

**PS04.09.13 IDENTIFICATION OF A PROTEIN BINDING SITE OF THE ALPHAVIRUS CAPSID PROTEIN AND IMPLICATION IN VIRUS ASSEMBLY.** Sukeyong Lee, Katherine E. Owen, Hok-kin Choi, Richard J. Kuhn and Michael G. Rossmann, Dept. of Biological Sciences, Purdue University, W. Lafayette, IN 47907

Many enveloped viruses exit cells by means of budding from the plasma membrane. The driving force for budding is the interaction of an inner protein core with transmembrane glycoproteins. The molecular details of this process are ill defined. Alphaviruses, such as Sindbis virus (SINV) and Semliki Forest virus (SFV), represent some of the simplest enveloped viruses and have been well characterized by structural, genetic and biochemical techniques. Although a high resolution structure of an alphavirus has not yet been attained, cryo-electron microscopy (cryo-EM) has been used to show the multilayer organization at 25 Å resolution (Cheng et al., 1995). In addition, atomic resolution studies are available of the C-terminal domain of the nucleocapsid protein (Choi et al., 1991) which has been modeled into the cryo-EM density (Cheng et al., 1995).

Three different crystal structures, containing five independent copies of Sindbis virus capsid protein (SCP), show that amino acids 108 to 111 bind into a specific hydrophobic pocket in neighboring molecules. It is proposed that the binding of these capsid residues into the hydrophobic pocket of SCP mimics the binding of the E2 glycoprotein carboxy terminal residues into the pocket. Mutational studies of capsid residues 108 and 110 confirm their role in capsid assembly. Structural and mutational analyses of residues within the hydrophobic pocket, residues 108 to 111 of the capsid protein and residues in the carboxy terminal region of E2 in Sindbis virus and Semliki Forest virus, suggest that budding results in a switch between two conformations of the capsid hydrophobic pocket. This is the first description of a viral budding mechanism in molecular detail.

Cheng, R.H., Kuhn, R.J., Olson, N.H., Rossmann, M.G., Choi, H.K., Smith, T.J. & Baker, T.S. (1995). Three-dimensional structure of an enveloped alphavirus with T=4 icosahedral symmetry. *Cell* 80, 1-20. Choi, H.K., Tong, L., Minor, W., Dumas, P., Boege, U., Rossmann, M.G. & Wengler, G. (1991). Structure of Sindbis virus core protein reveals chymotrypsin-like serine proteinase and the organization of the virion. *Nature* (London) 354, 37-43.

**PS04.09.14 X-RAY STRUCTURE OF CARNATION MOTTLEVIRUS AT 3.2 Å RESOLUTION** A. M. Mikhailov, E. Yu. Morgunova, B. K. Vainshtein, Institute of Crystallography, Moscow, Russia, D.I. Stuart, E.E. Fry, Oxford University, Oxford OX1 3QU, UK, Z. Dauter, K.S. Wilson, EMBL Outstation, DESY, Hamburg, Germany

The structure of the Carnation Mottle Virus (CMtV) capsid protein has been determined at 3.2 Å resolution by the method of molecular replacement. The virion consists of the protein shell, a molecule of genome RNA and two short non-genome RNA fragments. The capsid is composed of 180 copies of the same protein with molecular weight of 37,787 Da arranged in accordance with T=3 icosahedral symmetry. X-ray data (140,483 unique reflections;  $R(I)_{\text{merge}}=8.2\%$ ; completeness=91%, sp.gr. I23;  $a=382.6$  Å;  $z=2$ ) were collected only from 4 crystals using the synchrotron radiation with an image plate as detector. The coordinates of TBSV were used as a searching model at 6 Å resolution. Refinement has been performed using XPLOR with 5-fold non-crystallographic symmetry constraints. An R-factor of 18.3% (r.m.s. deviation from ideality for bond distances are 0.021 Å and for angles are 4.15°) in the resolution range 6.0-3.2 Å (using 140,248 unique reflections) for the final model of CMtV, containing 7,479 independent non-hydrogen atoms, three calcium ions and three sulphate ions. No water molecules are included in the model. The structural and biochemical results lead us to consider an alternative assembly pathway.

**PS04.09.15 CRYSTALLIZATION AND PRELIMINARY X-RAY STRUCTURE INVESTIGATION OF THE PORCINE VESICULAR EXANTHEMA VIRUS (S-72 STRAIN).** A.M. Mikhailov, Institute of Crystallography, Moscow, Russia; V.A. Perevozchikov, Institute of Animal Protection, Vladimir, Russia; A.N. Kornev, Institute of Cell Biophysics, Pushchino, Russia

The Sakhalin-72 (S-72) virus belongs to the viruses causing vesicular diseases comprise the Calicivirus genus of the Picornaviridae family. The S-72 virus was isolated in the Sakhalin region. Studies of the reactions of diffuse precipitation and neutralization in a tissue culture with porcine convalescent serums demonstrated that immunologically, virions of this strain are significantly different, and in some instances totally different, from the porcine vesicular disease viruses of other strains. An particle of S-72 with an outside diameter of 35±2 nm has well defined projections above the capsid surface. The molecular mass of the S-72 virion is 10×10 Da. The genome of the virus is an infectious RNA plus strand of molecular mass 2×10 Da. A protein sheath of the S-72 is formed by a single structural protein with molecular mass 65±5 kDa. The icosahedral capsid with the triangulation number T=3 is formed by 180 "copies" of protein molecules. The crystals were grown in the "sitting drop" mode. The optimum precipitant is a 1 M ammonium sulfate solution in a 0.2 M Tris-HCl buffer at pH 7.8. The crystallization solution with a volume of 20 ml was composed of 10 ml of the 1% viral suspension, 9 ml of the precipitant solution, and 1 ml of PEG 300. The crystal growth time was 2-4 weeks at 20-22 C. These are the crystals for which the diffraction range extends to 2.7-2.9 Å resolution. The packing of the virions in the crystal can be described by the sp.gr.P23 with the parameter of the unit cell  $a=413$  Å and  $Z=1$ . The asymmetric unit contains fifteen protein molecules of the capsid. In most cases the angular width of the reflections was equal to 0.3.

**PS04.09.16 POLIOVIRUS 3C PROTEASE CRYSTALLIZATION AND STRUCTURE SOLUTION.** S. C. Mosimann<sup>1</sup>, M. Chernai<sup>1</sup>, S. Plotch<sup>2</sup>, Y. Gluzman<sup>2</sup>, M. N. G. James<sup>1</sup>, <sup>1</sup>MRC Group in Protein Structure and Function, Department of Biochemistry, University of Alberta, Canada T6G 2H7, <sup>2</sup>Wyeth Ayerst Research, Division of American Home Products, Pearl River, New York, 10965

The 3C gene product of poliovirus is a cysteine protease required for viral infectivity[1]. The 3C protease is responsible for the bulk of the processing of the viral polyprotein into its individual proteins[2]. Recombinant poliovirus 3C protease has been expressed in *E. coli*, solubilized from inclusion bodies and purified to homogeneity. Large single crystals of poliovirus 3C protease have been grown from hanging drops containing (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> solutions. The crystals are orthorhombic (space group P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>), the unit cell parameters are  $a=78.95$  Å,  $b=116.30$  Å,  $c=47.14$  Å,  $\alpha=\beta=\gamma=90^\circ$  and there are two molecules of 3C protease in the asymmetric unit. The calculated solvent content is 55% and the specific volume is 2.64 Å<sup>3</sup>/Da[2]. A complete 2.5 Å X-ray intensity data set ( $R_{\text{merge}}=0.052$ ) has been collected using monochromated CuK $\alpha$  radiation from a rotating anode source and San Diego Multiwire Systems proportional counters. To date, one exceptional isomorphous derivative (uranyl acetate) has been identified and electron density maps calculated with the SIR phases clearly reveal the two molecules in the asymmetric unit. Solvent flattening and histogram matching using the program DM[3] have significantly improved the quality of the SIR electron density maps. The anomalous signal from the uranyl acetate derivative and the presence of right-handed  $\alpha$ -helices have been used to confirm the correct hand of the SIR density maps. At present, the poliovirus 3C protease sequence is being fit to the SIR electron density maps.

[1] Baum *et al.*, *Virology* 185, 140 (1991)

[2] Ypma-Wong *et al.*, *Virology* 166, 265 (1988)

[3] Cowtan, Joint CCP4 & ESF-EACBM Newsletter 31, 34 (1994)

[4] Matthews, *J. Mol. Biology* 33, 491 (1968)