independent P8 molecules are superimposed on one of the P3 core-capsid protein. Cα trace of the P8 molecule is shown in a stick model. Start and end of the two induced-fit loops, shown in gray stick, are indicated in terms of residue number.

Keywords: virus coat proteins, virus assembly, viral structure and function

P04.13.311

Acta Cryst. (2008). A**64**, C328

The structure of baculovirus intracellular polyhedrin crystals reveals homoplasy of viral polyhedra

Fasseli Coulibaly[1], Elaine Chiu[1], Keiko Ikeda[2], Sascha Gutmann[3], Peter W Haebel[4], Clemens Schulze-Briese[3], Hajime Mori[5], Peter Metcalf[1]

[1]University of Auckland, School of Biological Sciences, Thomas Building, 2A Symonds Street, Auckland, Auckland, 1001, New Zealand, [2]Protein Crystal Corporation, Osaka, Japan, [3]Swiss Light Source at Paul Scherrer Institute, Villigen, Switzerland, [4]Altana Pharma AG, Konstanz, Germany, [5]Kyoto Institute of Technology, Kyoto, Japan, E-mail：f.coulibaly@auckland.ac.nz

Because insect viruses often remain in soil or leaves for prolonged periods before finding suitable hosts, they have evolved unique strategies to preserve their infectivity in such conditions. The most striking of these survival strategies are polyhedra, crystals of the viral polyhedrin protein which form a tough matrix protecting virus particles. Virus particles embedded in polyhedra can remain infectious for decades in the soil but, once ingested by new larvae, polyhedra readily dissolve in the alkaline environment of mid-guts initiating a new infectious cycle. Recently, the first atomic structure of polyhedra revealed the architecture of such infectious crystals produced by the silkworm cypovirus, a RNA virus belonging to the Reoviridae family. To understand how this strategy evolved in the viral world, we have engaged in the structural analysis of polyhedra produced by baculoviruses, DNA viruses completely unrelated to cypoviruses. I will present the 2.3Å structure of baculovirus polyhedra determined by X-ray crystallography from crystals 5-10 micrometers in diameter purified from infected cells. These crystals belong to the *I*23 space group with cell edge parameters of 103Å, just like cypovirus polyhedra. They are also made of polyhedrin trimers and extremely dense and robust except in alkaline conditions. Despite these functional and structural similarities, baculovirus and cypovirus polyhedrin proteins are unrelated and the way they pack in polyhedra is strikingly different. This evolutionary convergence to very similar crystalline architectures from different building blocks is reminiscent of the wide use of the icosahedral symmetry in virus particles.

Keywords: virus assembly, virus evolution, microcrystals

P04.13.312

Acta Cryst. (2008). A**64**, C328

Insight into viral inhibition of apoptosis - Structures of myxoma virus M11L and vaccinia virus F1L

Marc Kvansakul[1], M F van Delft[1], H Yang[1], E F Lee[1], W D Fairlie[1], P E Czabotar[1], J M Gulbis[1], S F Fischer[1], M A Perugini[2], D C Huang[1], P M Colman[1]

[1]The Walter and Eliza Hall Institute of Medical Research, STRUCTURAL BIOLOGY, 1G ROYAL PARADE, Parkville, Victoria, 3050, Australia, [2]Department of Biochemistry and Molecular Biology, The University of Melbourne, Parkville, Victoria 3010, Australia, E-mail：kvansakul@wehi.edu.au

Programmed cell death (apoptosis) is a critically important mechanism that enables multicellular organisms to eliminate damaged, infected or unwanted cells. The Bcl-2 family of proteins, which contains both pro- and antiapoptotic members, plays a central role in regulating apoptosis. The two proapoptotic members Bax and Bak are activated in response to apoptotic stimuli and play a pivotal role by triggering the release of pro-death factors by a series of unknown conformational events that result in mitochondrial membrane permeabilization (MMP). In healthy cells, Bax and Bak are held in check by antiapoptotic family members such as Bcl-2, Bcl-xL and Mcl-1. Apoptotic stimuli result in the release of proapoptotic BH-3 only proteins that neutralize antiapoptotic Bcl-2, thus freeing Bak and Bax to cause MMP. Apoptosis is recognised as a key innate immunity defence mechanism, and viruses have developed different strategies to ensure their survival in the face of host immune responses. Viral Bcl-2 homologs are deployed by a number of viruses to prevent cells from apoptosis during infection. Myxoma virus (MV) and vaccinia virus (VV), which both belong to the poxviruses, encode numerous anti-apoptotic proteins, but lack obvious Bcl-2 homologues. The MV protein M11L and the VV protein F1L have been identified as major virulence factors that locate to the outer mitochondrial membrane, lack sequence similarity to any other protein and have been shown to inhibit apoptosis. We have determined the crystal structures of free M11L and M11L in complex with a Bak 26-mer peptide as well as the crystal structure of F1L, and investigated their antiapoptotic properties. Our analysis provides new insight into the mechanism by which MV and VV subvert host apoptosis to ensure virus survival.

Keywords: apoptosis, protein homology, viral protein

P04.13.313

Acta Cryst. (2008). A**64**, C328-329

Structure of influenza H5N1 nucleoprotein and its interaction with RNA

Pang-Chui Shaw[1], Andy Ka-Leung Ng[1], Hongmin Zhang[2], Jiahuai Wang[2], Kemin Tan[3], Shannon Wing-Ngor Au[1]

[1]Chinese University of Hong Kong, Department of Biochemistry, The Chinee University of Hong Kong, Shatin, Hong Kong, NT, China, [2]Department of Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA, [3]Midwest Center for Structural Genomics and Structural Biology Center, Argonne National Laboratory, Argonne, IL, USA, [4]Department of Microbiology, The Chinese University of Hong Kong, Shatin, Hong Kong, NT, China, E-mail：pcshaw@cuhk.edu.hk

Influenza is a contagious respiratory illness causing annual epidemics. The threat of a pandemic outbreak of influenza virus H5N1 has become a major concern worldwide. The nucleoprotein (NP) plays both structural and functional roles in influenza viruses and represents an attractive drug target. Here we report the 0.33nm crystal structure of H5N1 NP, which is composed of a head domain, a body domain and a tail loop. Our structure resolves the important linker residues (residues 397-401, 429-437) that connect the tail loop with the remainder of the molecule and a flexible, basic loop (residues 73-91) located in an arginine-rich groove surrounding Arg150. Using surface plasmon resonance, this basic loop and arginine-rich groove, but mostly a protruding element containing Arg174 and Arg175, were found to be important in RNA binding. A possible mechanism by which NP associates with RNA is as follow. First, the flexibility of the basic loop (residues 73-91) may allow it to sample the environment and capture RNA. The captured RNA could

then deliver into the arginine-rich groove. Second, our data show that the region centered around the protrusion is crucial for RNA binding and presumably is the major RNA binding site. The side chains of the arginine residues in this region are pointing towards each other, suggesting that this region may clamp the RNA into the groove. Third, we have found that an arginine rich region at the other end of the groove is also important for RNA-binding. Since 24-27 RNA nucleotides bind to an influenza NP molecule, the RNA is expected to make further contacts with NP in addition to binding along the arginine-rich groove. This work may lead to the design of inhibitors for perturbing the transcription and replication of influenza virus.

Keywords: infectious diseases, nucleoprotein, influenza virus

P04.13.314

Acta Cryst. (2008). A**64**, C329

Co-crystallization and X-ray studies of HIV-1 Vpr-Importin-alpha and Vpr-inhibitor complexes

Akira Sanjoh[1], Yuko Tatsumi[1], Hideyuki Miyatake[2], Naoshi Dohmae[2], Go Matsuda[3], Yoko Aida[3]
[1]Protein Wave Corporation, R&D Division, Bldg. Lab Wing/5F, 1-7 Hikaridai, Seika-cho, Soraku-gun, Kyoto, 619-0237, Japan, [2]Biomolecular Characterization Team, RIKEN, Wako, Saitama, Japan, [3]Viral Infectious Disease Research Unit, RIKEN, Wako, Saitama, Japan, E-mail：asanjoh@pro-wave.co.jp

Viral protein R (Vpr) of HIV-1 is a small nuclear protein (14KDa) of 96 aa and has 3 regions consisting of aH1 (residues between 17 and 33), aH2 (38 and 50) and aH3 (56 and 77). Vpr plays various roles in viral infection and cellular functions, and is also known as one of the possible mediators of the nuclear localization of preintegration complex. In a previous study, we showed that Vpr interacts with Importin-a through the aH1 and aH3 regions and that the interaction via aH1 is essential for entry into the nucleus but also for HIV-1 replication of macrophages. Crystal structures of the Vpr in complexes with Importin-a and inhibitors will therefore lead to discovery of novel lead compounds of HIV-1. Vpr (17-74 and 17-81 residues) and Importin-a were expressed as recombinant GST fusion proteins in E.coli. Both proteins were purified by glutathione sepharose 4B column chromatography, and GST was cleaved by Prescission protease. Vpr was further purified by applying Electro-Eluting system with non-reduced condition. After loading to size exclusion column, each protein was buffer exchanged and concentrated to 5mg/mL (Vpr) and 10mg/mL (Importin-a), respectively. Crystallization conditions were determined and optimized in each protein. Co-crystallization and X-ray diffraction trials are under way to determine complex crystal structures of Vpr-Importin-a and Vpr-Inhibitors.
[1] Nitahara-Kasahara Y., Kamata M., Yamamoto T., Zhang X., Miyamoto Y., Muneta K., Iijima S., Yoneda Y., Tsunetsugu-Yokota Y. and Aida Y. J. Virol., 81,5284, 2007. [2]Kamata M., Nitahara-Kasahara Y., Miyamoto Y., Yoneda Y., Aida Y., J. Virol., 2005, 79,3557. [3]Iijima S., Nitahara-Kasahara Y., Kimata K., Zhong Zhuang W., Kamata M., Isogai M., Miwa M., Tsunetsugu-Yokota Y., Aida Y., Virology, 2004,327,249.

Keywords: HIV-1, preintegration complex, protein-inhibitor co-crystallization

P04.14.315

Acta Cryst. (2008). A**64**, C329

New rearrangement in GroEL due to a 22 rotation between the heptameric rings

Aintzane Cabo Bilbao[1], Arturo Muga[2], Diego Guerin[3]
[1]CIC bioGUNE, Structural Biology, Parque Tecnologico, Edificio 800, Derio, Bizkaia, 48160, Spain, [2]Unidad de Biofisica, University of the Basque Country, Barrio Sarriena s/n Leioa, Bizkaia, Spain, [3]Facultad de Ciencias, University of the Basque Country, Barrio Sarriena s/n Leioa, Bizkaia, Spain, E-mail：acabo@cicbiogune.es

Folding, trafficking, maintenance, and degradation of proteins are all processes that depend on the assistance of molecular chaperones. Chaperones are proteins whose function is to assist other proteins in achieving proper folding. Many are heat shock proteins, that is, proteins expressed in response to elevated temperatures or other cellular stresses. Among these, GroEL is a double-heptameric 800 kDa toroid, made of identical subunits that contains two central cavities, one in each ring that can accommodate proteins up to 60 kDa. GroES is a single-ring heptamer that binds to GroEL in the presence of ATP or ADP. In this way, the complex GroES-GroEL forms a hydrophobic cavity where the substrate is folded and is subsequently returned to the medium. Interactions between the two rings in GroEL result in the allosteric regulation of ATP hydrolysis, binding, and release of folding substrates and the cochaperonin GroES. In order to gain information about the signalling pathway associated to cooperativity in this protein and to better understand the role of the interface in the allosteric communication, two different mutants that lack negative cooperativity were studied: GroELE434K and GroELE461K. Crystallographically solved structures of these mutants explain the role of the interface between the rings in the allosteric communication and help to describe the conformational changes that are the cause of the different behaviour of the mutants. On the other hand, regions that stay unaltered during the functional cycle were found. The studies conclude that: i)together with en-bloc domain movements, allosterism is held in GroEL by the combination of rigid and deforming regions within subunits and ii) salt bridge pathways control allosteric communication in GroEL.

Keywords: GroEL, chaperonin, allosterism

P04.14.316

Acta Cryst. (2008). A**64**, C329–330

Characterization of the Munc18-Syntaxin protein interaction

Michelle P Christie[1], Shu-Hong Hu[1], Linda H L Lua[2], Andrew E Whitten[3], Jill Trewhella[3,4], David E James[5], Jennifer L Martin[1]
[1]Institute for Molecular Bioscience University of Queensland, Chemical and Structural Biology, 306 Carmody Road, St Lucia, QLD, 4072, Australia, [2]Australian Institute for Bioengineering and Nanotechnology University of Queensland, St Lucia, QLD, 4072, Australia, [3]Bragg Institute, Australian Nuclear Science and Technology Organization, Menai, NSW, 2234, Australia, [4]School of Molecular and Microbial Sciences University of Sydney Camperdown, NSW, 2006, Australia, [5]Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst Sydney, NSW, 2010, Australia, E-mail：m.christie@imb.uq.edu.au

The SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins facilitate vesicle docking and fusion by forming SNARE complexes. These complexes are formed by the interaction of cognate SNAREs found on opposing membranes during fusion. The Sec/Munc (SM) family of proteins are a group of