with their dynamic "plus" ends facing the cell tips and their "minus" ends overlapping at the cell middle. Although the main protein factors involved in interphase microtubule organization have been identified, an understanding of how their collective interaction with microtubules leads to the organization and structures observed in vivo is lacking. We present a physical model of microtubule dynamics that aims to provide a quantitative description of the self-organization process. First, we solve equations for the microtubule length distribution in steady-state, taking into account the way that a limited tubulin pool affects the nucleation, growth and shrinkage of microtubules. Then we incorporate passive and active crosslinkers (the bundling factor Ase1 and molecular motor Klp2) and investigate the formation of IMA structures. Analytical results are complemented by a 3D stochastic simulation.

2603-Pos Board B573

Mechanical properties of a complete microtubule from all-atom Molecular Dynamics simulation

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Microtubules (MTs) are the largest type of cytoskeletal filament, and are essential in processes ranging from mitosis and meiosis to flaggelar motility. Many of these functions depend critically on the elastic properties of the MT, but the axial Young's and shear moduli have not been directly measured in experiments, which have instead measured flexural rigidity or radial elastic properties. Molecular Dynamics (MD) can reveal mechanical characteristics of biopolymers inaccessible to experiment, as well as the microscopic mechanisms underlying them, on the single-molecule level. However, while the atomic structures of alpha- and beta-tubulin have been solved, the only published structures of a complete MT are cryo-electron microscopy (cryo-EM) maps far from atomic resolution. To build our all-atom model, we used a 3-D energy potential based on a cryo-EM map as a target for the crystallographic tubulin dimer structure. By applying forces derived from this potential in an MD simulation, tubulin was made to adopt an MT conformation, yielding an all-atom model of a complete MT. Utilizing periodic boundary conditions and custom anisotropic pressure control, we could simulate the stretching and compression of an effectively infinite MT, while a force script was used to apply shear stress, thereby allowing individual determination of the elastic moduli. This work demonstrates the utility of Molecular Dynamics for determining the elastic properties of biological filaments despite the lack of a crystallized filament, opening the door to the study of other biopolymers.

2604-Pos Board B574

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Microtubule is filamentous structure supporting intracellular transportation as well as many other cell functions including the mitosis and axonemal motility. Since the function is closely related with the structural basis, it is crucial to understand the detailed properties of structure with nm resolution. Although the molecular structure of tubulin has been revealed (Löwe et al, 2001), our interest here is in the molecular flexibility and mobility in situ in functioning microtubules under physiological conditions. To know such dynamic features of microtubules, X-ray fiber diffraction analysis would be one of the most powerful tools. In the present study, we used our new method (2386-Pos/B601, Biophys. Meeting 2008) to complete quick aligning of taxol-stabilized porcine-brain microtubules (T240-B, Cytoskeleton, Denver) and small-angle X-ray diffractions were observed at the BL45XU beam line of SPring8 (wavelength 0.09 nm, camera length 1-2 m, 23-25°C). With our new aligning method, quick aligning within 5 s was accomplished and the observed angular deviation estimated from spreading of equatorial signals was <5 degrees. The present method is more advantageous for quick structural analys under physiological conditions being compared with previous aligning techniques (Mandelkow et al., 1977; Bras et al., 1998). We observed clear layer line diffraction signals of 4.1 and 2.0 nm representing the longitudinal regularity (meridional diffractions) of tubulin units. Almost the same layer line signals reflecting the helical arrangement of tubulin were also obtained in the axonemes of sea-urchin sperm flagellar observed exactly under the same flow-aligning conditions. For the equatorial signals, we obtained 0.051+0.001(J01), 0.86+0.002(J02), 0.13+0.003(J03) and 0.17+0.003(J13) nm-1 respectively. By comparing these data with those by the simulation of diffraction pattern of 13-protofilaments with the 3-start lattice arrangement of globular tubulin (data not shown), we estimated the diameter of microtubules (to the center of tubulin) to be 12.5+0.3 nm (n=4).

2605-Pos Board B575

The Microscopic Origins of Rheology of Microtubule Solutions and Actin-Micotubule Composites

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We have investigated the viscoelastic properties of microtubule solutions and composite networks of microtubules and F-actin using multiparticle tracking one- and two-point microrheology. The viscoelastic properties of microtubules as reported from two-point microrheology agree with the macroscopic measurement at high frequencies, but show a discrepancy at low frequencies, at time scales on the order of a second. A composite of F-actin and microtubules has viscoelastic behavior between that of F-actin and pure microtubules. We also show that the Poisson ratio of the composite, measured by the length-scale dependent two-point microrheology, is robustly smaller than that of the F-actin network alone, suggestive of a compressibility induced upon the addition of microtubules to the F-actin network.

Microtubular Motors II

2606-Pos Board B576

Elucidation Of Structural States Of Dimeric Motor Domain Of Dynein Using Cys-light Construct

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Cytoplasmic dynein is a motor protein that moves unidirectionally along a microtubule utilizing energy released by ATP hydrolysis. Introduction of probes such as fluorescent dyes that report structural changes and protein-protein interactions at particular locations will help to elucidate the molecular mechanism of force generation by dynein. For the site-directed labeling, we have replaced potentially reactive cys residues in the motor domain of Dictyostelium cytoplasmic dynein (the 380kDa fragment) with other amino acid residues without much affecting its motor activities. By using this cys-light dynein, we can insert a reactive cys residue in a specific, pre-selected location and selectively label the newly introduced cys residue with a fluorescent dye. To test the usefulness of this cys-light dynein, we introduced a reactive cys residue at the stalk head or at the stalk base of the dynein motor domain and then labeled it with Cy3 or Cy5. Introduction of the reactive cys residue and the subsequent Cy3/Cy5 labeling did not significantly affect microtubule-activated ATPase activity of cys-light dynein, suggesting the successful Cy3/Cy5 labeling of the stalk head or the stalk base. We then dimerized these Cy3 and Cy5 labeled cys-light dyneins by using a hetero-dimerizer to examine if the two stalks align closely in the dimeric dynein motor domain. The FRET measurements between the Cy3 and Cy5 labels showed that the two stalks actually stay closely in the dimer.

2607-Pos Board B577

How Does The Dimeric Cytoplasmic Dynein Processively Walk on a Microtubule?

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Cytoplasmic dynein is a two-headed molecular motor, which can take hundreds steps along a microtubule (MT). Although the mechanism of this processive motion remains poorly understood, it is generally assumed that each of the two heads alternatively produces force on MT to move forward. To elucidate the mechanism of this processive motion, we expressed the hetero-dimeric construct of dynein motor domain, in which one domain completely lost its ATP-binding activity due to the K/T mutation in the Walker A motif in its AAA1 module (P1T mutation). Our single-molecule motility assays showed that the hetero-dimer of the wild type and the P1T mutant (Wild/P1T) moved processively on MT with its velocity approximately half of that of the wild-type homo-dimer. Because one head of the Wild/P1T hetero-dimer cannot bind ATP, its processive motion suggests that the ''chemical gating'' is not necessarily required for the processive stepping, but some type of ''mechanical gating'' may be responsible for it. We then examined if the intramolecular tension through the tail domain linking the two motor domains is responsible for this ''mechanical gating''. We inserted a Gly-rich flexible linker with 20 or 40 residues between the tail domain and the hetero-dimerizer to reduce the tension. Unexpectedly, the Wild/P1T hetero-dimer with the flexible linker moved processively; their run length and velocity were similar to those of the hetero-dimer without the flexible linker. These

results suggest that the tension through the tail domain does not play a critical role in the processive motion. The direct interaction of the two AAA rings in the motor domain may be responsible for the ''mechanical gating'' to sustain alternative steps of the two motor domains on MT.

2608-Pos Board B578

In Vitro Reconstitution of Dynamic, ER-like, Nanotubular Networks, and of Small, Tubulo-Vesicular Transport Entities by Interactions of Cytoplasmic Dynein and Spectrin with Liposomes

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Cells contain intricate networks of membrane tubes of nanoscale dimensions, such as the endoplasmic reticulum (ER). Much smaller tubular entities, derived by extraction from donor compartments (e.g., those emerging from recycling endosomes or the trans-Golgi network) or generated by vesicle fusion (e.g., the ER-to-Golgi transport units), function in intracellular transport. Different mechanisms are thought to underlie the morphogenesis of the complex, tubular ER network, and the formation of the small tubular transport entities that travel along microtubules. Here, we show that the molecular machinery that powers retrograde vesicle motility in neurons can interact with membranes to generate these different types of tubes. We reconstituted in vitro elaborate networks of interconnected membrane tubes (with ER-characteristic ring closures and three-way junctions), as well as freely moving, stable tubes and tubulo-vesicular clusters, from mixtures of the minus-end motor, cytoplasmic dynein, its regulatory complex, dynactin, the anchoring protein, spectrin, and liposomes containing acidic phospholipids, in the presence of microtubules and ATP. The tubulo-vesicular clusters contained trains of spherical liposomes attached to a small tube via elastic linkers, likely maintained together through a supravesicular spectrin meshwork that encompasses both the tube and the associated vesicles. Recruitment of dynein-dynactin and spectrin from the cytosol to liposomes was stimulated by phospholipase D-induced conversion of neutral phospholipids to acidic forms, and by activation of small GTPases. We conclude that similar mechanisms underlie the generation of ER-like tube networks and small tubular transport entities. Both may be generated and maintained by the action of soluble microtubule motor complexes and anchoring proteins, which bind to phospholipids, and do not require membrane proteins. Supported by March of Dimes grant 1-FY04-240 and NIH grant R01GM068596.

2609-Pos Board B579

Dynein Stepping Flexibility as a Mechanism for Optimal Trafficking in the Cell

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Single molecule experiments have revealed that even under conditions of no load, backward stepping constitutes about 20 percent of cytoplasmic dynein's steps. Sideward steps are also common, and the motor's step size distribution is very broad. Such stepping flexibility might allow dynein to efficiently navigate the crowded cellular environment and avoid obstacles. However, the high speed and processivity of the motor implies strong coordination of its two heads. The idea of head coordination through a direct physical interaction seems plausible based on structural considerations, but such a mechanism raises the question of how tight coordination and stepping flexibility are simultaneously accomplished. We use physical reasoning and mathematical modeling to explore mechanisms that optimize these two opposing motor properties.

2610-Pos Board B580

Drag-brake Mechanism Of A Spindle Motor kinesin-5

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In most eukaryotic systems, kinesin-5 motors are absolutely required for mitosis and meiosis, where they drive engagement and subsequent sliding apart of the antiparallel half-spindles, whilst antagonising and/or collaborating with other motors. It was previously demonstrated that the kinesin-5 motor efficiently slows down other motors such as kinesin-1 and Ncd, suggesting it can generate resistive force as well as motive force (Crevel, I.M., Alonso, M.C., and Cross, R.A. 2004. Curr. Biol. 14, R411-412 and Tao, L., Mogilner, A., Civelekoglu-Scholey, G., Wollman, R., Evans, J., Stahlberg, H., and Scholey, J.M. 2006. Curr. Biol. 16, 2293-2302). Subsequent in vivo work has born this out: depletion of kinesin-5 causes faster spindle extension in Caenorhabditis elegans (Saunders, A.M., Powers, J., Strome, S., and Saxton, W.M. 2007. Curr. Biol. 17, R453-454) and causes up to 5 times longer and much more branched axons in neurons (Myers, K.A., and Baas, P.W. 2007. J. Cell Biol. 178, 1081-1091). The molecular mechanisms of these dual functions are poorly understood. Using mutagenesis, we show here that we can increase or decrease the drag-force component, with reciprocal effects on microtubule sliding velocity. In particular, we report a microtubule-binding-deficient mutant of Xenopus Eg5 with substantially reduced drag that slides microtubules 50% faster than wild type. Another mutant, in the kinesin-5 neck linker, shows increased drag together with decreased velocity. Our results suggest that whilst strongly-bound to the microtubule, Eg5 crossbridges are tuned to divide their time between force-generating states that drive microtubule sliding, and force-holding states that resist sudden or over-rapid microtubule sliding in cells.

2611-Pos Board B581

Millisecond Time-lapsed Monitoring of ATP Hydrolysis by Human Eg5 Kinesin: Real-time Dynamics of Conformation and Chemistry *in vitro*

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The hydrolysis of ATP is one of the key chemical reactions in life. Its mechanistic details in biological systems have been a challenge to unravel, as chemical changes in proteins during ATP hydrolysis go hand-in-hand with a series of large-scale conformational alterations. Monitoring the dynamics of chemistry and structure has been experimentally intractable, and requires application of novel methods. Herein, we present time-lapsed monitoring of an *in vitro* ATP hydrolysis reaction by a kinesin motor protein with 180-millisecond time resolution. Kinesin proteins are one of three major categories of motor proteins, capable of using ATP hydrolysis to power force generation and subsequent movement along cytoskeletal elements in cells. Our model protein system is human Eg5 (HsEg5), a Kinesin-5 motor protein participating in the spindle pole segregation during mitosis in higher eukaryotes. Truncated to its monomeric motor domain, we purified active HsEg5 and confirmed its ability to hydrolyze ATP. To monitor dynamic structural and chemical changes during ATP hydrolysis *concomitantly*, we used difference Fourier-transform infrared (FTIR) spectroscopy with HsEg5 kinesin samples, triggering initiation of the ATPase reaction by UV-photolysis of caged ATP. Interpretation of these biological data was guided by model compound data on ATP derivatives. The time-lapse data highlighted resolution of two distinct sets of conformational changes: a series of HsEg5 structural changes that precedes ATP hydrolysis and a set of structural alterations that occurs upon onset of ATP hydrolysis. Thus, we conclude that we have the first direct observation of dynamic conformational changes caused by the ATP binding in any kinesin motor protein. Secondly, the structural modifications that occur HsEg5 when ATP hydrolysis is initiated are different than those in the substrate-binding step.

2612-Pos Board B582

Crystal Structure of HsEg5 in Complex with S-trityl-L-cysteine

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Eg5 is a motor kinesin involved in the formation of the bipolar mitotic spindle which is essential for the completion of mitosis. The discovery of a class of allosteric Eg5 inhibitors has raised the possibility of a novel approach for the treatment of cancer. Monastrol and S-trityl-$_L$-cysteine (STC) are two well-characterized inhibitors of Eg5 known to prevent ADP release by the motor domain, with the latter compound being a more potent inhibitor of Eg5 than the former. We have determined the 2.5 Å resolution crystal structure of the Eg5 motor domain in complex with STC and Mg•ADP. STC interacts with Eg5 *via* a pocket formed by helices α2, α3 and loop L5, and induces conformational changes within the Eg5 motor domain similar to those seen with bound monastrol. The necklinker is positioned in the ''docked'' conformation seen in the monastrol-bound Eg5 structures, and the switch I and II regions also adopt conformations similar to those observed for bound monastrol. Moreover, STC contacts with Eg5 differ from those seen in monastrol-bound crystal structures. Monastrol contacts with Eg5 are largely mediated by nonpolar surfaces of the drug. During STC binding, contact between the motor domain and polar surfaces of the drug is increased in relation to that of monastrol by over 20 Å^2. STC binding occludes approximately 80 Å^2 more of the Eg5 surface from solvent access than does monastrol. Site-directed mutagenesis and coupled biochemical assays monitoring ATP hydrolysis were used to examine whether these residues involved in polar interactions between STC and Eg5, and residues altered in solvent accessibility upon STC binding to Eg5,