switching system into the microtubules enables high efficient regulation of kinesin motor activity.
In this study, we have done the experiments on incorporation of photochromic molecule into wild type microtubules and gliding assay of them under UV and VIS light irradiation. The gliding speed of the wild type microtubules modified with PAM or 3,3-dimethyl-6'-nitro −1-[2- [3-(3- pyrroline −2,5-dion) −1-yl] propanoyloxy]ethylindoline −2-spiro-2'(2H)- chromene (MASP) did not change. The results suggested that modification of the intrinsic reactive cysteine residues on the surface of microtubules does not affect motor activity of kinesin. Therefore, currently we are trying to prepare mutant microtubules that have an additional single reactive cysteine residue at the region near the kinesin binding site.

2439-Pos Board B425
Preparation and Polymerization of Tubulin of Ginkgo Biloba and its Interaction with Rice Plant Kinesin
Seigo Iwata, Nozomi Umezu, Kazunori Kondo, Shinsaku Maruta.
Previously, we have expressed novel rice plant specific kinesins and studied their biochemical characterization. The plant kinesins showed very unique properties. Especially ATPase activities of the kinesins were relatively much lower than that of conventional kinesin. Moreover, the kinesins did not show motile activity on the microtubules prepared from porcine brain. Although the structure of tubulin is well conserved, it is demonstrated that the plant tubulin has different characteristics from tubulin derived from animal. Therefore, plant kinesin may be more compatible with plant tubulin than animal tubulin. In this study, we prepared the plant tubulin from the pollen of *Pear* and *Ginkgo biloba*. The tubulin was extracted from the acetone powder of pollen. Subsequently, the tubulin was purified with DEAE Sephadex A-50 chromatography, Sephacryl S-300 gel filtration, and Mono Q anion exchange chromatography. And we also prepared the plant tubulin from the leaf of *Ginkgo biloba* utilizing the TCA/Phenol method.
Polymerization of the purified plant tubulin to microtubule was monitored by measuring the increase of absorption at 350 nm. Negative staining electron microscopic analysis revealed the microtubule configuration.
In preliminary experiments, the ATPase activity of rice plant kinesin K16 was activated by plant tubulin more significantly than that of animal tubulin. These results suggested that plant kinesin we have prepared is more compatible to plant kinesins than kinesin derived from animal.

2440-Pos Board B426
Katanin P60 and Fidgetin, Variations on Microtubule-Severing Mechanisms
Juan D. Diaz.
The dynamic properties of microtubule polymers are tightly regulated by the cell, often through the use of microtubule-associated proteins (MAPs). A novel class of microtubule-associated protein causes the severing of microtubules and are called microtubule severing enzymes. These proteins belong to the AAA+ family of ATPases. Katanin was the first microtubule severing enzyme identified. The catalytic subunit of Katanin, p60, has a role in cell division, cell motility, and regulated microtubule length and dynamics in mitotic and interphase cells. Other severing enzyme families include spastin and fidgetin. Fidgetin has yet to be proven to be a Severing enzyme in vitro. We performed the first single molecule characterization of katanin and fidgetin in vitro. We find that both katanin and fidgetin depolymerize microtubules at low concentration, but sever at high concentration. The depolymerization is faster from the plus end for katanin, but from the minus end for fidgetin. Using single molecule imaging, we find that, oligomerization, binding and diffusion depends on p60 nucleotide state. In contrast with katanin, fidgetin severs preferentially on GMPCPP-tubulin compared to GDP-tubulin. Finally, fidgetin presumably removes extended regions of protofilaments from the microtubule without cutting entirely through the microtubule, an ability called ''protofilaments stripping''. Both katanin and fidgetin are capable of severing microtubules, but their biophysical abilities and locations on microtubules are distinct from each other.

2441-Pos Board B427
A New Microtubule Gliding Assay Analysis of Microtubule Persistence Length
Lu Yu, Brian L. Van Hoozen, Carol E. Bodnar, **Douglas S. Martin**.
Microtubules are cytoskeletal polymers which play a role in cell division, cell mechanics, and intracellular transport. Each of these functions requires microtubules that are stiff and straight enough to span a significant fraction of the cell diameter. As a result, the microtubule persistence length, a measure of stiffness, has been actively studied for the past 15 years. Curiously, short microtubules are 10-50 times less stiff than long microtubules. In order to study this phenomenon, we have developed a new kinesin-driven microtubule gliding assay analysis of persistence length. By combining sparse fluorescent labeling of individual microtubules with single particle tracking of individual fluorophores, we tracked microtubule gliding trajectories with nanometer-level precision. By varying the surface density of kinesin, we measured the persistence length of microtubules on length scales from 100s of nanometers to 10s of micrometers. Microtubule persistence length increased from about 200 micrometers to 3 mm across these scales, consistent with previous experiments at short or long length scales. The scale dependence of persistence length we measured supports models in which protofilaments are loosely coupled near microtubule tips, becoming tightly coupled far from the tip.

2442-Pos Board B428
Microtubule Motility in Crowded Conditions in vitro
Lynn Liu, Oana Ursu, Jennifer Ross.
Microtubules and their associated proteins form a complex network essential to cellular trafficking. Motor proteins such as kinesin-1 travel on microtubules to transport membrane bound vesicles across the crowded cell. Many *in vitro* assays only study the biophysical properties of microtubules and motors at low density. In the gliding assay low concentrations of fluorescently labeled microtubules are visualized gliding as the immobilized motors push them. By adapting this assay to high density, gliding assays can study the physical properties of these biomolecules in a more physiological setting. We find that gliding velocity is is dependent on both motor and microtubule concentrations. Until a critical concentration is reached, the gliding velocity increases with increasing motor concentration. Conversely, increasing the microtubule concentration causes an increase in the gliding velocity. At the maximum microtubule concentration, self organized patterns such as vortices appear. These different behaviors give insight into how crowded conditions, such as those in the cell, might affect motor behavior and cytoskeleton organization.

2443-Pos Board B429
Enclosing, Deformable Membrane Aids in Focusing and Stabilizing the Mitotic Spindle
Christopher C. Poirier, Yixian Zheng, **Pablo A. Iglesias**.
Mitosis involves the interaction of microtubules, motor and associated proteins to assemble a mitotic spindle. Recent evidence suggests that the assembly of the mitotic spindle maybe be aided through the contributions of an external membranous matrix. A multi-agent computational model was constructed to study the possible mechanisms by which an external membrane could aid and influence the assembly of the mitotic spindle. A systematic study of the model parameters examined the effect motor and crosslinker proteins at varying concentrations have on the elongation rate and assembly of a mitotic spindle. The effect on spindle assembly and overall spindle length by varying membrane elasticity, microtubule length and microtubule orientation was also investigated. We show that overall; an enclosing, elastic membrane around the mitotic assembly machinery helps to focus microtubule minus ends, and provides a resistive force to regulate the size of the assembled spindle.

2444-Pos Board B430
VDAC and Tubulin are Similarly Abundant Cellular Proteins: Consequences for Tubulin Regulation of Mitochondrial Permeability and for Mitochondrial Outer Membrane Structure
Adrian Begaye, Ralph Nossal, **Dan L. Sackett**.
Tubulin binding to the mitochondrial outer membrane (MOM) was first reported many years ago but the function was unknown; we showed recently that dimeric tubulin binds to VDAC (mitochondrial porin), the major metabolite channel in the MOM, and that this results in closing the channel to ATP-ADP exchange, with a consequent drop in oxidative phosphorylation (PNAS (2008) 105:18746). This could act as a regulator of normal oxidative metabolism, but excess tubulin binding could also lead to changes in the inner membrane potential, and possibly lead to mitochondrial swelling and triggering of apoptosis. To evaluate the plausibility of this mechanism for either regulation of normal metabolism or induction of apoptosis, the concentrations of tubulin and VDAC in the cell need to be known, as a huge discrepancy in either direction could make the mechanism unlikely. We have used quantitative Western blots to measure the concentrations of tubulin and VDAC (and actin and GAPDH) in total cell extracts from a number of cell lines. As expected, we found tubulin to be an abundant protein (~1.0 % of total protein), but we also found VDAC to be more abundant than perhaps commonly thought (~0.4 % of total protein). Thus we conclude that tubulin regulation of VDAC permeability is a plausible mechanism. Further experiments are underway to test this mechanism and its role in induction of apoptosis by chemotherapy agents, especially microtubule-directed ones. In addition the abundance of VDAC means that a substantial fraction of the MOM surface area in mammalian cells is filled by VDAC.

2445-Pos Board B431
The Rigidity of Aging Microtubules
Taviare L. Hawkins, Matthew Mirigian, M. Selcuk Yasar, Jennifer L. Ross.
Microtubules are the most rigid filament of the cytoskeleton. They define the shape of the cell, function as routes for intracellular transport, and aid during