microtubule organization. First, sliding velocity scales with initial microtubule-overlap length. Second, the width of the final stable overlap scales with microtubule lengths. Our analyses reveal how nanometer-sized proteins can decode micron-scale geometrical features of antiparallel bundles to define the structure and mechanics of microtubule-based architectures.

2499-Pos Board B515

Kinesin Binding Expands and Stabilises the GDP-Microtubule Lattice

Daniel Peet, Nigel Burroughs, **Robert A. Cross**.
CMCB, Warwick University, Coventry, United Kingdom.

Kinesin-1 is a walking machine that steps processively towards the fast growing (plus) ends of microtubules, hauling molecular cargo to specific reaction sites in cells. Recent work hints that kinesin-1 may also play a role in modulating the structure and stability of its microtubule track, but results are conflicting and mechanisms as yet unclear. By capping dynamic microtubules with GMPCPP tubulin, tethering them in a microfluidic flow and introducing kinesin, we have found that strong-state (ATP- and apo-) kinesin-1 motor domains inhibit the shrinkage of GDP-MTs by up to 2 orders of magnitude and expand their lattice spacing by 1.6%. Our data reveal an unexpected new mechanism by which the mechanochemical cycles of kinesin and tubulin interlock, allowing motile kinesins to influence the structure, stability and mechanics of their microtubule track.

2500-Pos Board B516

Mechanism of Microtubule Stabilization by Kinesin-5

Geng-Yuan Chen[1], Ana B. Asenjo[2], Hernando J. Sosa[2], William O. Hancock[1].
[1]Biomedical Engineering, Pennsylvania State University, State College, PA, USA, [2]Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, NY, USA.

In addition to their capacity to slide apart antiparallel microtubules during spindle formation, the mitotic kinesin-5 motor Eg5 has been shown to pause at microtubule plus-ends and enhance microtubule polymerization (Chen and Hancock, Nature Comm. 2015:8160). The goal of the present work is to understand the Eg5 microtubule polymerase mechanism by studying how the motor alters the lateral and longitudinal tubulin-tubulin interactions that stabilize the microtubule lattice. Transient kinetics and single-molecule tracking experiments demonstrate that dimeric Eg5 motors reside predominantly in a two-head-bound strong-binding state while stepping along the microtubule (Chen *et al.*, JBC 2016:291(39), 20283-94). This suggests that when Eg5 pauses at a growing microtubule plus-end, the motor acts as a two-head-bound ''staple'' to stabilize the longitudinal bonds of incoming tubulin dimers. The on-rate for Eg5 binding to free tubulin is slow, suggesting that end-bound Eg5 motors do not bind free tubulin in solution; rather they stabilize incoming tubulin dimers that have bound to the plus-end. Because tubulin in the microtubule lattice resides in a ''straight'' conformation, while free tubulin resides in a ''kinked'' conformation, a second (non-mutually exclusive) model is that Eg5 stabilizes the straight conformation of tubulin. Consistent with this, monomeric Eg5 motors, which bind to the microtubule lattice without crosslinking tubulin dimers, also stabilize microtubules against depolymerization. Furthermore, the affinity of Eg5 for free tubulin is reduced in the presence of ''wedge inhibitor'' drugs that stabilize the kinked conformation of tubulin; conversely Eg5 binding to tubulin diminishes drug binding. Thus, we propose a microtubule polymerase mechanism in which binding by one Eg5 motor domain at the microtubule plus-end straightens tubulin dimers and stabilizes lateral tubulin-tubulin bonds, while the second Eg5 head binds incoming tubulin and acts as a staple to stabilize longitudinal tubulin-tubulin bonds and enhance microtubule growth.

2501-Pos Board B517

Molecular Requirements for the Transition from Lateral to End-on Microtubule Binding and Dynamic Coupling

Ekaterina L. Grishchuk[1], Manas Chakraborty[1], Anatoly V. Zaytsev[1], Maxim Godzi[2], Ekaterina Tarasovetc[1], Ana C. Figueiredo[3], Fazly I. Ataullakhanov[2].
[1]Dept. of Physiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA, [2]Center for Theoretical Problems of Physicochemical Pharmacology, Russian Academy of Sciences, Moscow, Russian Federation, [3]Chromosome Instability & Dynamics Laboratory, Universidade do Porto, Porto, Portugal.

Accurate chromosome segregation relies on the ill-understood ability of kinetochores to convert their lateral microtubule attachment into the microtubule plus-end association, capable of the processive motion with tubulin dynamics. Here we report that this transition can be recapitulated in vitro using only two components: the plus end-directed kinesin CENP-E and the microtubule wall-binding Ndc80 protein. CENP-E's primary role is to establish the end configurations for Ndc80, while Ndc80 mediates the maintenance of end attachment. To gain insights into the molecular requirements for end-conversion we paired CENP-E with other microtubule-binding proteins. Ska1, CENP-E Tail, EB1 and CLASP2 differed in their ability to retain the microtubule ends, and none performed as robustly as Ndc80. Likewise, a non-mitotic transporter Kinesin-1 failed to support the Ndc80-mediated end-conversion, implying that the pair of CENP-E kinesin and Ndc80 is optimally suited for this activity. To investigate the underlying mechanistic differences between these motors and MAPs, we developed a Brownian dynamics model for the molecular ensembles of proteins engaged stochastically in walking and diffusion on the microtubule wall. Modeling demonstrates that the observed differences in the end-retention by different MAPs can be largely attributed to their different residency times and rates of diffusion on the microtubule wall. The model also recapitulates the strikingly different behavior of Kinesin-1 and CENP-E, suggesting that it is rooted in their distinct force-detachment characteristics. Following a model prediction, we were able to achieve robust end-conversion with Kinesin-1 by amending its dynamic response via the reduced ATP concentration. Together, our results argue strongly that microtubule end-conversion is an emergent property of the ensemble of transporting motors and diffusing MAPs, limited by the microtubule end boundary. We propose that similar mechanism ensures microtubule end conversion at mitotic kinetochores.

2502-Pos Board B518

Microtubule Structural State Recognition by End Binding Protein 1

Taylor A. Reid[1], Courtney Coombes[1], Holly Goodson[2], Melissa K. Gardner[1].
[1]University of Minnesota, Minneapolis, MN, USA, [2]Notre Dame, South Bend, IN, USA.

Microtubules are structural polymers that participate in a wide range of cellular functions and are the site of localization and activity for a host of proteins. End binding protein 1 (EB1) localizes primarily to GTP-bound tubulin subunits at the growing ends of microtubules, where it facilitates interactions with other key cellular proteins. However, reports of sublocalization of EB1 within the GTP-rich region at growing microtubule ends suggest that there may be an additional layer of regulation for EB1 binding to the microtubule. Using both bulk and sub-microtubule correlation TIRF microscopy experiments, we found that, independently of nucleotide state, EB1 exhibits preferential binding for non-lattice structures such as those found at microtubule ends or defects. To predict the mechanism for this preference, we performed 3D molecular diffusion simulations, and found that the unique binding location of EB1 at the pocket-like interface between four adjacent tubulin dimers results in a form of structural state recognition due to a high steric hindrance to binding within the lattice, which is reduced at microtubule ends or lattice defects. Additionally, our experiments and simulations using a tubulin face binding protein resulted in elimination of microtubule structure recognition, suggesting a general principle for protein association to cellular polymers based on the location and conformation of the binding interface.

2503-Pos Board B519

Structural Model for Preferential Microtubule Minus End Binding by CAMSAP CKK Domains

Joseph Atherton[1], Kai Jiang[2], Marcel Stangier[3], Yanzhang Luo[4], Shasha Hua[2], Klaartje Houben[2], Guido Scarabelli[5], Agnel Joseph[1], Anthony Roberts[1], Barry Grant[6], Maya Topf[1], Michel Steinmetz[3], Marc Baldus[2], Anna Akhmanova[2], **Carolyn Moores**[1].
[1]Birkbeck College, London, London, United Kingdom, [2]Utrecht University, Utrecht, Netherlands, [3]Paul Scherrer Institut, Villigen, Switzerland, [4]Utrecht University, U, Netherlands, [5]University of Michigan, Ann Arbor, MI, USA, [6]University of California, San diego, San diego, CA, USA.

Microtubules are polar polymers, with minus and plus ends exhibiting differential dynamics and regulated by different cofactors. The evolutionarily conserved calmodulin-regulated spectrin-associated protein (CAMSAP) family are minus end binding proteins that modulate minus end microtubule dynamics. All CAMSAPs share a characteristic conserved CKK microtubule binding domain, which defines their minus end specificity. However the mechanism of this specificity is not understood. To shed light on this question, we determined the CKK structure by X-ray crystallography and characterised CAMSAP1 and 3's CKK binding site on microtubules by cryo-EM. The CAMSAP CKK binds at a previously undescribed binding site at the microtubule intra-dimer inter-protofilament interface. TIRF microscopy was used to confirm the contributions of conserved residues at the CKK-microtubule interface. The nucleotide state of tubulin was also shown not to influence minus-end specificity. Interestingly, CKK binding imposes a right-handed 'supertwist', on microtubule protofilaments, even on the taxol-stabilized microtubules used for

our reconstructions. Further cryo-EM investigations, including of a CKK mutant, and another CKK domain with reduced minus-end specificity, supports the possibility that subtle alterations in CKK positioning relative to tubulin polymer confer minus-end specificity. Cryo-electron tomography of minus-ends reveals curved lattice to sheet regions that retain lateral protofilament interactions and present unique minus-end tubulin conformations to which CAMSAPs may preferentially bind.

2504-Pos Board B520

Structural Changes in Tau Underlie Static and Diffusive Binding to the Microtubule Lattice

Alisa Cario, Jamie Stern, Christopher L. Berger.
University of Vermont, Burlington, VT, USA.

The microtubule associated protein (MAP) Tau, primarily expressed in neurons, is known to have a variety of functions such as regulation of microtubule dynamics, modulation of kinesin motor motility and participation in signaling cascades. Research on the microtubule binding behavior of Tau reveals that Tau binds to the microtubule surface in an equilibrium between static and diffusive states. These functional states have been shown to be important in regulating kinesin motility during axonal transport. However, the structural relationship between the states has not been characterized. Therefore, using Total Internal Reflection Fluorescence (TIRF) microscopy, we developed a three-color imaging assay to study the structural changes underlying Tau's dynamic behavior while bound to the microtubule surface using single molecule Fluorescence Resonance Energy Transfer (smFRET). Additionally, Alternating Laser Excitation (ALEX) is used to distinguish between single labeled populations and low FRET efficiency states. We have generated three 3RS-Tau FRET constructs to measure distances between distinct locations within Tau, between the N and C termini (N-C), between the microtubule binding repeats and the C terminal (3-C), and between the N terminal and the microtubule binding repeats (N-3). Initial studies indicate 3RS-Tau possesses distinct N and C termini interactions that allow for static versus diffusive binding. The examination of additional interactions will define overall structural changes in Tau on the microtubule surface. The smFRET-ALEX approach we have developed has applications for studying differences in other highly dynamic MAPs as well.

2505-Pos Board B521

The Effect of Site-Specific Tau Mutations on Microtubule Bundle Structures

Christine Tchounwou.
UCSB, Goleta, CA, USA.

Microtubules (MTs) are biological nanostructures that exhibit GTP-hydrolysis controlled polymerization-depolymerization transitions which enable many important cellular functions. In neuronal axons, inherently dynamic MT architectures are functionalized by binding of the MT-associated phosphoprotein tau. Changes in tau-MT associations have been noted in the literature as a consequence of post-translational modifications to tau such as hyperphosphorylation leading to the hallmarks of several neurodegenerative disease states. We hypothesize that post-translational alterations to specific residues in close proximity to the MT-binding region of tau may influence how tau mediates MT architectures in axons. Furthermore, we demonstrate that purification of site-specific mutagenic tau is now readily achievable with expression of mutant recombinant tau containing a poly-His-tag. Similar to phosphoprotein tau, we have previously reported that biologically relevant polyamines such as spermine also directly modulate electrostatic interactions between MTs leading to unique bundling architectures. Here we probe dynamical MT bundling architectures in the presence of site-specific mutagenic tau with and without polyamines using transmission electron microscopy (TEM) and small angle x-ray scattering (SAXS).

2506-Pos Board B522

Role of Anti-tau Antibodies on Microtubule Polymerization and Stability

Iva Ziu, Matthew Imhof, Saba Anwar, Sanela Martic.
Chemistry, Oakland University, Rochester, MI, USA.

Tau is an intrinsically disordered protein which in neuronal cells promotes the polymerization and stability of microtubules (MT). In neurodegenerative diseases, tau undergoes post-translational modifications (PTMs), misfolds and forms toxic aggregates. Currently, tauopathies remain without a cure. Immunotherapies targeting tau protein in animal models induced clearance of tau pathology. However, the mechanisms of antibody-based inhibition of neurodegeneration remain mostly unclear. We have previously reported on the inhibition of tau aggregation *in vitro* by antibodies to non-phosphorylated tau441. We also have shown that phosphorylation of tau441 at Ser199 by GSK-3ß protein kinase may be inhibited by antibodies to phosphorylated tau. Here, we evaluated the polymerization of tubulin in the presence of non-phosphorylated tau and antibodies to non-phosphorylated tau [targeting epitopes in the N-terminus, R4, and C-terminus]. ELISA was used to determine antigen-antibody binding affinities. The microtubules were characterized by transmission electron microscopy. Tubulin fluorescence polymerization assay indicated that the antibodies reduced tubulin polymerization. In the presence of tau441, the tubulin polymerization was rescued even in the presence of antibodies. In addition to MT formation, the stability of paclitaxel-stabilized MT was also evaluated. Data indicate that tau protein and its antibodies play regulatory and/or competitive roles in MT formation/stability.

2507-Pos Board B523

N-terminal Inserts Impact the Global Conformation of Tau and the Tau-Tubulin Complex

Kristen McKibben[1], Elizabeth Rhoades[2].
[1]Biochemistry and Molecular Biophysics Graduate Group, University of Pennsylvania, Philadelphia, PA, USA, [2]Department of Chemistry, University of Pennsylvania, Philadelphia, PA, USA.

Tau is an intrinsically disordered, microtubule associated protein best known for its role neurodegenerative tauopathies such as Alzheimer's disease. Tau's physiological roles include stabilizing microtubules and regulating microtubule dynamic instability. There are six different isoforms of tau arising from alternative splicing of two acidic N-terminal insert regions (0N, 1N, and 2N) and the second microtubule-binding repeat (3R and 4R). While significant attention has been given to differences in the interactions of 3R and 4R isoforms with tubulin and microtubules, less is known about the role of the N-terminal inserts. In this study, we seek to understand the impact of the N-terminal inserts on the global conformation of tau in solution and upon binding to tubulin using single molecule Förster resonance energy transfer (smFRET) spectroscopy. Our measurements show that the overall global architecture between the N-terminal isoforms is similar in solution. However, there are also isoform specific differences; within the N-terminus, there is an inverse correlation between the average per residue distance and the number of inserts. Upon binding to tubulin, there is a large expansion of the region between the N-terminus and the third microtubule-binding repeat. While the magnitude of this expansion per residue is similar in 2N4R and 0N4R, 1N4R shows a greater per residue expansion. Combined with the solution data, this suggests there is a short sequence or sequences in the N-terminal inserts responsible for maintaining a homologous conformation between isoforms in solution that is disrupted upon binding to tubulin. Additional studies are required to pinpoint this region and its corresponding interacting sequence.

2508-Pos Board B524

Disparate Roles of Alpha and Beta CTTs in Microtubule Severing

Rohith Anand Varikoti.
University of Cincinnati, University of Cincinnati, Cincinnati, OH, USA.

Microtubules (MTs) are responsible for major cellular processes like cell motility, transport, and mitosis. Modulation of their structure and function in in vivo conditions is achieved by various microtubule associated proteins (MAPs). Among these MAPs are the MT severing enzymes, which belong to the ATP-dependent homo-hexamerases of ATPases associated with various cellular activities (AAA^{+}) family of enzymes. These enzymes are known to bind to MTs in various orientations and for functional reasons they should bind to the acidic residue rich carboxy-terminal tails (CTTs) of tubulins. CTTs are known to be crucial for the severing of MTs, but the actual mechanism requiring CTTs is unknown. The current view is that severing proteins bind to CTTs and thread them through the center pore of the hexamer, thus facilitating severing. Using a combination of docking studies with molecular dynamics simulations and Normal Mode Analysis (NMA), we investigated the binding and dynamics of different CTT isoforms on severing proteins. We found that binding of Beta isoforms is preferred over Alpha isoforms, which recapitulates the behavior of katanin binding to CTTs from the experimental literature. Using the structural information resulting from docking for dynamic simulations can subsequently give insight into how the action of severing enzymes resembles that of AAA^{+} proteins in general, which involves the cooperative action of monomers or groups of monomers in a hexamer to perform the unfolding and translocation of substrates.

2509-Pos Board B525

Modulation of Macromolecular Biological Structures by Divalent Ions

Bretton J. Fletcher[1], Chaeyeon Song[1], Phillip Kohl[1], Peter Chung[1,2], Herbert P. Miller[1], Youli Li[1], Myung Chul Choi[3], Leslie Wilson[1], S.C. Feinstein[1], Cyrus R. Safinya[1].
[1]UCSB, Santa Barbara, CA, USA, [2]University of Chicago, Chicago, IL, USA, [3]KAIST, Daejeon, Republic of Korea.

Divalent ions can mediate condensation of highly charged biological macromolecular assemblies by producing attractions and suppressing repulsions between like-charged structures. Combining small-angle x-ray scattering with x-ray