

Structural delineation of stem-loop RNA binding by human TAF15 protein

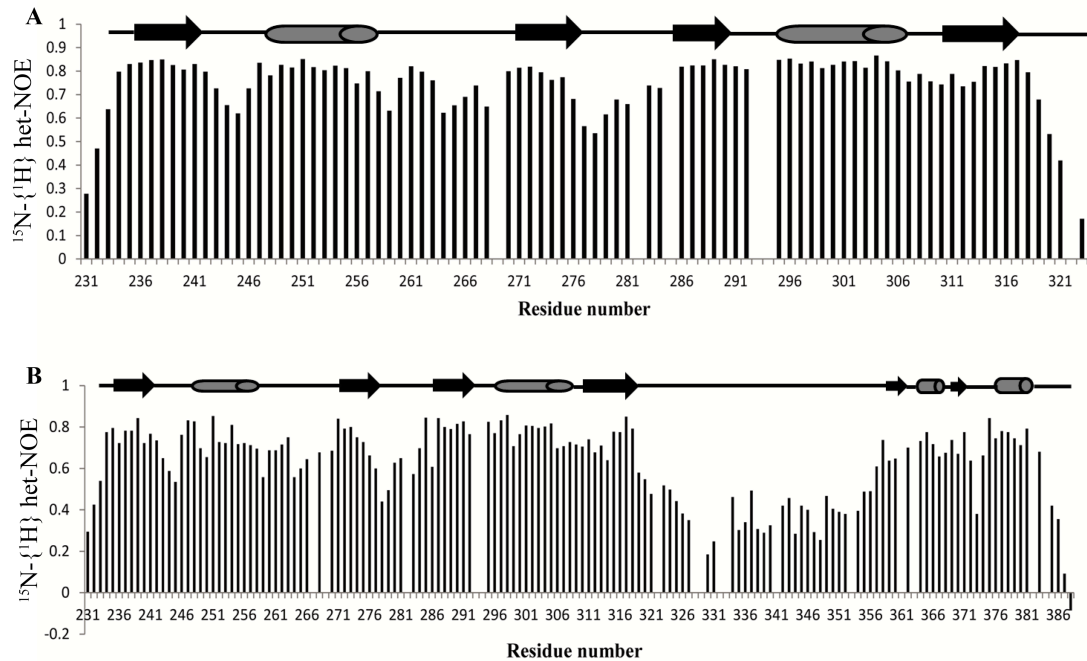
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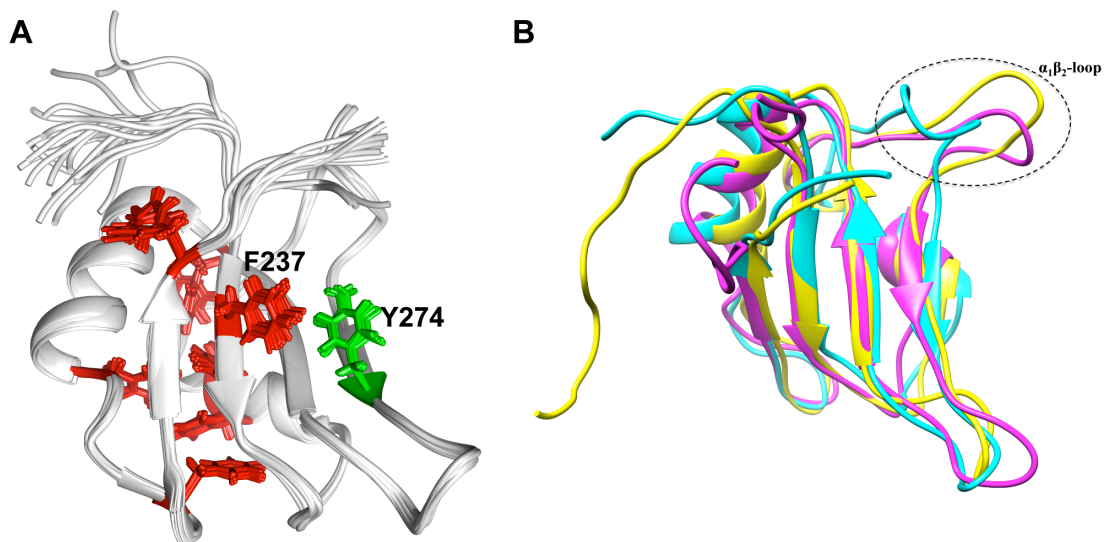
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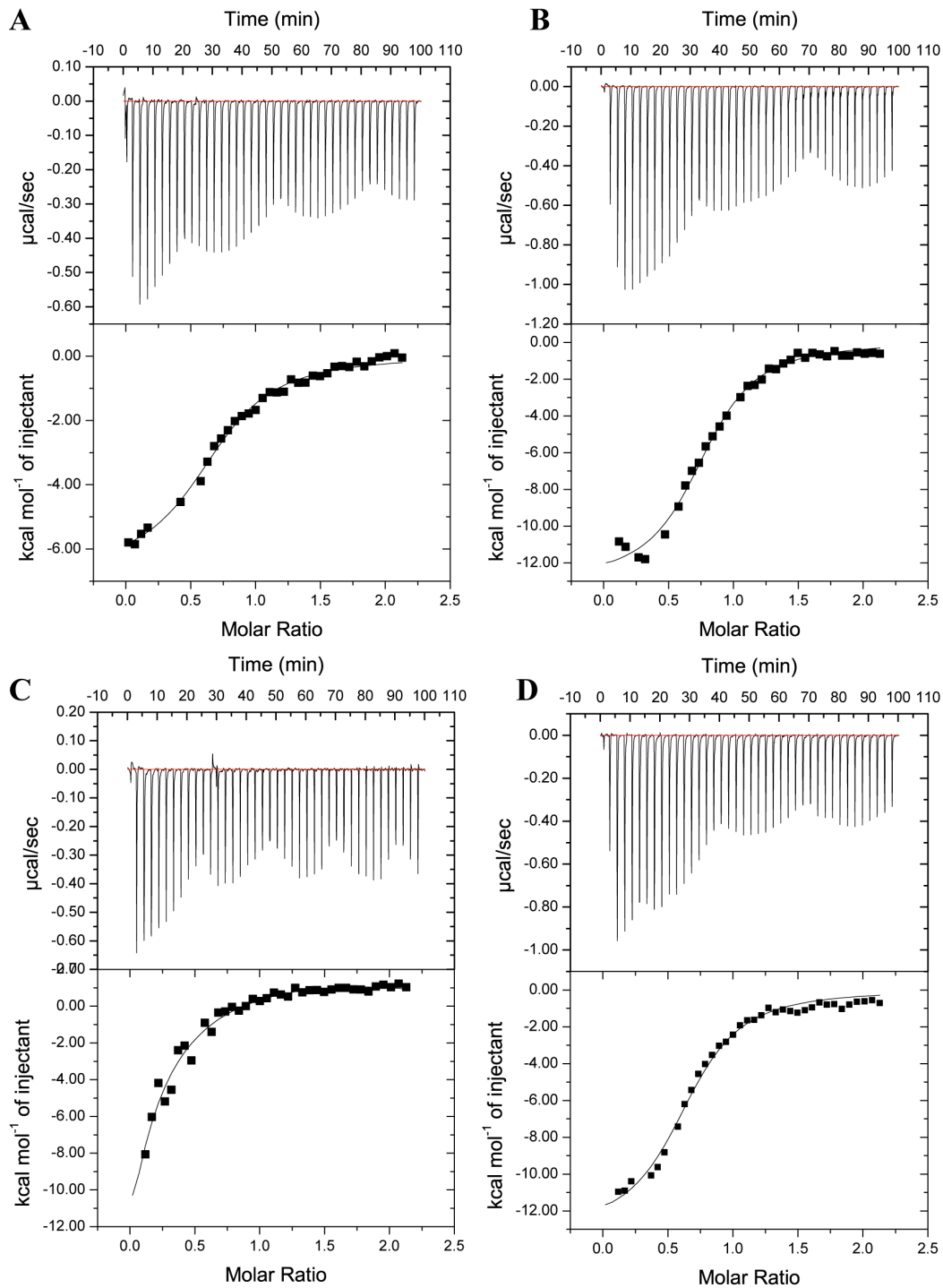
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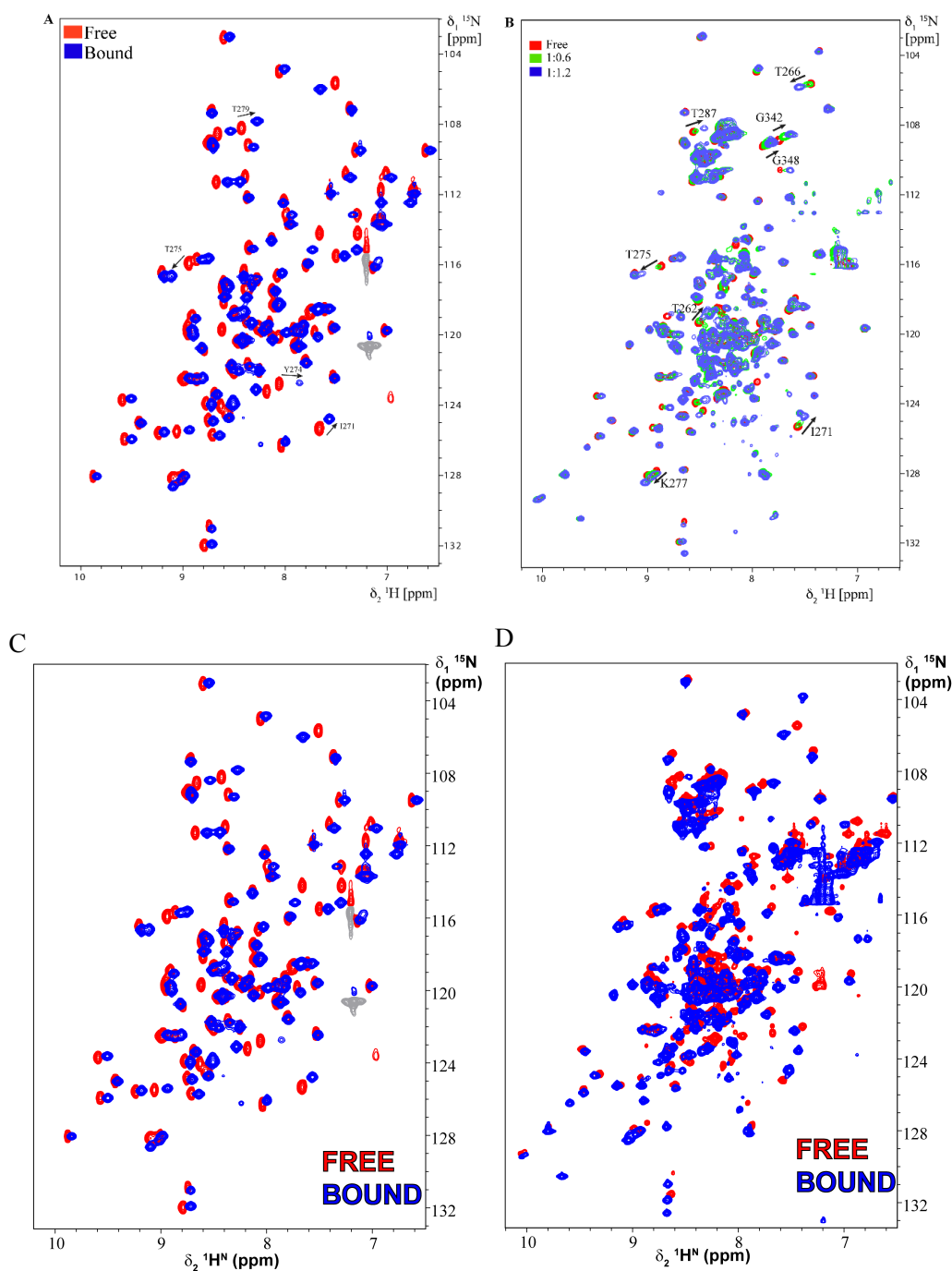
Supplementary figure S1. $^{15}\text{N}\{-^1\text{H}\}$ heteronuclear NOEs of TAF15 showing (A) highly structured RRM punctuated by few dynamic regions and (B) dynamic RGG motif linker between the structured RRM and RanBP2 type ZnF domains. The secondary structural elements are indicated on top (thick arrows indicate β -strands, cylinders indicate the α -helices and the loops and the disordered regions are indicated by thick line segments).



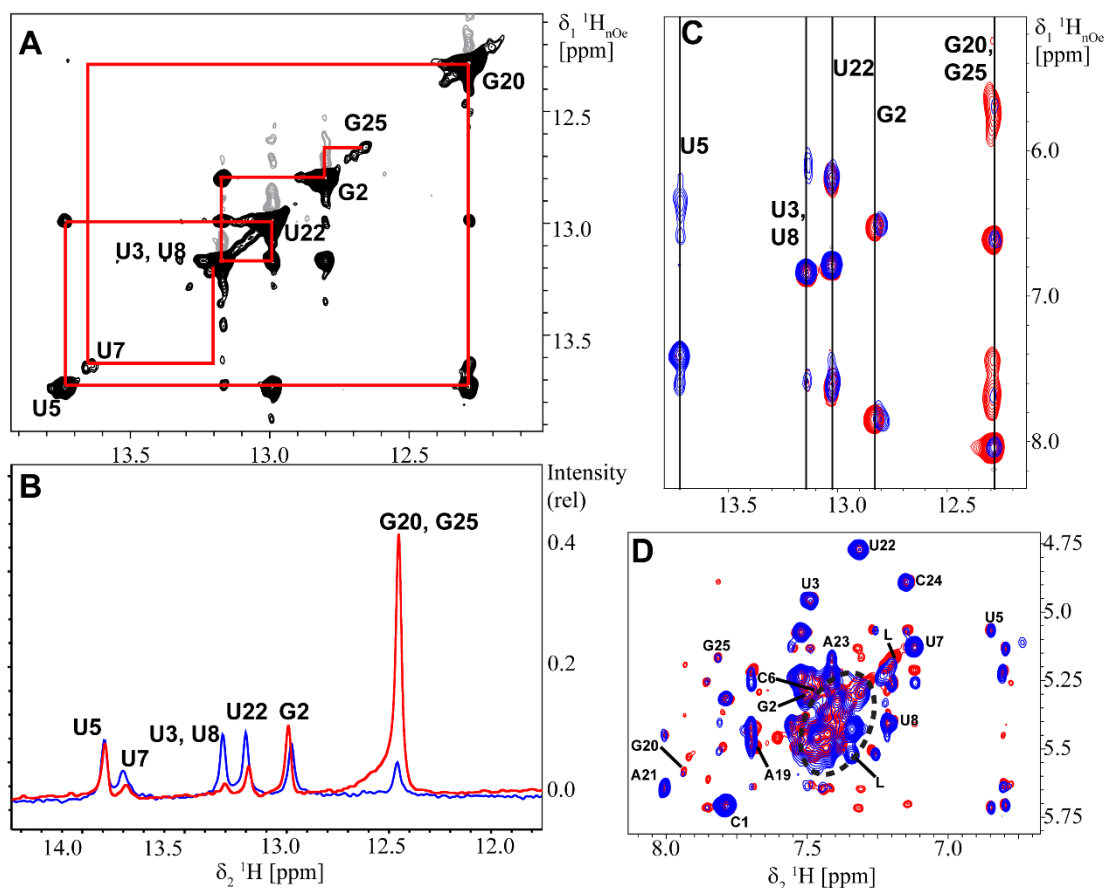
Supplementary figure S2. (A) Distribution of aromatic residues on TAF15 showing phenylalanines (red) and a single tyrosine (green). F237 and Y274 are the only surface-exposed aromatic residues on the β -sheet. (B) Structural alignment of TAF15-RRM (cyan) with FUS-RRM (magenta), PDB ID 2LCW and EWS-RRM (yellow), PDB ID 2CPE.



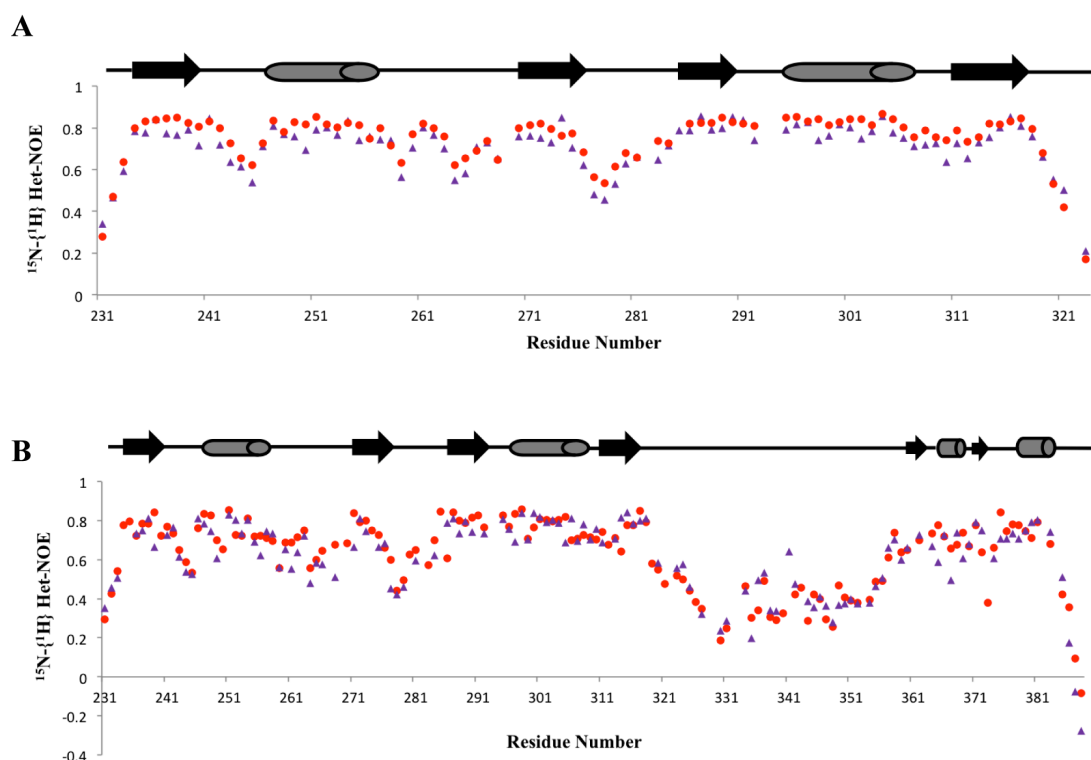
Supplementary figure S3. Binding isotherms and non-linear curve fitting for the titration of 1 mM *SON* stem-loop RNA in 100 μM solutions of (A) TAF15-RRM (B) TAF15-RRM-RanBP2 (C) FUS-RRM and (D) FUS-RRM-RanBP2 protein constructs.



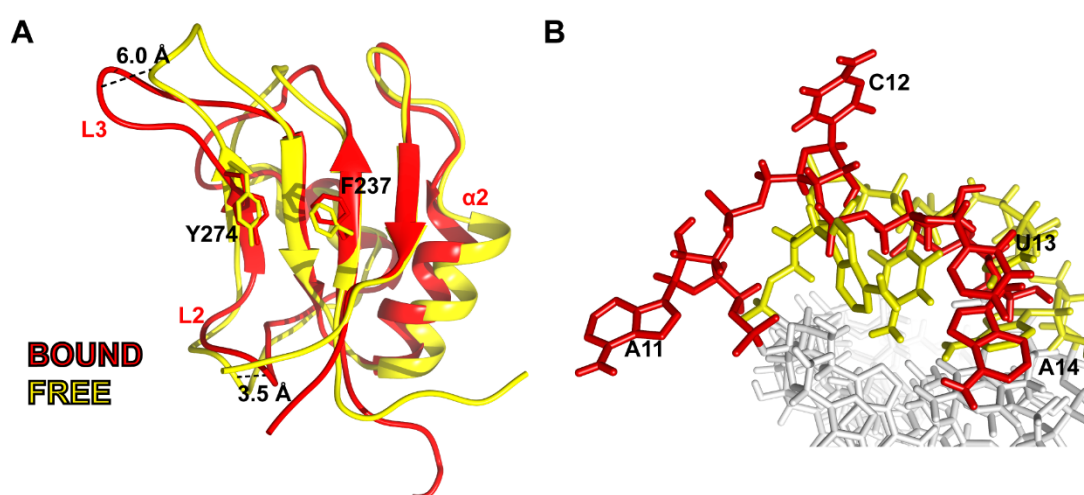
Supplementary figure S4: (A) Overlay of 2D [$^{15}\text{N}, ^1\text{H}$] HSQC spectra of unbound and 1:1.2 bound TAF15-RRM with *SON* stem-loop RNA measured at 298 K. (B) 2D [$^{15}\text{N}, ^1\text{H}$] TROSY spectra of TAF15-RRM-RanBP2 showing the progression of peaks upon titration with increasing molar stoichiometry (1:0.6 and 1:1.2) of *SON* stem-loop RNA measured at 298 K. CSPs for the same are shown in Fig. 2A (RRM) and 2C (RRM-RanBP2). (C) Overlay of 2D [$^{15}\text{N}, ^1\text{H}$] HSQC spectra of unbound and 1:1.2 bound TAF15-RRM with *SON* stem-loop RNA measured at 298 K. (D) Overlay of 2D [$^{15}\text{N}, ^1\text{H}$] HSQC spectra of unbound and 1:1.2 bound TAF15-RRM-RanBP2 with *SON* stem-loop RNA measured at 298 K.



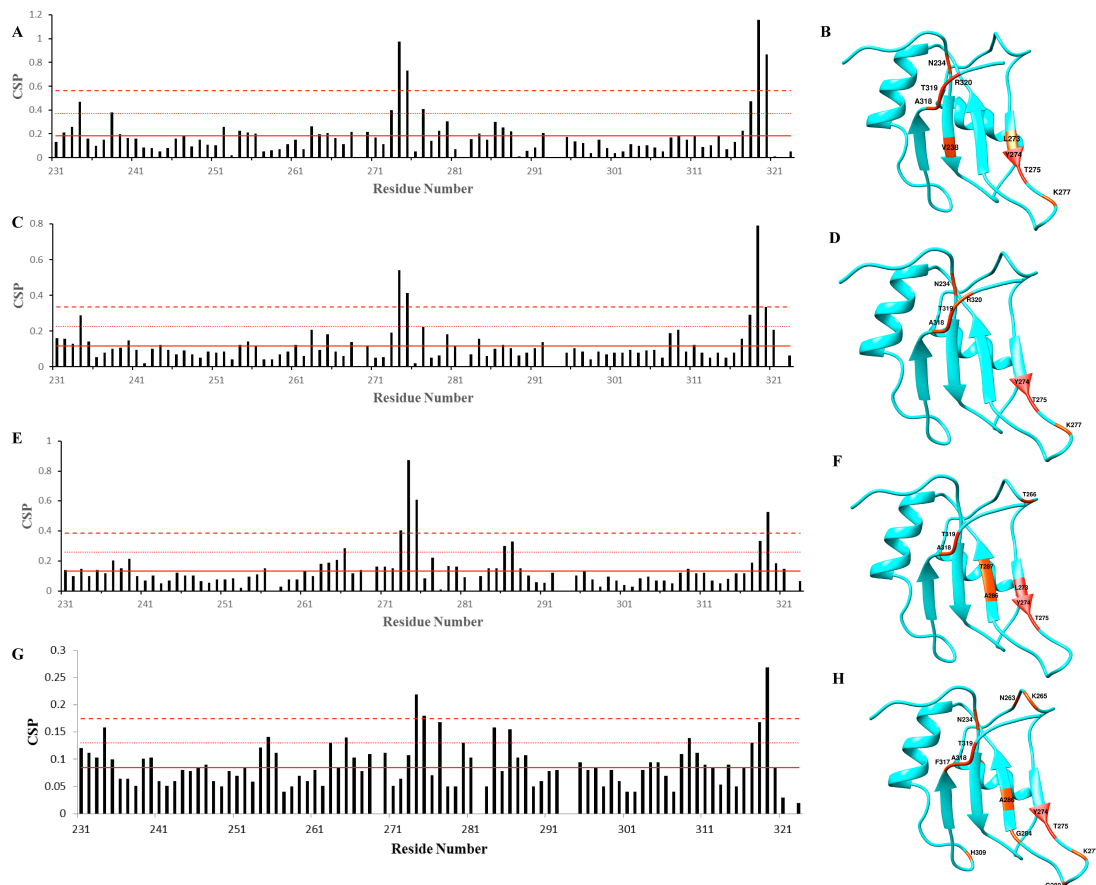
Supplementary figure S5. (A) 2D [$^1\text{H}, ^1\text{H}$]-NOESY spectrum ($t_{\text{mix}} = 350$ ms) of *SON* stem-loop RNA in H_2O at 273 K depicting a sequential ‘walk’ across uracil H3 and guanine H1 resonances of the RNA stem. (B) Overlay of 1D ^1H spectra for free (red) and TAF15-RRM-bound *SON* stem-loop RNA (blue) in H_2O at 298 K with imino resonances labeled. (C) Overlay of 2D [$^1\text{H}, ^1\text{H}$] NOESY spectra ($t_{\text{mix}} = 350$ ms) in H_2O at 298 K of free (red) *SON* RNA and complex of $U\text{-}^{13}\text{C}, ^{15}\text{N}$ TAF15-RRM with unlabeled RNA (blue) showing imino cross-peaks with base $\text{H}^{\text{Aro}}/\text{H}^{\text{Amino}}$ and sugar protons. (D) Overlay of 2D [$^1\text{H}, ^1\text{H}$] NOESY spectrum of free (red) *SON* RNA and 2D ^{13}C - $[\omega_1, \omega_2]$ -filtered [$^1\text{H}, ^1\text{H}$] NOESY ($t_{\text{mix}}=350$ ms) in D_2O at 298 K of $U\text{-}^{13}\text{C}, ^{15}\text{N}$ TAF15-RRM with unlabeled RNA (blue) showing changes in $\text{H}^8\text{-H}^{1'}$ (A/G) and $\text{H}^6\text{-H}^5$ (C/U) cross-peaks. Peaks arising from RNA loop are denoted by the letter ‘L’.



Supplementary figure S6. Comparison of $^{15}\text{N}\{-^1\text{H}\}$ heteronuclear NOEs of **(A)** TAF15-RRM and **(B)** TAF15-RRM-RanBP2 upon binding to *SON* stem-loop RNA. Red filled circles indicate the free protein constructs while blue filled triangles indicate their bound counterparts.



Supplementary figure S7: Structural rearrangements in TAF15-RRM and *SON* stem-loop RNA on binding. While the RRM shows movements of α_2 helix, loops L2 and L3 with a rearrangement of Y274 and F237 **(A)**, the RNA shows the adoption of a more extended state **(B)**.



Supplementary figure S8: Chemical shift perturbation histograms of TAF15-RRM upon binding to 1.2 molar equivalents of (A) 5' GGUGUG 3', (C) 5' CCUCUG 3', (E) 5' GGCUGCG 3' and (G) 5' GCUGCUG 3' RNAs. The solid, dotted and dashed horizontal red lines indicate averaged CSP, one and two standard deviations from the averaged CSP respectively. The panels, B, D, F and H show CSPs mapped on to the solution structure of TAF15-RRM of corresponding histograms A, C, E and G. Residues exhibiting CSPs are colored in a gradation of red to orange-red. The color scheme is; orange red and red for one and two standard deviations from the averaged CSP respectively.