[1]. Tat is critical for viral replication and is a potential HIV-1 vaccine candidate [2]. This intrinsically disordered protein is present in the extracellular medium and can enter neighbouring cells, in which it harbours functions involved in the pathogenicity of HIV by interacting with different cellular and viral biological partners.

A monoclonal antibody termed 11H6H1, specific to the N-terminal region of Tat, was selected to decipher the structural, biological and immunological properties of Tat. This N-terminal region, which contains an immunodominant epitope recognized by B-lymphocytes, could play a role in the immunosuppressive function of Tat. The equilibrium dissociation constants $K_D$ of full-length Tat and Tat N-terminal fragments complexed with 11H6H1 were estimated by competitive ELISA. The $K_D$ value in the nanomolar range reveals a high affinity between the partners and an interaction sufficiently strong to perform crystallographic experiments of complexes. Tat contains a single tryptophan residue, Trp11, located in the N-terminal region. We show that the substitution of Trp11 by a phenylalanine completely abolishes the binding of 11H6H1, whereas the transactivating activity of Tat is preserved. The minimal epitope recognized by 11H6H1 was experimentally restricted to the 9-mer peptide $P^{6}KLEPWKHP^{14}$ centered on Trp11 which is essential for recognition.

The crystal structures of this 9-mer peptide and of the overlapping 15-mer peptide were determined in complex with Fab' 11H6H1 at 2.4Å and 2.1Å resolution, respectively. Tat is intrinsically disordered and can undergo induced folding upon association with a biological partner. Our crystallographic study reveals that the two Tat peptides are lodged in the U-shaped groove of the Fab' antigen-binding site and adopt a standard type I β-turn conformation. The central Trp11 that is critical for Fab' recognition is further stabilized by π-stacking interactions. The fold adopted by the epitope upon binding with 11H6H1 has been compared with a recent crystallographic study of Tat in complex with the nuclear transcription factor p-TEFb [3]. The two folds share similar features. We will discuss the structural and biological consequences of this observation for HIV pathogenesis.

The nature of the residues of Tat interacting with the antibody, the molecular basis of these interactions, and the natural variability of these residues across HIV-1 strains give new insights for the molecular basis of immune escape of Tat from the humoral immune response, and thus for Tat-based vaccine development. As Tat remains an important component of anti-HIV vaccines, accumulating structural data for Tat proteins or peptides in complex with other Tat-specific antibodies remains an important issue for vaccine development.

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**Keywords: intrinsically disordered protein, crystal structure, induced folding**

## MS29.P07

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**Structure and RNA recognition in recombinant STNV capsids**

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We have expressed a recombinant form of the coat protein of satellite tobacco necrosis virus (STNV) in *E. Coli* using a codon-optimized gene, and shown it assembles spontaneously into capsids closely resembling the wild-type virus. The T=1 virus-like particles (VLPs) package the recombinant RNA transcript, and conditions have been established for disassembly and reassembly *in vitro*. *In vivo* in *E.coli* VLP assembly is dependent on the presence of the coat protein N-terminal helix, and *in vitro* it requires RNA. We have solved the X-ray crystal structure of the VLP refined it to $R/R_{free}$ 17.4/20.7% at 1.4Å resolution. It is very similar to wild-type STNV structure with no evidence of ordered RNA. Icosahedral symmetry constraints were removed to reveal small differences between subunits, presumably owing to crystal packing.

We collected low resolution X-ray data in the range 140-6Å, and the 60-fold averaged electron density map clearly shows well ordered RNA fragments lodged near the inside surface of the capsid, close to basic clusters of N-terminal triple helices that extend into the interior of the particle. The RNA consists of a 3 bp helical stem, with a single unpaired base at the 3' end and probably consists of a number of short stem-loops, where the loop region is disordered. The arrangement of the RNA is different from that observed in other satellite viruses.

Using immobilised coat protein monomers placed under reassembly conditions with 'free' coat protein subunits, we have prepared a range of partially assembled coat protein species for RNA aptamer selection. SELEX directed against the RNA-binding faces of the STNV coat proteins resulted in the isolation of a clone, B3, that matches the STNV1 genome in sixteen out of twenty-five (16/25) nucleotide positions, including across a statistically significant 10/10 stretch. This latter region folds into a stem loop displaying the loop motif ACAA, and for B3 this region binds STNV coat protein. Analysis of the other aptamer sequences reveals that the majority can be folded to stem loops displaying versions of this motif. Using a sequence/secondary structure search motif we then analysed the genomic sequence of STNV1 identifying 30 stem loops displaying the sequence motif. The implication is that there are many stem loops in the genome carrying essential recognition features for binding the STNV coat protein. The predicted genomic RNA fold is only 8/30 such stem loops are thermodynamically favoured, implying that assembly may occur on nascent RNA transcripts where folding can be controlled kinetically.

**Keywords: virus, RNA, recognition**

## MS29.P08

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**Structure of PB1-PB2 subunit interface of inflenza A virus RNA polymerase**

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Influenza is an infectious disease caused by the influenza virus. The symptoms such as a high temperature, chills, headache and so on are evident throughout the incubation period of about three days. Usually the symptoms disappear in a few weeks as the virus is cleared by the immune system, and there are no long-term consequences to health. However, sometimes highly pathogenic influenza viruses arise and cause pandemics, which may result in a large loss of human life. Recently a highly infectious H1N1 type virus appeared and caused a world-wide pandemic. It was also reported that humans had been

infected by highly pathogenic H5N1 type and H7N7 type avian influenza. There is a clear need for new anti-influenza drugs to combat such viruses, especially as resistance to the current drugs Tamiflu and Relenza is already widespread.

The RNA polymerase is a highly attractive target for new drugs since it plays a variety of essential roles in the viral life-cycle and is more highly conserved than other viral proteins. We concentrated our research efforts on structural studies of the RNA polymerase complex in order to assist structure-based drug design. Influenza virus RNA-dependent RNA polymerase is a hetero-trimer consisting of subunits called PA, PB1, and PB2. PB1 interacts directly with PA and PB2, but PA and PB2 do not. RNA polymerase uses a "cap-snatching" mechanism to produce viral mRNA. Host cell mRNA is cleaved to yield a cap-bearing oligo-nucleotide which can be extended using viral genomic RNA as a template. The cap binding and endonuclease activities are only activated once viral genomic RNA is bound. This requires signaling from the RNA-binding PB1 subunit to the cap-binding PB2 subunit, and the interface between these two subunits is essential for the polymerase activity.

We have defined this interaction surface by protein crystallography, and tested the effects of mutating contact residues on the function of the holo-enzyme[1]. This novel interface is surprisingly small, yet it plays a crucial role in regulating the 250 kDa polymerase complex, and is completely conserved among avian and human influenza viruses.

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**Keywords: structure, influenza virus, RNA-polymerase**

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**Crystal structure of the viroplasm matrix protein P9-1 of *Rice Black Streaked Dwarf Virus***

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*Rice black streaked dwarf virus* (RBSDV), which belongs to the genus *Fijivirus* in the family *Reoviridae*, can replicate both in plants and in an invertebrate insect vector. RBSDV is transmitted to rice, maize, barley and wheat exclusively by the small brown planthopper, after multiplication of the virus in the insect. RBSDV is an icosahedral double-shelled particle of approximately 75 to 80 nm in diameter and contains 10 segments of dsRNA (S1 through S10 in order of their mobility during SDS-PAGE). The genome encodes six putative structural proteins and six putative non-structural proteins. S9 comprises approximately 1.9 kb in size and encodes two ORFs, S9-1 and S9-2, separated by an intercistronic region. The ORF S9-1 at the 5′ end of the S9 encodes a 39.9 kDa protein, namely P9-1. The P9-1 protein of RBSDV accumulates in viroplasms within the cytoplasm of infected cells. They are discrete, punctate viral inclusions, which appear to play an important role in viral morphogenesis and are commonly found in viruses in the family *Reoviridae*. Crystallographic analysis of P9-1 revealed structural features that allow the protein to form dimers via hydrophobic interactions. Each dimer had C-terminal regions, resembling arms, that extended to neighboring dimers, thus uniting sets of four dimers by lateral interaction via hydrophobic interactions to yield cylindrical octamers. Positively and negatively charged patches on the side surfaces of the octamers suggested that octamer might bind laterally to other octamers via electrostatic complementarity. Furthermore, distribution of a positively charged region inside and negatively charged regions on the outer surface of each octamer suggested the three-dimensional stacking of octamer nets, when positively charged areas make contact with negatively charged regions composed of four neighboring octamers. Our structural analysis of P9-1 predicts that this protein has the intrinsic ability to form dimers, octamers, a lateral net of octamers and a three-dimensional viroplasm, as confirmed by the formation of viroplasm-like inclusions when P9-1 was expressed *in vivo* in the absence of other viral proteins and the absence of such inclusions when P9-1 was expressed without its C-terminal arm.

**Keywords: viroplasm, RBSDV, reoviridae**

## MS29.P10

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**Crystallization and preliminary X-ray diffraction analysis of the RNA-dependent RNA polymerase of *Thosea asigna* Virus**

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*Thosea asigna* virus (TaV) is a positive-sense, single stranded RNA (ssRNA) virus that belongs to the *Betatetravirus* genera within the *Tetraviridae* family. The genome of TaV consists of an RNA segment (5,700 nucleotides) with two open reading frames, encoding the replicase and capsid protein. The particular TaV replicase does not contain N7-methyl transferase and helicase domains but includes a structurally unique RNA-dependent RNA polymerase (RdRp). All RdRps share a closed "right hand" architecture with fingers, palm and thumb sub-domains, encircling the active site. Sequence analyses identified five ordered sequence motifs (A-B-C-D-E) within the palm subdomain that are conserved in all virus replicases [1]. However, in TaV RdRp motif C is located upstream the motif A. This sequence permutation yields a palm fold in which the canonical structural elements show a non-canonical connectivity (C-A-B-D-E). The permuted palm architecture is also found in double stranded RNA (dsRNA) viruses of the *Birnaviridae* family. Here we report the preliminary crystallographic studies of TaV RdRP. The purified enzyme, produced in insect cells infected with a recombinant baculovirus vector, was crystallized by the sitting drop vapour-diffusion method using PEG 8K and Litium sulphate as precipitants. Two different crystal forms were obtained: native RdRP crystallized in space group P21212 and diffract up to 2.1 Å and the RdRP-Lutetium derivative belong to C2221 space group, diffracting up to 3.0 Å resolution. Data collected at $Lu^{3+}$ absortium maximum ($\lambda$= 1.3404 Å) were suitable for structure determination by single wavelength anomalous dispersion (SAD) technique. The structure of TaV RdRP represents the first structure of a non-canonical RdRP from ssRNA virus. The structural similarities observed between the TaV and Birnavirus RdRps, particularly in the three dimensional organization of their non-canonical palms [2], is a structural evidence that strongly supports the existence of a common ancestor between these apparently unrelated viruses.

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