

Supporting Information

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**RNA Dynamics by Design: Biasing Ensembles Towards the Ligand-Bound State\*\***

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## Supplemental Methods

**Sample preparation and resonance assignments.** Uniformly  $^{13}\text{C}/^{15}\text{N}$  labeled TAR<sup>GC</sup> was prepared by run-off *in-vitro* transcription using synthetic double-stranded DNA containing the T7 promoter and RNA sequence of interest (*Integrated DNA Technologies*). Elongated and non-elongated TAR<sup>GC</sup> constructs were purified by 15% (w/v), and 20% (w/v) denaturing polyacrylamide gel electrophoresis containing 8M urea and 1x TBE, respectively, followed by electroelution in 20 mM Tris pH 8 buffer and EtOH precipitation. The resultant RNA pellet was dissolved and exchanged into NMR buffer (15 mM sodium phosphate, 0.1 mM EDTA, and 25 mM NaCl at pH ~6.4) using a Centricon Ultracel YM-3 concentrator to a final concentration of ~0.5-1.0 mM (Millipore Corp.). All NMR samples contained 10% D<sub>2</sub>O. All experiments were conducted in NMR buffer at 298 K on an Avance Bruker 600 MHz NMR spectrometer equipped with a triple-resonance 5 mm cryogenic probe. The TAR<sup>GC</sup> NMR spectra were assigned using conventional NMR methods employing exchangeable 3D  $^1\text{H}$ - $^{15}\text{N}$  NOESY-HSQC and non-exchangeable 2D  $^1\text{H}$ - $^{13}\text{C}$  NOESY-HSQC, 2D HCN, 2D IP-COSY experiments. Argininamide (ARG, Sigma Aldrich) and Neomycin B (NEOB, MP Biomedicals) titrations were performed by sequentially adding ~2uL of concentrated ARG and NEOB samples to 0.1 mM TAR<sup>GC</sup> and TAR-ARG, up to final ligand concentrations of 2.5 mM and 0.8 mM, respectively. 2D HSQC spectra were collected at each titration point.

**RDC measurements and order tensor analysis.** RDCs were measured in TAR<sup>GC</sup> and EI-TAR<sup>GC</sup> as previously described<sup>[1,2]</sup> using ~7mg/ml and ~23 mg/ml of Pf1 phage order medium<sup>3</sup>, respectively (Table S1). The RDCs measured in the two helices of TAR<sup>GC</sup> were subjected to an order tensor analysis (Table S2).<sup>[4-6]</sup> RDCs measured in E-AU-TAR<sup>GC</sup> and E-GC-TAR<sup>GC</sup> were normalized ( $L=0.66$ ) as previously described<sup>[2]</sup> to take into account differences in the degree of alignment arising from use of a slightly different Pf1 phage concentration. The normalized RDCs measured in each helix were combined in the order tensor analysis. Due to deviations from Watson-Crick geometry, the A22-U40 and terminal G17-C45 base-pairs were excluded from the analysis. The program AFORM-RDC<sup>[6]</sup> was used to estimate errors in the order tensor arising from “structural noise” and RDC measurement uncertainty. A-form helices were constructed using Insight II (Molecular Simulations, Inc), noting that the propeller twist angles had to be corrected from +15° to the standard A-form value of -15°.<sup>[6]</sup>

**Measurements of resonance intensities and  $K_d$ s.** Resonance intensities were measured and normalized to a baseline value of 0.1 as described previously for EI-TAR and EI-TAR-ARG<sup>7</sup>. TAR-ARG Dissociation constants were calculated from the change in weighted average chemical shift for each titration point using the equation<sup>[8]</sup>,

$$\delta_{obs} = \delta_{free} + (\Delta\delta_T) \times \left( \left( [Arg]_T + [RNA]_T + K_d \right) - \frac{\sqrt{\left( [ARG]_T + [RNA]_T + K_d^2 \right) + \left( 4[ARG]_T [RNA] \right)}}{2[RNA]_T} \right),$$

in which  $[ARG]_T$  is the total ARG concentration,  $[RNA]_T$  is the RNA concentration based on UV absorbance at 260 nm,  $\Delta\delta_T$  is the difference in chemical shifts between the free and ligand-associated states (in ppm),  $\delta_{obs}$  is the observed chemical shift (in ppm), and  $\delta_{free}$  is the chemical shift in the free state (in ppm). The data was fit using the Origin software (OriginLab Corporation) in which  $\Delta\delta_T$  and  $K_d$  were allowed to float.

**SUPPLEMENTARY TABLES**

**Table S1.** RDCs measured in EI-TAR<sup>GC</sup>, TAR<sup>GC</sup>-ARG, and TAR<sup>GC</sup>.

<b>Residue</b>	<b>Bond</b>	<b>EI-TAR<sup>GC</sup></b>	<b>TAR<sup>GC</sup>-ARG</b>	<b>TAR<sup>GC</sup></b>
G17	(C8H8)	18.0	NA	-1.3
G17	(N1H1)	-14.6	-10.1	0.7
G17	(C1'H1')	NA	NA	NA
G18	(C8H8)	NA	0.0	NA
G18	(C1'H1')	NA	NA	NA
G18	(N1H1)	-18.5	-3.9	NA
C19	(C5H5)	NA	NA	-2.4
C19	(C6H6)	11.3	NA	NA
A20	(C2H2)	25.2	8.4	2.3
A20	(C8H8)	21.0	30.5	11.7
A20	(C1'H1')	NA	NA	-19.2
G21	(C8H8)	24.5	44.7	19.4
G21	(C1'H1')	NA	-48.0	-17.2
G21	(N1H1)	-20.2	-22.8	9.3
G22	(C2H2)	NA	NA	NA
G22	(C8H8)	23.8	NA	20.9
G22	(C1'H1')	2.2	NA	8.9
G22	(N1H1)	-16.5	-18.7	4.5
U23	(C5H5)	NA	37.7	-0.9
U23	(C6H6)	3.6	38.4	5.4
U23	(C1'H1')	-4.5	37.9	0.2
C24	(C5H5)	-3.8	-6.5	-3.7
C24	(C6H6)	0.9	6.4	1.9
C24	(C1'H1')	-5.2	-10.2	-2.9
U25	(C5H5)	-0.5	1.5	0.4
U25	(C6H6)	-1.8	-14.8	0.1
U25	(C1'H1')	-2.1	-7.3	-1.3
G26	(C8H8)	23.2	26.9	12.4
G26	(C1'H1')	NA	NA	-10.9
G26	(N1H1)	NA	NA	3.2
A27	(C2H2)	20.7	33.2	12.5
A27	(C8H8)	20.7	25.2	7.0
A27	(C1'H1')	NA	-8.0	-6.5
G28	(C8H8)	NA	25.6	8.1
G28	(C1'H1')	NA	-13.9	-12.0
G28	(N1H1)	NA	-18.3	6.4
C29	(C5H5)	NA	NA	6.1
C29	(C6H6)	12.6	37.4	17.7
C29	(C1'H1')	NA	NA	NA
U31	(C5H5)	18.0	31.3	14.3
U31	(C6H6)	19.9	31.2	16.9
U31	(C1'H1')	-13.7	-21.5	-8.8
U32	(C5H5)	NA	25.4	10.4
U32	(C6H6)	12.1	15.3	2.9
U32	(C1'H1')	19.2	25.2	9.0
C33	(C5H5)	6.0	-6.1	1.0
C33	(C6H6)	13.2	12.1	4.1
C33	(C1'H1')	1.0	-33.6	-8.5
G34	(C8H8)	22.6	24.1	7.7

G34	(C1'H1')	20.1	11.6	5.2
G34	(N1H1)	-24.9	NA	7.1
G36	(C8H8)	25.7	46.6	21.5
G36	(C1'H1')	-11.2	NA	NA
G36	(N1H1)	NA	-21.5	7.8
C37	(C5H5)	20.8	NA	20.7
C37	(C6H6)	14.0	NA	NA
C37	(C1'H1')	NA	NA	-8.0
U38	(C5H5)	23.2	NA	18.8
U38	(C6H6)	NA	23.7	7.1
U38	(C1'H1')	-13.2	-20.2	-9.3
U38	(N3H3)	-11.1	-11.4	4.3
C39	(C5H5)	NA	NA	13.0
C39	(C6H6)	14.6	NA	13.5
C39	(C1'H1')	NA	-38.5	NA
C40	C6H6	15.9	NA	19.2
C40	(C5H5)	NA	NA	9.6
C40	(C1'H1')	NA	NA	-13.1
C41	(C5H5)	22.6	NA	10.9
C41	(C6H6)	NA	NA	NA
U42	(C5H5)	22.4	37.7	16.7
U42	(C1'H1')	NA	-7.3	NA
U42	(N3H3)	-14.5	-17.5	6.4
G43	(C8H8)	15.1	26.9	14.5
G43	(C1'H1')	NA	NA	NA
G43	(N1H1)	NA	-7.2	-2.0
C44	(C6H6)	NA	NA	-2.7
C44	(C5H5)	NA	NA	18.5
C45	(C6H6)	NA	NA	-0.9
C45	(C5H5)	NA	17.8	6.7
C45	(C1'H1')	NA	-16.6	-6.7
G-21	(C8H8)	19.6	NA	NA
C+21	(C5H5)	21.8	NA	NA

**Table S2.** Statistics for order tensor analysis of RDCs measured in the helices of EI-TAR<sup>GC</sup> and TAR<sup>GC</sup>-ARG using idealized A-form helices as input coordinates. Shown are the number of RDCs ( $N$ ) used in the order tensor determination, the root-mean-square deviation (RMSD) and correlation coefficient ( $R$ ) between measured and back-predicted values, asymmetry parameter ( $\eta$ ), generalized degree of order ( $\vartheta$ ), internal generalized degree of order ( $\vartheta_{\text{int}}$ ), and inter-helical bend angle ( $\beta$ ). Errors in  $\vartheta$ ,  $\vartheta_{\text{int}}$ ,  $\eta$ , and  $\beta$  are obtained using the program AFORM-RDC and take into account both RDC measurement uncertainty and local structural noise in the idealized A-form helix.<sup>[6]</sup>

	Helix	$N$	RMSD (Hz)	$R$	$\eta$	$\vartheta(\times 10^{-3})$	$\vartheta_{\text{int}}$	$\beta^\circ$
E-TAR <sup>GC</sup>	I	19	4.1	0.98	0.19±0.08	1.04±0.04	1.02±0.1	11.9±6.5
	II	12	3.2	0.98	0.16±0.10	1.06±0.10		
TAR <sup>GC</sup> - ARG	I	13	1.9	0.99	0.36±0.21	1.89±0.21	0.88±0.14	17.7±6.0
	II	14	4	0.99	0.26±0.17	2.15±0.20		

SUPPLEMENTARY FIGURES

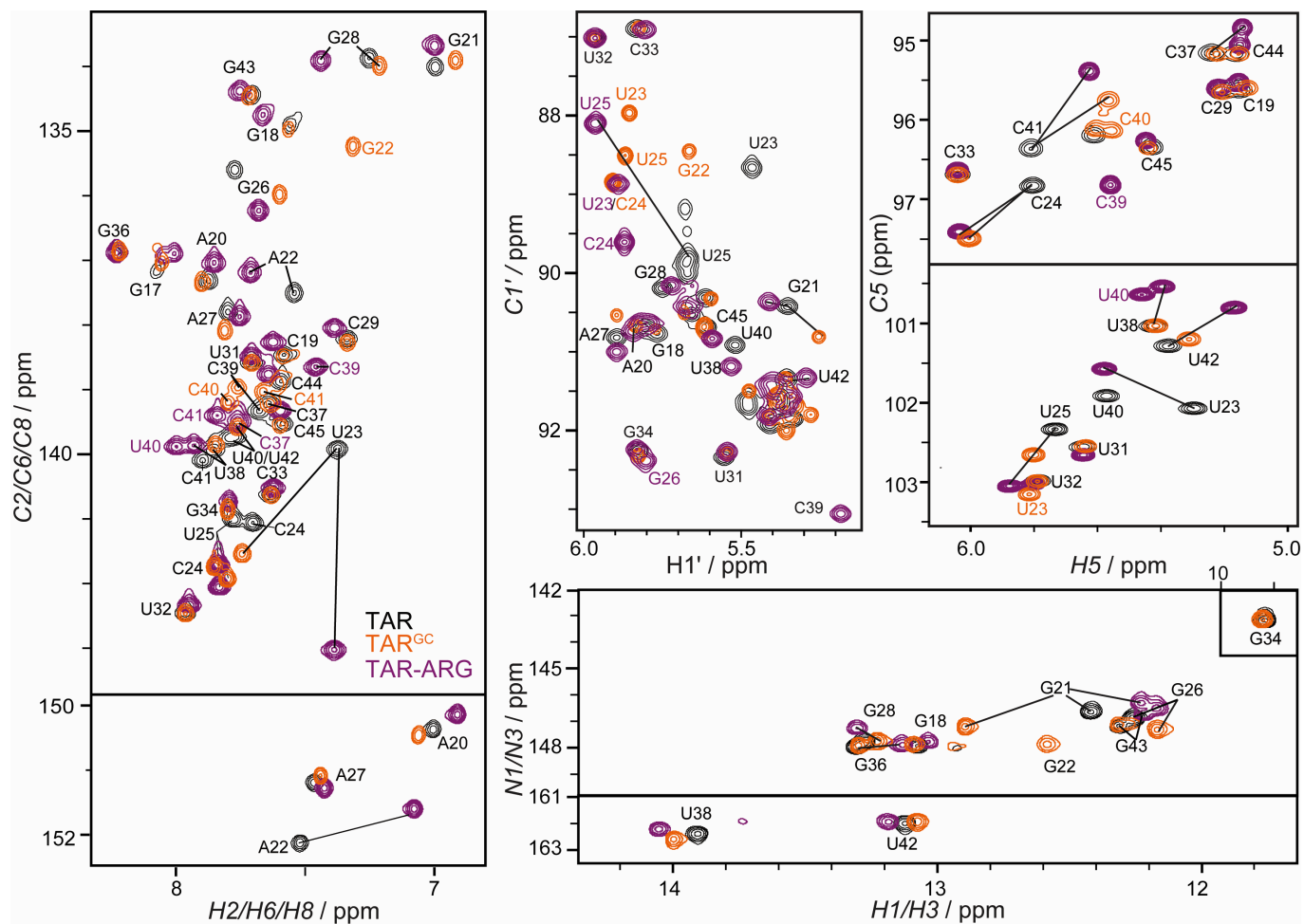
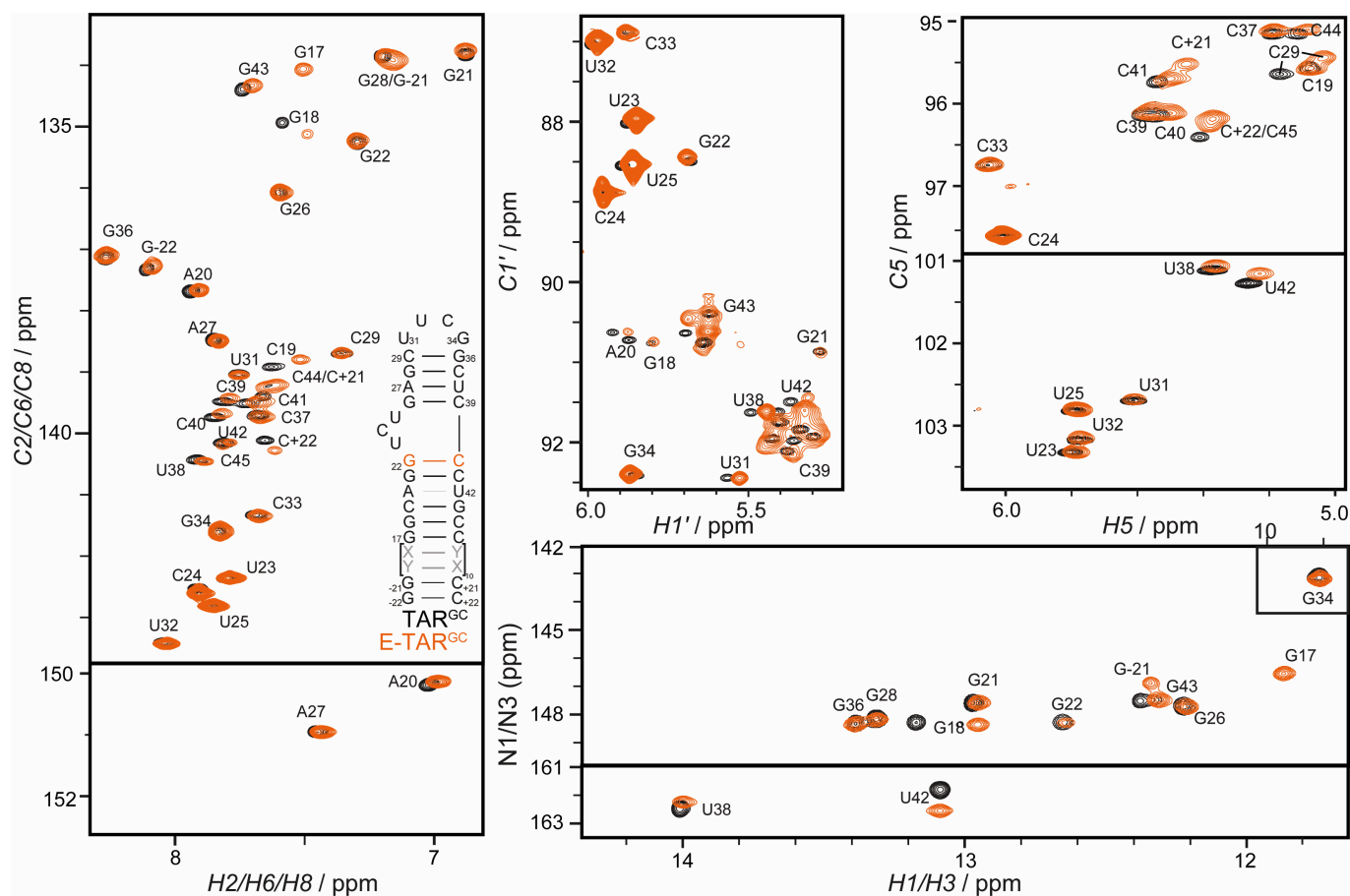
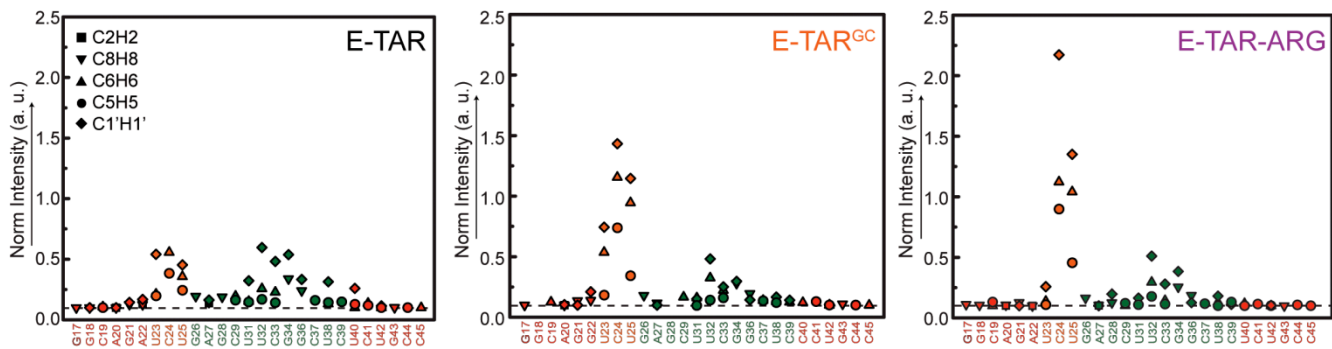


Figure S1. 2D CH and NH HSQC spectra of TAR (black), TAR<sup>GC</sup> (orange) and TAR-ARG (purple)





**Figure S2.** 2D CH and NH HSQC spectra of TAR<sup>GC</sup> (black) and EI-TAR<sup>GC</sup> (orange) demonstrating that elongation does not affect the structural and dynamical integrity of TAR<sup>GC</sup>. Significant perturbations are primarily localized at residues near the site of elongation.



**Figure S3.** Normalized resonance intensities measured in 2D HSQC spectra of EI-TAR, EI-TAR-ARG, and EI-TAR<sup>GC</sup>. Residues in helix I, helix II, and the bulge are colored-coded red, green, and orange respectively.



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