# ASPECTS OF 7SK SNRNA IN HIV1 DRUG DISCOVERY

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**ABSTRACT.**

RNA plays crucial roles in the pathogenesis of many diseases and has gained a lot of interest as atool for functional genomics. It is equally important as a promising therapeutic approachfor the treatment of various diseases, e.g. HIV-1 infection, diabetes, etc. In the present study, we focused on the 7SK small nuclear RNA (snRNA), which is abundant with 331-nucleotide. It has one of the important functions as a transcriptional regulator during the elongation phase of HIV-1 virus. Immunodeficiency virus (HIV) exploits host's cellular proteins during its replicative cycle and latent infection. The positive transcription elongation factor b (P-TEFb) is a key cellular transcription factor critical for these viral processes.7SK RNA binds to HEXIM1regulatory domain and promotes the binding of the HEXIM C-terminal domain to cyclic T1/T2 of. P-TEFb shows little CTD kinase activity during its sequestering with 7SK and HEXIM1; it indicates that 7SK snRNA, in collaboration with HEXIM1, functions as an inhibitory factor of P-TEFb. Successive viral replication of requires recruitment P-TEFb by HIV-1 TAT protein for the completion of the viral RNA transcription process. Thus, one of the burning hypotheses is 7SK snRNA and HIV-1 protein Tat interaction. The study reports the evidence of strong binding interaction of 7SK snRNA and HIV-1 protein Tat with promising data of 2D NMR spectroscopic studies and ITC (isothermal calorimetric analysis) and future direction of drug designing.

**BACKGROUND MATERIALS AND OTHER FINDINGS.**

HIV-1 transcriptional elongation is regulated by 7SK small nuclear RNA which forms a multi-subunit complexes (7SK RNA) with hexim-1 and PTEF-b (positive transcription elongation factor b) and thus inhibit kinase activity of PTEF-b. PTEF-b is essentially required by RNA polymerase II (pol II) to maintain its processevity for the generation of full-length mRNA transcripts for the HIV-1 life cycle. It is postulated that Tat mimics hexim and interacts with loop 1 of 7SK snRNA (7SK-SL1) during recruitment of PTEF-b.

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We report the solution structure of the 7SK-SL1-Tat complex, which demonstrated a direct interaction of Tat with 7SK-SL1 with a high affinity with a nano-molar range (1 nM). It supports that Tat may compete with hexim (HIV-1 protein) for same binding site of 7SK-SL1. Tat berthed specifically in two unique arginine sandwich motifs in 7SK-SL1. Our findings suggest direct interaction of Tat to 7SK-SL1 is obligatory for the recruitment of PTEF-b for the comprehensive HIV-1 replication.

HIV-1 transcription is highly regulated by 7SK snRNA which facilitating the inhibition of the P-TEFb (a general positive transcription elongation factor b) by pausing the elongation stage of pol II and averts it from generation of a full-length mRNA [1,2] which leads to the nascent non-infectious virus particle. P-TEFb is required at the elongation phase for the phosphorylation of the largest subunit of the C terminal domain of pol II and triumph of mRNA pre-mRNA processing [3].

At present, P-TEFb is also considered a global transcriptional elongation factor important for the expression of most RNA pol II-regulated genes [1]. A model for TAR RNA-Tat-PTEF b Ternary Complex is presented in Figure-1. Figure-1, showed that Tat (HIV-1) protein recruits cellular PTEF-b from 7SK RNA and form a ternary complex with HIV-1 TAR (RNA). TAR-Tat-PTEF-b ternary complex mediates HIV-1 transcriptional elongation through phosphorylation of serin (amino acid) of C terminal domain of RNA polymerase II (Pol II).

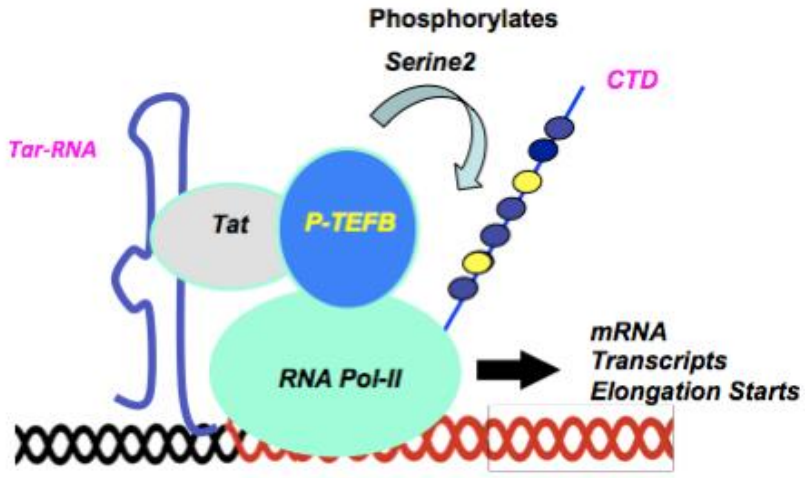


**Figure 1:** Model of TAR-Tat-PTEF b Ternary Complex

7SK snRNA is highly structured, abundant and noncoding RNA which plays a pivotal role in transcriptional regulation, it is highly conserved in vertebrates and in human it and contains 332 nucleotides with 4 stem loop. Stem loop 1 and 4 are manifested for their importance in the transcriptional regulation. Secondary structure of 7SK stem loop 1 is presented in **Figure 2,** a total three bulges e.g. AU, CU and CU are marked as Site S1, Site S2 and Site S3 respectively. Probable UAU, CGC and CGC triple base platforms are presented with doted lines.

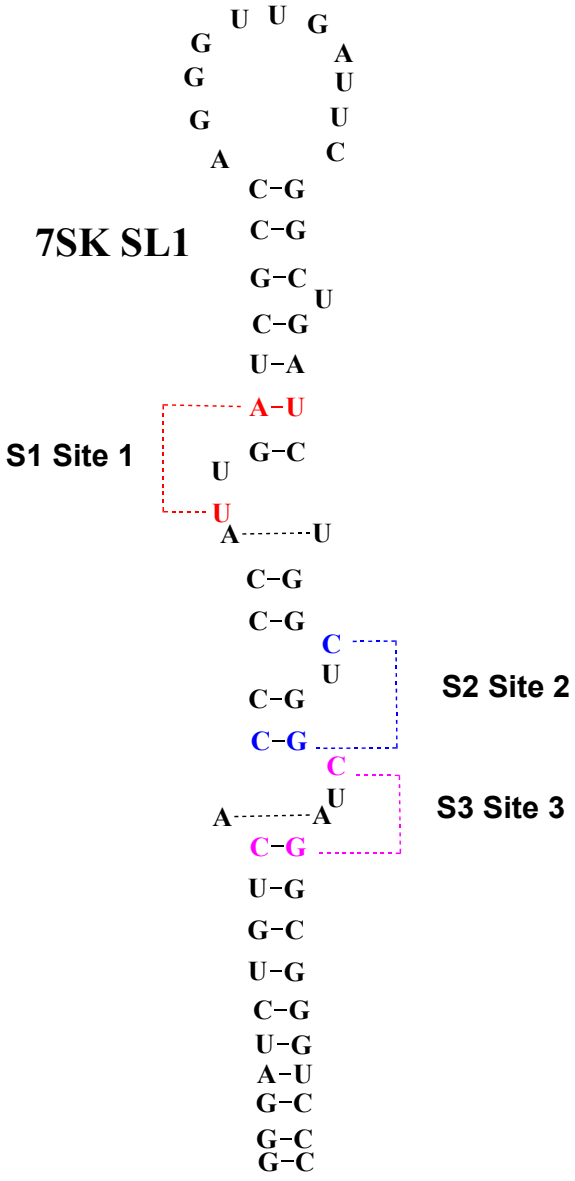


**Figure 2:** Secondary Structure of 7SK SL1

7SK snRNA provides a firm scaffold to P-TEFb to assemble its RNA polymerase II regulatory machinery, it leads to a reversible association of PTEF-b with 7SK snRNP along with the a

HEXIM dimer to a region near 5' end of 7SK snRNA. 7SK snRNA stabilizes P-TEFb and HEXIM interaction [2] and stipulates a kinase inactive state of P-TEFb [4]. In this inactive state HEXIM is bounded with Cdk9 /Cyclin T1 heterodimer domain of P-TEFb in 7SK snRNA. The mechanism anticipated as same as ternary complex produced by TAR RNA-Tat and P-TEFb where Zn finger 2 domain of the Tat activation domain binds with Cyclin T1 of P-TEFb and RNA binding region of Tat is arginine rich and is responsible to bind with TAR RNA. We anticipated similar Tat-7SK-SL1 structure where arginine rich region of Tat interacted with 7SK-SL1 as in Tat-TAR complex.

We here report Tat-7SK-SL1 complex as well as free 7SK-SL1 solution structure, the imino spectra of free 7SK-SL1 is presented in **Figure 3.** Imino spectra showed imino/imino region of a noesy spectra showing that the base pairs of the 7SK snRNA are formed correctly and the spectra is appropriate for solution of the free 7SK SL1 RNA structure and complex TAT-7SK SL1 RNA complex formation. Dashed line represent NH/NH sequential assignment.

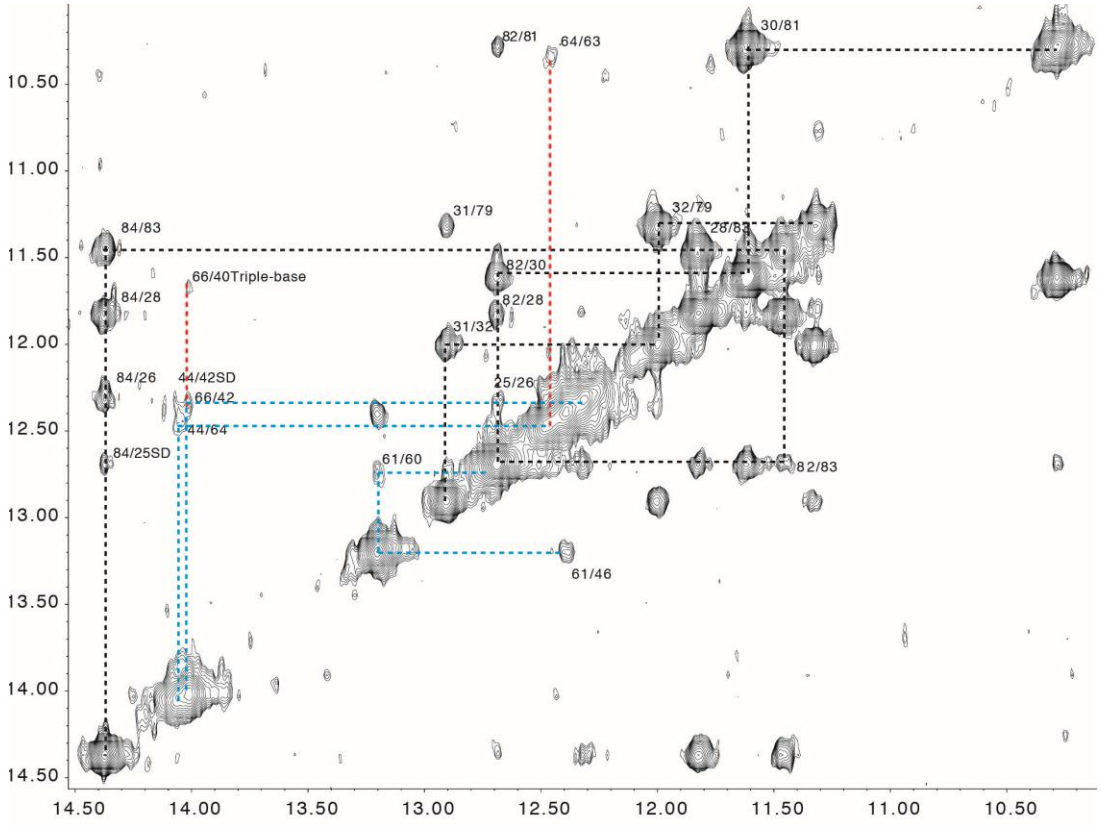


**Figure 3:** Imino Spectra of Free 7SK-SL1

The Tat binds with the 7SK-SL1 in a highly specific order and this binding is attributed by specific sequence of Tat RNA as free arginamide failed to show any kind of binding to the 7SK-SL1. ITC (Isothermal Titration Calorimetric) assay showed also a very high binding interaction with Tat peptide and 7SK snRNA, presented in **Figure 4.**

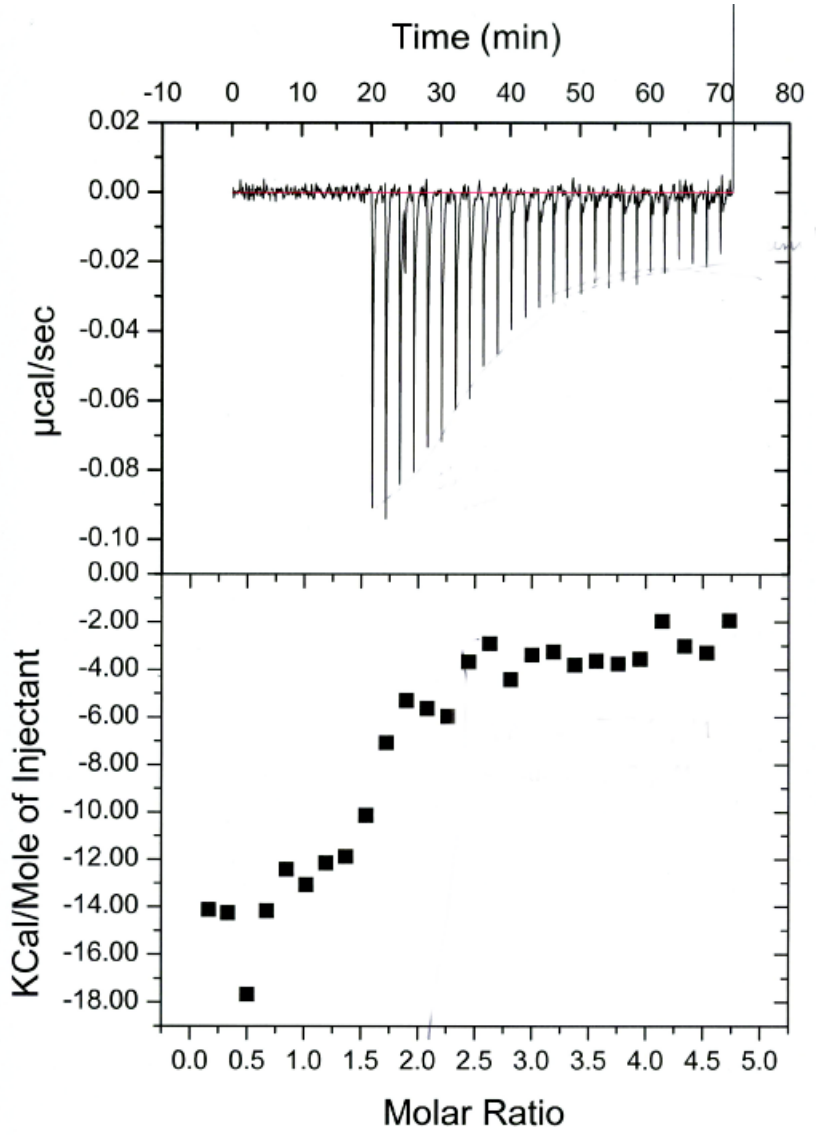


**Figure 4:** ITC Experiment

Isothermal Titration Calorimetric (ITC) assays were performed on 7SK-SL1 with TAT peptide, indicates a high binding affinity of TAT peptide to7SK-SL1 (Kd = ~50 nM, n=~1.36). It clearly

depicted that tat peptide is directly interact with 7SK SL1 and this is very important for the HIV pathogenesis.

The Tat-7SK-SL1 complex showed two different arginine sandwich motifs in 7SK-SL1 where two different arginine residues of Tat intruded with a high specificity affinity (nano molar range).

On the other hand free 7SK-SL1 structure showed presence of only one preformed arginine sandwich motif (CGC triple base) one of the arginine (156) of Tat, docked in this arginine sandwich pocket by succeeding a lock and key mechanism, which is very rare in bimolecular interaction till to date. This CGC preformed triple base motif is analogous to our previously reported arginine sandwich motif in 7SK-SL4.

Bulge nucleotides (U40-U41) are found stacked into the helix of free 7SK-SL1 structures. Comparing the free and complex structure it is clearly depicted that UU (40-41) un-stacked from the helix upon recognition of arginine residue (152) of Tat during complex formation and lead to a dynamic conformational change, which creates an arginine sandwich platform with UAU base triple to provide a firm attachment with the Tat-7SK-SL1.

7SK-SL1 bulge nucleotides experience a dynamic conformational reshuffling and briefly access arginine upon recognition, such dynamics is a striking phenomenon to understand Tat-7SK-SL1 interaction. This phenomenon reflects the conformation of TAR RNA where upon arginine binding, the bulge changes conformation, and essential nucleotides for binding form a base-triple interaction that stabilizes arginine hydrogen bonding [(5)].

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