[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 $P^6KLEPWKHP^{14}$ 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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### 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**

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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