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Supplemental Information

Structural Determinants for Geometry and Information Decoding of tRNA by T Box Leader RNA

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Inventory of Supplemental Information

***Supplemental Figure 1* – The overall Stem I_{86}-$tRNA^{Gly}$ structure and density maps to illustrate the quality of the structure. Figures are related to statistics shown in Table 1.**

***Supplemental Figure 2* – The overall structural details of the Stem I_{86}-$tRNA^{Gly}$ complex crystal structure. Figures are related to the overall structure shown in Figure 1.**

***Supplemental Figure 3* – Comparison of the Stem I_{86}-$tRNA^{Gly}$ structure to other solution (SAXS and NMR) and crystal structure data. Figures are related to Figure 2, highlighting possible structural change.**

***Supplemental Figure 4* – Analysis and modeling of potential tRNA modification sites onto the complex structure. Figures are related to detailed T box RNA-tRNA interactions shown in Figure 3.**

Supplementary References

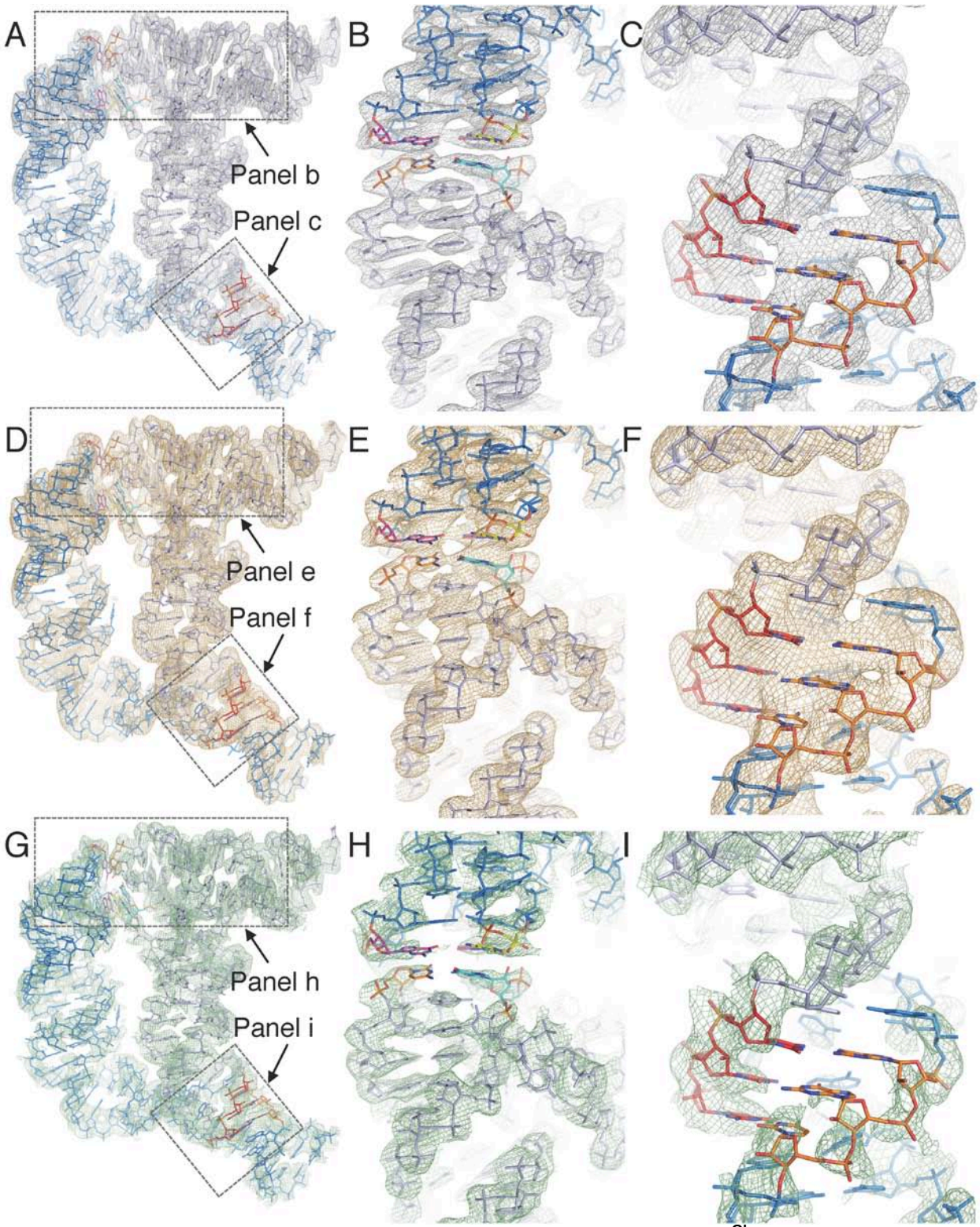

Figure S1. Electron density for chains A and B in the Stem I_{86}-$tRNA^{Gly}$ complex, related to Table 1. Maximum likelihood weighted $2F_o$-F_c maps are shown using B-factor sharpening in Phenix (grey, contoured at 1.2σ, panels A-C) and directly from Phenix.refine (orange, contoured at 1.2σ, panels D-F). A simulated annealing composite omit map generated using Autobuild (green, contoured at 0.8σ, panels G-I).

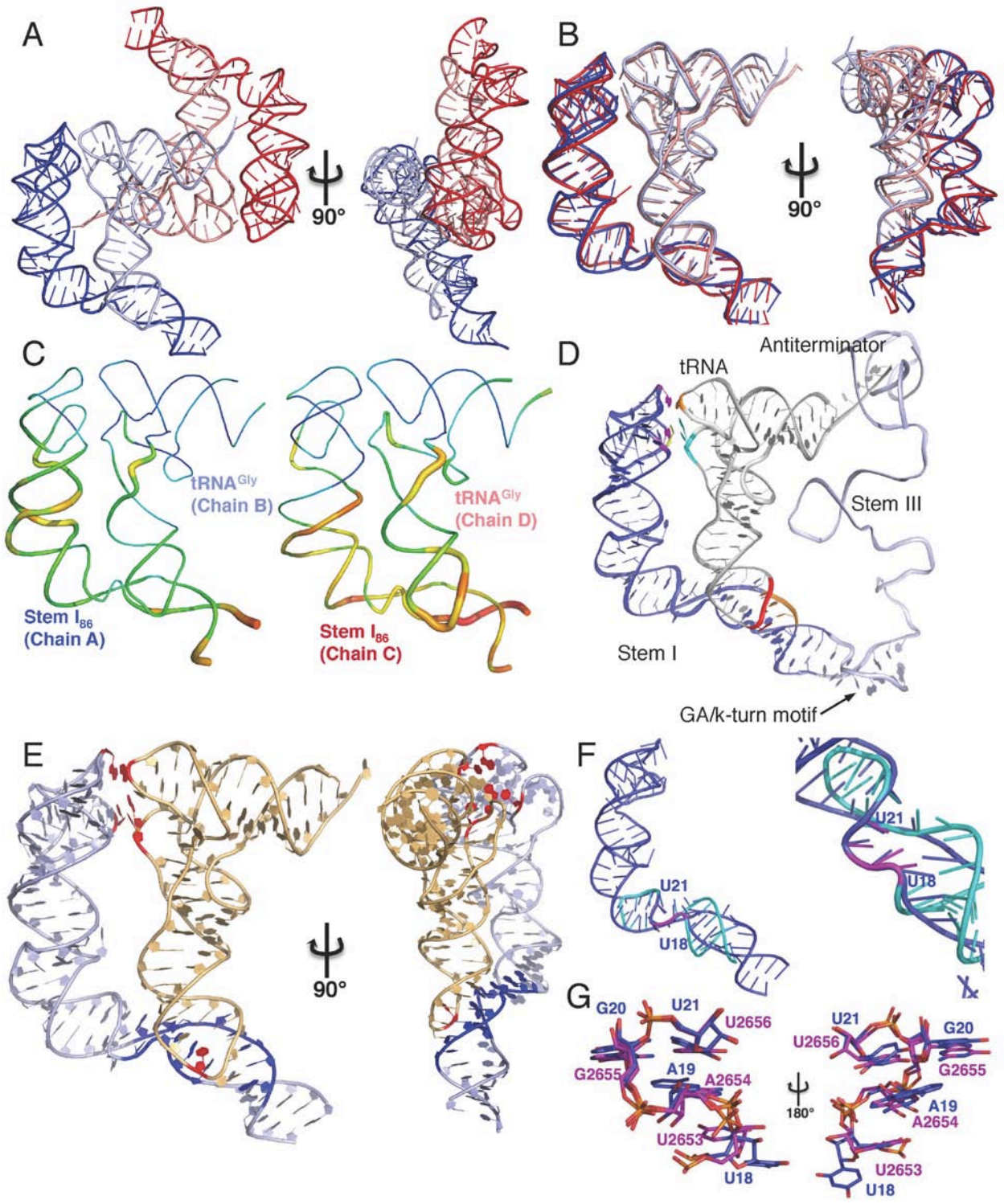

Figure S2. Stem I_{86}-$tRNA^{Gly}$ co-crystal structure, related to Figure 1. (**A**) Structure of two Stem I_{86}-$tRNA^{Gly}$ complexes in the asymmetric unit showing the placement of chains A (Stem I_{86}, blue), B ($tRNA^{Gly}$, light blue), C (Stem I_{86}, red) and D (tRNAGly, light red). (**B**) Superposition of the two Stem I_{86}-$tRNA^{Gly}$ complexes in the asymmetric unit, colored as in Part A. (**C**) Temperature factor distribution in both complexes revealing that the chain A-B complex is more ordered in the crystal lattice than the C-D complex. B-factors are illustrated on a tube diagram

from highest (red, maximum 252 $Å^2$) to lowest (blue, minimum ~70 $Å^2$). (**D**) Model for full-length T box riboswitch-tRNA interactions. Stem I_{86} is shown in dark blue with modeled regions in light blue and tRNA in grey. (**E**) Biochemical results mapped to the Stem I_{86}-$tRNA^{Gly}$ structure. Prominent UV-induced cross-linking sites (red) and complex-induced SHAPE protection (dark blue) agree closely with the structure (Grigg et al., 2013). SHAPE protection sites overlap with the UV crosslinking sites in the tRNA D/T loop contacting interface (top), and are displayed in red only. **(F)** Comparison between S-loops in the T box Specifier Loop and the Sarcin-Ricin domain from 23*S* rRNA. The T box RNA (blue) and the *E. coli* 23S Sarcin-Ricin domain (cyan) are shown as cartoons, with the S-loop highlighted in magenta (PDB ID: 483D). Structures were superimposed by least squares fitting between all atoms in the four conserved S-loop nucleotides. The motif starts with a *trans* Hoogsteen (H) to sugar edge (S) pair between $A22^{Tbox}$ and $G82^{Tbox}$, followed by a *trans* WC/H pair, $U21^{Tbox}$-$A83^{Tbox}$. The two non-WC pairs lead to an abrupt turn at $G20^{Tbox}$ that is stabilized by two hydrogen bonds from its N2 amine to $A83^{Tbox}$ OP1 and $U21^{Tbox}$ O4 and followed by a *trans* H/H pair between $A19^{Tbox}$ and $A84^{Tbox}$. (**G**) Stick view of the nucleotides forming the S-loop, colored as in Panel F.

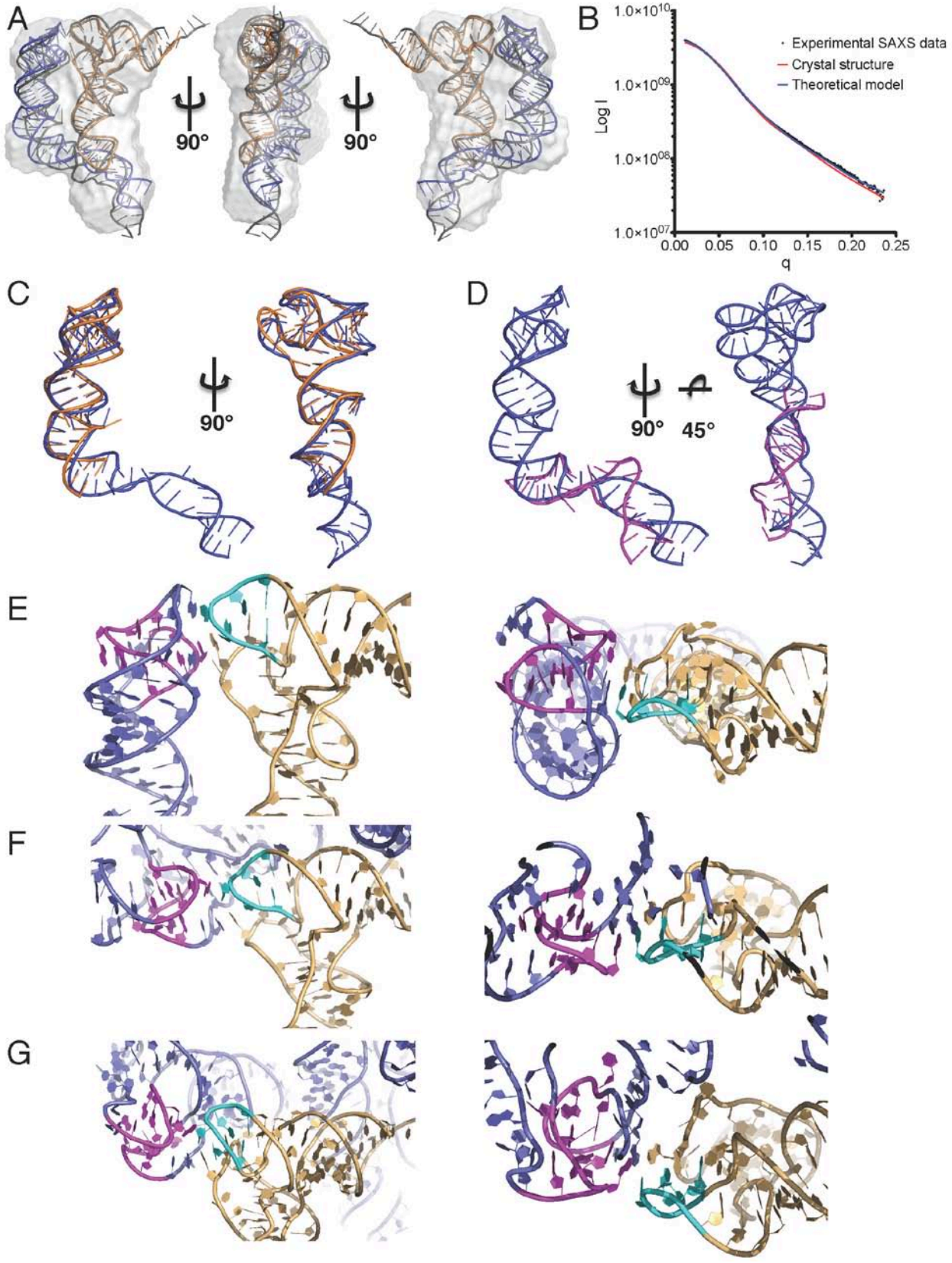
A
90°
90°
B
1.0×10^10
1.0×10^09
1.0×10^08
1.0×10^07
Log I
0.00
0.05
0.10
0.15
0.20
0.25
q
Experimental SAXS data
Crystal structure
Theoretical model
C
90°
D
90°
45°
E
F
G

Figure S3. Comparison of Stem I_{86} complex structure to previously determined structures, related to Figure 2. (**A**) Superposition of the structure onto model complex used in SAXS reconstructions (Grigg et al., 2013). The Crystal structure is shown in blue (Stem I_{86}) and orange ($tRNA^{Gly}$) with the model in dark grey. The SAXS reconstruction is shown as a light grey surface. (**B**) A calculated SAXS curve for the Stem I_{86}-$tRNA^{Gly}$ structure (red) was determined using the FoXS server (Schneidman-Duhovny et al., 2010) and is shown on experimental (black dot) and theoretical complex (cyan) curves, determined previously (Grigg et al., 2013). The crystal structure and theoretical model both agree well with the experimental data and have calculated chi values of 12.2 and 5.5, respectively. (**C**) Stem I_{86} (blue) superimposed on StemI_{57} (PDB ID: 4JRC, orange), with an r.m.s.d of 2.1 Å over all C3' atoms. The relatively high r.m.s.d for the analogous regions are a result of slightly altered hinge angles between segments (L3/4, P4/5) rather than localized structural differences. (**D**) Stem I_{86} (blue) superimposed on the specifier loop from the *Bacillus subtilis tyrS* T box RNA NMR structure (PDB ID: 2KZL, magenta). Given the large structural differences, superpositions were generated by least squares fitting of all atoms surrounding the S-turn motif (A17-G23). A comparison of the interlocking T-loops in: (**E**) T box Stem I_{86}-tRNA, (**F**) RNase P-tRNA, and (**G**) Ribosome L1-tRNA interfaces. Binding RNA (blue) and tRNA (light orange) are shown as cartoons with the interlocking T-loops highlighted in magenta and the tRNA T-loop highlighted in cyan.

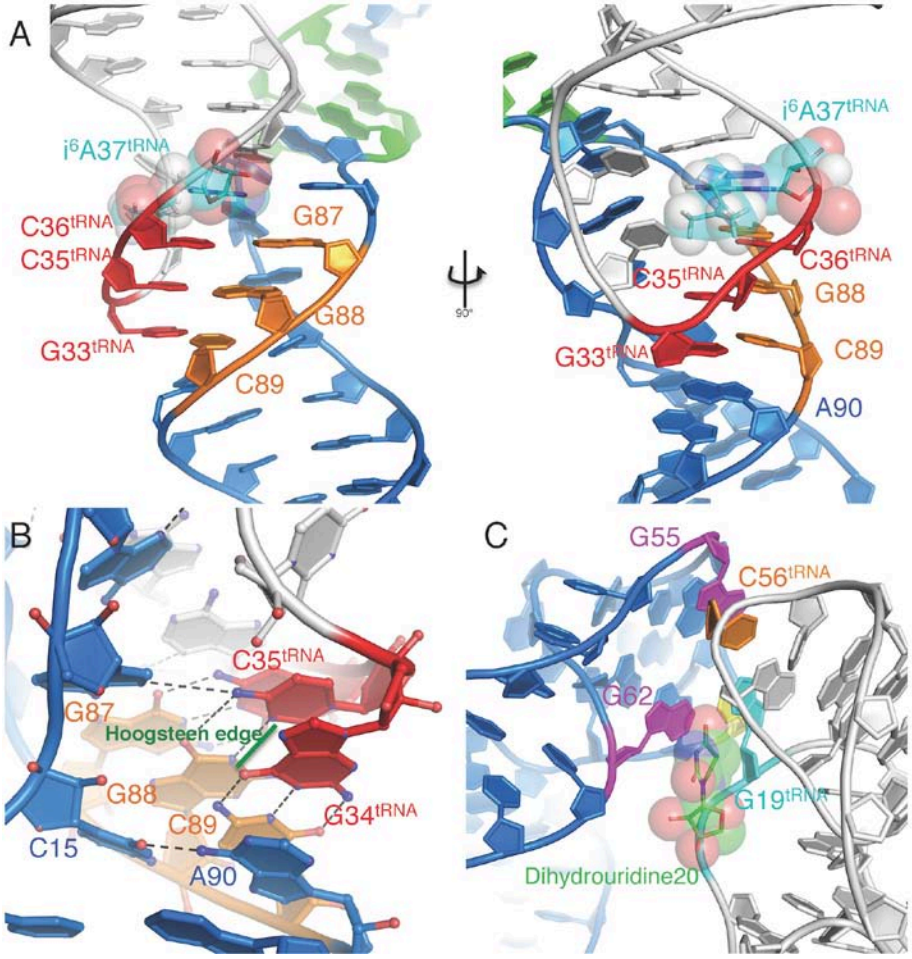

Figure S4. Common tRNA modifications are accommodated in the Stem I_{86}-$tRNA^{Gly}$ complex, related to Figure 3. (**A**) Model of N6-dimethylallyl adenine tRNA modification at $A37^{tRNA}$ **(**i^6A_{37}, cyan). The modification is directed away from the T box-tRNA interactions and would easily be accommodated. (**B**) G34 is commonly modified along its Hoogsteen edge (ie. Queuosine). In the complex, this region is directed into a cavity above C15 and the P2 helix and would be expected to accommodate such modifications. (**C**) U20 may be modified as dihydrouridine. This modification does not affect its hydrogen bonding interaction with G19, and because it is tilt-stacked against G62, distortion of its pyrimidine ring, as the result of modification, is not expected to have a deleterious effect.

References

Grigg, J.C., Chen, Y., Grundy, F.J., Henkin, T.M., Pollack, L., and Ke, A. (2013). T box RNA decodes both the information content and geometry of tRNA to affect gene expression. Proc Natl Acad Sci U S A 110, 7240-7245.

Schneidman-Duhovny, D., Hammel, M., and Sali, A. (2010). FoXS: A Web server for Rapid Computation and Fitting of SAXS Profiles. Nucl Acids Res 38 Suppl:W540-544.