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.

[1] Holmes, I.H. Archives of Virology (1994).
[2] Prasad, B.V.V. et al., J.Virol., 66, 2135-2142 (1994).
[3] Grimes, J. et al., Nature, 373, 167-170 (1995).
[4] Burroughs, N. et al., Virology, 210, 217-220 (1995).

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

protruding quite prominently, forming deep valleys at the pseudo-threefold axes between the A, B, and C subunits. The quasi-equivalent A, B, and C subunits adopt the jellyroll fold and are very similar in structure. The N termini, which had been found on the outside of the virion immunogenically, are found in the interior of the virion, and the first 26 residues of the A subunit are disordered. The N termini of the B and C subunits, which are completely visible, interact at the interior of the pseudo 6-fold axes, forming annuli. The C termini are exterior to the virion. All three histidines present in the coat protein are found on the inside of the virion, and may confirm the prediction that these residues bind RNA.

MS04.09.06 STRUCTURE OF HUMAN RHINO VIRUS 50 AT 1.8 Å E. Blanc[1], R. S. Alexander[2], M. McMillan[3], D. C. Pevear[4], V. Giranda[5], M. S. Chapman[1], [1]Institute for Molecular Biophysics and Department of Chemistry, Florida State University, Tallahassee, Fl 32306, [2]Dupont Merck Pharmaceutical Co., Wilmington, De 19880, [3]Eastman Kodak Co., Rochester, NY 14650, [4]Viropharma Inc., Malvern, Pa 19335, [5]Abbott Laboratories, Abbott Park, Il 60064-3500

Human Rhino Virus 50 (HRV50) belongs to one of the largest and most important families of viral pathogens: the picornaviridae. Rhinoviruses are among the smallest RNA-containing viruses; their genome is surrounded by a protein shell made of 60 copies of the asymmetric unit, each containing 4 polypeptide chains. As the major cause of the common cold, these viruses have already been extensively studied, and several structures have been determined. HRV50 is of special interest for two reasons: firstly, it is one of the more representative serotypes, of use in the design of broad spectrum drugs against all rhinoviruses. Secondly, there is the opportunity to study the structure and drug interactions at unusually high resolution, 1.8 Å.

The structure refinement has been performed mainly in real-space, using a new technique [Chapman (1995) Acta Crystallogr. **A51**: 69-80] that accounts for the resolution of the experimental data, and can be 50 times faster than conventional reciprocal-space refinement. The method takes advantage of accurate phases that result from application of 15-fold non-crystallographic symmetry. Moreover, the method makes it tractable to refine such large structures against all experimental data simultaneously. Prior comparaisons [Chapman & Rossman (1996) Acta Crystallogr. **D**: in press] with canine parvovirus have demonstrated that the quality of a real-space refined virus is at least as good as refined in reciprocal space.

MS04.09.07 STRUCTURE-BASED DESIGN OF PEPTIDE PRESENTATION ON A VIRAL SURFACE: THE CRYSTAL STRUCTURE OF A PLANT/ANIMAL VIRUS CHIMERA AT 2.8Å RESOLUTION T. Lin[1], C. Porta[2], G. Lomonossoff2, J. E. Johnson[1] [1]Department of Molecular Biology, The Scripps Research Institute, 10666 N. Torrey Pines Rd., La Jolla, CA and [2]Department of Virus Research, John Innes Centre, Colney Lane, Norwich NR4 7UH, UK

A genetically engineered icosahedral plant virus, cowpea mosaic virus (CPMV), was employed as an expression and presentation system to display a 14 amino acid linear antigenic epitope found in a capsid protein of human rhinovirus 14 (HRV14). Gram quantities of the CPMV/HRV14 chimera were made in plants and purified particles were crystallized in a form isomorphous with wild-type CPMV. The 2.8 Å resolution structure of the chimera shows that the inserted loop is well ordered and that, if the loop were intact, a PHE residue of CPMV would be placed in a hydrophilic environment. The resultant strain must make the loop an attractive substrate for endogenous plant proteases, as roughly 80% of the inserted polypeptides are cleaved, allowing the PHE to be partially buried. Altering the PHE to an ARG should relieve the stress, reducing the propensity for cleavage and increasing the likelihood that the peptide will assume a structure closely similar to its structure in HRV14. Successful crystallization of other CPMV chimeras in forms isomorphous with the native virus suggests that this is a viable system for structure-based design of peptide presentation.

PS04.09.08 STRUCTURAL STUDIES OF AN HRV14:HIV-1 V3 LOOP CHIMERA THAT INDUCES NEUTRALIZING ANTIBODIES AGAINST HIV-1. Edward Arnold, Dawn A. Resnick, Jianping Ding, Sheila C. Geisler, Greg Kamer, and Gail Ferstandig Arnold, CABM and Rutgers University Chemistry Department, 679 Hoes Lane, Piscataway, NJ 08854

Human rhinovirus 14 (HRV14) has been engineered for use as a live-virus vaccine against human immunodeficiency virus (HIV-1). An HRV14:HIV-1 chimeric virus, designated DN-6, contains an immunogenic region from the V3 loop of the gp120 surface glycoprotein of HIV-1 transplanted into the neutralizing immunogenic site II of HRV14. DN-6 is strongly neutralized by anti-HIV-1 antibody preparations and elicits the production of antisera that potently neutralize HIV-1 in cell culture (Resnick *et al.*, 1995). The crystal structure of DN-6 will reveal the conformation of a V3 loop epitope that is able to elicit a neutralizing response against HIV-1.

Crystals of DN-6 were obtained that diffract X-rays to 2.8 Å resolution. A native dataset measured at CHESS from cooled crystals is 44.8% complete to 3.5 Å resolution (238,364 independent reflections, R_{merge}=11.7%) and from a flash-frozen crystal is 61.4% complete to 3.5 Å resolution (322,463 independent reflections, R_{merge}=14.7%). The crystal symmetry and unit cell dimensions are similar to those of the orthorhombic crystal forms of HRV14 (Erickson *et al.*, 1983) and poliovirus type 1 Mahoney (Hogle *et al.*, 1985). Surprisingly, although the cell dimensions are very similar, the packing is different from that of the poliovirus 1 Mahoney crystals and the space group is $P22_12_1$ instead of $P2_12_12$. The reflections were indexed accordingly for the standard space group choice $P2_12_12$ with a=378.4, b=354.7, and c=317.7 Å.

Rotation and translation functions indicated a particle rotation of 93.0° (cooled) or 94.2° (frozen) around the z axis relative to a standard icosahedral orientation and the particle center position to be close to z=0.25. Rigid body refinement using the HRV14 native coordinates (Rossmann *et al.*, 1985; Arnold & Rossmann, 1988) yielded an R-factor of 0.34 for 15-3.5 Å resolution for the cooled dataset. Phase improvement by 30-fold noncrystallographic symmetry averaging is underway. Electron density is well ordered for the majority of the protein shell. We are in the process of interpreting the density in the chimeric region in terms of the structure of the transplanted HIV-1 V3 loop immunogen.

PS04.09.09 STRUCTURE OF COXSACKIEVIRUS B1 COMPLEXED WITH AN ANTIVIRAL AGENT. Karen N. Lentz[1], Allen D. Smith[1], Sheila C. Geisler[1], Stu Cox[2], Jason DeMartino[2], V. Girijavallabhan[2], John O'Connell[2], and Edward Arnold[1], [1]Center for Advanced Biotechnology and Medicine, and Rutgers University Department of Chemistry, 679 Hoes Lane, Piscataway, NJ 08854, [2]Antiviral Chemotherapy, Schering-Plough Research Institute, 2015 Galloping Hill Road, Kenilworth, NJ 07033

Coxsackievirus B1 (CVB1) is a member of the picornavirus family and causes a wide variety of human diseases. These include common colds, mild febrile illness, encephalitis, and acute myocarditis. The picornaviruses are small (approximately 300 Å