Proper organization of the microtubule cytoskeletal network is required to perform necessary cellular functions. Network organization is achieved through regulating by microtubule associated proteins (MAPs) that control microtubule dynamics by selectively stabilizing and destabilizing microtubules. Stabilizing MAPs are relatively well understood, while less is known about destabilizing MAPs, such as severing enzymes. Katanin, the first discovered microtubule severing enzyme, is a AAA+ enzyme that oligomerizes into hexamers and uses ATP hydrolysis to sever microtubules. Using single molecule biophysics techniques in vitro we investigate how katanin and its severing activity can be regulated by the type of microtubule, free tubulin, ATP concentration, and the neuronal MAP tau isoform 4R. This work provides evidence that katanin activity is regulated by common cellular conditions that will ultimately affect microtubule organization in cells.

**Molecular Basis for Age-Dependent Acetylation by Tubulin Acetyltransferase**

Jeffrey Spector, Agnieszka Zvyk, Alexandra M. Deaconescu, Max Valenstein, Benjamin Goodman, Vaslilla Kormendi, Nikolaus Grigorieff, Antonina Roll-Mecak, Co-First Author, NIH/NINDS, Bethesda, MD, USA, Co-First Author, HHMI Janelia Farm, Ashburn, VA, USA, Brown University, Providence, RI, USA, NIH/NINDS, Bethesda, MD, USA, HHMI Janelia Farm, Ashburn, VA, USA, NHBLI, Bethesda, MD, USA.

Microtubules are subject to a diverse array of posttranslational modifications. The majority of these modifications occur on the exterior surface of the microtubule. Acetylation of Lysine 40 is unique in that it occurs in the lumen of the microtubule. This modification is a marker for old, stable microtubules that are resistant to depolymerization. Tubulin acetyltransferase (TAT) is the enzyme responsible for this modification. Here we employ X-ray crystallography, electron microscopy, structure-based functional assays, single molecule imaging and first-principles modeling to understand how this unique enzyme is able to gain access to the microtubule and selectively modify microtubules that are long lived. Single molecule TIRF imaging of TAT-GFP reveals that TAT is undergoing 1-D diffusion along the microtubule and that the acetylation pattern is not biased for microtubule ends. The interaction time with the microtubule is unchanged when the microtubule surface is decorated with microtubule binding proteins or when the tubulin C-terminal tails are removed. However, TAT scanning is not observed when the luminal acetylation site is missing. First-principles modeling demonstrate that the slow catalytic rate of the enzyme and the access to the luminal modification site is rate-limiting. Consistent with this, our X-ray structure of TAT reveals an active site not optimized for efficient catalysis. Thus, the rapid diffusion of TAT coupled with its low catalytic rate gives cells a mechanism for selectively marking long lived microtubules and evenly deposit the acetylation mark along their length. Our insights into TAT mechanism have broader implications for proteins that reside in the microtubule lumen.

**Microtubule Electrodynamics Associated with Vibrational Normal Modes**

Michal Cifra, Daniel Havelka, Marco A. Deriu, Ondrej Kacera, 1Bioelectrodynamics, Inst. of Photonics and Electronics, Academy of Sciences of CR, Prague, Czech Republic, 2Department of Innovative Technologies, Institute of Computer Integrated Manufacturing for Sustainable Innovation, University of Applied Sciences and Arts of Southern Switzerland, Manno, Switzerland.

Cytoskeleton is a crucial integrating component of cellular functions. Microtubules, as a part of cytoskeleton, are involved in such functions, serving as transport track, signaling substrate and also are fundamental for cellular division. While chemical, mechanical and electrostatic nature of microtubule function in these cellular processes is being researched, current picture of all underlying types of interactions is far from complete.

First, a microtubule lattice is reconstructed from combined RCSB Protein DataBank 1TUB and JFF tubulin structure and interlocking alpha-tubulin loops between adjacent protofilaments are remodeled to obtain physiological conformation. We analyze normal modes of anisotropic elastic network microtubule model obtained by coarse-graining the atomic model and using rotating block approximation. Further, we map the atomic charge distribution upon atomic trajectories of the first 30 normal modes, which lie in GHz spectral region. Approximating the oscillating atomic charges as a Hertzian dipoles, we calculated and visualized electrodynamic field around the microtubule for each normal mode.

Based on the results, we suggest that the electrodynamic field around microtubule can provide interactions of different nature than pure electrostatic interactions since the ionic screening is diminished for frequency the electric >10 MHz. Significance of these unique theoretical predictions is planned to be verified in the upcoming experimental studies. Exploitation of microtubule electrodynamic properties could open paths to novel tools for microtubule manipulation with prospects in new therapeutic and diagnostic methods in the future.

**TRIM50 Interacts with Microtubules to Facilitate Vesicle Trafficking in Gastric Parietal Cells**

Kristyn N. Guumper, Mingzhai Sun, Jaqing Huang, Pei-Hui Lin, Miyuki Nishi, Hiroshi Takeshima, Jianjie Ma.

1Department of Surgery, Davis Heart and Lung Institute, Columbus, OH, USA, 2Department of Electrical and Computer Engineering, The Ohio State University, Columbus, OH, USA, 3Kyoto University, Kyoto, Japan.

The tripartite motif (TRIM) family of proteins consists of over 75 different genes and serve as regulators of many critical aspects of health and disease including, wound healing, secretion, and apoptosis. Our lab showed previously that MG53, a member of the TRIM-family proteins, played an essential role in repair cell membrane injury. TRIM50 is the closest homologue to MG53 and has dissimilar cellular function. The molecular mechanisms that underlie the different functions for MG53 and TRIM50 are unknown. We know that TRIM50 is predominantly expressed in the stomach, and participates in vesicle trafficking during acid secretion in gastric parietal cells. Recently, our lab has shown MG53 vesicle trafficking is driven by the molecular motor non-muscle myosin II-A. In this study, we used intracellular vesicle tracking to identify how TRIM50 associated vesicles move within the cell, distinguishing between diffusive and directional ballistic motion. We demonstrated that vesicles associated with TRIM50 do not exhibit diffusive motion; rather, they exhibit directed motion, which requires microtubules. Additionally, we showed that TRIM50 knockdown cells expressing the microtubule depolymerizing agentnocodazole or colchicine abolished the directed motion of the TRIM50-associated vesicles. We also performed a pull-down assay to identify the molecular motor that drives TRIM50 trafficking. Overall, our data suggest TRIM50-associated