PS04.09.17 IMPLICATIONS FOR THE TRIMERIC STRUCTURE OF THE SIV MATRIX ANTIGEN. @Zihe Rao, @Elizabeth Fry, $Ian Jones, $Polly Roy and @David Stuart (@The Laboratory of Molecular Biophysics, Rex Richards Building, South Parks Road, Oxford OX1 3QU, UK. $NERC Institute of Virology and Environmental Microbiology, Mansfield Road, Oxford OX1 3SR, UK.)

The crystal structure of the SIV matrix antigen (MA) has been determined at 100K (to dmin 2.1Å) and 293K (to dmin 2.2Å) by multiple isomorphous replacement and cross-averaging[1]. The refined models (R-factors 18.0% and 16.4% for the 100K and 293K structures respectively) are highly similar (r.m.s deviation 0.18Å between C_α atoms) but in some places the electron density is better defined for the 100K structure.

MA has roles delineated in targetting Pr55 Gag to the plasma membrane, facilitating incorporation of the virus envelope glycoprotein (portions of which also form trimers[2]) and assembly of the Pr55 Gag shell. The structure revealed that when forced into a higher order assembly in the crystal, the MA subunits form a trimer. This oligomer may correspond to a basic building block in the Gag shell, explaining many of the biological properties of the protein . It has since been corroborated by a crystallographic analysis of its HIV homologue[3] (50% sequence identity).

The three-dimensional structure also suggests a model for retroviral Gag shell assembly. These implications and future possibilities for inhibitor design will be discussed

1)Z.Rao, A.Belyaev, E.Fry, P.Roy, I.Jones, D.Stuart. Nature 378, 743-747 (1995).
2)S.Blacklow, M.Lu, P.Kim. Biochemistry (in press).
3)C.Hill, D.Worthylake, D.Bancroft, A.Christensen, W.Sundquist. PNAS (in press).

PS04.09.18 REOVIRUS CORES: PROGRESS TOWARD A STRUCTURE. Karin M. Reinisch*, Max L. Nibert†, Stephen C. Harrison*. *Department of Molecular and Cellular Biology and Howard Hughes Medical Institute, Harvard University, Cambridge, MA 02138,†Department of Biochemistry and Institute for Molecular Virology, University of Wisconsin-Madison, Madison, WI 53706

We will describe our progress toward a structure determination for cores of reovirus. Reovirus consists of a segmented ds RNA genome surrounded by two concentric protein shells. When the outer shell of reovirus is proteolytically removed, the viral core becomes active as a RNA processing machine. Cores of a strain T3D reassortant have been crystallized in spacegroup F432, a=1250Å. Crystals at cryogenic temperatures diffract to 6Å at the F1 beam line at CHESS. Our efforts at data collection and phasing will be described.

PS04.09.19 CRYSTAL STRUCTURE OF COXSACKIEVIRUS A9. John Tate*, Michael Smyth, Timo Hyypiä** and David Stuart, Laboratory of Molecular Biophysics, Oxford, U. K. and **Department of Virology, University of Turku, Turku, Finland, *Present address: The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, California, 92037, U.S.A

Coxsackievirus A9 (CAV9) has been purified and crystallized in the presence of an antiviral compound and its structure determined.

Crystals belong to space group C2 with cell dimensions a=488.5Å, b=358.5Å, c=306.5Å and β=128.1°. There is half a virion in the asymmetric unit giving rise to 30-fold non-crystallographic symmetry. Oscillation images have been collected and processed to 4.2Å, yielding approximately 20% of possible reflections to this resolution.

The structure solution used molecular replacement with a model composed from human rhinovirus 14 and poliovirus. An electron density map was calculated using all available data, and cyclic 30-fold averaging performed. Following 50 cycles the R factor was 15.5%. An atomic model has been fitted into this electron density map using the published sequence.

The major proteins VP1-3 show the same eight-stranded ß-barrel motif as the other picornaviruses and spherical plant viruses. A striking canyon is the dominant feature of the surface, with adjacent loops similar to poliovirus. A hydrophobic pocket lies within VP1 below the canyon floor and contains electron density commensurate with the antiviral compound (WIN51711) which was necessary for capsid stabilization during the crystallization step. A discrete node of density at the exterior of the 5-fold annulus may indicate a calcium binding site, while density on the interior of the capsid surrounding the 5-fold may be due to the myristate of VP4.

It is known from the CAV9 sequence that the carboxy-terminal of VP1 is extended by approximately 15 amino acids when compared to the closely related coxsackie B viruses, and this region contains the RGD motif implicated in receptor binding. In our structure to date, the 19 terminal residues have not been modelled due to ambiguities in the electron density.

We await data to higher resolution which will elucidate some of these ambiguities.

PS04.09.20 CRYSTAL STRUCTURE OF HUMAN RHINOVIRUS 3 AND COMPARISON WITH OTHER RHINOVIRUSES. Rui Zhao, Marcia Kremer, Richard Kuhn, Michael Rossmann, Dept. of Biological Sciences, Purdue University, W. Lafayette, IN 47907, USA, Daniel Pevear, Vincent Giranda, Jennifer Kofron, Mark McKinlay, Former Sterling Winthrop Pharmaceutical Research Division, 1250 S. Collegeville Rd., PO Box 5000, Collegeville, Pennsylvania 19426 0900, USA

Human Rhinovirus (HRV) is the major causative agent of common cold in humans and consists of over 100 different serotypes. These can be roughly divided into a major and minor group according to their cellular receptors. The crystal structures of HRV14 and HRV16, major receptor group rhinoviruses, as well as HRV1A, a minor receptor group rhinovirus, have been previously determined. Sequence comparisons had shown that HRV14 seemed to be an outlyer among rhinoviruses. Furthermore, HRV14 was the only virus with no cellular "pocket factor" in a hydrophobic pocket thought to regulate viral stability. The structure of HRV3, another major receptor group virus, was determined by the Molecular Replacement method. The amino acid sequence of HRV3 capsid proteins was obtained through cDNA cloning of the HRV3 RNA genome. Suprisingly, the structure and amino acid sequence of HRV3 are very similar to HRV14. Like HRV14, it also had no bound "pocket factor". A structural basis for the different antigenic and stability properties displayed by HRV3 has been proposed, and the implications of the similarity between HRV3 and HRV14 will be discussed.

There is non-protein electron density on the vital five-fold axes of all known rhinovirus crystal structures. Difference electron density maps between EGTA-soaked crystals of HRV14 as well as HRV16, and their corresponding native structures show that this density is a EGTA-chelatable ion. Analysis of the coordination geometry indicates that the ions in HRV3, HRV14 and HRV1A are likely to be Ca^{++}, and the ion in HRV16 could be Zn^{++}. These cations may play a role in regulation of the rhinovirus stability, although the loss of the ion itself seems not enough to lead to viral disassembly.