Neuraminidase (NA) is one of the two glycoproteins on the influenza virus membrane. Its role is to enhance viral mobility via hydrolysis of the glycosidic linkage between a terminal sialic acid residue and the adjacent carbohydrate moiety on the host receptor. We have determined the crystal structure of native type B neuraminidase and the type B neuraminidase-sialic acid complex from several type B influenza strains. The native crystal type B neuraminidase structure is similar to the six β-sheet propeller fold found in type A neuraminidase. The sialic acid complex crystal structure indicates that the bound sialic acid is in the half-chair conformation, with planar density around C2, and no density for OH2. The complex structure supports the hypothesis that the enzymatic hydrolysis of terminal sialic acid by neuraminidase may be catalyzed by formation of a stabilized transition state species, not by nucleophilic attack from a proton donor. Further structural studies of type B neuraminidase complexed to several Abbott compounds showing neuraminidase inhibition activities are ongoing.

MS-03.02.06

CD4-HIV RECEPTOR by Jia-huai Wang*, You-wei Yan, P.J.Garrett,Jin-huan Liu and Stephen C. Harrison, Departmentof Biochemistry and Molecular Biology, Harvard University, Howard Hughs Medical Institute

The CD4 transmembrane glycoprotein on the surface of T-cell is a critical component in the cellular immune responce machinary. Ironically, human CD4 has become better known, because it is the receptor for HIV. We have been carrying out structural studies of various fragments by x-ray crystallographic method. The structure of N-terminal two domain fragment (CD4 (1-182) fragment) has been determined and refined to 2.2A resolution.

The CD4(1-182) contains two abutting immunoglobulin-like domains. Domain 1(D1) bears typical Ig V-type character, whereas domain 2(D2) is an interesting variation. It has an intrasheet disulfide bond instead of a usual intersheet bond across β-barrel. The relative packing of two sheet also shifts compared to the normal Ig fold. Between two domains there is an extensive hydrophobic interface. The last β-strand of D1 extends uninterruptedly to form the first strand of the D2. Together it makes a distict rigid concatenated domain connection. It is belived that this kind of domain organization should exists in other cell surface proteins as well.

The interaction between CD4 and HIV envelop protein gp120 is restricted to the very N-terminal domain of CD4 molecule. Key components of the binding site include :(a)The unique protrusion of C'-C" corner (in particular the Phe43), supported by the bulky sidechain of Trp62. This Trp62 is situated in the middle of an α-helix, which is a insertion in CD4 as opposed to any other Ig superfamily members. (b)A patch of positively charged residues surrounding Phe43. The MHC molecule binding site is, on the other hand, much more extended, involving both D1 and D2. We propose that the zigzagged surface of the first two domains of CD4 is complementary to a notched surface of class II MHC molecule, formed by two domains in its β-chain.

MS-03.02.07 THE STRUCTURE OF A TYPE C FOOT-AND-MOUTH DISEASE VIRUS AT 3.5Å. R. Abu-Ghazaleh‡, W. Blakemore‡, S. Curry‡, E. Domingo§, E. Fry†, T. Jackson‡, A. King‡, S. Lea†, M. Mateu§, J. Newman‡ and D. Stuart*†. †Laboratory of Molecular Biophysics, Oxford University, U.K. ‡AFRC Institute for Animal Health, Pirbright, Woking, U.K. §Centro de Biología Molecular, Madrid, Spain.

We have determined the structure of a serotype C (isolate C-S8c1) foot-and-mouth disease virus (FMDV) at 3.5Å resolution by X-ray crystallography. The overall structure of the virus is seen to be similar to that previously determined for O_1BFS (Acharya, R. *et al.* (1989), *Nature (London)*, **337**, 709-716.). There are significant changes in the structure of some antigenically important external loops and in some of the less well ordered regions involved in protomer-protomer contacts. The structure aids interpretation of a mass of antigenic results. New features seen in the C-S8c1 structure include visualisation of the N-terminal residues of VP2 and extra density around the interior of the 5-fold axes of the virion which may be interpreted (by comparison with the structure of Polio virus; Chow, M. *et al.* (1987), *Nature (London)*, **327**, 482-486.) as the myristate moiety bound to the N-terminus of VP4.

The GH loop of VP1 (the 'FMDV loop') is of major interest as the dominant antigenic site and location of the putative receptor binding residues. The flexibility of this loop is regulated by a disulphide bond in type O_1 virus (the loop becomes ordered, and therefore visible crystallographically on reduction of the disulphide; Logan, D. *et al.* (1993), *Nature (London)*, In press.). Despite lacking the disulphide this loop is disordered in the C virus (and also in two serotype A FMDVs we have studied) suggesting flexibility of the loop is advantageous to the virus. Possible rôles for this flexibility will be discussed.

PS-03.02.08 DECONVOLUTION OF DATA FROM INTIMATELY TWINNED CRYSTALS OF FMDV. By S. M. Lea* and D. I. Stuart. Laboratory of Molecular Biophysics, Oxford University, U.K.

Processing of FMDV data in space group I23 requires division of the data into two subsets (Fry, E., Acharya, A. and Stuart, D. (1993). *Acta Cryst.* A**49**, 45-55.) corresponding to the two ways of indexing the I23 lattice which are geometrically indistinguishable (i.e. placing the virion on a specific 3-fold axis related by a 90° rotation about a particle 2-fold). Within a crystal particles are all in the same relative orientation but the choice is random between crystals. Each crystal may therefore be indexed as h,k,l or k,h,l. By comparison to a reference set the data can be divided into two streams and processed separately until (following postrefinement) the indices of one of the streams are modified and the 2 data sets merged. Data collected from a mAb-escape derived FMDV mutant (G67) appeared to crystallize isomorphously with the parent virus (O_1K) (Curry, S. *et al.* (1993), *J. Mol. Biol.* **228**, 1263-1268); I23, a=345Å, however data from these crystals correlated poorly with the reference set, the correlation coefficient for either indexing scheme against the parent virus data being less than 0.5. This suggested extra 4-fold symmetry which is geometrically impossible for an icosahedral virus. However, statistically the data appeared to belong to point group 432. Assuming that in each crystal all viral 2-folds are arranged randomly with respect to all other 2-folds with the ratio of the two orientations 50:50 the data would have apparent 4-fold symmetry. Processing

therefore progressed in PG432 (R_{merge} 0.11 and 0.19 for two data sets). Difference maps were calculated using "pseudo" 432 data sets produced using I23 virus data folded into the 432 asymetric unit with one of two models for the disordering:

1. Random arrangement of the the 2–folds throughout the crystals (vector mean of phased I23 structure factors models the observed data). **2.** Mosaic blocks of the crystal contain similarly oriented particles, but arrangement of 2–folds between different mosaic block is random (arithmetic mean of I23 intensities provides model).

Model **2** provided an excellent match to the observed G67 data. The pseudo 432 data for the parent virus were scaled in resolution shells to the G67 data then the I23 asymetric unit regenerated by duplicating the records to write h,k,l and k,h,l. Difference maps were calculated a set of accurate phases for the native virus in space group I23. Averaging the difference maps to impose icosahedral symmetry then revelaed the structural differences between the parent and mutant viruses. However the nature of the disordering suggested a possible route for deconvolution of the data by back–transformation of a map obtained in the way described above to provide estimates of the true I23 structure factors. Our observed data provide a series of constraints in reciprocal space:

$$2(F_{OBS})^2=(F_{23,hkl})^2+(F_{23,khl})^2$$

These constraints are applied to improve our estimates for the intensities. We have constructed an iterative process of map calculation and constraint application. Using synthetic test data the correlation coefficient between the reconstructed F_{23} and the true values rose from 0.70 to 0.97 over 6 cycles. A two cycle application improved the G67 maps. Since this procedure provides a rather general method for convoluting entirely overlapped reflections we intend to investigate its general use in deconvoluting wavelength overlaps that occur using the Laue technique.

PS–03.02.09 ICOSAHEDRAL SYMMETRY OF A DOUBLE-SHELLED RICE DWARF VIRUS BY THE ROTATION FUNCTION. By Y.Morimoto*[1], K.Tomizaki[1], T.Tsukihara[1], T.Omura[2], M.Koizumi[3], H.Mizuno[4] and H.Kano[4], [1]The University of Tokushima, Japan, [2]National Agriculture Research Center, Japan, [3]National Institute of Sericultural and Entomological Science, Japan, [4]National Institute of Agrobiological Resources, Japan,.

Rice Dwarf Virus (RDV), a phytoreovirus, from *Nephotettix* species infects systemically rice and wheat plant and causes stunting or dwarfing of plant bodies. The virus being a double-shelled particle is approximately 700 Å in diameter and the molecular weight of 6.52 x 10^7. A total of 540 identical protein subunits are on the particle surface, suggesting T=9 icosahedral symmetry of the capsid. It is of great interest to study the large and complicated virus in structure of double-shell arrangement and in the function of virus. Structure determination may permit improvement of immunization efficiency and enhancement of immunological activity by modifying the structure of the antigen, the surface of the virus coat.
The virus has been crystallized in the cubic space group *I*23 with a=789Å. Two particles are included in a unit cell. Diffraction experiments were carried out with a macromolecular Weissenberg camera at Photon Factory, Japan. Camera length was 860mm, wavelength 1.04Å. Oscillation angle 1.5°. Intensity data were obtained from three native crystals with an index searching procedure and merged into an independent data set (~10Å).
Self-rotation function was calculated at 20Å resolution with the integration radius of 100Å using Crowther's program (The Molecular Replacement Method, 1977, 173-178). The rotation function showed icosahedral symmetries such as 2-, 3- and 5-fold axes with significant peaks higher than 60% of the ideal height. Some of 2- and 3-fold axes are coincident to those of the crystallographic symmetry. The RDV particles are located on the special positions of the Wyckoff notation "a" in the cell. From the particle size (~700Å) and the cell dimension, it suggests that the viruses are packed with the closest distances in the *I*23 lattice.

PS–03.02.10 CRYSTALLIZATION AND CHARACTERIZATION OF COXSACKIEVIRUS B3 (CVB3). By Jodi K. Bibler*, Marcia Kremer, Liang Tong and Michael G. Rossmann. Department of Biological Sciences, Purdue University, West Lafayette, IN 47906.

Coxsackievirus B3 (CVB3) is a small, icosahedral, single-stranded RNA virus belonging to the enterovirus genus within the picornavirus family. The enteroviruses include poliovirus, the coxsackie A and B viruses, hepatitis A virus and the echoviruses. The coxsackie B viruses are causative agents of a wide variety of mild to severe diseases and are the most common agents known to cause viral myocarditis. CVB3-Gauntt causes myocarditis in mice and provides an excellent animal model in which to study virus structure-function and drug therapy.
CVB3 crystallizes in a primitive monoclinic space group (a = 574.6Å, b = 302.1Å, c = 521.6Å, β = 107.7°) with two virions in the asymmetric unit. The crystals diffract well to 2.7Å resolution and 66% of the X-ray diffraction data has been collected to 3.0Å. Systematically weak reflections and the self-rotation function confirms pseudo R32 symmetry. This pseudo R32 symmetry orients and positions each particle in the monoclinic cell near face-centered positions. The resultant packing arrangement is equally consistent with space groups P2 or $P2_1$. Currently, work is underway to establish the deviations from R32 packing for both independent particles in the monoclinic asymmetric unit and to determine the true space group (P2 or $P2_1$). These parameters must be accurately known in order to determine and extend phases to the resolution limits of the available data and to successfully utilize the high order of non-crystallographic redundancy (120 fold) during molecular replacement real space averaging.

PS–03.02.11 PURIFICATION AND CRYSTALLOGRAPHIC STUDY OF AFRICAN HORSESICKNESS VIRUS CORE PROTEIN VP7. By A. K. Basak*, J. Grimes, P. Roy and D. I. Stuart. Laboratory of Molecular Biophysics, Oxford University, U. K.

African horsesickness (AHS) is a disease of caused by a dsRNA Orbivirus transmitted by gnats. Nine different serotypes of the virus have been identified. AHS virions, in common with their paradigm, bluetongue virus (BTV), consist of seven structural proteins (VP1-VP7) and three non-structural proteins (NS1-NS3). AHSV is very similar to BTV both in morphology and biochemical properties. The full length cDNA coding for core protein VP7 of the (AHSV-4) virus has been cloned and expressed in insect cells using a baculovirus expression system. This protein is the major component of the inner capsid of the virion and is thought to be present in 780 copies in the intact virus.
We have purified the protein and crystallized it in 2.5M urea. The protein is very hydrophobic in nature and has been crystallized at 20°C by vapour diffusion using the sitting drop method. The crystals exhibit tetragonal symmetry with unit cell dimensions a=b=157.10Å c=57.90Å $\alpha=\beta=\gamma=90°$, and belong to space group I4. A complete native data set to 3.0Å spacings has been collected inhouse on a Marresearch imaging plate. We hope to solve the structure by the molecular replacement method using the homologous structure of BTV-VP7 (Basak, A.K.; Grimes, J.M.; Roy, P. and Stuart, D.I.; this meeting).