Elucidating transactivation in HIV

one structure at a time

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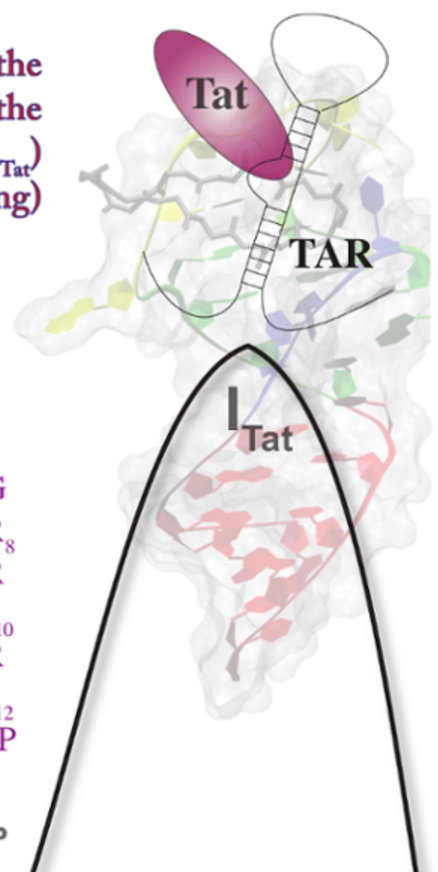
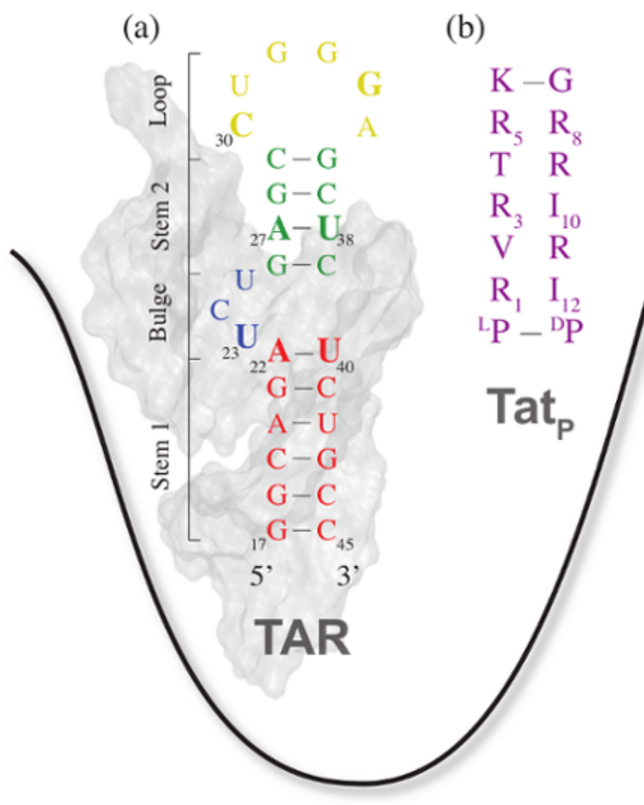
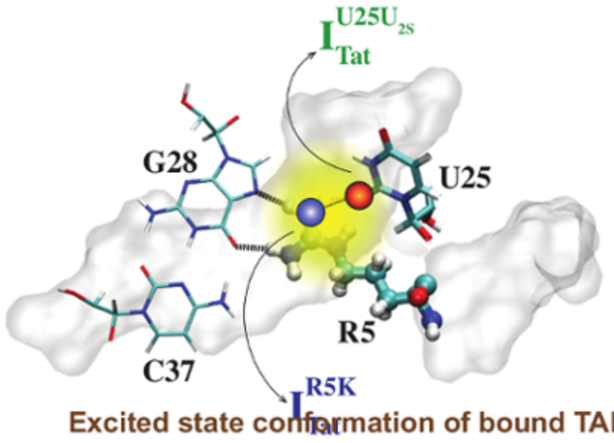
HIV hijacks the human transcription machinery to make multiple copies of its own genome. This process, known as transactivation, is crucial in the HIV infection cycle and has thus become the object of focused scientific attention in the past two decades - both for understanding its molecular mechanism and for the development of anti-HIV drugs.

I. We present for the first time, the characterisation and validation of the structure of an intermediate state (I_{Tat}) on the pathway of release (and binding) of Tat to TAR.





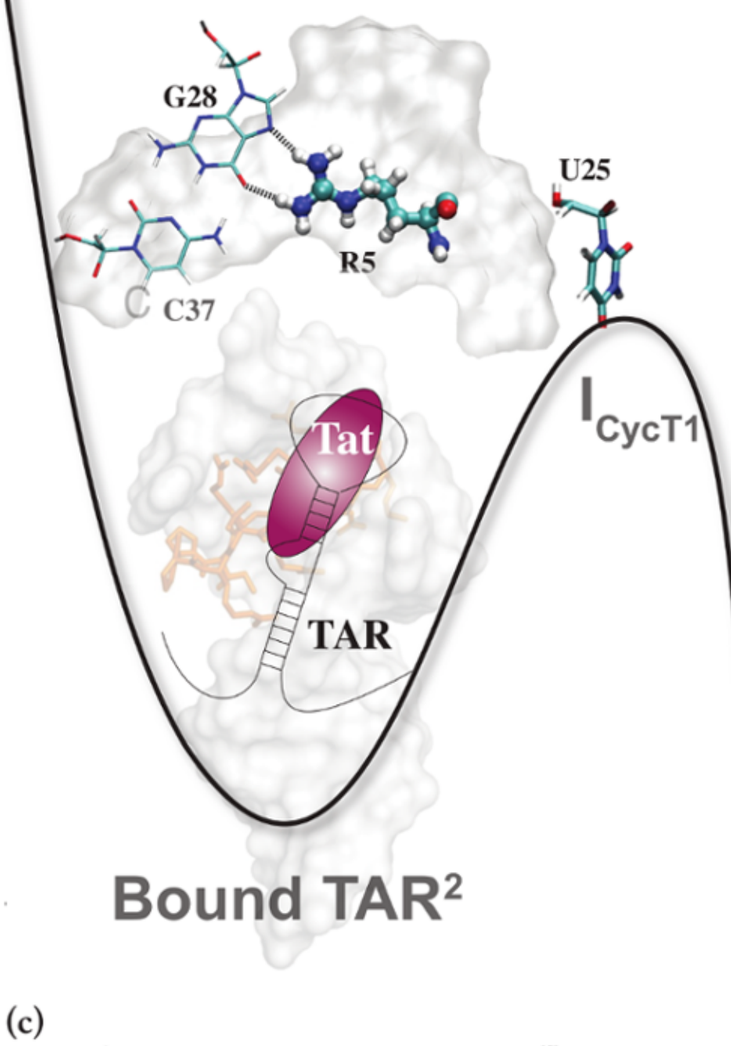
To identify the intermediate I_{Tat} on the pathway of Tat binding to free TAR , we contructed the conformation landscape of the Tat_P bound TAR via the "Θ RDC restrained Replica Averaged Metadynamics (RAM)" simulation[3-6] (see flyer).



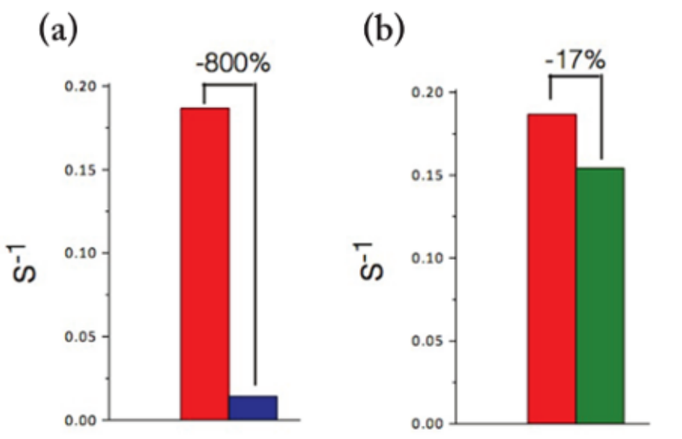
Excited state conformation of bound TAR

Destabilising this interaction via intelligient mutant design should decrease the rate of release of the Tat peptide from TAR.

Canonical RNP interaction in bound TAR

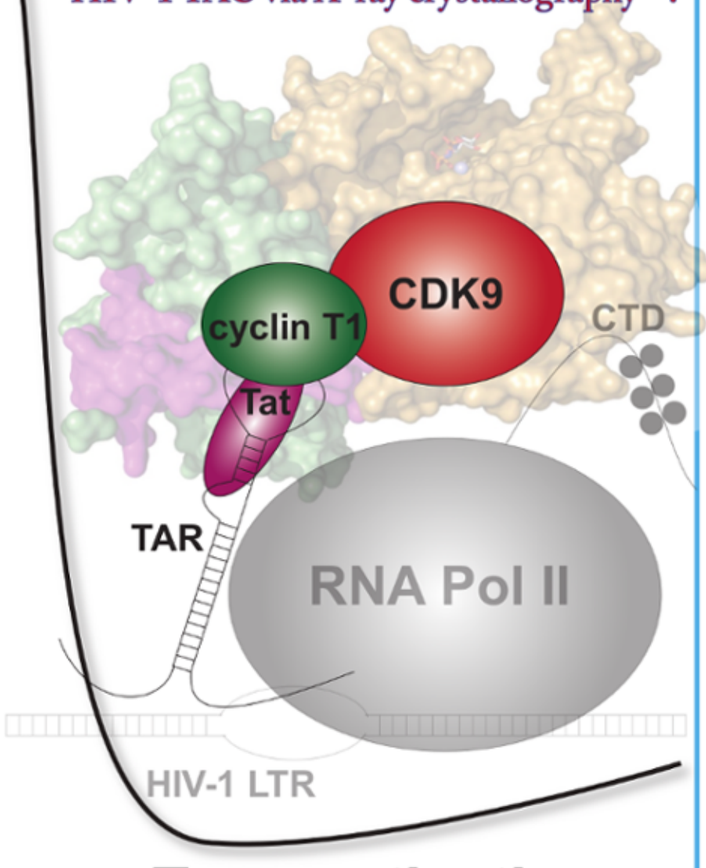


The I_{Tat} is stabilised by a non-native H-bond between the RNA and the peptide.

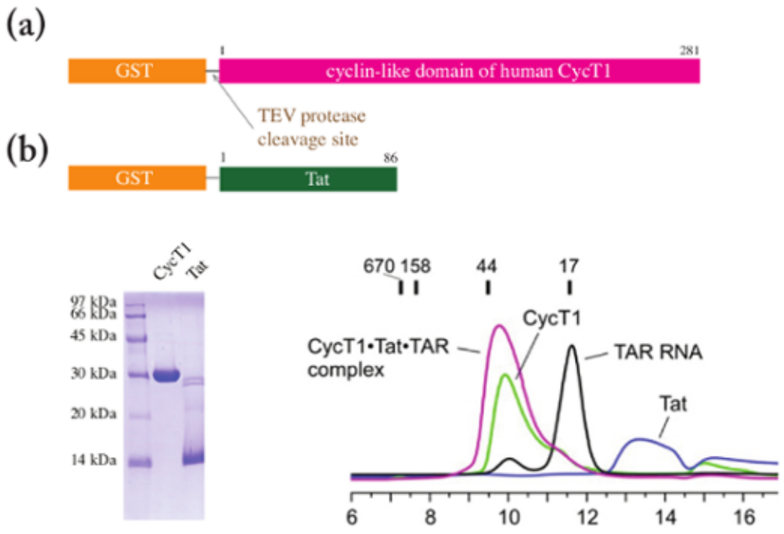


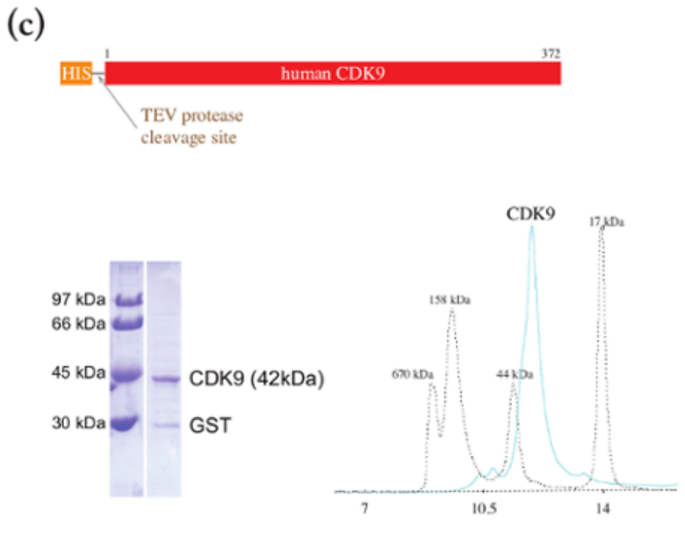
K_{off} calculated via Surface Plasmon resonance experiments for the mutants (a) I_{Tat}^{R5K} (blue) and (b) I_{Tat}^{U25U2S} (green) is less than that for the wild type (red), thus validating our structure of the proposed $I_{Tat.}$[7]

II. For the next phase of the project, I will be working towards obtaining a comprehensive structure of the whole HIV-1 TAC via X-ray crystallography[8-9].



To reconstitute a stable and homogeneous HIV-1 TAC in solution, we purified (a) human CycT1 and (b) HIV-1 Tat from bacterial expression, (c) human CDK9 from Sf9 cells using the baculovirus expression system and obtained chemically synthesised HIV-1 TAR.





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