Microtubules, Their Motors and Associated Proteins I

1769-Pos Board B499
Tubulin Heterodimers Reversibly Dissociate with Moderate Kinetics as Demonstrated using Sedimentation Velocity Analytical Ultracentrifugation
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A number of studies have reported reversible αβ-tubulin heterodimer dissociation with Kd > 10^-8 M, with presumably rapid but undetermined kinetics. However, in a recent study an extremely tight association between subunits (Kd < 10^-10 M), as well as a slow dissociation rate of the monomers was reported (with a half time for dissociation > 3 hours). In this work we have revisited the thermodynamics and kinetics of rat brain tubulin (RBT) dissociation by taking advantage of sedimentation velocity analytical ultracentrifugation methodology (SV-AUC) developed in recent years. In particular, a combination of optical detection systems was used to cover a wide range of protein concentrations: pseudo-absorbance optics (pABS-AUC) for micro- and submicromolar concentrations, and the newly developed fluorescence optics (FDS-AUC) for nanomolar concentrations. SV-AUC data was modeled using SEDFIT software using the heteroassociation model A+B1AB to compute the limiting sedimentation coefficients of the dimer and monomer species as well as the equilibrium dissociation constant. In our experimental conditions, RBT heterodimer sediments with s20,w = 5.1 s, while the monomer species co-sediments with s20,w = 2.9 s. According to the heteroassociation model, the RBT heterodimer dissociates with Kd = 5.5 x 10^-8 M. From the analysis of sedimentation profiles we find the characteristic dissociation time constant to be on the order of hours. In conclusion, the αβ-tubulin heterodimer displays reversible dissociation, moderate Kd, and moderate dissociation kinetics.

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Studying the Structural Origins of Microtubule Dynamic Instability through Combining Computational Modeling and cryoEM
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Despite the importance of microtubule dynamic instability for their function in cell division and for the effect of anticancer drugs like taxol, understanding this macroscopic behavior at the structural level has been so far hindered by the limitations in cryoEM resolution available for this system. To gain mechanistic molecular understanding of the dynamic nature of microtubules, we have used real space refinement helical reconstruction to obtain cryoEM maps of microtubules in various ligand-bound states: GMPCP, (a non-hydrolyzable GTP analog), GDP, (dynamic microtubules), and GDP+taxol (drug-stabilized). These maps, ranging from 4.5 to 5 Angstrom resolution, represent the most detailed description of alpha and beta tubulin in the microtubule lattice to date. In order to analyze the differences between the maps, we performed molecular refinement using the software Rosetta guided by the EM density, as well as information from available crystal structures. With this new approach, we were successful in obtaining well-converged ensembles of models that fit their respective maps significantly better than they fit the maps of other ligand-bound states. We find that major differences between the GMPCPP and GDP-bound microtubules is a compression along the longitudinal axis, accompanied by subtle structural rearrangements within the asymmetric dimer. This compression is not observed when comparing the GMPCP and GDP+taxol bound structures, which are highly similar. The M-loop involved in lateral contacts between tubulins is modeled well in all of the maps by a short helical structure that is similar to that described recently in the zanpanolide bound crystal structure of Steinmetz and colleagues. More detailed structural analysis is underway and will be presented at the conference.

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Katanin Regulates Microtubule Dynamic Instability
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Microtubule length regulation is required for proper spindle length and positioning, cilia formation, and axonal outgrowth. For correct microtubule organization to occur, microtubule associated proteins (MAPs) bind to microtubules and control the dynamics. Despite much work on stabilizing MAPs affecting microtubule dynamics, few studies have investigate the role of destabilizing MAPs on microtubules. Katanin, a known destabilizing MAP and the first-discovered microtubule severing enzyme, is a AAA- enzyme that oligomerizes into hexamers and uses ATP hydrolysis to sever microtubules. We seek to measure the effects of katanin severing on microtubule dynamic instability in vitro, away from confounding cellular factors. We will use Total Internal Reflection Fluorescence (TIRF) microscopy on fluorescent microtubules and purified MAPs to reveal that katanin is unable to sever dynamic microtubules, and instead modulates microtubules by changing the rates of growth and depolymerization. Our experiments also indicate that katanin does not modify the catastrophe rate of microtubules. Further work is required to disclose how katanin may work with stabilizing MAPs to control microtubule length.

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Label-Free Observation of Single Microtubules by Means of SHG Microscopy
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The microtubule is a dynamic filamentous polymer involved in various important cell processes including cell division, cell migration and organelle transport. The dynamics of microtubules highly correlate with its structure. Despite of extensive studies, however, detecting detail structure of a single microtubule in living cell is still very challenging. Here we succeeded to visualize single microtubules by optical second-harmonic generation (SHG) microscopy which is one of powerful tools to characterize macroscopic structure of molecular assemblies in liquid. The technique allows us to detect nonlinear optical scattering signal arising from an electric polarization in a microtubule which is related to alignment of tubulins so that we can get information about structure of a microtubule without any labeling to sample. We demonstrate the results of SHG imaging of single microtubules in vitro using a home-built optical system. During the experiments, we found that photo-induced SHG (PISHG) signal from cover slips became dominant source of non-negligible background noise, and decreased signal-to-noise ratio. To suppress the PISHG, we used cover slips of highly-pure SiO2. As a result signal-to-noise ratio has been dramatically improved compared with the case we used borosilicate glasses, and we have successfully obtained clear images of microtubules. Furthermore, we have performed polarization-resolved measurements and obtained quantitative data which could be used to evaluate the structures of microtubules.

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Modeling the Microscopic to Macroscopic Dynamics of Actively Streaming Microtubule Suspensions
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Suspensions of cytoskeletal filaments driven by molecular motors in vitro are a new form of active soft matter. We developed a multi-scale theory of active microtubule suspensions driven by crosslinking motors. At short length scales we use Brownian dynamics simulation of rigid filaments with explicit mobile crosslinks to study the microscopic development of active stresses in this system, and find the novel observation that generation of destabilizing extensional stress occurs both for antiparallel and parallel filament pairs. We describe how extensional stress generation for parallel pairs occurs, in contrast to previous theoratical predictions that it must be zero. The microscopic simulations allow us to compute the key active stress parameter describing stress generation needed in our mesoscopic kinetic theory that describes the nonlinear dynamics and pattern formation in active microtubule suspensions, where the active stresses exerted upon the fluid can induce hydrodynamic instabilities and a large-scale streaming flow. When the suspension is bounded on a liquid-liquid interface, we show that the streaming microtubule nematic exhibits self-generation and annihilation of defects. Our results are similar to the experimental results of Sanchez et al. (2012).

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Microtubule Real-Time Microtubule Spool Formation in a PDMS Microfluidic Device
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The motor-driven self-assembly of microtubule rings and spools has been reported by several research groups and attributed to one of two potential mechanisms: pinning by inactive motors or mechanical strain from oligomer...