MS04.13.06 STRUCTURAL STUDIES OF THE MOTOR PROTEIN KINESIN BY IMAGE RECONSTRUCTION AND X-RAY CRYSTALLOGRAPHY. E. Mandelkow, F. Kozielski, A. Marx, E. Schonbrunn, S. Sack, V. Biou*, A. Thompson**, M. Thormahlen, E.-M. Mandelkow. Max-Planck-Unit for Struct. Mol. Biol., c/o DESY, D-22603 Hamburg, Germany; *Eur. Sync. Rad. Fac. (ESRF); **Eur. Mol. Biol. Lab. (EMBL), F-38042 Grenoble, France

Kinesin, a microtubule-activated ATPase, is a cytoplasmic motor which moves vesicles or organelles towards the distal end of microtubules. We have expressed several fragments of kinesin head domain in E. coli and used it for structural and biochemical studies of the microtubule-kinesin interaction. Open questions include: (a) Which tubulin subunit binds to kinesin? (b) What is the stoichiometry of binding? (c) How does the decoration by kinesin reveal the underlying microtubule lattice? (d) What is the polarity of the kinesin binding, and that of the tubulin subunits in a microtubule? Structural questions were addressed by image reconstruction of opened up microtubule walls decorated with several kinesin constructs, and by X-ray crystallography of the kinesin head domain. The reconstructions of the decorated and two-dimensionally ordered "tubulo- kinesin-complex" yields a low resolution (2nm) picture of how kinesin and tubulin interact with one another, including the tubulin lattice, binding stoichiometry, and polarity. Alternatively, the kinesin head can be induced to form well-ordered 3-dimensional crystals suitable for X-ray crystallography. The solution of the structure makes use of single isomorphous replacement including anomalous diffraction at different wavelengths, using a mercury derivative of kinesin. In addition, MAD data were obtained from derivatives in which methionines were replaced by seleno-methionines. The interpretation of the electron density will be presented.

MS04.13.07 CRYSTAL STRUCTURE OF THE KINESIN MOTOR DOMAIN REVEALS A STRCTURAL SIMILARITY TO MYOSIN F. Jon Kull, Elena P. Sablin, Rebecca Lau, Robert J. Fletterick, Ronald Vale, Department of Biochemistry and Biophysics, UCSF, San Francisco, CA 94143

Kinesin is the founding member of a large superfamily of microtubule-based motor proteins that perform force-generating tasks such as organelle transport and chromosome segregation. In this study, the crystal structure of the human kinesin motor domain with bound Mg-ADP was determined to 1.8 Å resolution by X-ray crystallography using two MIR derivatives, EMTS and 2'-iodo-ATP. Crystals grow in the orthorhombic space group $P2_12_12_1$ with one monomer per unit cell (48.5 x 67.9 x 113.0 Å). The motor consists primarily of a single α/ß arrowhead-shaped domain with dimensions of 70 x 45 x 45 Å. Unexpectedly, this motor exhibits a striking similarity to the core of the catalytic domain of the actin-based motor myosin. Although kinesin and myosin have virtually no amino acid sequence identity and exhibit distinct enzymatic and motile properties, our results suggest these two classes of mechanochemical enzymes evolved from a common ancestor and share a similar force-generating strategy.

MS04.13.08 THREE-DIMENSIONAL STRUCTURE OF THE MOTOR DOMAIN OF NCD, A KINESIN-RELATED MOTOR WITH REVERSED POLARITY OF MOVEMENT. Elena P. Sablin*, F. Jon Kull*, Roger Cooke*, Ronald D. Vale*@#, and Robert J. Fletterick*#. Departments of *Biochemistry/Biophysics and #Pharmacology and the Howard Hughes Medical Institute@ University of California, San Francisco, CA USA

The motor domain of the kinesin homolog NCD that is required formeiotic chromosome segregation in Drosophila has been crystallized in the presence of MgATP using polyethylene glycol as the precipitant. The crystals belong to the orthorhombic space group I222 with unit cell dimensions a=127.1 Å, b=122.26 Å and c=68.0 Å, and there is one NCD molecule per asymmetric unit. The structure of the NCD motor domain complexed with MgADP was solved using multiple isomorphous replacement method. X-ray diffraction data for the native NCD crystals and five isomorphous derivatives were measured at -170 C using R-AXIS IIC image plate detector. The current NCD model has been refined to 2.5 Å with an R value 22.4% and includes 321 amino acid residues along with MgADP and 66 water molecules. Structural comparison between the NCD and kinesin motor domains show that they are remarkably similar in structure and likely share a common microtubule binding site. Moreover, structural and functional comparisons of NCD, kinesin, myosin and G proteins reveal that these NTP-ases may utilize a similar strategy of changing conformation between NTP and NDP states. A general model for converting a common gamma-phosphate sensing mechanism into opposite polarities of movement for kinesin and NCD is proposed.

Protein Design & Engineering

MS04.14.01 ADVENTURES IN X-RAY STRUCTURE DETERMINATION OF PROTEIN DESIGNS. David Eisenberg, Gilbert G. Privé, Nancy L. Ogihara, Manfred S. Weiss, Daniel H. Anderson, Laura Wesson. UCLA-DOE Laboratory of Structural Biology and Molecular Medicine. Box 951570, UCLA, Los Angeles, CA 90095-1570 USA.

The goal of protein design is to create a sequence of amino acids that folds into a predetermined three-dimensional structure. In our lab, X-ray structures have been determined for four designed proteins. In all four cases, the structure displays some elements of the design, but in three of the cases, the structures also disclose major surprises. A 12-residue peptide designed to self-associate into a four-alpha helical bundle did in fact form a bundle of alpha helices in two crystal structures, one at low pH and one at high pH. The low pH form turned out to be a bundle of six helices with 32 symmetry, rather than the designed four-alpha helical bundle. The same peptide at high pH self associates into an amphipathic wall of alpha helices. The crystal is formed from alpha helices that hydrogen bond head to tail into columns which in turn pack side-to-side into infinite sheets. Within each sheet, the alpha helices run antiparallel and are closely spaced (8-9 Å). The sheets are more loosely packed against each other (13-14 Å). Each sheet has apolar leucine sidechains projecting from one face and charged lysine and glutamate sidechains from the opposite face. The result is a packed protein bilayer, with alternating polar and apolar interfaces.

The structures of helical bundles designed as two-stranded coiled-coils also offered surprises. One such structure was found to be a three-stranded coiled-coil with two helices running in one direction, and the third in the opposite direction. A sequence with minor variations turned out to be a parallel three-stranded coiled-coil. In the crystal, this coiled-coil stacks head to tail to form a continuous trimeric superhelix.