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.

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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.

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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).

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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

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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.

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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