Structure of HIV-1 TAR in Complex with a Lab-Evolved Protein Provides Insight into RNA Recognition and Synthesis of a Constrained Peptide that Impairs Transcription

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Natural and lab-evolved proteins often recognize their RNA partners with exquisite affinity. Structural analysis of such complexes can offer valuable insight into sequence-selective recognition that can be exploited to alter biological function. Here we describe the structure of a lab-evolved RNA recognition motif (RRM) bound to the HIV-1 *trans*-activation response (TAR) RNA element at 1.80 Å-resolution, which represents the first high-resolution crystal structure of intact TAR to our knowledge. Importantly, the complex reveals that a trio of arginines within the evolved β2-β3 loop penetrates deeply into the major groove to read conserved guanines while simultaneously forming cation-π and salt-bridge contacts. The observation that the evolved RRM engages TAR within a double-stranded stem is atypical compared to most RRMs. Mutagenesis, thermodynamic analysis and molecular dynamics validate the atypical binding mode and quantify molecular contributions that support the exceptionally tight binding of the TAR-protein complex ($K_{D,App}$ of 2.5 ± 0.1 nM). These findings led to the hypothesis that the β2-β3 loop can function as a standalone TAR-recognition module. Indeed, a "stapled" β2-β3-loop peptide still binds TAR ($K_{D,App}$ of 1.2 ± 0.5 μM) and a shorter variant significantly weakens TAR-dependent transcription in HeLa nuclear extracts. Our results provide a detailed understanding of TAR molecular recognition and suggest a new role for lab-evolved RRMs as a platform to target other disease-relevant RNAs. Targeting the TAR RNA element has implications for functional cure efforts aimed at locking HIV-1 into a latent state.