vesicles are associated with dynein, a microtubule-associated molecular motor, to power vesicle trafficking related to acid secretion in gastric parietal cells. This study provides a clue in how TRIM50 is involved in vesicle trafficking and how the deletion of TRIM50 involved in the gastrointestinal issues observed in patients with Williams Syndrome.

2260-Pos Board B397
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 homogenous 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.

2261-Pos Board B398
Dynamics of Microtubule Networks with Antiparallel Crosslinkers
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Microtubules constitute the most rigid element of the cytoskeleton. They are responsible for the morphology of cells, and constitute the tracks for transport of cargo. Interactions between microtubules, motor proteins, and microtubule associated proteins drive very important mechanisms in the cell, such as cell division, cell motility, cell homeostasis, and cell signaling. We seek to understand how such complex, energy-consuming biological networks self-organize by studying in vitro microtubules bundled by MAP65, in kinesin gliding assays. We find that large networks can break into smaller, cell-like networks that can mimic types of cell motility. Dynamics of these networks change with varying concentrations of MAP65 and microtubules.

2262-Pos Board B399
Kinetochore Dynamic Structure at Super-Resolution Accuracy and Mechanical Stiffness Revealed in vitro by Combined Tirf and Optical Tweezers
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The kinetochore is a protein complex that interfaces spindle microtubules with chromosomal DNA. During mitosis, kinetochores establish correct bi-oriented attachment of spindle on sister chromatids, and mediate the segregation of sister chromatids in anaphase. Throughout this process, kinetochores remain attached on the plus ends of the microtubules, and exert force to the centromere DNA. The significance of the tension in silencing the spindle assembly checkpoint signal remains a central unsolved question in mitosis. Recent advances in isolating kinetochore particles from budding yeast enables the application of direct and controlled mechanical perturbation in vitro. Meanwhile, by fluorescently labeling kinetochores particles, the subunits can be accurately localized. We used a combined instrument of optical tweezers, total internal reflection fluorescence microscopy, and differential interference contract microscopy to measure the super-resolution structural change induced by an external tension exerted by attached microtubule. In addition to confirming the linear structural organization, the stiffness of kinetochore subunits is also directly quantified in this work. This information is useful to reveal the role of intra-kinetochore stretch in the mechanism of generating spindle assembly checkpoint signaling.

2263-Pos Board B400
Asymmetric Friction of Non-Motor Maps can lead to their Directional Motion in Active Microtubule Networks
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Cells utilize dynamic biopolymer networks to carry out mechanical tasks during diverse processes. These cellular processes require microtubules to be organized into distinct structures, such as asters or bundles. Within these dynamic motifs, microtubule-associated proteins (MAPs) are frequently under load, but how force modulates these proteins' function is poorly understood. Here, we combine optical-trapping with TIRF-based microscopy to measure the force-dependence of microtubule interaction for three non-motor MAPs (NuMA, PRC1, and EB1) required for cell division. We find that frictional forces increase non-linearly with the velocity of MAP motion across microtubules and depend on filament polarity, with NuMA's friction being lower when moving towards minus-ends, EB1's lower towards plus-ends, and PRC1 exhibiting no directional preference. Mathematical models predict, and experiments confirm, that MAPs with asymmetric friction can move directionally within actively fluctuating microtubule pairs that they crosslink. Our findings reveal how non-motor MAPs can generate frictional resistance in dynamic cytoskeletal networks via micromechanical adaptations whose anisotropy may be optimized for MAP localization and function within cellular structures.

2264-Pos Board B401
The Determination of Young's Modulus for Microtubules Stabilized with Taxol and Analysis of Vibrational Modes
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We present here the effects of Taxol, a cancer drug, on the intracellular mechanisms associated with dynamic instability of microtubules. Since Taxol affects the stability of microtubules, the general process of microtubule polymerization and depolymerization will be studied closely throughout this research project. By analyzing the dynamic instability of microtubules, the effects of Taxol on the microtubules can be used to elucidate the complex functioning of Taxol within the cell. The observations and discoveries of the research can have revolutionary effects in the fields of life science and medicine.
The short-term goal of this project involves analysis of the thermal fluctuations of a single Taxol-stabilized microtubule. From our measurement of the elastic properties for Taxol-stabilized microtubules we can gain insight on vibrational modes of the microtubules. We propose that the vibrational modes of microtubules will vary based on the Taxol concentration at which they are grown and diluted. Then, by comparing the vibrational modes to the resonant frequency of the Taxol-stabilized microtubules, we will relate the dynamic instability of the microtubules to the change in vibrational mode. Therefore, the ultimate goal of the research is to acquire more knowledge about the function of Taxol in order to discover more effective cancer treatment methods. These findings will elucidate the confounding enigma that plagues humanity (cancer) and will lead to further advancements in cancer therapy research. Based on current findings, the microtubules grown with higher Taxol concentrations have lower Young's Modulus values.

2265-Pos Board B402
Regulation of Tau Dynamics by Phosphorylation in the Squid Giant Axon
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Tau is a conformationally dynamic microtubule-associated protein expressed at high levels in neurons. It is localized primarily in the axonal compartment where it has been implicated in a number of intracellular functions, including microtubule stabilization, cross-linking between the cytoskeleton and plasma membrane, acting as a scaffold for a variety of signaling molecules, and modulating the axonal transport process. Tau's myriad of functions are likely to be related to its conformationally dynamic structure, but the structure/function relationships within this molecule remain poorly defined. Additionally, Tau is known to be regulated by phosphorylation at numerous Ser/Thr and Tyr sites throughout the molecule, but the effects of phosphorylation on Tau's structural dynamics are also unclear. Previous in vitro work in our lab has shown that Tau interconverts between static and mobile (diffusive) states on the microtubule surface in an isoform and microtubule-lattice specific manner (McVicker et al., (2014) Cytoskeleton 71:184). To further extend these studies into a physiologically relevant environment in which Tau is naturally regulated by phosphorylation, we have used single-molecule imaging to examine the dynamic behavior of fluorescently-labeled Tau on the surface of microtubules within the isolated axoplasm of the squid giant axon. Tau maintains its isoform-specific ability to interconvert between static and diffusive states on the microtubule surface under these conditions. Furthermore, we demonstrate phosphorylation influences Tau's dynamic behavior on the microtubule surface, e.g., inhibition of CDK5 by Roscovitine results in a significant shift in Tau's dynamic equilibrium towards the diffusive state. These studies establish the isolated axoplasm of the squid giant axon as a novel model system for studying Tau dynamics under physiologically relevant conditions, and provide new insight into the role of phosphorylation in regulating Tau's structural dynamics on the microtubule surface.