mutations to tau may alter the balance of tubulin binding and microtubule assembly that is necessary for maintaining a dynamic microtubule cytoskeleton.

740-Pos Board B509
The Effects of Nanomolar Concentrations of Taxol on Microtubule Polymerization
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Microtubules are intracellular polymers that assemble from heterodimeric αβ-tubulin subunits. Polymerizing microtubules exhibit dynamic instability, a GTP-hydrolysis-driven phenomenon in which they alternate abruptly between phases of growth and relatively rapid shortening. The kinetics of tubulin subunit exchange at the microtubule tip, which are crucial to processes such as mitosis, are affected by the chemotherapeutic drug taxol, but the precise mechanism of action at therapeutic concentrations remains unclear. An understanding of how taxol alters exchange kinetics may assist in the development of new chemotherapeutic drugs.
We observe microtubules polymerized at 4.5 micromolar tubulin and at 1-480 nanomolar taxol using total internal reflection fluorescence microscopy (TIRFM), achieving improvements in spatial and temporal resolution of, respectively, 1 and 2 orders of magnitude compared to previous taxol studies. We measure microtubule length changes to within 25 nm at 8 Hz, and we detect changes in the structure of the microtubule tip. We find that taxol potently affects microtubule growth at concentrations as low as 10 nM. At these concentrations we observed higher variability in growth rate than seen under control conditions, especially on long timescales (on the order of 100 seconds). In some cases, microtubules switched between normal growth and periods of very slow growth. Further, we have found that 10 nM taxol almost completely suppresses rapid shortening, and beginning at 100 nM taxol, the tubulin on and off rates gradually decrease, though the microtubule net growth rate (excluding periods of very slow growth in taxol-microtubules) remains constant.

741-Pos Board B510
The Effect of Tubulin Acetylation on Microtubule Structure and Dynamics
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The post-translational acetylation of tubulin is a highly conserved process, but the effect of this modification on microtubule structure and function remains unclear. We have previously reported that the organization and localization of acetylated microtubules changes as epithelial cells undergo apico-basal polarization, suggesting that these microtubules may play a role in the trafficking of cargoes to specific subcellular domains in these cells. As the site of acetylation is thought to be in the lumen of the microtubule, it is unlikely that acetylation can directly affect the interactions between microtubules and molecular motors. Rather, we hypothesize that acetylation may alter the structure and/or dynamics of microtubules, which in turn may affect motor traffic. We have found that in MDCK epithelial cells, there is a persistent pool of soluble acetylated tubulin. When microtubules are de-polymerized by either nocodazole or cold treatment, this acetylation is not rapidly reversed, and the soluble tubulin remains acetylated. We found that this acetylated tubulin is polymerization-competent, and here we present data describing the polymerization rates and structural effects of acetylation. Finally, we have used a recombinant histone deacetylase 6 (HDAC6) enzyme to examine the effects of deacetylation in vitro. Together, these studies will shed light on the effect of the post-translational acetylation of tubulin on microtubule structure and function.

742-Pos Board B511
High-Throughput Screening Assay for Modulators of Tubulin Tyrosine Ligase
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A posttranslational modification that is unique for the protein tubulin is tyrosination/detyrosination of the C-terminus of α-tubulin. Detyrosination of the genetically encoded C-terminal tyrosine by an unidentified carboxypeptidase is followed by retyrosination catalyzed by the enzyme tubulin tyrosine ligase (TTL). Proper functioning of tyrosination/detyrosination cycle is required for cell vitality. In particular, TTL downregulation and increase of detyrosinated tubulin correlate with increased tumorigenesis and tumor invasiveness. Exogenous modulators of TTL may restore tyrosinated tubulin and impair tumor progression. The only method currently available for studying such modulators requires use of radioactive ligands. Here, we present an assay suitable for high-throughput screening of TTL effectors, in which the readout is fluorescence- or immunochemistry- based. In this assay, detyrosinated tubulin and TTL are allowed to react with a potential modulator in the presence of 3-formyltyrosine (3fY), a known substrate for TTL. The ligand's ability to compete with 3fY is quantified by the fluorescence signal generated when the protein bound aldehyde group covalently reacts with a suitably derivatized fluorophore. In addition, we have established an immunochemical detection method in which biotin hydrazide is used instead of a fluorophore. The extent of biotinylation is then detected using Streptavidin-HRP conjugate. Both these detection methods generate comparable results. Thus, we have developed a versatile, sensitive, non-radioactive high- throughput screening assay that can be employed to examine TTL modulators.

743-Pos Board B512
Flexibility Analysis of the Microtubules Polymerized in the Presence of Photochromic Nucleotide Analogues
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Azobenzene is a photochromic molecule that undergoes rapid and reversible isomerization between the *cis*- and *trans*-form in response to ultraviolet (UV) and visible (VIS) light irradiation, respectively. Previously, we have incorporated azobenzene derivative into functional region of ATP driven motor protein and succeeded to control microtubule dependent ATPase activity reversibly by photo-irradiation. The results suggested that the photo-control system using photochromic molecules might be applicable to other functional biomolecules. It is known that tubulin utilize GTP to polymerize and the GTP is entrapped within the GTP binding site of tubulin. And the microtubules composed of GDP bound tubulin easily depolymerized. Interestingly, it was demonstrated the structure of the GTP analogue GMP-CPP bound microtubule is squigglier configuration than that of regular GTP bond microtubule. In the present study, we have employed photochromic nucleotide analogue, phenylazobenzoyl-iminoethyl-tri-Pphosphate (PABITP) in order to photo-control flexibility of microtubules. The PABITP bound microtubules showed much more flexible shape change during gliding on the in-vitro motility assay than regular GTP bound microtubule. The effect of photo-isomerization of PABITP entrapped within the site of microtubules induced by UV and VIS light irradiation for the flexibility of microtubules was also examined.

744-Pos Board B513
Topological Phonon Modes and their Role in Dynamic Instability of Microtubules
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Microtubules (MTs) are self-assembled hollow protein tubes playing important functions in live cells. Their building block is a protein called tubulin, which self-assembles in a particulate 2 dimensional lattice. We study the vibrational modes of this lattice and discuss the existence of topological vibrational modes localized at MTs edges. Since these modes are robust against the large changes occurring at the edges during the dynamic cycle of the MTs, we can build a simple mechanical model to illustrate their role in dynamic instability and the effect of taxol.

745-Pos Board B514
A New Directionality Software Tool Reveals Muscle Microtubule Pattern Alterations
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Cytoskeletal components have distinct patterns, whose modifications may indicate pathological changes. However, the changes may be difficult to assess visually. For example, recent data show that microtubules play a role in Duchenne Muscular Dystrophy pathology. We and others have reported, earlier, that the microtubules of the mdx (dystrophin-null) mouse are perturbed. Although it was possible to assess microtubule patterns in fibers of adult fast muscles (EDL) visually, it was not possible to do so in fibers from slow muscles (Soleus), which have a more tangled microtubule pattern. We need a robust tool to analyze the digital images, more specifically to detect the directionality of the microtubule network. The most commonly used tool is based on the Tamura method, which is calculated from 3 x 3 edge filters. This approach mainly focuses on local image features while neglecting the global features. We find that this method is not well suited to the structure of microtubules. We have therefore developed a new directionality detection method utilizing texture features. The software generates a graphic by calculating the texture correlation for each orientation. Both local and global features contribute to the final results. A direct visual comparison

of the produced images makes it easy for the user to distinguish subtle variations in pattern. A directionality score is also calculated based on the peak sharpness in the plot. This new tool was used to compare microtubules in the soleus muscle of wild-type, mdx, utrophin-null and double knockout mice. The directionality scores revealed differences among these mouse lines which could not be appreciated visually and which paralleled the differences observed in EDL muscles.

746-Pos Board B515

Measuring Microtubule Polarity in Spindles with Second-Harmonic-Generation Microscopy

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Microtubule polarity, the fraction of microtubules that point in one direction versus the other, varies throughout spindles. Near the poles, most microtubules point with their plus ends away from the pole, while in the middle of the spindle, equal number of microtubules point in either direction. Antiparallel microtubules in the spindle midzone are thought to be important for determining the localization of proteins that drive spindle elongation in anaphase. While in vitro experiments have suggested mechanisms connecting antiparallel microtubules, protein localization, and spindle elongation, it has been difficult to establish the validity of these models in vivo. A major challenge is that current methods for measuring microtubule polarity, based on electron microscopy, are extremely labor intensive, challenging to combine with protein localization studies, and only provide static snapshots.

Building off of approaches that have been pioneered in neuroscience by Watt Webb's group, we have developed a method using non-linear optical microscopy - second harmonic generation and two-photon fluorescence - to map microtubule polarity throughout spindles. This new technique allows us to quantitatively and nondestructively measure the polarity of microtubules in spindles at high resolution, and study how the distribution of polarity changes over the course of anaphase. We will present a detailed description of the methodology and preliminary results on the temporal evolution of microtubule polarity in the first mitotic spindle of C. elegans.

747-Pos Board B516

Visualization of Individual Cryptophycin-Tubulin Rings by Electron Microscopy Tomography

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The antimitotic drug Cryptophycin-1 (Cr) arrests cell growth by destabilizing microtubules. When added in vitro to either dimeric tubulin or preformed microtubules, Cr induces the formation of single protofilament rings that are highly monodisperse, having a diameter of ~28nm and thickness ~4 nm, and composed of eight tubulin dimers. Here we apply negative stain tomography [Fera, et al, J. Comp. Neurol., 2012] to Cr-tubulin rings. This new technique, which utilizes a beam stable organo-tungsten negative stain, allows construction of tomograms resolving the individual components of the protofilament rings without averaging. Molecular structure of Cr-tubulin rings is revealed in series of en face virtual sections ~4 Å thick positioned parallel to the plane of Cr-tubulin rings. We repeated the observations with rings obtained from tubulin-S, which lack the C-terminal acidic tail filaments that face the center of the Cr-tubulin rings. These tail filaments, normally on the outside of the microtubule and important for MAP and motor protein binding, have a diameter of ~1 nm and significant conformational freedom, making them impossible to detect by averaging methods. Thus, comparison of tomograms from rings of tubulin and tubulin-S will gather further information about these filaments. We also investigate ring-ring interactions in samples of higher concentration to probe their material properties.

748-Pos Board B517

Thermal Noise Imaging Indicates a New Regime of Length-Dependent Persistence Length in Microtubules

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Microtubules perform a diverse set of essential functions within the cell. Most of these functions are mechanical and, therefore, their mechanical properties and the extent to which these properties are tunable are of wide interest. It is known that the persistence length of microtubules depends on factors, such as type and concentration of associated proteins, binding of stabilizers, and their length. We present a new method for measuring microtubule persistence length using a recently developed technique known as three-dimensional thermal noise imaging. Thermal noise imaging is a three-dimensional scanning probe technique capable of probing soft matter at physiological conditions on the nanometer scale. Transverse fluctuations of microtubules have been imaged with thermal noise imaging, and subsequent analysis reveals mechanical properties of the filaments. A novel assay that allows for the isolation of single, grafted microtubules provides a method for measuring the persistence length of microtubules for contour lengths as small as 500 nm. Such a length regime has proven difficult to study using other techniques. We present data that indicates a length regime of microtubules in which the persistence length is significantly smaller than has been measured in longer filaments.

749-Pos Board B518

p150Glued Regulates Microtubule Dynamics in Neurons through Tandem Tubulin-Binding Domains

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Regulation of microtubule dynamics is critical, particularly in neurons, where defects may contribute to neurodegeneration. Here, we demonstrate in vitro using TIRF microscopy and in primary neurons using live-cell imaging that the p150Glued subunit of dynactin regulates microtubule dynamics. To modify dynamics, p150Glued must be dimerized, and it must bind soluble tubulin, an interaction that we find requires tandem CAP-Gly and basic domains. p150Glued is alternatively spliced in vivo, with the full length isoform including both these domains expressed primarily in neurons. Accordingly, depletion of p150Glued in a non-polarized cell line does not alter microtubule dynamics, while p150Glued RNAi in neurons leads to a dramatic increase in microtubule catastrophe. Strikingly, a Parkinson syndrome-associated mutation blocks this microtubule-stabilizing activity both in vitro and in neurons. Together, our data reveal that p150Glued plays a crucial role in promoting microtubule stability in neurons, and that defects in this function may lead to neurodegeneration.

750-Pos Board B519

Obscurin-Dependent Localization of Ankyrin B is required for the Organization of Sub-Sarcolemma Microtubules, Localization of Dystrophin, and Sarcolemmal Integrity in Skeletal Muscle

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Obscurin is a large myofibrillar protein that contains several interacting modules, one of which mediates direct binding to muscle-specific ankyrins. Interaction between obscurin and the muscle-specific ankyrin sAnk1.5 has been found to regulate the organization of the sarcoplasmic reticulum in striated muscles. The organization of dystrophin and β-dystroglycan at costameres is mediated by additional muscle-specific ankyrin isoforms, namely ankB and ankG, localized at the sub-sarcolemma level. In particular, ankB is responsible for the assembly of sub-sarcolemma microtubules required for the correct delivery of dystrophin and β-dystroglycan at costameres. On this basis, we investigated whether obscurin might be involved in the sub-sarcolemma localization of ankB and ankG, and eventually in the assembly of dystrophin and β-dystroglycan at costameres in skeletal muscle fibers. We found that in mice deficient for obscurin, ankB was displaced from its localization at the M-band, while localization of ankG at Z-disk was not affected. In obscurin KO mice, the sub-sarcolemma microtubule cytoskeleton was disrupted and localization at costameres of dystrophin, but not of β-dystroglycan, was markedly reduced. In addition, these mutant mice displayed sarcolemmal fragility and lower forelimbs muscle strength. Altogether, these results support a model where obscurin, by targeting ankB at the M-band, contributes to the organization of sub-sarcolemma microtubules, localization of dystrophin at costameres and to maintenance of sarcolemmal integrity. Accordingly, obscurin appears to represent a multifunctional anchoring protein that on one hand establishes interactions with sarcomeric proteins and on the other hand enables complex formation with extra-sarcomeric proteins, like the muscle-specific ankyrin isoforms, that help to connect the sarcomeres with the SR and with the sub-sarcolemmal cytoskeleton.