observed. From the KCl-extraction experiments, we concluded that 24 nm layer-line signals were from outer dynein arms (ODAs). From the detailed analysis of 24 nm signals, we found that ODAs in a rigor state were arranged in parallel planes perpendicular to the axonemal axis, but in a relaxed state they were converted into another stable arrangement, *i.e.,* arranged along helical lines around axonemes (3.3±1.8-start helices) or in parallel planes oblique (19±2.7 degrees) to the axonemal axis. It implies that there are two stable specific dispositions of peripheral doublet microtubules (MTs) that are converted into one another depending on the cross-bridging states between ODAs and MTs. In the case of oblique or helical arrangements of ODAs, interactions between the central pair MTs and spoke heads would be dominant. We also determined the diameter of ODA center positions in the axonemes to be 135 nm that showed no obvious changes between in rigor and relaxed states.

2362-Pos Cross Linking Of Kinesin Using Bifunctional Photochromic Compounds Results In Photo-regulation Of Atpase Activity

Eiichi kobayashi, Masafumi D. Yamada, Masato Ito, Shinsaku Maruta

Soka University, Tokyo, Japan.

Board B477

We have previously demonstrated the possible application of an azobenzene derivative in regulating a conformational change in skeletal muscle myosin. The bifunctional SH reactive azobenzene derivative, 4,4 -azobenzene-dimaleimide (ABDM), was incorporated into the SH1-SH2 region of skeletal muscle myosin subfragment-1 (S1), which is a potential energy-transducing site. Myosin S1 was modified with ABDM, and the global conformational change of S1 induced by the cis-trans isomerization of the cross-linked ABDM in response to UV/VIS light was investigated. It was shown that the cis-trans isomerization of ABDM promotes a swing in the lever arm of S1 in a direction opposite to that induced by ATP binding. In the present study, we have cross-linked kinesin using 4,4-azobenzene-dimaleimide (ABDM) in order to photo-control the ATPase activity. The mutants of kinesin motor domain A247C, T242C, A244C, L249C, A252C, G272C, and S275C, which have a single cysteine residue in L11 or L12, have been prepared. The mutants were cross-linked intermolecularly by ABDM at high efficiency 70–90%.The microtubules dependent ATPase activity of the cross-linked kinesin mutants A247C, T242C, A244C, L249C, G272C, S275C did not change on alternate UV-VIS light irradiation. On the other hand, A252C cross-linked by ABDM showed significant alteration of ATPase activity between UV and VIS light irradiation.

2363-Pos Impact Of C-terminal Truncation On Cytoplasmic Dynein

Naoki Numata[1], Tomohiro Shima[1], Anthony Roberts[2], Takahide Kon[1], Reiko Ohkura[1], Kazuo Sutoh[1]

[1] *University of Tokyo, Tokyo, Japan,*
[2] *University of Leeds, Leeds, United Kingdom.*

Board B478

Cytoplasmic dynein is a motor complex responsible for diverse cellular processes within eukaryotic cells, including mitosis, Golgi dynamics and the retrograde transport of various vesicles and organelles along the microtubule network. The motor unit of cytoplasmic dynein consists of three structurally distinct domains: a catalytic head, a cargo-carrying tail, and a microtubule-binding stalk. The head comprises six concatenated AAA+ (ATPases associated with diverse cellular activities) modules arranged in a ring-like structure, and a C-terminal sequence with unknown structural fold. While the ATP binding/hydrolysis mechanism of the multiple AAA+ modules has been well characterized, how the C-terminal sequence contributes to the head structure and the activity is poorly understood. In this study, we attempted to reveal the functional role of the C-terminal sequence, using a C-terminally truncated motor derived from Dictyostelium cytoplasmic dynein.

We show that the complete removal of the C-terminal sequence (406 residues (46kDa)) caused loss of all the motor activities (microtubule-dependent ATPase, ATP-sensitive microtubule binding, and microtubule sliding activities), despite maintaining the ring-like appearance as revealed by EM. On the contrary, partial removal of the C-terminal sequence (283 residues (30kDa)), the sequence missing in Saccharomyces Cerevisiae and other fungi, had little influence on the activity. We are now characterizing the motile activity of this construct at a single molecule level.

Microtubules & Microtubule-associated Proteins

2364-Pos The Structure of Kinesin-13 Rings and Its Functional Implications

Dong-Yan Tan, Ana Asenjo, Vito Mennella, Hernando Sosa

Albert Einstein College of Medicine, Bronx, NY, USA.

Board B479

Kinesin-13 proteins are key players during mitosis in that they effectively regulate microtubule dynamics by inducing microtubule depolymerization. This function of kinesin-13s is distinct from conventional kinesins, which are motile proteins that transport cargoes along the microtubule tracks. We are interested in understanding the structural basis for the unusual behavior of the kinesin-13s. In our previous studies, we have observed kinesin-13s form a novel structure of rings/spirals around microtubules in the presence of AMP-PNP, a non-hydrolysable analog of ATP. Three-dimensional structural analysis on negative-stain micrographs suggested the rings/spirals consist of a curved protofilament ring on the outside and two circumferences of kinesin-13 motors in the inside. One of these circumferences interacts with the curved protofilament and the other interacts with the microtubule surface. The structural model also predicts the existence of the motor-motor interaction to maintain the radial integrity of the ring complex. However, our recent data obtained by cryo-electron microscopy and helical reconstruction techniques suggests a different structural model of the ring complex.

The primary reconstruction suggests that only one kinesin-13 motor circumference exist inside the ring, which is responsible for interacting with both the microtubule lattice and the outermost curved protofilament. On the other hand, our live cell imaging

experiments suggested the existence of kinesin-13 rings *in vivo* and studies on the kinesin-13 mutants indicate a correlation between the protein's depolymerization activity and its ability to form oligomeric rings.

2365-Pos Force Generation by GTP- and GMPCPP- Microtubules: from Single Microtubules to Cooperative Bundles

Julien Husson, Liedewij Laan, Laura Munteanu, Marileen Dogterom

AMOLF, Amsterdam, The Netherlands.

Board B480

Microtubules (MTs) play a key role in many functions of the cell. They participate in the positioning of chromosomes during cell division during which dynamic parallel bundles interact with the kinetochore. The dynamics of these bundles derive from the coupled dynamics of single microtubules. Microtubules polymerized in the presence of guanosine triphosphate (GTP) undergo a stochastic switch between slow growth and fast shrinkage termed dynamic instability. Microtubules growing in the presence of the slowly hydrolysable GTP analogue GMPCPP grow without undergoing catastrophes, i.e. switching to a shrinking phase. In order to better understand the force generation by a parallel MT bundle and the influence of GTP hydrolysis on it, we first measured in vitro with optical tweezers the forces that a GTP MT bundle generates when placed against a rigid micro-fabricated barrier. We observed that MTs composing the bundle share the force applied to the bundle. The bundle still undergoes catastrophes, but can reach higher forces than a single MT can. Single MT stall forces were distinguishable in the data and showed that a MT bundle can reach a maximum force which is the sum of the maximum forces generated by the individual MTs in the bundle. We performed simulations based on a simple stochastic model of a bundle and the known effects of force on the dynamics of single MTs. The predictions obtained for both the bundle catastrophe behavior and the bundle stall force are in good qualitative agreement with experimental observations. Additional measurements on GMPCPP MT bundles showed the same linear addition of single MT maximum forces, but without the occurrence of catastrophes. Ongoing single-MT measurements will enable a direct quantitative comparison of stalling forces of single MTs in the presence of GTP or GMPCPP.

2366-Pos The Effect of Microtubule-associated-protein (MAP) Tau on the Size Distribution and Mechanical Stability of Microtubules Probed by Synchrotron X-ray Diffraction

M.C Choi[1,2], U. Raviv[1,3], H. Miller[1], M. Gaylord[1], L. Wilson[1], M. W. Kim[2], S. Feinstein[1], C. R. Safinya[1]

[1] *UCSB, Santa Barbara, CA, USA,*
[2] *KAIST, Daejon, Republic of Korea,*
[3] *Hebrew University of Jerusalem, Givat Ram, Israel.*

Board B481

In neurons, microtubules (MTs), 25nm protein nanotubes, are used extensively as tracks for transporting nutrients and cellular components between the cell body and its processes (axons and dendrites). The microtubule-associated-protein (MAP) tau regulates microtubule assembly and, in a poorly understood manner, interactions between microtubules. Tau dysfunctions and altered tau-MT interactions result in MT depolymerization, and tau tangles, which is implicated in a large number of neurodegenerative diseases. We describe our recent findings which show that the size distribution and the enhanced mechanical stability due to tau bindings are dependent on tau isoforms.

Supported by DOE DE-FG02-06ER46314, NSF DMR-0503347, NIH GM-59288, NIHI RO1-NS35010, and USPHS NS13560.

2367-Pos Manipulation And Organization Of Microtubules Using AC Electrokinetics

Maruti Uppalapati, Ying-Ming Huang, Thomas N. Jackson, William O. Hancock

Pennsylvania State University, University Park, PA, USA.

Board B482

AC electrokinetics provides a novel tool for manipulating and organizing microtubules in solution. By fabricating microelectrodes on glass substrates and generating AC electric fields across solutions of microtubules in low ionic strength buffers, we were able to collect and align bundles of microtubules and to measure electrical properties of microtubules in solution. We found that AC electric fields result in electroosmotic flow, electrothermal flow and dielectrophoresis of microtubules, which can be controlled by varying the solution conductivity, AC frequency, and electrode geometry. By mapping the solution conductivity and frequency over which positive dielectrophoresis occurs, the apparent conductivity of taxol-stabilized bovine brain microtubules in PIPES buffer was measured to be 250 mS/m. AC electroosmotic flow occurs at frequencies less than 1 MHz, while electrothermal flow occurs is observed at all frequencies. We have optimized experimental conditions such that electrohydrodynamic flows are minimized, and microtubules are reliably attracted to the electrodes by positive dielectrophoresis. At field strength of 3 x 10^6 V/m and a frequency of 5 MHz, microtubules align at the edges of the electrode.

By using novel electrode geometry with opposed electrodes (15 μm wide) placed 10–20 μm apart, two bundles of microtubules are created along with an overlap zone containing filaments with mixed orientations. By selectively patterning motors on the electrodes, these bundles can be sorted for polarity resulting in bipolar organization of microtubules similar to a mitotic spindle. This structure can be used to assay the function of mitotic kinesins and their cooperative effects, providing a crucial link between single molecule investigations on individual motors and the complex dynamics driven by multiple motor species in cells and cell extracts.

2368-Pos Intrinsic Bending and Structural Rearrangement of Tubulin Dimer: Molecular Dynamics Simulations and Quasiharmonic Analysis

Yeshitila Gebremichael[1], Jhih-Wei Chu[2], Gregory Voth[3]

[1] *Biomedical Engineering Department, Wayne State University, Detroit, MI, USA,*
[2] *Department of Chemical Engineering, University of California at Berkeley, Berkeley, CA, USA,*
[3] *Center for Biophysical Modeling & Simulation, Department of Chemistry, University of Utah, Salt Lake City, UT, USA.*

Board B483

Microtubules are long polymers of αβ-tubulin heterodimers. They undergo a process known as dynamic instability in which ends of microtubule switch stochastically between phases of slow growth and rapid shrinkage. The molecular mechanism inducing the depolymerization of microtubule has been attributed to the hydrolysis of GTP nucleotide bound to the b-tubulin. GTP hydrolysis is thought to cause microtubule instability by promoting outward curving of protofilaments constituting the microtubule lattice. The bending of the protofilaments is associated with the structural transformation of tubulin dimer from straight to curved conformations. However, the intrinsic bending of the dimer remains illusive. The present study employs molecular dynamics (MD) simulations and quasiharmonic analysis (QHA) to reveal the intrinsic bending as well as the local structural rearrangements of unassembled tubulin dimer as the dimer relaxes from its lattice constrained, straight conformation of zinc-induced tubulin sheet. The intrinsic bending of GDP-tubulin is examined by using the straight crystal structure of GDP-bound tubulin from tubulin sheet as a starting structure. The effect of the nucleotide state on the dimer bending is investigated by the introduction of g-phosphate into the b-tubulin to form GTP-bound tubulin. Both GDP-bound and GTP-bound tubulin dimers are found to have curved conformations, but with a smaller bending seen in the GTP-tubulin than in the GDP-tubulin. The perturbation induced through the introduction of g-phosphate is posited to play a role of straightening the intra-dimer bending. The local structural rearrangements due to the bending mode of motion of the dimer reveals that, one of the three functional domains, the intermediate domain, exhibits significantly lower bending deformation as compared to the others, signifying a dynamical connection to the functionally defined domains.

Visual Receptors

2369-Pos Influence of Lipid Membrane Composition on the Kinetics of Rhodopsin Activation by Time-Resolved UV-Visible Spectroscopy

James W. Lewis[1], Karina Martínez-Mayorga[2], Istvan Szundi[1], Jacqueline Epps[1], Victor U. Constantino[3], David S. Kliger[1], Michael F. Brown[4]

[1] *Department of Chemistry and Biochemistry, University of California, Santa Cruz, CA, USA,*
[2] *Torrey Pines Institute for Molecular Studies-PSL, Fort Pierce, FL, USA,*
[3] *Department of Biochemistry and Molecular Biophysics, University of Arizona, Tucson, AZ, USA,*
[4] *Department of Chemistry, University of Arizona, Tucson, AZ, USA.*

Board B484

The process of vision starts with *cis-trans* isomerization of the retinal cofactor of rhodopsin, which is a membrane protein prototype of the GPCR superfamily. Changes in lipid composition of membranes may crucially influence the activity of the embedded proteins [1]. Time-resolved UV-visible spectroscopy is a powerful technique where one can measure changes in the absorbance of a sample after perturbation by a light pulse. It has been employed to study the kinetic mechanism of rhodopsin photolysis[2]. Using an optical multichannel analyzer (OMA) we explore here the influence of the physicochemical properties of the membrane lipid bilayer on rhodopsin activation. Time-resolved absorbance changes from 1 microsecond to 50 milliseconds after excitation by 7 nanosecond pulses of 477 nm light were measured at 30 °C in several reconstituted membrane preparations of rhodopsin (DOPC, 25% DOPC:75%DOPE, egg PC, and POPC; all 100:1 lipid to rhodopsin ratio). Measurements were made at pH 5.5, pH 6.5 (high and low salt), and pH 8 in order to determine how the pK of the Meta I_{480}-Meta II equilibrium and other aspects of the photointermediate formation scheme were affected by the lipid membrane environment. Results show that membrane composition strongly affects the pK with all lipids studied having a lower equilibrium pK than for the native lipids. These differences are originate from membrane properties due to the polar head group and acyl chain composition of the lipid bilayer. Other aspects of photointermediate formation were more subtly affected by the changes in membrane composition, and no apparent heterogeneity was seen in any of these reconstituted samples.

References

[1]. A. V. Botelho *et al.* (2006) *Biophys. J.* ***91***, 4464.
[2]. I. Szundi *et al.* (1998) *Biochemistry* ***37***, 14237.

2370-Pos Membrane Lipid Modification of Rhodopsin Activation Studied by FTIR Spectroscopy

Reiner Vogel[1], Karina Martinez-Mayorga[2], Mohana Mahalingam[1], Victor U. Constantino[3], Michael F. Brown[4]

[1] *Biophysics Group, Albert-Ludwigs University, Freiburg, Germany,*
[2] *Torrey Pines Institute for Molecular Studies-PSL, Fort Pierce, FL, USA,*
[3] *Department of Biochemistry and Molecular Biophysics, University of Arizona, Tucson, AZ, USA,*
[4] *Department of Chemistry, University of Arizona, Tucson, AZ, USA.*

Board B485

Rhodopsin is the prototype of the GPCR superfamily of membrane proteins, and its photochemical function is strongly infuenced by the lipid composition [1]. Structural changes of both the protein and retinylidene ligand are conveniently monitered by FTIR spectros-