**PS04.08.12 CRYSTALLOGRAPHIC INVESTIGATIONS OF ERYTRHROCRUORIN FROM LUMBRICUS TERRESTRIS.** by Kristen Strand and William Royer, Jr., Program in Molecular Medicine and Dept. of Biochemistry and Molecular Biology, University of Massachusetts Medical Center, Worcester MA 01605.

Lumbricus hemolglobin is an extracellular respiratory protein complex located in the hemolymph of the common earthworm where it functions to transport oxygen and carbon dioxide. It is composed of four unique heme binding polypeptides (abcd chains) and three linker chains which are required for assembly of the entire molecule. We are investigating the crystal structure of the entire molecule and isolated subunits in order to learn the mechanism for the self-limited assembly of a cooperative complex from more than 200 polypeptide chains. We have recently crystallized the abcd assemblage in 2.2 M phosphate buffer pH 6.7. These crystals show symmetry of the space group C2221 and diffraction corresponding to at least 2.8 Å resolution with cell constants of a=138.2 b=171.1 and c=201.2 Å. We have also grown crystals in which the Calcium has been replaced with various Lanthanides. The modulation of diffraction intensities at different wavelengths due to anomalous scattering of these lanthanides will be used to solve the phases problem. The structure of the abcd complex will then be fitted into cryo-electron microscopy images of Lumbricus erythrocruorin in order to provide initial phases for the whole molecule crystal diffraction data.

**PS04.08.13 X-RAY STRUCTURE OF EUKARYOTIC E3, LIPOAMIDE DEHYDROGENASE, FROM YEAST.** T. Toyoda and A. Takenaka, Department of Life Science, Tokyo Institute of Technology, Yokohama, Japan; and T. Sekiguchi, Department of Fundamental Science, Iwaki Meisei University, Fukushima, Japan.

α-Keto acid dehydrogenase complex is a family of well-organized multienzyme systems which are composed of three kinds of enzymes, E1, E2, and E3. Depending on the substrates, E1 and E2 are varied to construct the different architectures with 532 symmetry for pyruvate dehydrogenase complex and 432 symmetry for α-ketoglutarate dehydrogenase complex in eukaryotes. The third component E3 (lipoamide dehydrogenase) is, however, commonly used among them. To reveal the structure of E3 which binds to both complexes and to elucidate the reaction mechanism, the crystal structure of E3 from yeast was solved by the molecular replacement method with diffraction data collected up to 2.98(max. 2.49)Å resolution using synchrotron radiation. The initial phases were improved by non-crystallographic symmetry averaging and solvent flattening of electron density. The atomic coordinates of the molecular model constructed by computer graphics were refined with molecular dynamics. The final R-factor is 19.0% at 2.49Å resolution. Compared with prokaryotic Gram negative bacteria E3 (A. vinelandii ) which binds only to complexes with 432 symmetry, large differences occur in the loop regions with insertion or deletion of amino acids. There are no differences in topology of the secondary structures. One β-sheet (246-276 residues) is changed the normal on the molecular surface. It is expected that E3s have different molecular surfaces between those incorporated into the cores with only 432 and with both 432 and 532 symmetries. A characteristic feature, found on an electrostatic molecular surface, may be concerned with such binding properties.

**PS04.08.14 CRYSTAL STRUCTURE OF A NEW HEAT-LABILE ENTEROTOXIN, LT-IIb, THAT CAN ADP-RIBOSYLATE Gs-ALPHA.** F. van den Akker[1,2], S. Sarfaty[1,3], E. Twiddy[4], R.K. Holmes[4], W.G.J. Hol[1,3], [1]Departments of Biological Structure and Biochemistry, [2]Biomolecular Structure Center, [3]Howard Hughes Medical Research Institute, University of Washington, Seattle, WA 98195, and [4]Dep. of Microbiology, University of Colorado Health Science Center, Denver, CO 80262, USA

The LT-IIb crystal structure is the latest addition to structures determined in the cholera toxin (CT) family which encompasses a common theme of conserved structural similarities despite little sequence identity. The LT-IIb subunit organization is identical to CT and heat-labile enterotoxin (LT-I): a catalytic A subunit, capable of ADP-ribosylating Gsalpha, and a B pentamer which serves to bind to the ganglioside receptor located on the outside of the target cells. The sequence similarity of LT-IIb and LT-I is substantial when comparing their A subunits but was undetectable when comparing their B subunits. The crystal structure was determined by single isomorphous replacement (SIR) using a $K_2PtCl_4$ derivative. An initial 15-5 Å SIR map, without anomalous data, showed features of a 5-fold arrangement of long rods. These rods could be superimposed onto the 5 long α-helices found in the LT-I B pentamer and provided the first evidence of structural similarity between the B subunits of the two toxins. Solvent flattening combined with phase extension and 5-fold averaging improved the electron density dramatically. The LT-IIb structure is currently refined to 2.25 Å resolution with an R-factor of 19.1% with good geometry. The B-pentamer of LT-IIb shows the same 'OB-fold' as the pentamers of LT-I, CT, and other AB5 toxin structures reported to date, such as pertussis toxin and shiga toxin. This constitutes a remarkable level of structural homology even in the absence of detectable sequence identity. The nature of the B pentamer pore, which is involved in binding the A subunit, among members of the cholera toxin family is very different except for a conserved ring of solvent accessible hydrophobic surface present in all members of the family. We speculate that this hydrophobic ring is critical for AB5 assembly in the periplasm of the pathogens producing these toxins.

## Viruses

**MS04.09.01 STRUCTURAL STUDIES OF POLIOVIRUS ASSEMBLY AND CELL-ENTRY INTERMEDIATES.** James M. Hogle, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, 240 Longwood Avenue, Boston, MA 02115

The high resolution structures of polio and related picornaviruses have provided considerable insights into those properties that are relevant to the extracellular form of the virus. It is known, however, that the virus undergoes significant conformational rearrangements during assembly and cell entry. We have recently described the structure of the empty capsid assembly intermediate of poliovirus (1). This form of the virus lacks the viral RNA and has yet to undergo the maturation cleavage of the immature capsid protein precursor VP0 to yield VP4 and VP2. The structure demonstrates that the cleavage of VP0 is required for the correct formation of a network of interactions of VP4 and the amino terminal extension of VP1 on the inside surface of the virion. Interestingly, those portions of the network which form only after VP0 cleavage involve normally internal portions of the capsid protein which are externalized during conformational changes which are induced by binding to the poliovirus receptor. These results, together with analysis a wide variety of poliovirus variants with altered stability and/or receptor interactions, have

given rise to a picture in which the virus is a metastable intermediate which links virus assembly and cell-entry. In this model the cleavage of VP0 and subsequent rearrangements of VP4 and the aminoterminal extension of VP1 trap the virus in the metastable form and prime it for subsequent structural rearrangements required for cell-entry. The receptor "catalyzes" these transitions by utilizing some of the energy which is released upon its binding to receptor to lower the activation barrier which traps the virus in this metastable state (2).

In order to further characterize this process we have undertaken a program of structural studies of intermediates which are thought to be important in the cell entry process using cryoelectron microscopy (in collaboration with Alasdair Stevens, Frank Booy, Benes Trus and David Belnap at NIH) and x-ray crystallography. Low resolution models for two such intermediates will be presented.

**MS04.09.02 THE STRUCTURE OF INTACT HUMAN RHINOVIRUS 14 COMPLEXED WITH Fab17-IA.** Thomas J. Smith, Elaine S. Chase, Timothy Schmidt, Norman H. Olson, Timothy S. Baker, Department of Biological Sciences, Purdue University, West Lafayette, IN 49707

Antibodies are a major component of the immune response to picornaviruses. It has long been contended that antibody neutralization is due to large structural changes in the capsid upon binding. To test this hypothesis, we have used crystallography and electron microscopy to determine the structure of intact human rhinovirus (HRV14) complexed with Fab17-IA. The atomic structures of Fab17-IA and HRV14 were first used to interpret the ~25Å resolution image reconstruction. This model was used to calculate initial phases to 8Å resolution for the data from a frozen Fab17-IA/HRV14 crystal. After phase extension to 4Å resolution, the structure clearly shows that the initial model was mis-positioned by up to 4Å in places, the HRV14 structure does not seem to change upon antibody binding, and that the CDR3 loop of the heavy chain moves to accommodate the epitope. This CDR3 movement had been predicted by molecular dynamics calculations.

**MS04.09.03 STRUCTURAL STUDIES ON ORBIVIRUSES.** J. Grimes[1], P. Gouet[1], A. Basak[5], G. Sutton[1,2], P. Roy[1,2], N. Burroughs[3], B.V.V. Prasad[4], P. Mertens[3] and D. Stuart[1], [1]Laboratory of Molecular Biophysics, Rex Richards Building, South Parks Road, Oxford, OX1 3QU, United Kingdom, [2]NERC Institute of Virology, Mansfield Road, Oxford, United Kingdom, [3]Institute for Animal Health, Pirbright, Surrey, United Kingdom, [4]Baylor College, Houston, Texas, USA, [5]Birkbeck College, Malct St., London, United Kingdom

We are studying the structures of some orbiviruses. These are animal viruses, which belong to the same family as the better known rotaviruses and reoviruses, which cause significant human disease[1]. Bluetongue virus is the classic orbivirus; it has a proteinaceous capsid from which an outer layer can be stripped away to reveal a 700Å core particle[2]. The core is robust and penetrates the host cell intact, it contains a small number of proteins with enzymatic activity and much larger numbers of VP3 and VP7. VP7 forms the outer surface of the core and is present at the level of 780 copies per core, arranged on a T=13 lattice[2]. We have determined, by X-ray crystallography, a number of structures of this molecule[3] (Grimes et al., unpublished). We have combined the information from the X-ray structures of VP7 with that from electron cryo-microscopy (Prasad, unpublished) and used simple fitting procedures to place the X-ray structure in the EM map.

We have separately crystallized the whole core of 2 serotypes: BTV-1 and BTV-10. BTV-1 crystallized in space group $P2_12_12$, a=798Å, b=825Å, c=756Å, BTV-10 crystallized in space group $P4_12_12$, a=b=1120Å, c=1592Å. Data have been collected at the SRS (UK) & ESRF (Fr) for BTV-1 and at the ESRF for BTV-10. Both structures have been solved at low resolution using the cry-EM phasing model. The resolution is being extended for both structures and the unusual architecture will be discussed.

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[3] Grimes, J. et al., Nature, 373, 167-170 (1995).
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**MS04.09.04 STRUCTURES OF INFLUENZA VIRUS PROTEINS.** Ming Luo, University of Alabama at Birmingham, 1918 University Blvd., Birmingham, AL 35294

We have determined the structure of type B influenza virus neuraminidase. Influenza virus infection remains to be an uncontrolled human disease which causes up to 20,000 death per year. Novel inhibitors guided by the crystal structure of NA from several virus strains have been developed and structures of NA complexed with various inhibitors are reported here. These compounds (benzoic acid derivatives) are aromatic in nature and offer the advantages of chemical stability and simplicity in chemical synthesis. They also have the potential to be orally active. 13 compounds have thus far been designed and synthesized. The most potent inhibitor synthesized so far has an $IC_{50}$ value around 2 μM in NA enzyme inhibition assays and was shown to reduce influenza virus HA titer in cell culture by 50% at a concentration between 1 - 10 μM. Since a large number of compounds representing different chemical classes have been prepared, they can be quickly screened when new strains emerge in a pandemic. If they are not effective, we can model the new strain based on existing NA structures and come up with new modifications. Since aromatic compounds are easier in chemical synthesis, this process can be fast.

In addition, we are working on the structure of other influenza proteins which can be potential targets for structure-based drug design. Currently, we have grown crystals of M1 and are in the process of determining the structure. M1 is relatively more conserved when compared with NA. With drugs targeted to different proteins, we have a better chance to handle a pandemic.

**MS04.09.05 THE STRUCTURE OF TURNIP YELLOW MOSAIC VIRUS (TYMV) AT 3.2 Å RESOLUTION.** M. A. Canady, S. B. Larson, J. Day, and A. McPherson, Department of Biochemistry, University of California, Riverside CA 92521 USA

The structure of turnip yellow mosaic virus has been solved to 3.2 Å using molecular replacement, multiple isomorphous replacement (MIR), and molecular averaging. Crystals were of space group $P6_422$ with unit cell dimensions a=b=515.5, c=309.4 Å. Native and heavy atom data were collected at Brookhaven National Laboratory at the University of California, San Diego. Using cowpea chlorotic mottle virus as a model, phases were computed and the map was averaged. A difference Fourier synthesis using the phases from the averaged map and data collected from two platinum derivatives allowed us to determine the positions of the heavy atoms. A polyalanine model was built into an averaged MIR map. Correct sidechains were built into an averaged map phased by the refined polyalanine model. The structure was refined using conjugate gradient minimization, simulated annealing, and individual restrained B factor refinement to an R-value of 18.7% with a free R-value of 19.3% to 3.0 Å. The structure of the virion at high resolution resembles the predictions made at low resolution, with the pentameric and hexameric coat protein assemblies