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Generation of Differentially Modified Microtubules using in vitro Enzymatic Approaches
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Tubulin, the building block of microtubules, is subject to chemically diverse and evolutionarily conserved post-translational modifications that mark microtubules for specific functions in the cell. Here we describe in vitro methods for generating homogeneous acetylated, glutamylated, or tyrosinated tubulin and microtubules using recombinantly expressed and purified modification enzymes. The generation of differentially modified microtubules now enables a mechanistic dissection of the effects of tubulin post-translational modifications on the dynamics and mechanical properties of microtubules as well as the behavior of motors and microtubule associated proteins.

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A Comparison of the Conformational Changes of Tau Isoforms in the Tau-Tubulin Complex
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Tau is an intrinsically disordered protein found in the axons of neurons, where it functions to maintain microtubules and stabilize microtubule growth. It is present in the human nervous system as six differentially spliced isoforms, most noticeably half of which possess four imperfect repeats (4R) within the microtubule binding region (MTBR) and the others possess three of these repeats (3R). For poorly understood reasons, tau can form intracellular aggregates known as neurofibrillary tangles (NFTs) which have been implicated in the pathology of Alzheimer's disease and other tauopathies. This aggregation of tau in disease is thought to be precipitated by altered interactions between tau and tubulin (or microtubules). Previous studies indicate that some tauopathies possess isoform-specific aggregates, leading us to hypothesize that differences in microtubule binding between isoforms may be important to understanding the isoform-specific transition to aggregation. To investigate this, we determined the average conformational changes of tau 3R and 4R isoforms upon binding to tubulin via single molecule Förster resonance energy transfer (smFRET). Our constructs were labeled at several sets of residues within the MTBR with donor and acceptor fluorophores to elucidate the changes in residue distance between tau's solution conformation and its tubulin-bound conformation. The results provide insight into differences in the topological features of the tubulin-bound isoforms. Moreover, they may also elucidate mechanisms of association and dissociation of tau to microtubules, relevant to the initiation of aggregation.

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Analyzing the Frequency of Thermally Fluctuating Segments of Microtubules
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Taxol is a drug used to treat cancer by stabilizing microtubules. The purpose of this research is to understand and explain how Taxol stabilizes microtubules and build a foundation upon which new discoveries involving cancer research can be made. We analyzed if Taxol affects the vibrational modes of microtubules by determining a frequency of Taxol-stabilized microtubules. Microtubules are grown, imaged, and analyzed by measuring the change of angle in radians of the end segments at 83ms intervals. The results depicted a sinusoidal movement of the end segment of the microtubule. From this, we found the resonant frequency by taking the Fourier Transform of the data and analyzing where the maximum peak occurred. The smaller peaks in the transform may be a result of the surrounding solution or internal fluctuations of the microtubule. We interpreted a 10.2 μm microtubule to have a frequency of 0.96 Hz. The process is repeated with microtubules of similar lengths, incubated with Taxol. We compared the resonant frequencies of the various lengths of microtubules and observed that there is a relationship between the length of a microtubule and its fundamental resonant frequency. The trend shows that as length of a microtubule increases, the fundamental frequency decreases.

Cytoskeletal Assemblies and Dynamics

2269-Pos Board B406
Effects of Added Divalent Counterions on the Properties and Behaviors of Microtubule Filaments
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Microtubules are polymeric cytoskeletal filaments that define the shape of eukaryotic cells and are widely involved in intracellular active transport. Physiological regulation of microtubule mechanics and dynamics may be achieved through electrostatics based on their strong polyelectrolyte nature. Here, we report on the effects of counterions on microtubules at concentrations below the like-charge bundling phase boundary. We first show that the persistence length (Lp) is significantly increased in the presence of physiologically relevant amounts of certain divalent salts (Mg^{2+}, Sr^{2+}, and Ba^{2+}). These observations are counter to theoretical expectations and experimental observations in similar systems where biological rod-like polyelectrolytes (e.g., DNA) are reported to present lower Lp values due to counterion-induced condensation. The increase in microtubule LP was attributed to screened coulomb interactions between the filament surface and the highly negatively charged C-terminal tails. Suppression of depolymerization was also observed in the presence of Ba^{2+} and in the absence of stabilization agents (e.g., paclitaxel). The observed correlation between structural stability and mechanical rigidity is consistent with prior work involving MAPs, which also affect dynamics through interaction with the C-terminal tails. Lastly, the counterion-induced increase in Lp also significantly affected the characteristics of kinesin-transport. Here the path trajectories of microtubules in the gliding motility assay transition from highly dispersed transport to deterministic transport following the addition of divalent ions. Overall these results establish a novel mechanism by which microtubules dynamics, mechanics, and interaction with molecular motors may be regulated by physiologically relevant concentrations of divalent salts.
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2270-Pos Board B407
Spatio-Temporal Model for Silencing of the Mitotic Spindle Assembly Checkpoint
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The spindle assembly checkpoint arrests mitotic progression until each kinetochore secures a stable attachment to the spindle. Despite fluctuating noise, this checkpoint remains robust and remarkably sensitive to even a single unattached kinetochore among many attached kinetochores; moreover, the checkpoint is silenced only after the final kinetochore-spindle attachment. Experimental observations showed that checkpoint components stream from attached kinetochores along microtubules toward spindle poles. Here, we incorporate this streaming behavior into a theoretical model that accounts for the robustness of checkpoint silencing. Poleward streams are integrated at spindle poles, but are diverted by any unattached kinetochore; consequently, accumulation of checkpoint components at spindle poles increases markedly only when every kinetochore is properly attached. This step-change robustly triggers checkpoint silencing after, and only after, the final kinetochore-spindle attachment. Our model offers a conceptual framework that highlights the role of spatiotemporal regulation in mitotic spindle checkpoint signaling and fidelity of chromosome segregation.

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A Bundle of Antiparallel Microtubules Connects Sister K-Fibers and Balances Forces within the Metaphase Spindle
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Chromosome segregation is driven by forces generated by motor proteins and microtubules (MTs). Although MTs are highly dynamic, the metaphase spindle can be considered as a stable state as all forces acting within it are accurately balanced. Here, we propose that a new class of spindle microtubules exists which contributes to the force map of the mitotic spindle. We named this population of MTs bridging microtubules (bMTs) as they, being a bundle

of antiparallel MTs, connect two sister k-fibers. To confirm the connection of the bMT bundle with the sister k-fibers, a laser cutting assay was developed, where the outermost k-fiber of the spindle was severed. After the cut, sister k-fibers moved together with the bMT bundle and behaved as a single object, revealing their connection. To test whether the forces in the spindle can be explained by including the bMT bundle, a theoretical model was developed. The model predicts that the thickness of the bMT fiber defines the magnitude of the forces within the spindle. To test this prediction, the thickness of the bMT fiber was increased by overexpression of tubulin and antiparallel MT crosslinking protein PRC1. This perturbation of the system resulted in a much faster movement of the severed k-fibers, confirming that the force map within the spindle was affected by thickening the bMT fiber. Furthermore, the experiments showed that the bMT bundle participates in anaphase A movement of the chromosomes. When the severed k-fiber did not reconnect to the spindle pole before the onset of anaphase, the sister chromatids, which were connected only to one pole, were able to move apart along the bMT. This finding reveals an alternative mechanism of chromosome segregation.

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The Kinetics of Fesselin (Avian Synaptopodin 2) Binding to Smooth Muscle Myosin is Dependent on Calcium-Calmodulin

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Fesselin (or avian synaptopodin 2) is an actin binding protein that nucleates actin filament formation (Leinweber et al. 1999; Beall et al. 2001) and bundles actin filaments (Schroeter et al. 2013). The nucleating activity is inhibited by Ca^{2+}-calmodulin (Schroeter et al. 2004). Fesselin also binds myosin with an affinity of 2 x 10^6 M^{-1} at 50 mM ionic strength (Schroeter & Chalovich 2005). That binding increases the length and width of myosin filaments, and reduces the rate of dissociation of myosin filaments and actin-myosin complexes by ATP (Kingsbury et al. 2013). Fesselin labeled with IANBD produced a fluorescence increase upon binding to smooth muscle myosin or S1. The apparent rate constant for the fluorescence change increased with increasing concentrations of myosin or S1 in a hyperbolic fashion (105 mM ionic strength, 10 deg C). Binding to myosin began with the rapid formation of a low affinity intermediate followed by a second step having an observed rate constant ($k_2 + k_{-2}$) of 30/sec. Several observations suggested that the binding of fesselin to myosin was affected by calmodulin and calcium. The apparent rate constants for both processes in the binding of IANBD-fesselin to S1 were 2-fold faster in the absence of calcium. Binding of IANBD fesselin to intact myosin was complex in the presence of calcium with a rapid increase in fluorescence followed by a slower decrease; that behavior was not observed in the presence of calcium but absence of calmodulin. Ca^{2+}-calmodulin may play roles in the formation of actin filaments, myosin filaments and actin-myosin complexes through fesselin.

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Force Generation and Contraction of Random Actomyosin Rings

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Cytokinetic rings and stress fibers generate effective contraction, but how organized respective actomyosin arrays are, and how semi-random actomyosin bundles generate contraction is not clear.

We investigate computationally the self-organization and contraction of an actomyosin ring that is completely disorganized initially. To this end, we formulate a detailed agent-based model for a 1D chain of cross-linked actin filaments forming a closed ring interspersed with myosin-II motor proteins.

The result of our numerical experiments is that in order to contract, 1) actin filaments in the ring have to turn over, 2) myosin motors have to be processive, and 3) filaments have to be sufficiently crosslinked. We find that contractile force and rate scale with myosin density and have a complex dependence on the actin density. The simulations indicate that the ring consisting of short filaments contracts rapidly but exerts little force, while the long filament ring generates significant force but contracts slowly. Our simulations predict, in agreement with experimental observations, that the rate of contraction is constant and the time of contraction is invariant with respect to the original ring size. Finally, the model demonstrates that with time, a pattern formation takes place in the ring worsening the contractile process. The more random actin dynamics are and the longer actomyosin ring stays disorganized the higher contractile force and rate it generates.

2274-Pos Board B411

Simulating Complex Mechanochemistry of Actin Networks

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Actin network dynamics within the cell cytoskeleton are a complex phenomenon that is important to many biological processes. To model the nonlinear mechano-chemistries driving cytoskeletal dynamics, we have developed a set of coarse-graining algorithms, integrated into a software package, which allows for stochastic simulations of growing and shrinking actin filaments, where the latter are treated as semiflexible polymers. In addition to polymerization and depolymerization processes, a multitude of other important chemical reactions can be taken into account, including binding and unbinding of cross-linkers and stepping and force generation of molecular motors. In addition to mechanical and chemical processes, transport of monomeric species is treated on equal footing. We have used this newly developed model to study the non-linear, far from equilibrium processes in the cytoskeleton, as well as in other active matter systems.

2275-Pos Board B412

Visualizing F-Actin Structure in Developing Zebrafish Zygotes to Supplement Viscoelastic Measurements

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The cell has developed an elaborate cytoskeleton that can undergo dramatic rearrangements in order to grow, divide, or move. In the one-cell stage of the zebrafish embryo, the cytoskeleton completely reorganizes to form the cellular compartment. We want to understand the link between the morphological structure of the cell's cytoskeleton and the mechanical properties of the cell. That is, we want to find out if rearrangements of filamentous cytoskeletal proteins like F-actin and microtubules lead to changes in cellular viscosity or elasticity. To do this, we need to correlate images of cytoskeletal structures with mechanical measurements. In this part of our project, we imaged the rearrangement of the F-actin network during cytoskeletal formation in the one-cell stage of the zebrafish embryo. These images will complement microrheology measurements of the viscoelasticity of the cell. To take images, we fixed the embryo and labeled the cellular F-actin with the dye phalloidin. We then imaged the mounted embryos on a confocal microscope to see the F-actin organization. Our images show that just before cellular cleavage, the F-actin has collected at the cellular periphery, similar to previous work.

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Visualizing the Compartmentalization of the Surface of Mammalian Cells by Cortical Actin with Superresolution

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The plasma membrane is a complex fluid where proteins move by diffusion, a process that is critical to biochemical reactions. Despite the membrane fluidity, distinct compartments transiently form on the cell surface, suggesting the plasma membrane maintains a dynamic ordered environment. Single-molecule methods show that membrane proteins exhibit transient confinement mediated by the actin cytoskeleton. However, direct visualization of the membrane compartmentalization by the underlying cortical actin structure is experimentally challenging because of the need for spatial resolution beyond the diffraction limit and high temporal resolution. In order to overcome these challenges, we employ dynamic photoactivated localization microscopy (PALM). We image the cortical actin with 40-nm resolution for continuous periods longer than one minute, while we simultaneously track individual membrane proteins that interact with the actin cytoskeleton. Kv2.1 and Kv1.4 ion channels are labeled with quantum dots to investigate the effect of cortical actin compartmentalization on protein diffusion using single-particle tracking. We find that individual ion channels are confined within compartments formed by the cortical actin for times up to several seconds. Nevertheless, molecules are observed to permeate through actin barriers at locations where the cytoskeleton is retracted from the plasma membrane. The analysis of compartment morphological properties reveals a broad distribution of compartment sizes. Further, the imaged actin cortex appears to form a fractal structure, which explains the observed subdiffusion of various membrane proteins over very broad time scales. Our data provide evidence showing that the restriction to lateral mobility by an actin random fractal is one of the fundamental physical mechanisms for the anomalous diffusion of transmembrane proteins.