were critical in the increased efficacy of Eg5 inhibition by this small molecule.

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The Homotetrameric Kinesin-5, KLP61F, Preferentially Crosslinks Antiparallel Microtubules
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The segregation of the genetic material during mitosis is coordinated by the mitotic spindle, whose mechanism of action depends upon the polarity patterns of its constituent microtubules (MTs). Homotetrameric mitotic kinesin-5 motors are capable of crosslinking and sliding adjacent spindle MTs, but it is unknown if they, or other motors, contribute to the establishment of these MT polarity patterns. Here we explored if the *Drosophila* embryo kinesin-5, KLP61F, which is thought to crosslink both parallel and anti-parallel MTs, displays a preference for the parallel or anti-parallel orientation of MTs. In motility assays, KLP61F was observed to crosslink and slide adjacent MTs, as predicted. Remarkably, KLP61F displayed a three-fold higher preference for crosslinking MTs in the antiparallel, relative to the parallel orientation. This polarity preference was observed in the presence of ADP or in ATP plus AMPPNP, but not in AMPPNP alone, which induces instantaneous rigor binding. Also, a purified motorless tetramer containing the C-terminal tail domains displayed an antiparallel orientation preference, confirming that motor activity is not required. The results suggest that, during the morphogenesis of the *Drosophila* embryo mitotic spindle, the crosslinking and sliding activities of KLP61F could facilitate the gradual accumulation of KLP61F within antiparallel interpolar (ip) MTs at the equator, where the motor could then generate force to drive poleward flux and pole-pole separation.

2614-Pos Board B584
Three-dimensional Nanometer Resolution Optical Tracking Reveals A Torque Component Present In Single-headed Kinesin
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We have developed a novel method for tracking microtubule rotation in three dimensions, which uses only one optical component, a prism, without modification of other aspects of a standard microscope. We applied our method to a conventional *in vitro* sliding assay by tracking streptavidin-coated quantum dots that are bound to a sparsely-biotinylated microtubule sliding across lawns of kinesin motors. Our method achieves nanometer accuracy and returns three-dimensional positional information. Using this method, we found that surface-attached Eg5 monomeric fragments (a member of the kinesin-5 sub-family of microtubule-based motors, which is essential for the assembly and maintenance of the bipolar spindle architecture *in vivo*) drove counterclockwise rotation of sliding microtubules around their axis. These corkscrewing motions have not been seen previously for kinesin-5, and it demonstrates that single kinesin-5 heads produce torsional force as well as axial sliding force. We also found that the rotational pitch was insensitive to microtubule geometry [1]. This short-pitch rotation by single-headed kinesin-5 molecules is strikingly similar to both that of a plus-end directed, non-processive single-headed kinesin-1 molecules [1, 2], which have a N-terminal motor domain, and that of a minus-end directed, non-processive double-headed kinesin-14 molecules [3], which have a C-terminal motor domain. A value of ~ 0.3 μm for the rotational pitch generated by theses three motors appears to represent a characteristic signature for non-processive motors. This suggests the possibility that a kinesin head possesses in common an inherent torque component. [1] Yajima J., Mizutani K. & Nishizaka T. Nat. Struct. Mol. Biol. (2008), [2] Yajima J. & Cross RA. Nat. Chem. Biol. 1 (2005) 338-41., [3] Walker RA., Salmon ED. & Endow SA. Nature 347 (1990) 780-2.

2615-Pos Board B585
Structure of the Kinesin13-Microtubule Ring Complex
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Kinesin-13 proteins are a group of motors that are not motile on microtubules, but instead catalyze the ATP-dependent depolymerization of microtubules *in vivo* and *in vitro*. Their functions are important for accurate chromosome segregation in mitosis. Our goal is to elucidate the structural basis of the mechanism-of-action of these motors by studying the interaction of kinesin-13s with microtubules.
Previously we have shown that the kinesin-13 motor domain (MD) in ATP-bound state has the unusual property to form rings/spirals around microtubules. We have recently obtained a medium resolution three-dimensional (3D) density map of the kinesin13-ring-microtubule complex by cryo-electron microscopy and image analysis. An atomic model of the complex has been built by docking the crystal structures of tubulin and a kinesin13 MD into the 3D map. Our model reveals a snapshot of the depolymerization mechanism by providing a 3D view of the complex formed between the kinesin13 MDs and a curved tubulin protofilament. It suggests that contacts mediated by kinesin13 class-specific residues in the putative microtubule-binding site stabilize intra-dimer tubulin curvature. In addition, a new tubulin-binding site on the kinesin13 MD was identified. Mutations at this class-conserved site selectively disrupt the formation of microtubule-associated ring complexes.

2616-Pos Board B586
Studies of the Interaction of a Kinesin-13 Protein with Microtubules
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Kinesin-13s are motor proteins involved in microtubule (MT) depolymerization and are important for regulating of MT dynamics during chromosome segregation in mitosis. Recently, it was proposed based on single molecule experiments that Kinesin-13s use diffusion on the lattice to reach the MT ends, where they accumulate and induce the depolymerization in an ATP dependent manner. Besides, we showed by Electron Microscopy that Kinesin-13s form rings and spirals around MT in AMPPNP state. Such behavior was never reported for others Kinesin family, which shows only regular MT decoration in identical conditions. Furthermore, observation that kinesin-13s accumulate on depolymerizing ends of a MT *in vivo* suggests that such rings might work by keeping kinesin-13s associated with the MT ends.
Here, we are using BSR-labeled KLP10A to investigate changes in orientation and mobility of Kinesin-13 bound to MT at different steps in the ATP hydrolysis cycle by Fluorescence Polarization Microscopy. Our results show that KLP10A is more disordered than Kinesin in all nucleotide conditions, except ADP state. We observed diffusion of KLP10A neck-motor constructs on MT in all nucleotide states except by Non-Nucleotide conditions. Further experiments with KLP10A motor-only constructs are important for the identification of the regions of the protein necessary for the diffusion movement. Interestingly, we observed events of KLP10A oligomerization during our diffusion experiments, which can be an evidence of the rings structures. A comparison between the diffusion profile of KLP10A in AMPPNP state with MT attached and detached from the slide bottom is important to reinforce the evidence for KLP10A oligomerization. Also, the accumulation of KLP10A on the depolymerizing MTs ends by single molecule measurements would favor the hypothesis of rings facilitating MT depolymerization. Overall, this work will provide a better understanding of the interaction of Kinesin-13 with MT.

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Measurement Of The Protein Friction Between The Yeast Kinesin-8 Kip3p And Microtubules
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Several proteins have been shown to undergo 'one-dimensional' diffusion along the surface of microtubules. Diffusion is thought to enhance the rate of targeting of proteins to the microtubule end for the depolymerizing kinesin-13 and the polymerase XMAP215, or to increase the processivity of kinesin-1 and dynein. According to the Einstein-Smolukowski relation, the diffusion coefficient, D, is related to the friction coefficient, *gamma*, according to $D = kT/gamma$. This relation, however, has not been experimentally tested for individual bio-molecules. We measured both the diffusional and frictional properties of single yeast kinesin-8 motor proteins,