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Neuronal microtubules (MTs) are 25 nm protein nanotubes used as tracks for intracellular trafficking of biomolecules, for example, those involved in transmitting signals between neurons. Distinct members of MAP tau isoforms regulate microtubule assembly and stabilization. Altered tau-MT interactions lead to MT depolymerization and tau tangles, which are implicated in a large number of neurodegenerative diseases. We describe our recent findings about the effect of human wild type MAP tau on interprotofilament and intermicrotubule interactions, by using synchrotron small angle x-ray scattering. Supported by DOE DE-FG02-06ER46314, NSF DMR-0503347, NIH GM-59288, NIHI RO1-NS35010 and NS13560.

2598-Pos Board B568

Comparison of Microtubule Dynamics for A- and B-Lattice Geometries

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Accurate quantitative interpretation of experimental data and prediction of the effects of microtubule-targeted anti-mitotic drugs require a detailed model of the events that occur at microtubule ends. Before searching the large parameter space of a model with few constraints on lattice symmetry, binding site configuration, GTP-hydrolysis rate, and oligomerisation state of the associating and dissociating species, we performed an extensive, systematic investigation into the dynamics of a series of simplified models with significantly smaller parameter spaces. The models had regular A or B-lattice geometries, tightly coupled GTP-hydrolysis, and association-dissociation events involving the formation or breakage of just two lateral bonds. GTP-hydrolysis weakened the two lateral bonds to the β-tubulin subunit by 4.6 k_BT, in either a balanced (+2.3 k_BT each) or an unbalanced way (+4.6 k_BT for one and 0 for the other bond). Association rate constants were 1 $\mu M^{-1}s^{-1}$, and dissociation rates were thus dependent on the lateral bond energies. We observed the following:

1. Values for C_C (the concentration of free tubulin-GTP at which the net growth is zero) varied from 1.2 to 80 μM
2. All configurations showed discernable phases of growth (G) and shrinkage (S) around their C_C
3. Effective growth rates at C_C (average growth rate during the G-phase divided by the maximum attainable growth rate at that C_C) varied from less than 0.1 in most of the B-lattice geometries to 0.9 in the A-lattices with a balanced effect of hydrolysis
4. G-phase lifetimes were relatively short (10-15 s), and growth was significantly more uniform in the balanced A-lattice geometries, compared with those in the unbalanced geometries (lifetimes > 100 s)

Thus, balanced A-lattice configurations support efficient growth on relatively unstable microtubule ends, whereas most other configurations grow less efficiently on more stable ends.

2599-Pos Board B569

Response of the Mitotic Spindle to Mechanical Force

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Cell division is an inherently mechanical process, from chromosome congression, error correction and segregation to furrow ingression. Although the molecules involved in cell division are becoming better characterized, surprisingly little is known about the underlying mechanical principles and interactions. The spindle is a complex assembly and its response to mechanical force should yield insight into its structure, the mechanisms governing its shape and size, and how forces are transmitted from the spindle apparatus to the chromosomes. Here, we develop an assay to mechanically flatten mitotic spindles in live mammalian cells and use fluorescence microscopy to monitor the response of the microtubule cytoskeleton and kinetochores. We show that, upon flattening, the spindle deforms asymmetrically: it widens rapidly as the kinetochore-microtubule bundles pivot around the poles, and lengthens slowly in a tubulin polymerization-dependent manner. Interestingly, spindle length can double reversibly under the mechanical perturbation, providing insight into spindle size determination. In addition, we find that kinetochore motion is robust to changes in spindle shape and size, and to forces resulting in drastic bends of kinetochore-microtubule bundles, suggesting that kinetochore motion is locally driven. Finally, the data point towards a framework where mechanical forces are locally transmitted and generated by the spindle and the method introduced provides a useful tool to probe mechanical interactions between spindle components.

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Challenges In Modeling Chromosome-driven Mitotic Spindle Formation

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A mitotic spindle is a regular structure within a cell, consisting of oriented microtubule fibers. It plays a fundamental role in chromosome separation during cell division. Forming a spindle pattern is a major structural step towards mitosis. We have developed a biophysical non-equilibrium thermodynamic model to describe in vitro chromosome driven spindle formation experiments in Xenopus extracts. Our modeling work, as well as the work of others such as Nédélec and collaborators, has shed considerable light on this process. Our modeling analysis has produced results that agree in several respects with experimental findings. We believe, however, that there are a number of challenges that must be addressed for spindle modeling to continue to be a useful tool for understanding this fundamental biological process. A biophysical model for spindle formation requires detailed biological hypotheses determining the behavior of key model elements. Current modeling work has shown some deficiencies in our understanding of particular problems. In particular, better biological hypotheses are needed to describe how molecular motors behave near the endpoints of microtubules and how those motors influence microtubule dynamic instability. We will detail what we believe are important problems needing better biological hypotheses. Accurate numerical modeling based on biophysical models of mitosis is challenging because the models must simultaneously represent thermal diffusion effects that happen in microseconds as well as spindle formation processes that take minutes or even hours. We will discuss our work on numerical algorithmic improvements that will greatly speed simulations without sacrificing biophysical model properties or numerical accuracy.

2601-Pos Board B571

Antimitotic agent alters MIP levels In breast cancer cells

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Progression through mitosis requires a balance of active microtubule-interacting proteins (MIPs) that stabilize and destabilize microtubules in mitotic spindles. Molecular interactions between MIPs and tubulin or microtubules are important for cell cycle progression. Insights into these interactions can contribute to understanding the mechanisms underlying cell cycle interference by antimitotic agents that halt cell cycle progression in mitosis. We examined the direct interaction of the antimitotic agent, vinblastine with tubulin and stathmin using AUC, in order to understand how changes in stathmin levels during the cell cycle might affect the cellular drug response. Vinblastine acts during G2/M phase of the cell cycle and reduces microtubule dynamics. At high doses it destabilizes microtubules in mitotic spindles. We found in vitro that stathmin reduces the potency of vinblastine. Vinblastine was found to compete for tubulin-stathmin oligomers, at the same time as it induced tubulin spiral formation. To extend these data to a cellular context, we investigated changes in intracelluar MIP levels in response to paclitaxel, an antimitotic agent known to stabilize microtubules at high concentrations. Using qRT-PCR we found that paclitaxel treatment of human breast cancer MCF7 cells leads to a significant reduction in MAP4 and stathmin mRNA levels. Interestingly, the levels return to pre-paclitaxel treatment levels after a 4-day drug washout, suggesting that paclitaxel alters transcript levels. We found that the ratios of MAP4/stathmin increased after or during drug treatment. These data suggest that changes in MIPs levels alter the cellular response to drugs. These results also suggest that disruption of the cell cycle by antimitotic agents can alter the relative amounts of MIPs and thus affect the balance needed for normal progression through the cell cycle. These results must be taken into account when modeling the cellular response to antimitotic drugs.

2602-Pos Board B572

Theoretical Description of Microtubule Dynamics in Fission Yeast During Interphase

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Fission yeast (S. pombe) is a unicellular organism with a characteristic cylindrical shape. Cell growth during interphase is strongly influenced by microtubule self-organization - a process that has been experimentally well characterised. The microtubules are organized in 3 to 4 bundles, called "interphase microtubule assemblies" (IMAs). Each IMA is composed of several

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

Dr

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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