

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^{GC} 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^{GC} 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% D₂O. 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^{GC} NMR spectra were assigned using conventional NMR methods employing exchangeable 3D ^1H - ^{15}N NOESY-HSQC and non-exchangeable 2D ^1H - ^{13}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^{GC} 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^{GC} and EI-TAR^{GC} as previously described^[1,2] using ~7mg/ml and ~23 mg/ml of Pf1 phage order medium³, respectively (Table S1). The RDCs measured in the two helices of TAR^{GC} were subjected to an order tensor analysis (Table S2).^[4-6] RDCs measured in E-AU-TAR^{GC} and E-GC-TAR^{GC} were normalized ($L=0.66$) as previously described^[2] 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^[6] 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°.^[6]

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-ARG⁷. TAR-ARG Dissociation constants were calculated from the change in weighted average chemical shift for each titration point using the equation^[8],

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

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), δ_{obs} is the observed chemical shift (in ppm), and δ_{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^{GC}, TAR^{GC}-ARG, and TAR^{GC}.

| Residue | Bond | EI-TAR^{GC} | TAR^{GC}-ARG | TAR^{GC} |
|----------------|-------------|----------------------------|-----------------------------|-------------------------|
| 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^{GC} and TAR^{GC}-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 (η), generalized degree of order (ϑ), internal generalized degree of order (ϑ_{int}), and inter-helical bend angle (β). Errors in ϑ , ϑ_{int} , η , and β 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.^[6]

| | Helix | N | RMSD (Hz) | R | η | $\vartheta(\times 10^{-3})$ | ϑ_{int} | β° |
|------------------------|-------|-----|-----------|------|-----------|-----------------------------|--------------------------|---------------|
| E-TAR ^{GC} | 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 ^{GC} -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

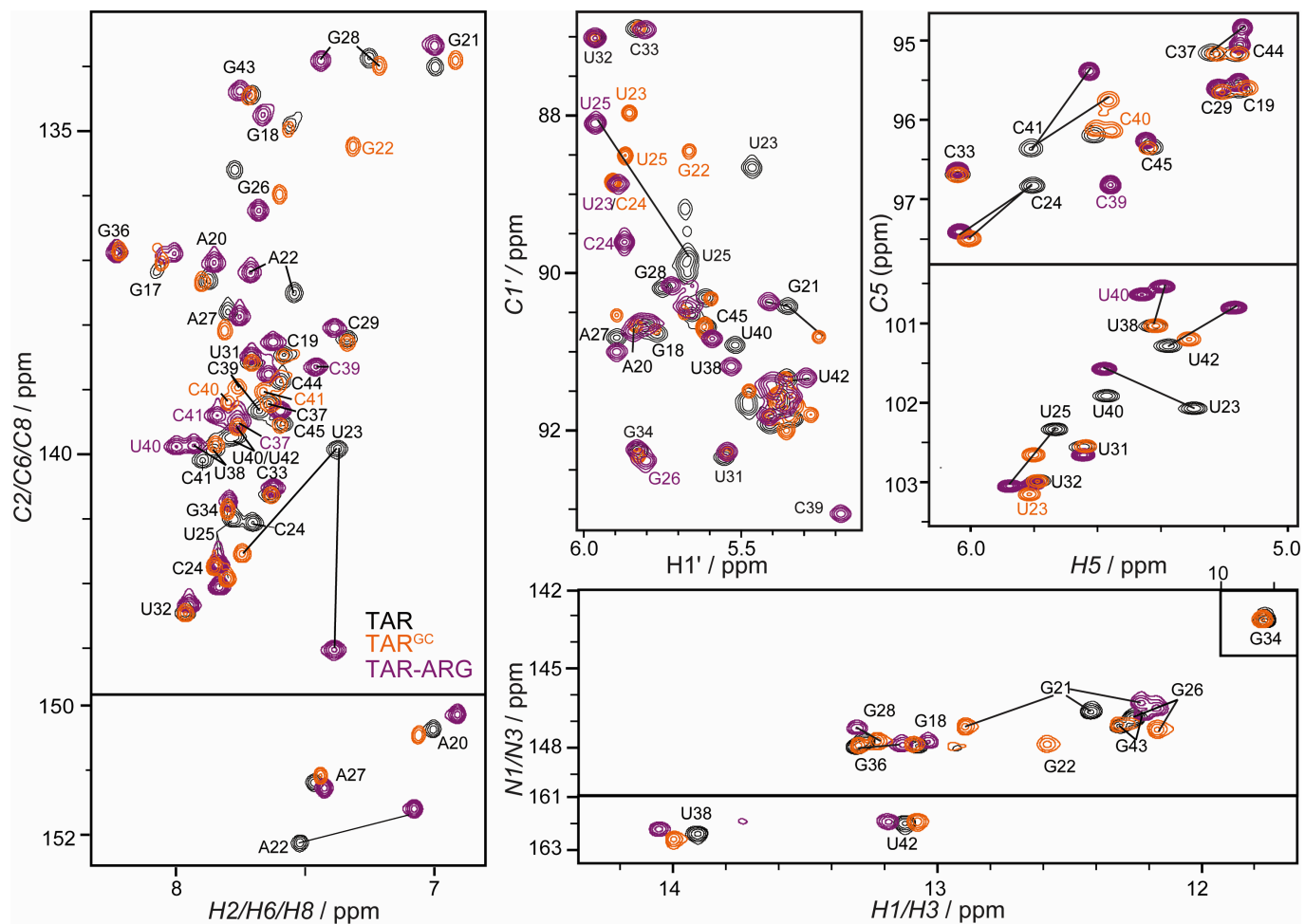


Figure S1. 2D CH and NH HSQC spectra of TAR (black), TAR^{GC} (orange) and TAR-ARG (purple)

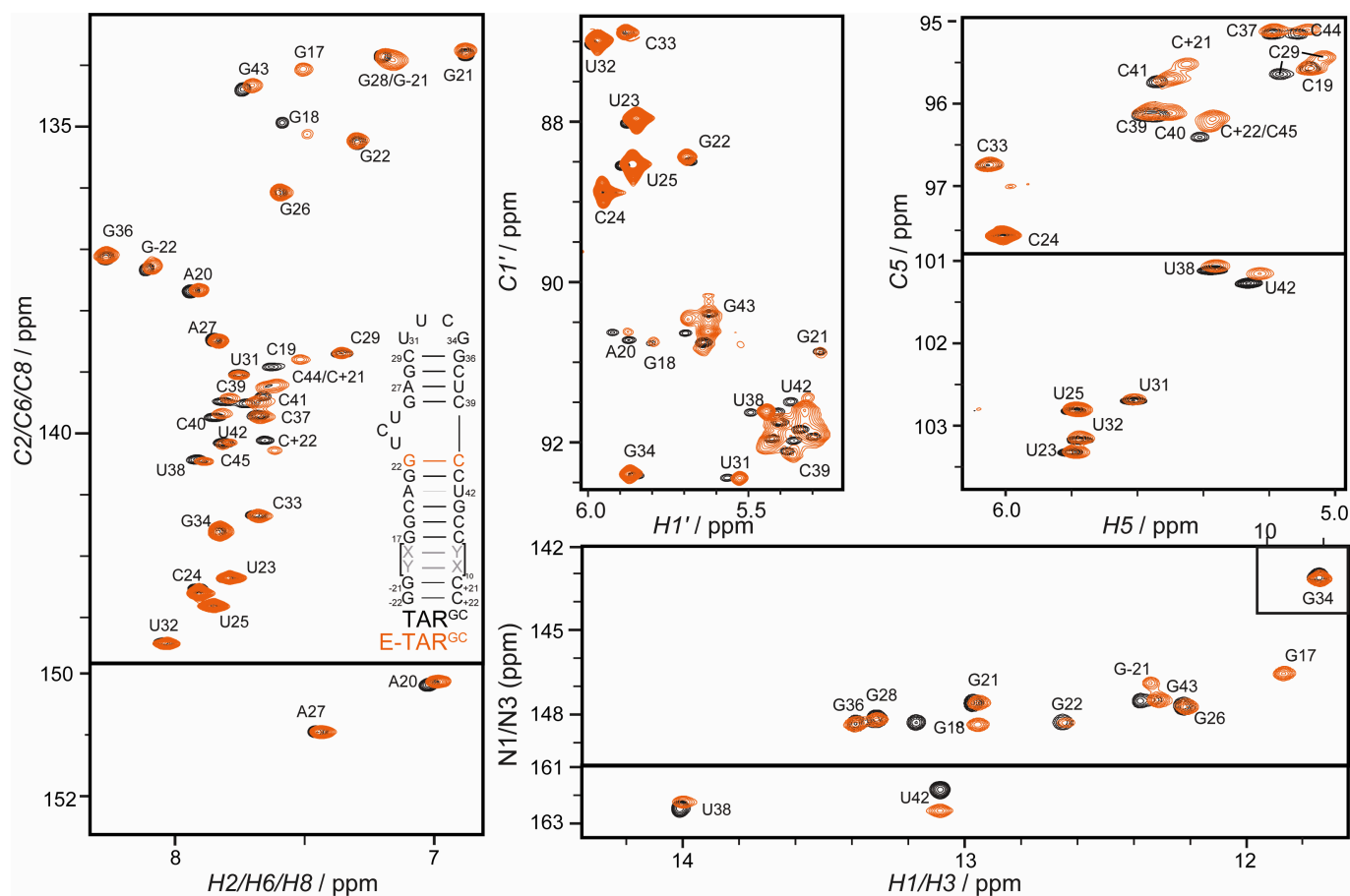


Figure S2. 2D CH and NH HSQC spectra of TAR^{GC} (black) and EI-TAR^{GC} (orange) demonstrating that elongation does not affect the structural and dynamical integrity of TAR^{GC}. Significant perturbations are primarily localized at residues near the site of elongation.

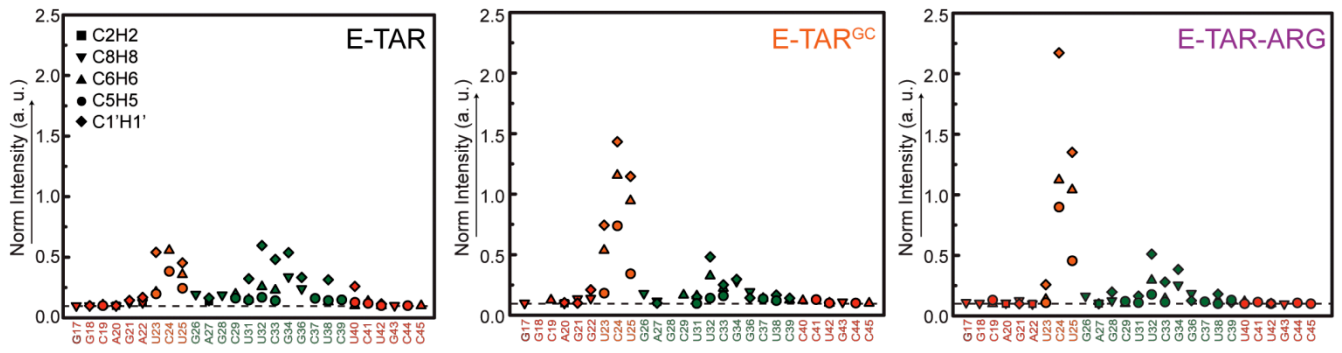


Figure S3. Normalized resonance intensities measured in 2D HSQC spectra of EI-TAR, EI-TAR-ARG, and EI-TAR^{GC}. Residues in helix I, helix II, and the bulge are colored-coded red, green, and orange respectively.

SUPPLEMENTARY REFERENCES

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