FA1-MS08-T01

Crystallography of virus capsids, the evolution of viruses, technology and therapy. David Stuart,
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I will summarize the limits of current technology for the crystallographic analysis of viruses and explore how far these limits might be pushed over the next few years, with some examples from current projects. I will also indicate with examples how the structural information we have might have an increasing impact on human and animal health as we start to integrate structural knowledge into the process of vaccine optimization.

FA1-MS08-T02

Structural insights into antibody-mediated neutralization of dengue virus. Joseph Cockburn[a], Erika Navarro Sanchez[a], Ana Goncalvez[b], Isabelle Staropoli[a], Fernando Arenzana-Seisdedos[a], Hugues Bedouelle[a], Ching-Juh Lai[b], Felix Rey[a], [a]*Institut Pasteur, Paris, 75015, France.* [b]*NIAID/NIH, Bethesda, MD 20892, USA.*
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Dengue is the foremost vector-borne viral disease in the developing world. The immunology of dengue virus (DENV) is complicated by the existence of four serotypes (DENV-1 through 4), with immunity against one serotype predisposing to severe disease in subsequent infections from the others. This is thought to involve enhancement of infection by weakly or non-neutralizing antibodies that cross-react with multiple dengue serotypes. DENV is an enveloped, plus-sense single-stranded RNA virus belonging to the *Flaviviridae* family. Virus ingress involves uptake by receptor-mediated endocytosis followed by low-pH induced fusion of the viral and endosomal membranes. The DENV Envelope (E) protein is responsible for receptor binding and membrane fusion, and is the principal viral antigen. The E protein ectodomain comprises three domains (DI-DIII), and elicits monoclonal antibodies (Mabs) with varying serotype specificities, neutralization potency and enhancement potential. A detailed understanding of the antigenic structure of the E protein is of vital importance to vaccine development. I will present our crystallographic studies of antibody-antigen complexes involving the DENV E protein and two Mabs with highly complementary properties. 4E11 is a murine antibody that neutralizes all four DENV serotypes by blocking cell attachment, binding to an epitope on DIII. We have solved crystal structures of a 4E11 scFv construct in complex with DIII from all four DENV serotypes, all to ~2Å resolution, revealing a novel mechanism of cross-reactivity. In a unique comparative study, we have related the atomic structures of these complexes to binding affinity and neutralization efficacy by serotype. The 4E11 epitope is located on an immunodominant portion of DIII and the structures reveal the molecular basis for this immunodominance. Previously, a murine Mab possessing a similar epitope to 4E11 was shown to neutralize DENV-2 by disrupting the virion architecture [1]. Our analysis provides new insights into the molecular details of how this proceeds and suggests that this mechanism is likely to be common to all antibodies binding to this portion of the E protein. The epitopes of strongly neutralizing human Mabs against DENV are of crucial importance to vaccine design but so far none have been described. The chimpanzee monoclonal antibody 5H2 is specific for DENV-4, and neutralizes the virus at a post-attachment stage of the entry pathway by binding to a novel epitope on DI. We have solved the crystal structure of its Fab fragment in complex with the intact, dimeric ectodomain of E and with DI, at 3.2Å and 2.7 Å resolution, respectively. The structure explains the serotype specificity and shows that 5H2 interferes with the conformational changes in the E protein that accompany membrane fusion. 5H2 is highly human-like, and human equivalents to 5H2 may therefore be important effectors of serotype-specific human humoral immunity to DENV. These findings may thus provide new strategies for vaccine design.

[1] Lok *et al.*, *Nat Struct Mol Biol.* 2008 Mar;15(3):312-7

Keywords: dengue, antibody, structure

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Crystal structure of gp V – the bacteriophage P2 cell puncturing device. Petr G. Leiman[a], Christopher Browning[a], Mikhail Shneider[b], [a]*Ecole Polytechnique Federale de Lausanne (EPFL), Switzerland.* [b]*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry (RAS), Moscow, Russia.*
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The needle or spike protruding from the center of the baseplate is a conserved feature found in all known contractile tail phages. It has been proposed that this needle punctures the outer membrane of the cell during phage infection and tail contraction [1]. The structure of one of these needles, the phage T4 gp5-gp27 complex, was reported earlier [1]. However, the structure of the membrane-interacting tip of the T4 needle is unknown because it is formed by a yet unidentified protein, which decorates the gp5 beta-helix [2].
We report here the crystal structure of gene product V (gpV) from the well studied bacteriophage P2 with a contractile tail. Using cryoEM, we show that gpV forms the needle of P2 tail and there is no other protein at the tip of the needle.
GpV is a long trimer and consists of two domains, the N-terminal OB-fold domain and the C-terminal triple-stranded beta-helix domain. Remarkably, the N-terminal half of P2 gpV structure superimposes onto the N-terminal part of T4 gp5 very well despite exhibiting only 11% sequence identity. The gpV beta-helix is different from that of gp5 in that it tapers strongly towards the C terminus and ends in a very sharp tip (only 10 Å in diameter), making the whole protein look like a sharpened pencil, further supporting its membrane penetrating function. There are three ions positioned on the trimer threefold axis near the 'sharpened' tip – Fe, Ca and Cl – all of which might be important for both, protein folding and membrane penetration during infection.

[1] Kanamaru S, Leiman PG, Kostyuchenko VA, Chipman PR, Mesyanzhinov VV, Arisaka F, and Rossmann MG. *Nature*, 2002. 415(6871): p. 553-557. [2] Kostyuchenko VA, Leiman PG, Chipman PR, Kanamaru S, van Raaij MJ, Arisaka F, Mesyanzhinov VV, and Rossmann MG. *Nat Struct Biol*, 2003. 10(9): p. 688-693.

Keywords: bacteriophage, beta-helix, membrane-binding protein

FA1-MS08-T04

Structure of an intramolecular chaperone mediating triple–β-helix folding. Eike C Schulz[a], Achim Dickmanns[a], Henning Urlaub[b], Andreas Schmitt[a], Martina Mühlenhoff[c], Katharina Stummeyer[c], David Schwarzer[c], Rita Gerardy-Schahn[c], Ralf Ficner[a].
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Protein folding is often mediated by molecular chaperones. Recently, a novel class of intramolecular chaperones has been identified in tailspike proteins of evolutionarily distant viruses, which require a C-terminal chaperone for correct folding. The highly homologous chaperone domains are interchangeable between pre-proteins and release themselves after protein folding. We solved the crystal structures of two intramolecular chaperone domains in either the released or the pre-cleaved form, revealing the role of the chaperone domain in the formation of a triple-β-helix fold [1]. Tentacle-like protrusions enclose the polypeptide chains of the pre-protein during the folding process. After the assembly, a sensory mechanism for correctly folded triple β-helices triggers a serine-lysine catalytic dyad to autoproteolytically release the mature protein. Sequence analysis shows a conservation of the intramolecular chaperones in functionally unrelated proteins presumably sharing triple β-helices as a common structural motif.

[1] Schulz EC, Dickmanns A, Urlaub H, Schmitt A, Mühlenhoff M, Stummeyer K, Schwarzer D, Gerardy-Schahn R, Ficner R., *Nat. Struct. Mol. Biol*. 2010 Feb; 17 (2), pp. 210-5.

Keywords: intramolecular chaperone assisted folding, triple-β-helix tailspike, autoproteolysis

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Structural studies of herpesvirus terminase. Marta Nadal[a,b], Phillipe Mas[c], Alexandre G. Blancoa[,b], Carme Arnan[a,b], Maria Solà[b], Darren Hart[c], Miquel Coll[a,b].
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During viral replication herpesviruses package their DNA into the procapsid by means of the terminase protein complex. In human cytomegalovirus (hespesvirus 5) the terminase is composed by main subunits UL89 and UL56. UL89 bears the nuclease activity, cleaving the long DNA concatemers into unit-length genomes of appropriate length for encapsidation, which occurs through the portal protein UL104. The structural and functional characterization of the herpes packaging proteins, which could assist in the discovery and development of novel antiviral molecules, has been hindered by the difficulties in expressing enough soluble material for structural analysis. We are using ESPRIT, a high throughput screening method, to identify soluble fragments of the terminase subunits from libraries of randomly truncated gene constructs. Employing this methodology a soluble fragment of subunit UL89 has been obtained. This fragment, which corresponds to the nuclease domain of the subunit, has been purified and crystallized. Its structure has been determined to 2.15 Å resolution and will be presented.

Keywords: herpesvirus, DNA packaging, terminase