View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector



uncertainties, we relied on recently developed laser trap and fluorescence-based assays (Akiyoshi et al. Nature 2010) in which individual native kinetochore particles purified from budding yeast are coupled to single microtubules. Neither phospho-mimetic mutations at kinase target sites on Dam1 nor a mutation that disrupts the Dam1 complex had a strong effect on the initial binding of kinetochore-coated beads to microtubules. By contrast, phospho-mimetic mutations on Ndc80 reduced binding 3-fold. In force-ramp assays with tip-attached kinetochores, phospho-mimetic mutations on either Ndc80 or Dam1 reduced rupture forces roughly 1.5-fold relative to wild type and phospho-deficient controls. Likewise, in force-clamp experiments, phospho-mimetic kinetochores detached up to 8-fold more quickly, depending on the level of force. Taken together, these results indicate that Ndc80 and Dam1 play distinct roles in kinetochore function. Initial binding is dependent mainly on Ndc80 and modulated strongly by phosphorylation of Ndc80. Tip coupling depends on both Ndc80 and Dam1 and it is modulated by phosphorylation of both.

### 3552-Pos Board B413

### Determining Structure-Mechanics Relationships of Viscoelastic Microtubule Networks through Microscale Manipulation

**Bugra Kaytanli**, Yali Yang, Jun Lin, Omar A. Saleh, Megan T. Valentine.
UCSB, Santa Barbara, CA, USA.

The microtubule cytoskeleton is essential in maintaining the shape, strength and organization of cells and its misregulation has been implicated in neurological disorders and cancers. To better understand the structure-mechanics relationships in microtubule networks, we measure the time- and force-dependent viscoelastic responses of entangled and crosslinked microtubule networks to precise microscale manipulation. We use magnetic tweezers devices to apply calibrated step stresses and measure the resultant strain as a function of time. To understand the molecular origins of network behavior, we use a newly-developed portable magnetic tweezers device to observe network morphology using a confocal microscope while simultaneously applying point-like stresses to embedded magnetic particles. Our results are important to understanding the role of the cytoskeleton in regulating cargo transport in vivo, as well as the basic physics of non-affine deformations in rigid rod polymer networks.

### 3553-Pos Board B414

### Analysis of the Projection Domain of the Microtubule Associated Protein Tau using Force Spectroscopy

**Jared T. Saunders**, Dennis S. D'Urso, Zachary J. Donhauser.
Vassar College, Poughkeepsie, NY, USA.

Microtubule associated proteins (MAPs) are an important class of biological molecules that stabilize and organize microtubules. The MAP tau has been the subject of particular attention recently because it plays a role in some neurodegenerative diseases. This study focused on tau's projection domain in order to gain a better understanding of how the functional properties of this region of the protein affect its behavior. Using force spectroscopy of tau immobilized on mica substrates, we evaluated three versions of the protein where the projection domain had been modified, and compared long-range interactions between proteins. We found that as increasingly larger portions of tau's projection domain are removed, the range of long-range interactions decreases. Varying experimental conditions also changed the behavior of the projection domain, which is evident when the measurements are conducted in a high ionic strength buffer. This condition essentially removed the long-range interactions and provided strong evidence that the observed interactions are primarily electrostatic in nature. We also demonstrate that careful control of the experimental system allows us to measure tau-tau interactions or the behavior of tau alone on the mica. Our data confirms that the projection domain can exert strictly (entropic) repulsive interactions and can take part in more specific electrostatic interactions, depending on the geometry of the system.

### 3554-Pos Board B415

### Katanin P60 Targets Microtubules with Defects

**Megan E. Bailey**, Margaret M. Morelli, J. Daniel Diaz, Jennifer L. Ross.
University of Massachusetts at Amherst, Amherst, MA, USA.

Microtubules form and reorganize a variety of networks in cells, including the mitotic spindle, cilia, and neuronal processes. The organization and remodeling of microtubule architecture is regulated by microtubule-associated proteins (MAPs). Microtubule severing enzymes are a novel class of MAPs that regulate filaments by cutting them anywhere along their length. Microtubule severing enzymes, such as Katanin, oligomerize into hexamers and hydrolyze ATP to sever microtubules. Previous work has shown that Katanin specifically targets defects in the microtubules. Using in vitro single molecule assays with GFP labeled Katanin p60, the catalytic subunit of Katanin, we show that Katanin severs microtubules made in high salt more frequently. High salt microtubules have been shown to have fewer than the typical 13 protofilaments and a high incidence of protofilament shifts. We also measured the activity of Katanin on subtilisin treated microtubules (normal and high-salt) which have the c-terminal tails of alpha and beta tubulin cleaved off. Using these types of microtubules has given us insight into what types of defects Katanin might target and a better understanding of its mechanism of severing.

### 3555-Pos Board B416

### Mechanics and Dynamics of Microtubules in the Presence of the EBs and MAP4

**Taviare L. Hawkins**[1], Karan S. Hingorani[1], Sarah Y. Dole[2], Anne Straube[3], Jennifer L. Ross[1].
[1]University of Massachusetts Amherst, Amherst, MA, USA, [2]Mount Holyoke College, South Hadley, MA, USA, [3]University of Warwick, Coventry, United Kingdom.

Microtubules are the most rigid cytoskeletal filaments, providing structure to the cell, the path for intracellular transport, and the machinery of the mitotic spindle. To perform these functions in the cell, the microtubule must be rigid yet dynamic. Both their rigidity and dynamicity are controlled by microtubule associated proteins (MAPs). We characterized the effects of MAP4 and the EBs on microtubules mechanics and dynamics in vitro.

### 3556-Pos Board B417

### Covalent Conjugation of Antibodies to Biomolecular-Motor Driven Shuttles

**Jenna Campbell**, Dibyadeep Paul, Katsuo Kurabayashi, Edgar Meyhofer.
University of Michigan, Ann Arbor, MI, USA.

Emerging diagnostic needs for the detection of wide arrays of biomarker molecules require the detection of ultralow concentrations of protein in small volumes of biological samples. In past work, a number of microfluidic devices have been developed in attempt to satisfy these needs. One technology incorporates a critical component of the cell's cytoskeletal system, kinesin and microtubules (MT), in engineered device structures to actively collect and concentrate functionalized microtubules into a small concentrator region. The biomolecular motor kinesin hydrolyzes ATP and converts this chemical energy into mechanical work by moving along microtubule filaments. Incorporating MTs coated with antibodies (Ab) into this concentrator device will allow for a large variety of analytes to be captured with high specificity and localized for detection. We use a heterobifunctional, 8.3Å long crosslinker to covalently attach a reduced thiol group on the heavy chain of the antibody to a free succinimidyl ester on the outer surface of the microtubule. This conjugation method prevents undesired cross-linked products (MT-MT, Ab-Ab) and is applicable to monoclonal, polyclonal and $F(ab)'_2$ antibodies, specifically anti-BSA and anti-TNF$\alpha$. The motility of the functionalized microtubule complex was not significantly impaired as the gliding velocity of the functionalized microtubules driven by Kinesin-1 from *Neurospora crassa* decreased by about 25%, lowering the average velocity of the complex from 2.28 μm/s to 1.71 μm/s. Importantly, image analysis of fluorescent antigens attached to functionalized MTs show that microtubules are conjugated with >100 antibodies/μm of length. Based on these results, we conclude that the high density of antibodies on the microtubules along with the high binding affinity of antigen suggests that these molecular shuttles coupled with fluorescent detection can be incorporated into concentrator devices to detect ultralow concentrations of specific biomolecules.

### 3557-Pos Board B418

### Sub-Diffraction Limited Imaging of Sliding Microtubules Based on Nanoscale Localization Sampling using Nanoantenna Arrays

**Donghyun Kim**[1], Kyujung Kim[1], Junichiro Yajima[2], Youngjin Oh[1], Wonju Lee[1], Shinsuke Oowada[3], Takayuki Nishizaka[3].
[1]Yonsei University, Seoul, Korea, Republic of, [2]University of Tokyo, Tokyo, Japan, [3]Gakushuin University, Tokyo, Japan.

We investigate sub-diffraction limited imaging of fluorescent subunits on sliding microtubules by nanoscale localization sampling (NLS). NLS is based on periodic circular nanoantenna arrays with diameter=300nm and period=1um that create locally amplified electromagnetic hot-spots through localization of surface plasmon. The localized near-field hot-spot samples microtubular movement in the time domain for improved spatial resolution. We have demonstrated enhancement of spatial resolution by four-times compared to conventional wide-field microscopy (figure below). The resolution enhancement was