1874-Pos
Highly Variable Microtubule Assembly Dynamics Reflect Near-Kilohertz Kinetics: Evidence Against Traditional Linear Growth Theory
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Microtubules are intracellular polymers that dynamically grow and shorten at their ends via the stochastic addition and loss of alpha-beta-tubulin heterodimers. The kinetics of tubulin assembly are central to the regulation of microtubule dynamics by microtubule-associated proteins and therapeutic drugs. Previously, rates of tubulin subunit exchange at the ends of growing microtubules have been estimated using a linear growth theory that assumes tubulin dissociation occurs at a constant rate regardless of the free tubulin concentration. However, by measuring the variance of microtubule assembly at the nanometer scale, we find that stochastic tubulin dynamics are an order of magnitude faster than previously estimated. This discrepancy is explained by molecular-level simulations showing that the tubulin dissociation rate during microtubule growth is not constant, but rather should increase with increasing tubulin concentration. This indirect effect is due to a concentration-dependent bias in simulated microtubule tip structure, as has been experimentally observed. Our analyses indicate that the published tubulin subunit addition and loss rates at growing microtubule ends *in vitro* have been consistently underestimated in the literature: the variance in the assembly rate *in vitro* is too high to be consistent with the previous low kinetic rate estimates, and we conclude that both tubulin addition and tubulin loss events occur on the millisecond time scale, far faster than the previously believed 10-1000 millisecond scale. More generally, we demonstrate that the fixed off rate originally used in the linear growth theory of Oosawa and assumed in most subsequent models, fails to describe the behavior of self-assembled polymers having both lateral and longitudinal bonding interactions between subunits.

1875-Pos
Filament Localization with Nanometer Accuracy
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Recent developments in optical microscopy and nanometer tracking of single fluorescent molecules (or alternative subresolution particles) have greatly enhanced our understanding of biomolecular processes in vivo and in vitro. In particular, fitting the intensity profiles of nanometer-sized objects to 2D-Gaussian models allows their two-dimensional localization with an accuracy in the one-nanometer range, primarily only limited by the number of photons collected. Here, we present a novel algorithm which adapts 2D-Gaussian models to precisely determine the contour, as well as the end points, of curved filaments whose structures are characterized by subresolution diameters and micrometer lengths. Utilizing surface-immobilized microtubules (diameter of 25 nm, densely labeled with fluorophores) we demonstrate positional accuracies of ~2 nm and ~15 nm when localizing the center line and the end points of the filament, respectively. We report on the application of the algorithm to determine (i) the dynamics of microtubule polymerization/depolymerization and (ii) the speed of microtubules gliding over motor-coated surfaces. Combined with methods to measure nanometer heights above substrate surfaces (such as fluorescence interference contrast or parallax), our algorithm - which is also readily applicable to fluorescently labeled actin or DNA/RNA filaments - presents a promising tool for optical 3D-nanometry.

1876-Pos
The Effect of Human Microtubule-Associated-Protein Tau and Ionic Strength on the Assembly Structure of Microtubules: Synchrotron X-Ray Scattering and Binding Assay Study
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Microtubules (MTs), a major component of the eukaryotic cytoskeleton, are 25 nm protein nanotubes with walls comprised of assembled protofilaments built from αβ heterodimeric tubulin. In neural cells, different isoforms of the microtubule-associated-protein (MAP) tau regulate tubulin assembly and MT stability. Using synchrotron small angle x-ray scattering (SAXS) and binding assay, we examine the effects of human MAP tau on the assembly structure of taxol-stabilized MTs. We find that tau regulates the distribution of protofilament numbers in MTs as reflected in the observed increase in the average radius of MTs with increasing the tau/tubulin molar ratio. Further, we describe that tau-MT interactions are mediated to a large extent via electrostatic interactions: the binding affinity of tau to MTs is ionic strength dependent. Supported by DOE DE-FG02-06ER46314, NSF DMR-0803103, NIH NS35010, NIH NS13560. (Ref) M.C. Choi, S.C. Feinstein, and C.R. Safinya et al. *Biophys. J.* 97; 519 (2009).

1877-Pos
A Fluorescent GTP Analogue as a Single Molecule Fluorescence Label of Microtubules
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Microtubules are cytoskeletal polymers which play a role in cell division, cell mechanics, and intracellular transport. Dynamic studies of microtubule function make use of fluorescent labels via antibodies, paclitaxel, and direct attachment to the tubulin protein. However, these labels suffer from drawbacks such as transient labeling, occlusion of functional sites on the microtubule surface, or structural non-specificity. Here we report a new, complementary fluorescent labeling technique that avoids these drawbacks. A fluorescently modified GTP analogue is used to polymerize microtubules from tubulin dimers. This GTP analogue binds selectively to the exchangeable GTP-binding site (E-site) on the tubulin dimer, which is available only during polymerization. The E-site affinity of this GTP analogue is about 100 fold weaker than that of GTP. Because this labeling technique places a bright fluorophore at a defined location within the microtubule lattice, it may facilitate observations of microtubule dynamics with increased precision.

1878-Pos
Kinesin-Calmodulin Fusion Protein as a Molecular Shuttle and Marker for Plus End of Microtubule
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In the present study, we have demonstrated that the novel molecular shuttle with reversible cargo-loading system by using calmodulin (CaM) and M13 peptide. We designed a kinesin (K560) chimera protein with CaM fused at the C-terminal tail region of K560 (K560-CaM). K560-CaM was expressed using an Escherichia coli expression system and purified. We successfully observed that K560-CaM transported quantum dot-conjugated M13 peptide along the microtubule in the presence of Ca2+ by the total internal reflection fluorescence microscopy. Reversible Ca2+dependent cargo-loading system was achieved by changing the Ca2+ concentration in the flow cell. K560-CaM was adsorbed onto the fluorescently unlabeled microtubule adhered on the glass surface in flow cell using non-hydrolyzable ATP analogue, AMP-PNP which stabilize the microtubule binding state of kinesin. Subsequently, Qdot-M13 was added in the presence of Ca2+ to be loaded on K560-CaM adsorbed on the microtubule. The fluorescence of Qdot-M13 loaded onto K560-CaM along a microtubule was observed after washing excess unbound Qdot-M13. When the Ca2+ solution in the flow cell was replaced by the Ca2+ free solution, Qdot-M13 was unloaded. Even after the several times alternate exchange of the solution in the flow cell with Ca2+ and EGTA solutions, the K560-CaM adsorbed onto microtubule by AMP-PNP showed stable Ca2+ dependent cargo loading. When excess ATP was added into the flow cell, K560-CaM-Qdot started to move along the microtubule. Interestingly, 145 seconds later, K560-CaM-Qdot accumulated at plus end of microtubule and showed fluorescent clumps as marker for plus end of microtubule.

1879-Pos
High Resolution Structural Characterization of the Human Spastin Protein and its Complex With Tubulin
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Hereditary spastic paraplegia (HSP) is a motor neuron disease caused by a progressive degeneration of the motor axons of the corticospinal tract. A number of different proteins have been implicated in the pathology of HSP, including mitochondrial proteins, a kinesin motor, spartin and spastin. Of the autosomal dominant cases of HSP, approximately 40% are caused by point mutations or exon deletions in spastin. Because of spastin's significant role in the development of HSP, there have been an increasing number of studies on this protein and its role in axonal degeneration. Recent studies have shown that spastin has a microtubule severing activity, and that this activity is linked to the manifestation of neurological disorders in Drosophila. Until recently very little structural information has been available for the spastin protein, and the mechanism by which it severs microtubules remains elusive. The goal of this study is to

determine the high resolution crystal structure of the human spastin protein both alone and in complex with a domain of tubulin. To this end, we have expressed and purified a construct of human spastin and a C-terminal tubulin construct, to which spastin binds. We have also crystallized spastin and collected a 3.3 angstrom data set and are now attempting to crystallize the spastin-tubulin complex. Visualization of such a complex will provide details as to how spastin binds to and severs microtubules, and will also help to explain how point mutations in the spastin gene lead to altered protein activity and a disease phenotype.

1880-Pos
Characterization of Tau Interactions with Lipid Vesicles
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A number of neurodegenerative diseases, including Alzheimer's disease, are associated with the deposition of aggregated tau protein in the form of neurofibrillary tangles (NFTs). Although a causal relationship between NFTs and disease has not been conclusively resolved, it is hypothesized that either the aggregates themselves or the process of aggregation is pathological. Tau is an intrinsically unstructured protein expressed in neurons, primarily functioning to stabilize and catalyze microtubule assembly. Studies indicate that tau binding of lipid vesicles may serve as a mimic of microtubule binding. We measured the affinity of the various constructs of tau encompassing the microtubule binding region to synthetic lipid vesicles using fluorescence correlation spectroscopy (FCS). Importantly, we observe that the binding behavior is dramatically affected by solution pH. At pH 7.4, the protein binds stably and we are able to extract a partition coefficient for both wild-type and the disease-associated point mutant, P301L. However, at low pH, binding to lipid bilayers triggers rapid aggregation of the tau fragments, which we were able to confirm as amyloid using Thioflavin T binding and electron microscopy.

1881-Pos
Tubulin is an Amphiphilic Protein Whose Interaction With Membranes is Regulated by its Charged Carboxy-Terminal Tails
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Tubulin is an acidic heterodimeric protein whose negative charges are significantly (~40% of total negative charge) located on the 10-15 residue long, glutamic acid-rich, unstructured, carboxy-terminal tails (CTT) found on both α- and β-subunits (though not on the monomeric γ-tubulin). Unsurprisingly, tubulin is a quite water-soluble protein. However, it has been consistently reported to be a component of highly purified membranes, including plasma membranes, intracellular membranes such as Golgi and mitochondrial outer membranes, and vesicle membranes as in clathrin-coated endocytic vesicles. In these preparations, tubulin is non-microtubular and is tightly associated with the membrane, often requiring detergent for solubilization. Tubulin has also been shown to associate tightly with liposomes make of purified lipid only, including neutral lipid. The exact mechanism of tubulin-membrane association has not been defined, nor has it been shown that there is only one mechanism. Tubulin could dock with lipid-embedded proteins, for example. We have shown that tubulin binds to VDAC in the mitochondrial outer membrane, with functional consequences for mitochondrial function, and this binding requires and is mediated by the CTT. Thus the CTT can enhance membrane binding of tubulin. This cannot be the mechanism for liposome interaction, since there is no protein present other than tubulin. We show by charge-shift electrophoresis and non-ionic detergent extraction that (a) tubulin behaves as an amphiphilic protein, and (b) the CTT can regulate interaction with amphipathic molecules.

Cell & Bacterial Mechanics & Motility II

1882-Pos
Mechanics of the Cell Nucleus as a Function of Lamin Expression in Granulocyte Differentiation
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The ability of cells to deform through narrow spaces is critical for processes ranging from immune function to metastasis. Neutrophils are the most abundant white blood cell, which are required to transit through spaces less than 1/5 of the cell's diameter. As the nucleus is typically the stiffest organelle in the cell, the lobulated shape of the neutrophil nucleus is thought to facilitate its transit. However, neither the mechanical properties of the nuclei, nor the mechanism underlying the transition from ovoid to lobulated nuclear shape, are fully understood. We used HL60 cells as a differentiable model system to study nuclear shape transitions and the effects on cell mechanics. To elucidate the effects of the nuclear envelope protein, lamin A, we genetically modified the cells to generate subpopulations of cells with well-defined lamin A levels. Quantitative image analysis revealed that increased lamin A expression inhibits the nuclear shape transition to the typical lobulated morphology. To determine the effect on whole-cell mechanics, we measured cell deformability by flowing cells through channels of a microfluidic device, monitoring transit time and nuclear deformation. In addition, we performed functional assays to test if the impaired transition in nuclear morphology is also associated with defects in phagocytotic function, and thus reflect overall impairment of differentiation due to increased lamin levels. These results help to elucidate the molecular mechanism of granulocyte differentiation, and may have possible implications for understanding reduced immune function in aging, where lamin A has been reported to accumulate at the nuclear envelope.

1883-Pos
Model for Microtubule-Actin Interactions in Growth Cone Motility
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A growth cone is a motile structure on the tips of axons that guides axon extension to synaptic targets during nervous system development. In order to translate chemotactic signals into a mechanical response, the microtubule and actin filaments in the growth cone self-organize into a motile lamellipodial structure. A meshwork of actin filaments in the lamellipodium are continually transported inward by myosin-driven forces, at a speed that matches actin polymerization at the leading edge. This creates a stationary actin treadmill when actin adhesion to the substrate is low, and allows leading edge protrusion when actin adhesion increases in response to guidance cues. A population of highly dynamic microtubules that explore the P domain in stochastic bursts of growth and shrinking have also been shown to play an essential role in growth cone steering. Cooperation between these two filament systems is known to be essential for directed motility. We present initial results from a theoretical model of the growth cone cytoskeleton in the lamellipodium, testing the hypothesis that dynamic microtubules and actin work cooperatively to guide growth cone motility. We simulate dynamically unstable microtubules that transiently attach to actin retrograde flow, actin-myosin-adhesion force balance, and test several scenarios for feedback between microtubules and actin. Our theoretical work is guided by direct visualization of actin and microtubule dynamics during growth cone advance with fluorescent speckle microscopy.

1884-Pos
Examining Mechanical Properties of Vertebrate Meiotic Spindles
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Accurate chromosome segregation during cell division relies on the self-organization of a dynamic multi-component apparatus, the microtubule-based bipolar spindle. Through genetic, biochemical, and cell biological approaches, the complete 'parts list' of the components, including microtubule-based motor proteins, which are responsible for the assembly and maintenance of the meiotic spindle are now available. However, how spindle self-organization is controlled through integration of forces generated by multiple biomolecular processes remains mysterious. Here we report a calibrated microneedle-based system that allows application of sub-nanoNewton forces at specific sites within the metaphase spindle assembled in Xenopus egg extracts. Our set-up allows direct force measurements and can be combined with multi-mode high resolution microscopy along with chemical perturbations of specific spindle components. Using this system we applied sinusoidal strain to the metaphase spindle, keeping the range $<5\%$ to minimize nonlinearities of the response. The stress response was measured over the frequency ranged between 0.01 Hz and 2 Hz. Based on the resultant stress-strain relationship we determined the frequency-dependent mechanical properties of the spindle. The spindle response showed a typical characteristic time-scale of ~50 s, at which the mechanical property changed from solid-like to liquid-like. Additional experiments examining stress relaxation, which may reflect the dynamics of structural reorganization, revealed a similar time-scale. This transition disappeared when the spindles were treated with AMPPNP, a slow-hydrolyzing ATP analog, suggesting that the characteristic time-scale is determined by an ATP-dependent processes within the spindle. The contribution of key mitotic motors such as dynein and kinesin is being examined by pharmacological or immunological perturbations. Together, these analyses will allow us discuss models for how forces in the self-organizing meiotic spindle are integrated.