

**FA1-MS08-T01**

**Crystallography of virus capsids, the evolution of viruses, technology and therapy.** David Stuart, *Division of Structural Biology, University Oxford.*  
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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<sup>a</sup>, Erika Navarro Sanchez<sup>a</sup>, Ana Goncalvez<sup>b</sup>, Isabelle Staropoli<sup>a</sup>, Fernando Arenzana-Seisdedos<sup>a</sup>, Hugues Bedouelle<sup>a</sup>, Ching-Juh Lai<sup>b</sup>, Felix Rey<sup>a</sup>, <sup>a</sup>*Institut Pasteur, Paris, 75015, France.* <sup>b</sup>*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

**FA1-MS08-T03**

**Crystal structure of gp V – the bacteriophage P2 cell puncturing device.** Petr G. Leiman<sup>a</sup>, Christopher Browning<sup>a</sup>, Mikhail Shneider<sup>b</sup>, <sup>a</sup>*Ecole Polytechnique Federale de Lausanne (EPFL), Switzerland.* <sup>b</sup>*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.