conformation that masks interactions of ligands with the head (Vh) and tail (Vt) domain. Upon activation, vinculin functions as a scaffold to regulate cellular events resulting in cell migration, cell survival and embryogenesis. Vinculin null cells display tumorigenic properties and mutation or loss of vinculin is associated with cardiac disease. The interaction between vinculin and actin plays a pivotal role in linking transmembrane receptors to the cytoskeleton, which, in turn, is important for controlling cellular cell morphology, force transmission and motility. Binding of F-actin to Vt causes a conformational change that induces formation of a cryptic dimer necessary for actin filament bundling. However, the conformational change that occurs and dimer that is formed is unknown. It is also unclear how vinculin recognizes PIP2, inserts into membranes and is regulated by this interaction. We have now obtained a sub-nanometer resolution reconstruction of the Vt/actin complex which sheds light on actin-induced conformational changes necessary for vinculin dimerization and actin filament bundling, and have integrated computational and experimental approaches to generate and test models for the actin-induced vinculin dimer and vinculin/PIP2 membrane interaction and assess their significance in vinculin function both in vitro and in cells.

2567-Plat
Towards a Model of the Tau-Tubulin Complex
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Tau is a microtubule-associated protein which functions to maintain microtubule stability as well as promote microtubule polymerization in the axons of neurons. Its self-association and deposition as neurofibrillary tangles is also one of the primary pathological features of Alzheimer’s disease and a broad array of other neurodegenerative disorders. We previously showed that altered interactions between tau and tubulin heterodimers are associated with impaired microtubule polymerization and that the interaction between tau and soluble tubulin may play an important role in both tau function and dysfunction. However, a detailed description of the tau-tubulin complex is lacking. This in part due to the challenges associated with characterizing this complex, in particular the highly dynamic character of the intrinsically disordered tau as well its ability to accelerate tubulin polymerization. Here, we studied the interaction between tau and tubulin heterodimers using fluorescence correlation spectroscopy (FCS) and acrylodan fluorescence which allow us to propose a detailed model of the complex. Our results provide insight into differential roles of the individual microtubule binding repeats in mediating the tau-tubulin interaction and further illuminate the mechanism by which tau promotes tubulin polymerization.

2568-Plat
Multiple Reversible Molecular Events at the Microtubule Tip Drive the Age-Dependent Microtubule Catastrophes
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A growing microtubule can undergo a catastrophe, a sudden stochastic switch to shortening. Catastrophe rate increases slowly during the first few minutes of microtubule elongation in vitro but then reaches a plateau, a phenomenon called microtubule “aging”. Two different models have been proposed previously to explain aging: one based on gradual accumulation of permanent wall defects and the other based on a progressive increase in tapering of the growing microtubule end. To examine these hypotheses we have used molecular dynamics approaches to simulate tubulin-tubulin interactions and the resulting evolution of the microtubule tip structure. Although the in silico microtubule does not accumulate any microtubule wall defects or show a monotonic increase of tip tapering, the model still exhibits realistic microtubule aging and the plateau of catastrophe rate. It also provides good quantitative description of other key experimental findings, including the effects of soluble tubulin concentration and sudden tubulin dilution. Soon after the initiation of tubulin assembly in this model, the average tip characteristics, such as the number of GTP tubulins and the extent of tip tapering, reach steady states. The tip, however, continues to fluctuate quickly and reversibly based on a large number of random conformational changes. Microtubule catastrophes result from rare coincidences of multiple short-lived molecular events, each of which does not precipitate a catastrophe on its own. These events promote fluctuations in tip composition and structure, most notably in the number of protofilament curls and lateral tubulin-tubulin contacts at the tip. We propose that microtubule aging is a property of the complex stochastic system, representing fluctuating microtubule tip, to evolve slowly and asymptotically towards the steady-state levels of occupancy of these rare configurations.

2569-Plat
Intrinsically Disordered Map Tau Mediates both Short-Range Attraction and Long-Range Repulsion between Microtubules
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Neurofibrillary tangles, the hallmark intracellular symptom of Alzheimer’s disease, are the aggregated form of hyperphosphorylated tau. To give insight on the diseased state, the complete understanding of the healthy, physiological function of tau is necessary but difficult due to the intrinsically disordered nature of the protein. All isoforms of tau are often found bound to microtubule surfaces, but the N-terminal tail (with isoform-dependent lengths) is thought to project off the microtubule surface and interact with the N-terminal tail of tau proteins on other microtubule surfaces. The forces effected by tau-coated microtubules in the physiological composition range were investigated using small-angle X-ray scattering (SAXS). Samples were osmotically stressed using depletants to replicate the packed axoplasmic environment in which tau and microtubules are found. In going from no coverage of tau to high coverage (1:10 tau-to-tubulin molar ratio, near the physiological limit) isoforms with longer N-terminal tails sterically stabilized microtubules, preventing bundling up to 10,000 Pa (in comparison, microtubule bundling at 1,000 Pa in absence of tau). In striking contrast, coverage by tau isoforms with the shortest N-terminal tails did not change the bundling pressure (≤ 1,000 Pa), even at high coverages (1:10 tau-to-tubulin molar ratio). Surprisingly, in the high-pressure limit, the polyampholytic nature of tau brought about a coverage-dependent and irreversible electrostatic attraction between microtubules. The unique manifestation of both short-range electrostatic attraction and long-range steric repulsion by tau on microtubules gives insight to both the physiological function of tau and the design of biologically-inspired materials with multiple interaction motifs.

2570-Plat
Eh1: A Highly Dynamic and Diffusive Microtubule +Tip-Tracking Protein
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We use the nonhydrolyzable GTP analogs GMPCPP and GTPγS to polymerize microtubules that recapitulate the plus-end binding behavior of EB1 along the entire length of the microtubule. Through the use of single-molecule TIRF imaging we find that EB1 is highly dynamic (with a sub-second characteristic binding lifetime) and continuously diffusive while bound to the microtubule. We measure the diffusion coefficient through linear fitting to mean-squared displacement of individually labeled proteins, and the binding lifetime by fitting a single exponential decay to the probability distribution of trajectory lifetimes. In agreement with measurements of other diffusive microtubule associating proteins, we find that the diffusion coefficient increases and the characteristic binding lifetime decreases with increasing ionic strength. We also find that the diffusion coefficient is sensitive to the choice of GTP analog: EB1 proteins bound to GTPγS polymerized microtubules have a diffusion coefficient half of that found with GMPCPP polymerized microtubules. To compare these single-molecule measurements to the bulk binding behavior of EB1, we use TIRF imaging to measure the intensity of microtubules coated with EB1-GFP as a function of EB1 concentration. We find that EB1 binding is cooperative and both the quantity of EB1 bound and the dissociation constant are sensitive to GTP analog and ionic concentration. The correlation between binding affinity and diffusion coefficient and the cooperative nature of EB1-microtubule binding leads to a decrease in diffusion coefficient with increasing EB1 concentration. Interestingly, we also find an increase in binding lifetime at high EB1 concentrations, consistent with attractive EB1-microtubule interactions driving the cooperativity.