ability) completely abolishes the effects of retigabine. This finding suggests that retigabine interacts with KCNQ channels via a hydrogen bond with the Trp indole group, a rarely demonstrated mode of drug:target interaction. Supporting this model, substitution with fluorinated Trp analogs can strengthen retigabine potency by increasing the indole N-H bond polarity. Lastly, the potency of numerous retigabine analogs for KCNQ3 channels was found to correlate with the negative electrostatic surface potential of a conserved carbonyl oxygen atom. These findings demonstrate the detailed chemical interactions and functional groups that likely underlie the effects of retigabine and other KCNQ activators, highlighting a rarely observed mode of drug interaction with multifunctional amphipathic Trp side chains. These stringent constraints for models of retigabine interactions with KCNQ channels may guide the rational development of improved retigabine derivatives.

Platform: Kinesins, Dyneins, and Other MT-based Motors

100-Plat
Kinesin-5 Acts as a Microtubule Stabilizer, Polymerase and Plus-Tip Tracker
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Kinesin-5 slides antiparallel microtubules apart during mitosis and is necessary for bipolar spindle formation. Besides its unique homotetrameric configuration, determined by the coiled-coil domain, kinesin-5 motor domains also possess specific properties optimal for their spindle organizing function. To study properties intrinsic to the kinesin-5 motor domain, we generated functional kinesin-5 dimers by fusing the kinesin-5 head and 18-residue neck linker to the coiled-coil rod of kinesin-1. In ATP this kinesin-5 dimer decorated plus-ends of taxol-stabilized microtubules and slowed depolymerization of GMPCPP microtubules. On dynamic microtubules, kinesin-5 dimer increased the microtubule growth rate by more than a factor of two and reduced the catastrophe frequency three-fold. These findings are consistent with kinesin-5 acting as a microtubule polymerase. To understand this polymerase mechanism, TIRF microscopy was used to visualize individual GFP-labeled kinesin-5 dimers on immobilized microtubules. Motors walked to the ends of microtubules and remained bound there for 7 seconds, much longer than the 0.1 s motor step time. We hypothesize that kinesin-5 promotes microtubule polymerization by stabilizing longitudinal interactions between tubulin subunits on a single protofilament. Consistent with this protofilament stabilization hypothesis, fluorescence analysis suggests that microtubule plus-ends are more tapered in the presence of kinesin-5. Furthermore, curved and looped ends were observed, which occasionally resolved into straight microtubules, consistent with kinesin-5 stabilizing long protofilament bundles. In cells, these end-binding and polymerase activities should enhance the ability of kinesin-5 to establish and maintain spindle pole separation during mitosis.

101-Plat
Why are Kinesin-2 KIF3AB and KIF3AC so Processive?
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Mammalian KIF3AC is a heterotrimeric kinesin-2 that is best known for roles in organelle transport and remodeling of the microtubule cytoskeleton in neuronal tissue, yet *in vitro* studies to characterize its single molecule behavior are lacking. We report that a bacterially expressed native sequence of KIF3AC is highly processive with run lengths matching those exhibited by conventional kinesin-1 K560 in comparative experiments. This result was unexpected because KIF3AC exhibits the canonical kinesin-2 neck linker sequence that has been reported to be responsible for shorter run lengths observed for kinesin-2 KIF3AB. However, a comparative KIF3AB engineered with native sequence also exhibited long run lengths suggesting that neck linker length is not the sole determinant of run length for native kinesin-2 motors. KIF3C contains a long extension in surface loop L11 not present in KIF3A or KIF3B, or other processive kinesins. L11 is a component of the kinesin microtubule interface and has been implicated in activation of ADP release upon microtubule collision. KIF3AC encoding a truncation in KIF3C L11 (KIF3ACL11) is even more processive than wildtype KIF3AC and more similar to KIF3AB, suggesting that L11 also plays a role in determining run length. Steady-state ATPase experiments show that shortening L11 does not alter the k_{cat}, consistent with the observation that single molecule velocities are not affected by this truncation. However, shortening L11 does significantly increase microtubule affinity in these dimers. Analysis of homodimeric KIF3CC and KIF3CCL11 reveal that both are processive but exceedingly slow. These results reveal that processivity can be regulated in part by L11, consistent with the interpretation that L11 becomes ordered upon microtubule collision to activate ADP release. These studies also expand our understanding of motor processivity and point to alternative structural and mechanistic modulators of processivity. Supported by NIH R37-GM54141.

102-Plat
SRC Kinase Phospho-Regulation of the Human Mitotic Kinesin Eg5
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Eg5 is a human kinesin-5 that drives spindle pole separation during the early phases of mitosis. While a substantial body of work has revealed the role that Eg5 plays during mitosis, relatively little is known about how Eg5 activity is regulated throughout the cell cycle. Our data shows that endogenous Eg5 in HEK cells is phosphorylated on tyrosine residues. In silico predictors suggest three tyrosines in the Eg5 motor domain as targets of Src kinase phosphorylation. We show that these residues are phosphorylated specifically by Src kinase, but not by Wee1 kinase in vitro. Furthermore, cells expressing Eg5 constructs with a phosphomimetic mutation of Y211 to glutamate (Y211E) show significantly higher percentages of monopolar spindles than cells expressing wild-type Eg5. The Y211E mutation also significantly decreases Eg5 ATPase rates and motility in vitro. The proximity of the potential phosphorylation sites to the binding sites of small molecule inhibitors targeting Eg5 suggested that phosphorylation may interfere with drug binding. Isothermal calorimetry experiments show that phosphomimetic Eg5 constructs have significantly decreased affinity for S-trityl-L-cysteine (STLC). Together, these data suggest a role for the mitotic kinase Src in regulating Eg5 throughout the cell cycle by directly altering its motor characteristics. Such a role for post-translational modifications has not yet been investigated in Eg5. Additionally, the effect of phosphomimetic mutations on STLC binding suggests that phosphorylation may decrease the efficacy of Eg5 inhibitors that are currently in clinical trials. Future work will seek to confirm Src kinase as a regulator of Eg5 activity in cells and investigate phosphorylation as a potential cancer resistance mechanism.

103-Plat
Emergence of Large-Scale Vortices of Microtubules Collectively Driven by Axonemal Dyneins
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Experimental systems have long been demanded for the study of collective motion often observed in biology (flocking of birds, cell migrations during development etc). In vitro motility assays commonly used in protein-motors' biophysics now fulfill the demand described above. Using the in vitro motility assays, we reported collective motion and emergence of vortices of microtubules (MTs) driven by axonemal dynein [Sumino et al., Nature 483, 448-452, 2012]. Recently, we found that under various experimental conditions, the collective motion of MTs can display nematic order, millimeter-scale meandering streams or millimeter-scale vortices. To explore the conditions causing such phase-shifts, we use different types of dynein (dynein c and g of Chlamydomonas flagella) and MTs with various mean lengths. Dynein c and g are capable of moving MTs on glass surface at velocities of ca. 12 and 6 μm/sec in the presence of 1 mM Mg-ATP at 23°C, respectively. Upon the addition of Mg-ATP, MTs start smooth gliding motions. Moving MTs often collide each other and upon these collisions they are aligned through nematic interactions. MTs-alignment gradually increases its size and finally forms streams meandering across a very large distance. MTs of less than 60-μm mean length finally generate vortices with 200-500 μm diameters. On the other hand, MTs of longer than 60-μm do not form vortices. The features of vortices generated by dynein g, i.e. number, diameter and time needed for their emergence, are different from those in dynein c. These results suggest that the vortex formation may reflect the mechanical properties of inner-arm dyneins and one parameter which can modify the phase is the length of MTs.