The myosin cross bridge is necessarily polymorphic: to understand muscle contraction one needs to understand how the shape of the cross bridge responds to the binding and hydrolysis of nucleotides [Rayment, Topic 04.13] and to the binding of actin. EM studies of the actomyosin complex show that the tail rotates on binding ADP [Milligan, Topic 04.13]. Since the complex between myosin cross-bridge and monomeric actin has not been crystallised it is imperative to get high resolution data from cryo EM reconstructions. One method of extending the resolution is by the use of an energy-filter microscope. Images with a resolution of 15-20Å have been obtained which allow a more detailed examination of the effect of actin-binding on the myosin cross-bridge. Fiber diffraction from orientated gels of decorated actin in the presence and absence of ADP can also be used to register changes in the actomyosin structure.

1. Kabsch *et al* (1990) *Nature* **347**:37-44.
2. Holmes *et al* (1990) *Nature* **347**:44-49.
3. Rayment *et al* (1993) *Science* **261**:58-65.
4. Schröder *et al* (1993) *Nature* **364**:171-174.

MS01.10.04 COMBINING CRYO-EM AND X-RAY DIFFRACTION STRUCTURE RESULTS FOR SPHERICAL VIRUSES. Michael G. Rossmann, Department of Biological Sciences, Purdue University, West Lafayette, IN 47907-1392

Cryo-EM structures of spherical viruses usually have a limiting resolution of around 22 Å. X-ray diffraction data are usually somewhat incomplete inside 25 Å resolution, on account of being obscured by the beam stop. Thus, phasing of the X-ray data, using the EM structure as a model, frequently lacks sufficient overlap to permit successful phase extension using the non-crystallographic symmetry. These problems can be alleviated both by carefully matching the radial scale of the EM image to the X-ray data and by assigning a crude atomic structure to the density. This can be based either on a rough structural interpretation or on suitably randomly placed atoms within the boundary of the EM density.

Interpretation of the EM density with reasonable models based on structural components of the virus requires checking for the uniqueness of the proposed fit. A variety of biological information can be used to support the results. Ross River virus (an enveloped +RNA virus) and ϕX174 (a ssDNA phage) assembly intermediates will be used as examples.

PS01.10.05 OPTIMAL MODELING OF ELECTRON MICROSCOPIC 3D RECONSTRUCTIONS USING COMPONENTS OF KNOWN ATOMIC STRUCTURE. Tang, J., Blanc, E., & Chapman, M.S.; Department of Chemistry and Institute of Molecular Biophysics, Florida State University, Tallahassee, FL 32306-3015, USA

Several large complexes beyond the reach of x-ray crystallography have recently been analyzed using a combination of electron microscopy and interpretation with crystallographically derived atomic models of their smaller components. Analyses include complexes of actin and myosin, relevant to muscle function; viruses with antibodies and cellular receptor fragments. The focus of our work is the development of intuitive computational methods that optimize the agreement between model and EM based 3D reconstructions by adjusting the positions and orientations of domains, and experimental parameters defining phase contrast and magnification.

A function has been derived through which it is possible to calculate from an atomic model, the appearance of an electron density map at any arbitrary experimental resolution [Chapman (1995) Acta Crystallogr. **A51**: 69-80]. This is the basis of a stereochemically restrained least squares refinement protocol that has been applied to 3 virus x-ray crystallographic structures. The method has been adapted to lower resolution EM through the approximation of electron scattering factors by a 4-term exponential series. Following attenuation by an isotropic approximation to the contrast transfer function, the electron density contribution of each atom is calculated by analytic Fourier transformation. The sum of these contributions is compared to the EM reconstruction at each point. The method is being tested on the cryo-EM images of a virus-Fab complex, but will also be applicable to other EM techniques such as tomography.

Other

PS01.11.01 AN EFFICIENT AND CONVENIENT METHOD FOR RECORDING LOW TEMPERATURE X-RAY DIFFRACTION PHOTOGRAPH. S. A. Chawdhury, Suvechcha 130/2, East Subidbazar, Sylhet 3100, Bangladesh

An experimental technique has been developed for taking x-ray diffraction photograph within the range of gaseous nitrogen temperature. This technique has not only made possible the study of single crystals of interesting substances which are liquids or gases at room temperature and the phase change of certain compounds but also made possible a convenient means of increasing the quantity and improving the quality of intensity data. A metal dewar of special design, the outer tube of which is of stainless steel and the inner tube is of German silver, was constructed. It was then fitted to the specially constructed liquid nitrogen container and connected to the goniometer. A steady temperature anywhere from room temperature down to -185°C or so can be obtained.

PS01.11.02 THE *Fddd* DIFFRACTOMETER: HARDWARE INNOVATIONS AND A STUDY OF $[Zn(H_2O)_6]$-$[C_6H_2(COOH)_2(COO)_2]$. R.C.B. Copley,[a] C.W. Lehmann,[a] J.A.K. Howard,[a] K. Wade,[a] G. Walker,[a] J.M. Archer,[b] and K.N. Trueblood.[c] [a]Dept. of Chemistry, University of Durham, Durham DH1 3LE, UK; [b]Institute Laue Langevin, BP 156X, F-38042 Grenoble, France; [c]Dept. of Chemistry and Biochemistry, UCLA, CA, 90024, USA.

The *Fddd* four-circle diffractometer has been developed to collect X-ray diffraction experiments at temperatures down to 9K and here we describe some hardware innovations and a study at five different temperatures on the compound $[Zn(H_2O)_6][C_6H_2(COOH)_2(COO)_2]$ (**1**). The diffractometer consists of: (i) a Siemens molybdenum rotating anode generator; (ii) Huber circles with offset chi; (iii) a Siemens Fast Scintillation Detector; and (iv) an APD '202' Displex cryogenic refrigerator.

The belt-driven rotating anode gives X-ray fluxes far superior to those obtained with a conventional X-ray tube. X-ray alignment requires precise movements of the 300kg circles and this is achieved using air pads attached to the base of the goniometer. When activated with compressed air, the pads 'float' above the polished surface of an aluminium tabletop and allow precise movements of the circles.

The steel braided gas lines between the Displex and the helium compressor are supported by a counter balance system. The stress on these lines has been reduced by attaching them to the Displex via rotating joints and by passing them through a metal ring 50cm above the Displex. The ring is supported by a framework attached to the chi circle. A compact vacuum gauge has been mounted through one of the four ports on the top of the cryostat and gives interesting information on the vacuums obtained within the Displex during an experiment. Crystals are mounted on 'sharpened' 0.3mm graphite pencil leads and a new sample mount has been designed.

X-ray diffraction data for **1** have been collected at 296, 210, 120, 50 and 9K. Full analysis of the ADPs at the different temperatures demonstrates the high resolution capabilities of the *Fddd* diffractometer.