Supporting Information

Cooperative RNA Folding Under Cellular Conditions Arises From Both Tertiary Structure Stabilization and Secondary Structure Destabilization

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Table S1. Melting temperatures of T7 tRNA^{phe}, its individual HF, and the SSS in the background of 0 mM Mg^{2+} derived from optical melting.

Melting Temperature (°C) in 0 mM Mg^{2+}						
Additive	**FL T7 tRNA^{phe}**	**Acceptor Stem**	**D SL**	**Anticodon SL**	**TΨC SL**	**Sum of SS**
None	53.2	56.8	60.5	63.0	58.3	59.6
20% PEG200	52.8	52.9	58.8	61.7	53.4	55.6
40% PEG200	50.5	45.5	44.0	60.0	44.0	48.4
20% PEG4000	45.5	57.8	64.9	66.7	56.8	61.3
40% PEG4000	64.0	59.3	64.6	67.6	60.0	62.2
20% PEG8000	66.0	68.1	69.0	72.5	72.4	70.4
40% PEG8000	54.0	57.0	51.0	68.8	60.5	58.9
20% PEG20000	--	60.5	61.5	57.0	57.0	57.0

All samples contain a background of 10 mM sodium cacodylate (pH 7.0) and 140 mM KCl.

Table S2. Melting temperatures of T7 tRNA^{phe}, its individual HF, and the SSS in the background of 0.5 mM Mg^{2+} derived from optical melting.

Melting Temperature (°C) in 0.5 mM Mg^{2+}						
Additive	**FL T7 tRNA^{phe}**	**Acceptor Stem**	**D SL**	**Anticodon SL**	**TΨC SL**	**Sum of SS**
None	57.8	57.1	(1) 60.5 (2) 67.8	67.8	59.6	63.4
20% PEG200	57.0	52.3	63.5	63.5	55.3	56.4
40% PEG200	59.1	44.6	51.5	55.6	50.3	49.3
20% PEG4000	63.0	57.7	78.0	67.1	57.2	58.2
40% PEG4000	65.5	59.3	64.6	67.7	61.0	62.3
20% PEG8000	64.0	59.0	55.1	67.6	57.4	58.4
40% PEG8000	66.0	57.2	54.2	66.7	53.6	57.7
20% PEG20000	64.0	58.5	--	62.0	58.5	58.5

All samples contain a background of 10 mM sodium cacodylate (pH 7.0) and 140 mM KCl.

Table S3. Melting temperatures of T7 tRNA^{phe}, its individual HF, and the SSS in the background of 2.0 mM Mg^{2+} derived from optical melting.

Melting Temperature (°C) in 2.0 mM Mg^{2+}						
Additive	**FL T7 tRNA^{phe}**	**Acceptor Stem**	**D SL**	**Anticodon SL**	**TΨC SL**	**Sum of SS**
None	65.1	59.7	66.5	69.7	62.0	63.4
20% PEG200	64.8	55.8	61.6	66.1	58.2	59.3
40% PEG200	64.1	50.4	58.4	54.6	61.4	51.9
20% PEG4000	69.5	59.3	64.6	67.6	61.9	62.3
40% PEG4000	87.5	58.0	52.5	67.6	61.0	62.3
20% PEG8000	69.0	59.8	62.7	70.1	61.1	64.3
40% PEG8000	64.0	57.6	59.5	66.5	61.0	60.6
20% PEG20000	70.0	58.0	--	69.0	58.5	62.5

All samples contain a background of 10 mM sodium cacodylate (pH 7.0) and 140 mM KCl.

Table S4. Evaluation of SAXS data fitting using FoXS and Supcomb.

SAXS Sample	**RMSD (Å)**
Buffer with 0 mM Mg^{2+}	6.56
Buffer with 0.5 mM Mg^{2+}	4.13
Buffer with 2.0 mM Mg^{2+}	3.30
20% PEG with 0.5 mM Mg^{2+}	3.55
20% PEG with 2.0 mM Mg^{2+}	2.75

The RMSD was found using the SUPCOMB alignments of the DAMAVER envelopes to the tRNA crystal structure.

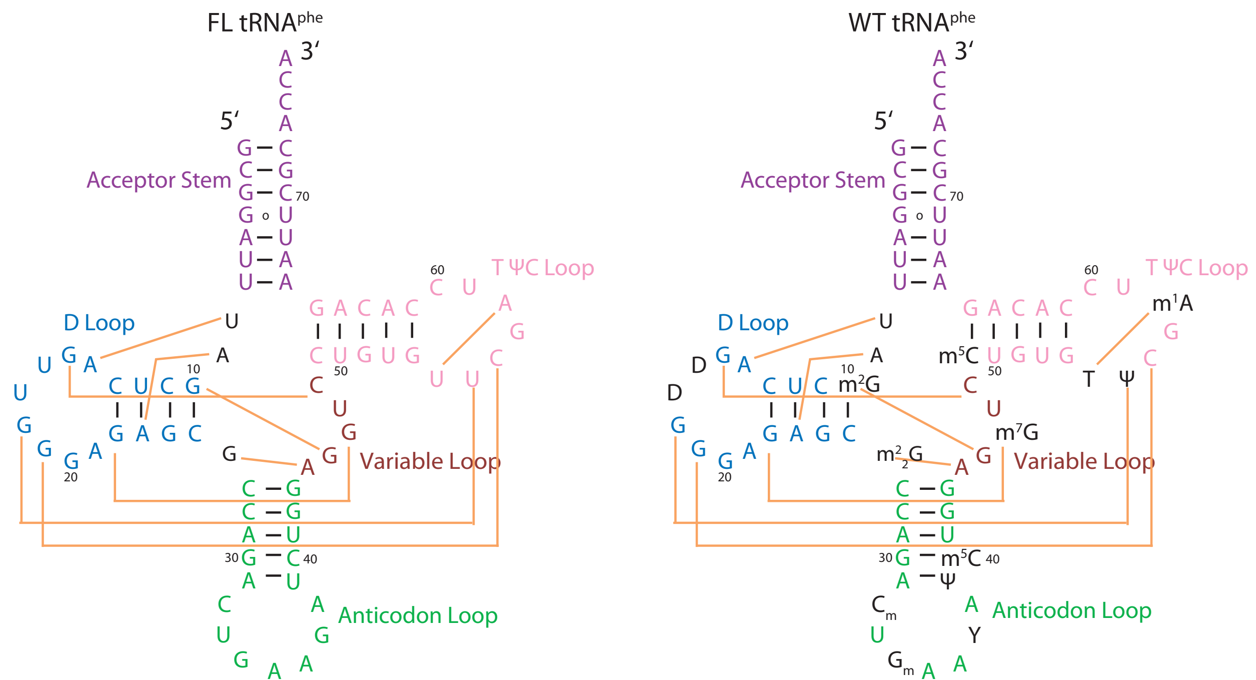

Figure S1. Secondary and tertiary structures of FL tRNA^{phe} and WT tRNA^{phe}. Tertiary contacts, yellow lines, are superimposed on *(left)* FL tRNA^{phe} and *(right)* WT tRNA^{phe}, which contains modifications (black bases). Coloring as per Figure 1.

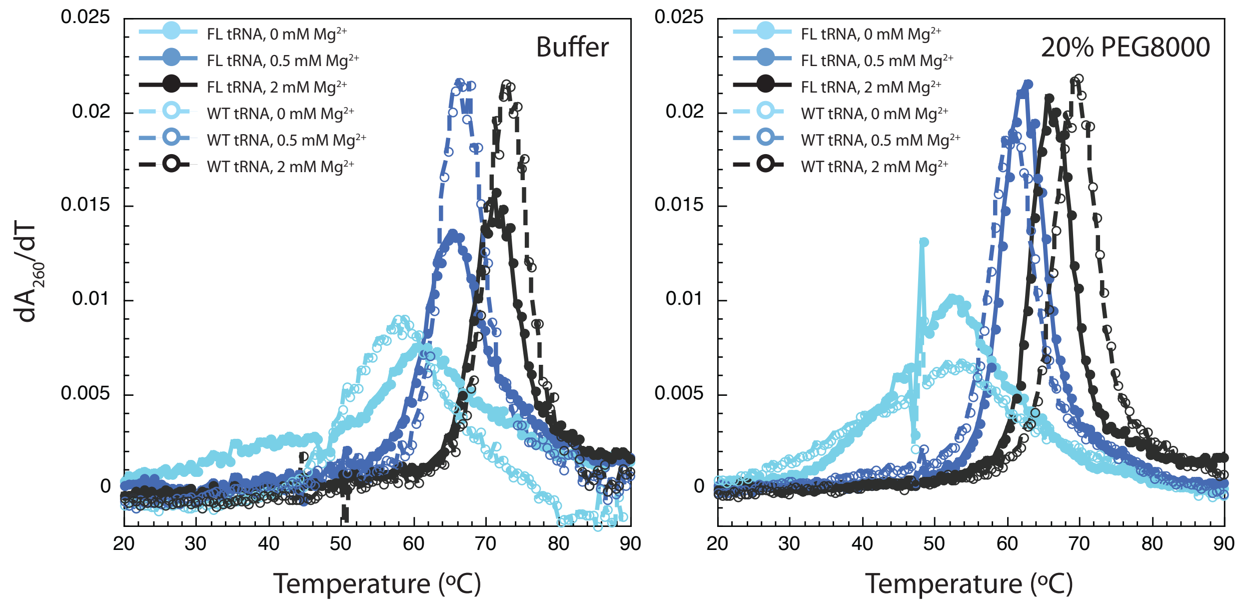

Figure S2. FL transcribed tRNA and WT tRNA behave in a similar manner in buffer and crowded conditions. In *(left)* buffer and *(right)* 20% PEG8000 FL tRNA (closed circles) and WT modified tRNA (open circles) have similar folding transitions at physiological concentrations of Mg^{2+}.

0.5 mM Mg^{2+}

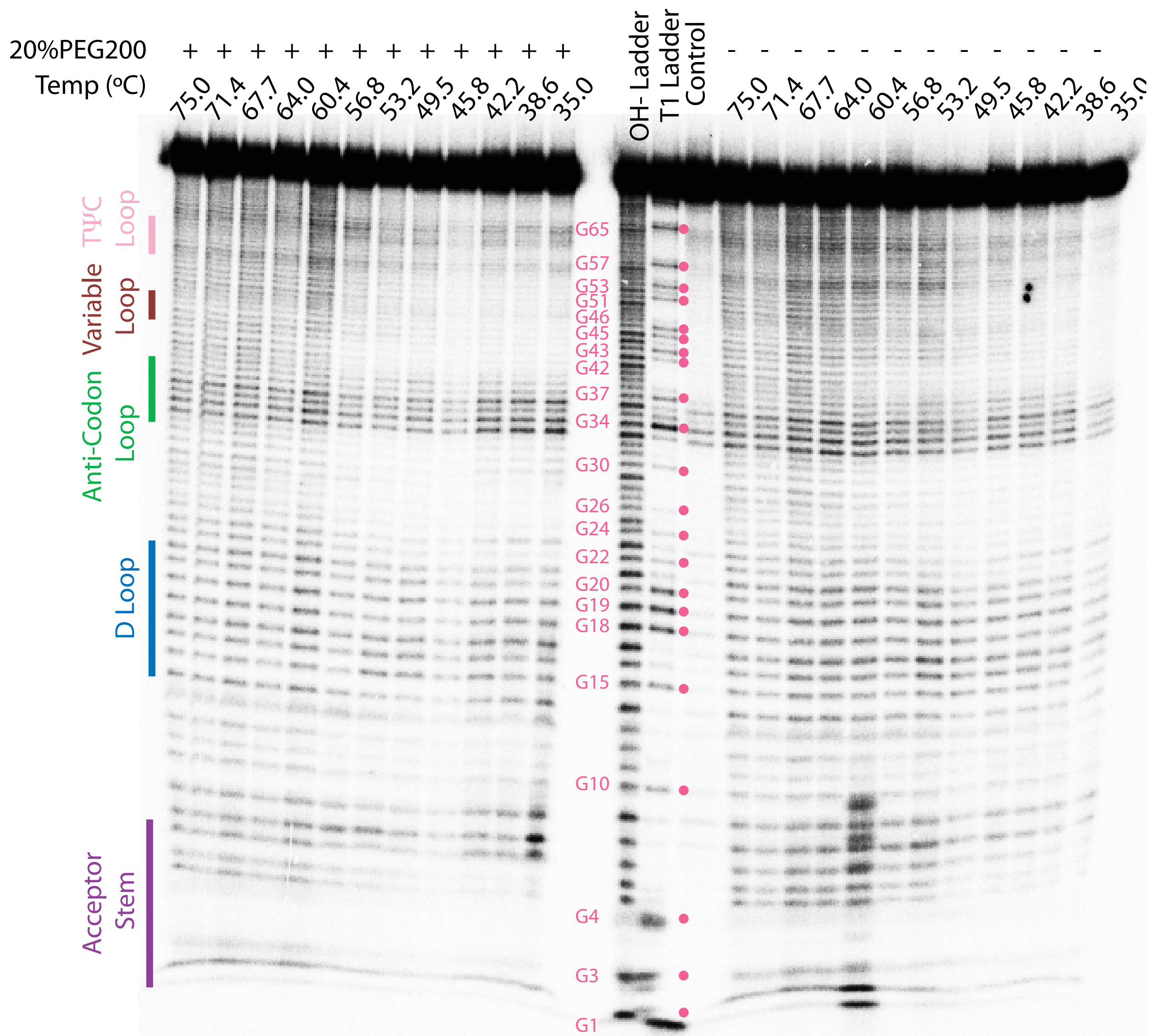

Figure S3. Temperature-dependent in-line probing (dT-ILP) PAGE gel of FL $tRNA^{phe}$ in buffer and 20% PEG200 with a background of 10 mM sodium cacodylate, 140 mM KCl, and 0.5 mM Mg^{2+}. Guanosines on the T1 ladder are marked with a pink dot, and the regions of the gel that contain nucleotides in the 3' of the acceptor stem, the D loop, anticodon loop, variable loop, and TΨC loop are noted. The colors of those regions match the colors in Fig. 1. At 35.0 °C, 38.6 °C, and 42.2 °C the 36 h time point was analyzed; at 45.8 °C, 49.5 °C, and 53.2 °C the 24 h time point was analyzed; at 56.8 °C and 60.4 °C the 5 h time point was analyzed; at 64.0 °C and 67.7 °C the 3 h time point was analyzed; and at 71.4 °C and 75.0 °C the 1 h time point was analyzed.

2.0 mM Mg^{2+}

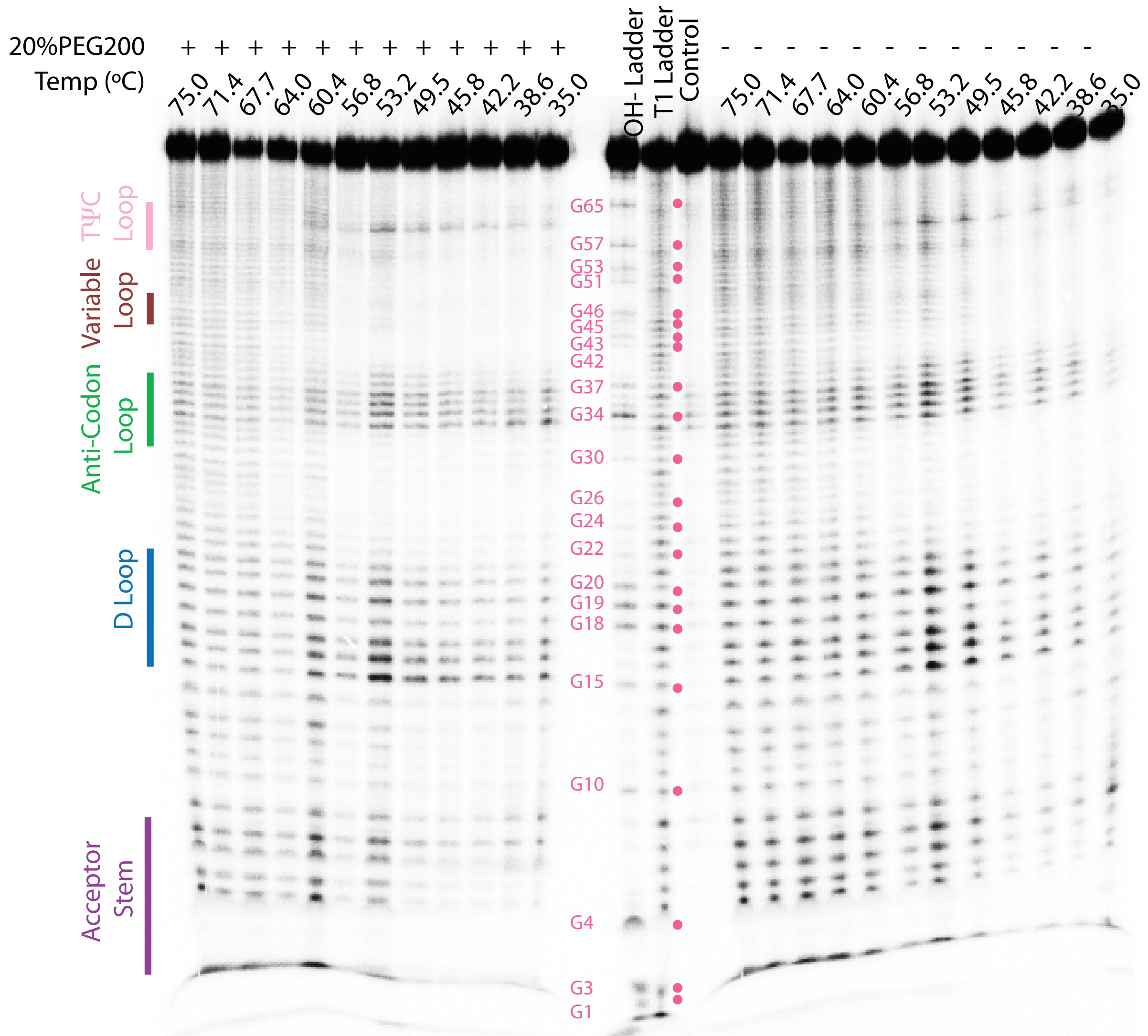

Figure S4. Temperature-dependent in-line probing (dT-ILP) PAGE gels of FL tRNA^{phe} in buffer and 20% PEG200 with a background of 10 mM sodium cacodylate, 140 mM KCl, and 2.0 mM Mg^{2+}. Guanosines on the T1 ladder are marked with a pink dot, and the regions of the gel that contain nucleotides in the 3' of the acceptor stem, the D loop, anticodon loop, variable loop, and TΨC loop are noted. The colors of those regions match the colors in Fig. 1. At 35.0 °C, 38.6 °C, and 42.2 °C the 36 h time point was analyzed; at 45.8 °C, 49.5 °C, and 53.2 °C the 24 h time point was analyzed; at 56.8 °C and 60.4 °C the 5 h time point was analyzed; at 64.0 °C and 67.7 °C the 3 h time point was analyzed; and at 71.4 °C and 75.0 °C the 1 h time point was analyzed.

2.0 mM Mg^{2+}

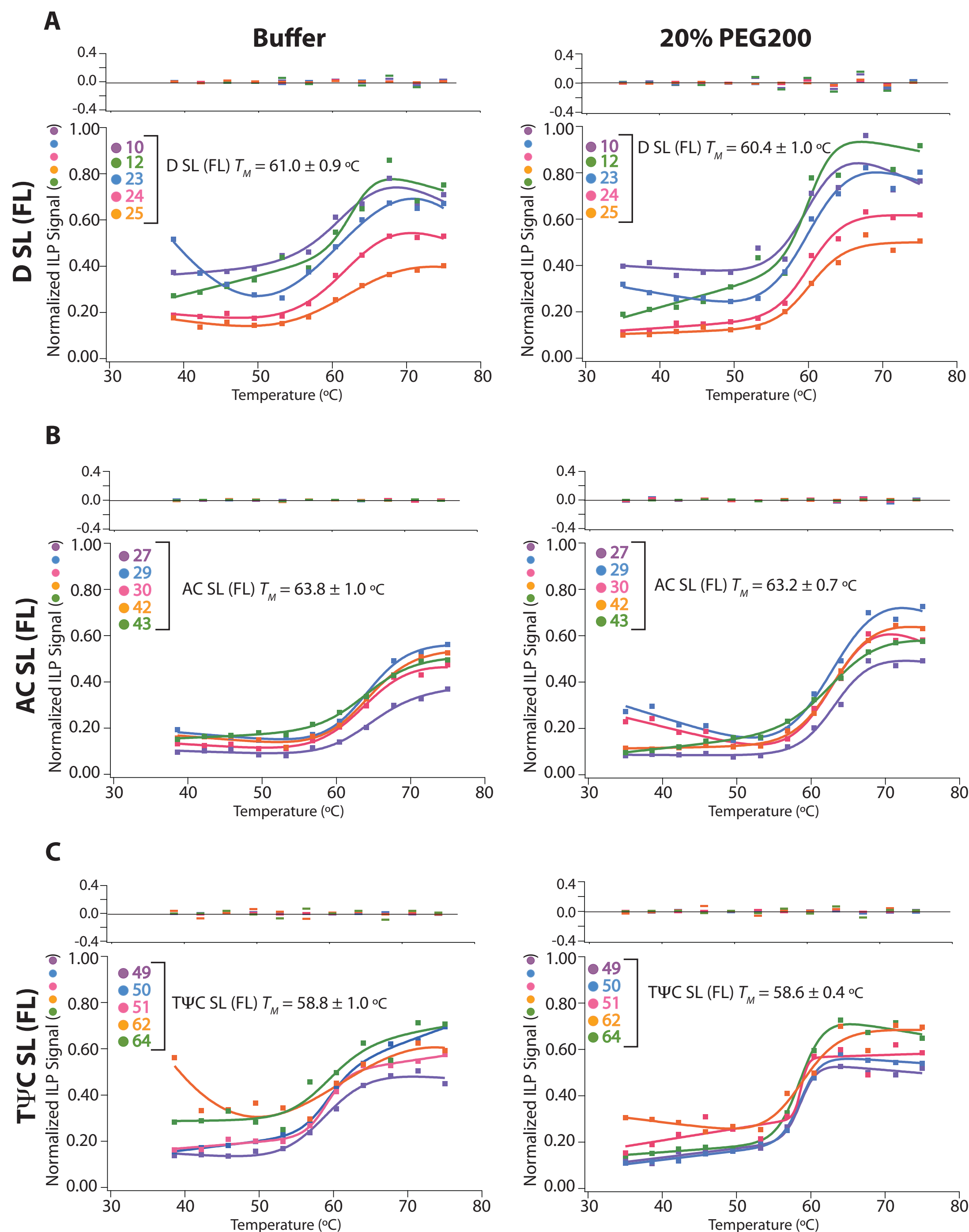

Figure S5. Helical stem fitting of temperature-dependent ILP data in buffer and 20% PEG200 2.0 mM Mg^{2+}. Helical fits (globally fit for nucleotides shown with bracket) were performed on buffer and 20% PEG200 samples to obtain a T_M for unfolding of each stem in *(A)* D SL, *(B)* AC SL, and *(C)* TΨC SL. The fit T_M's and the residuals of the fits are provided in each figure and in Table 1.

2.0 mM Mg^{2+}

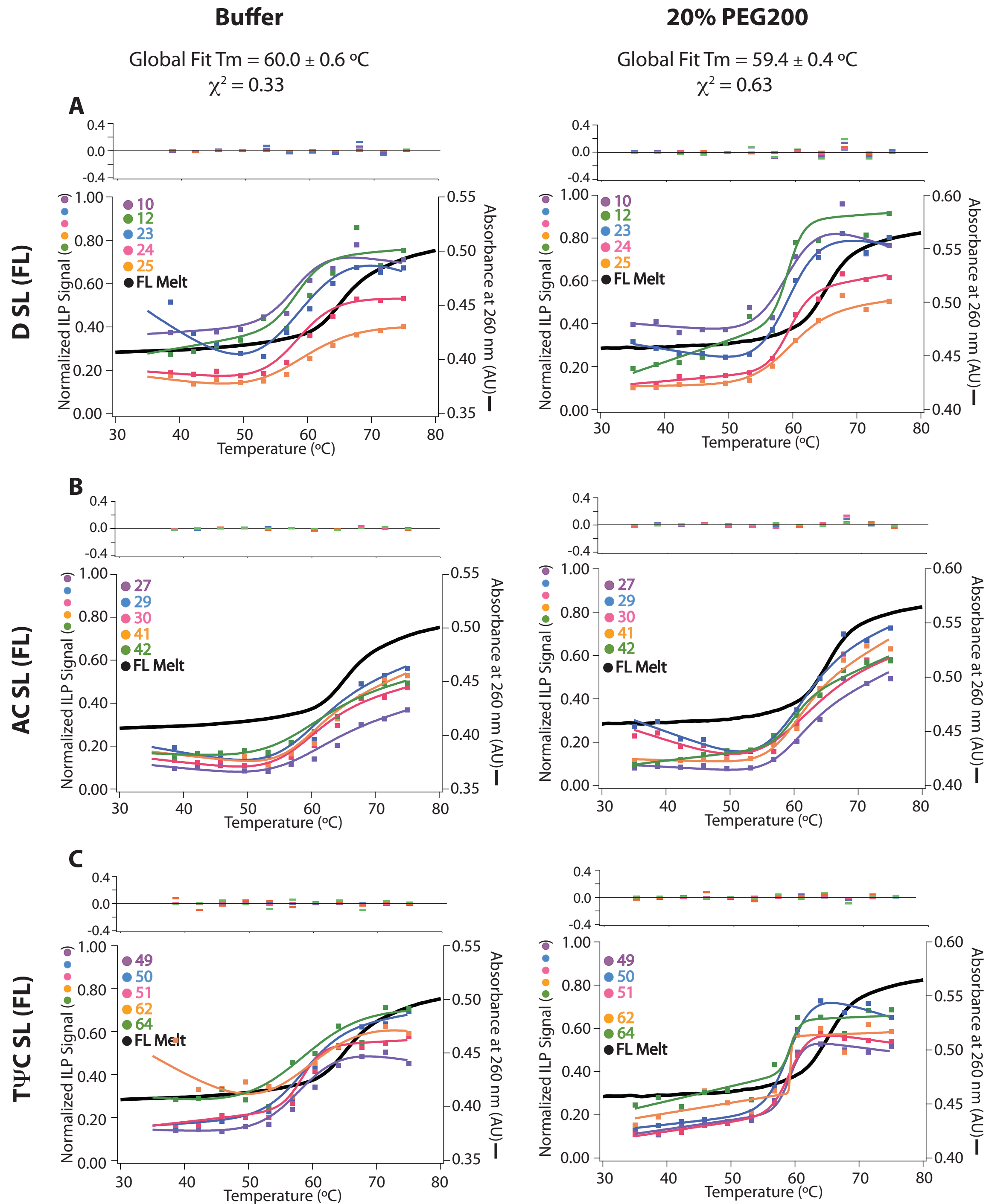

Figure S6. Global fitting of temperature-dependent ILP data in buffer and 20% PEG200 with 2.0 mM Mg^{2+}. The same ILP data from Figure S5 were fit globally across each nucleotide in each stem for a single T_M of the RNA and to look for two-state behavior. The T_M values and the residuals from the fits are provided in each figure and in Table 1.

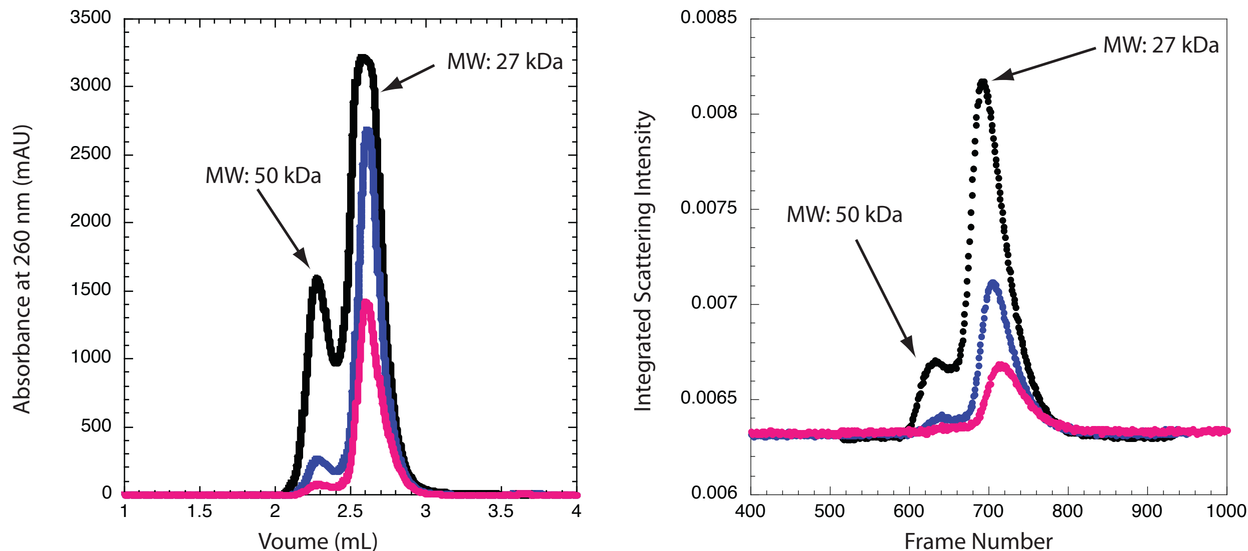

Figure S7. Uv-vis detection and scattering intensity of in-line SEC SAXS experiments of FL tRNA in buffer with 0.5 mM Mg^{2+}. *(Left)* Absorbance-detection at 260 nm and *(Right)* Integrated scattering intensity of size-exclusion traces of FL tRNA in buffer with 0.5 mM Mg^{2+} at 0.2 (pink), 0.4 (blue), and 0.6 mg/mL (black). The molecular weight of both peaks as determined from the integrated intensity in BioXTAS RAW is labeled. The left peak has a higher molecular weight (~50 kDa) and is attributed to the formation of a dimer, and the right peak has a lower molecular weight (~27 kDa) very close to that expected of the monomer (estimated 25 kDa). In the 0.2 mg/mL curves, trace amounts of the dimer peak are absorbance-detected, but are not scattering detected. The curves of 0.6 mg/mL RNA (black) were offset to align with the other curves; during the 0.6 mg/mL experiment a bubble was found in the SEC line and the beam had to be turned off, therefore the time of exposure and volume eluted was offset.

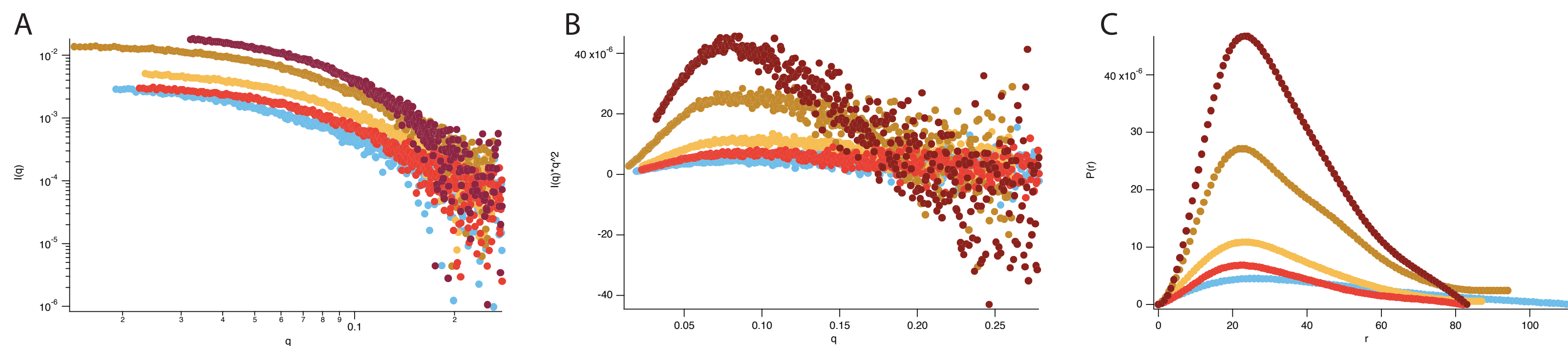

Figure S8. Small angle X-ray scattering data fitting of FL tRNA in buffer and 20% PEG8000 in the background of low, physiological Mg^{2+} and crowding. *(A)* Scattering curves, *(B)* Kratky plots, and *(C)* P(r) plots of FL tRNA in buffer with 0 (blue), 0.5 (light yellow), and 2.0 mM Mg^{2+} (red), and in 20% PEG8000 with 0.5 (dark yellow) and 2.0 mM Mg^{2+}(maroon). The data without PEG8000 were collected by SEC-SAXS with the 2.0 mM Mg^{2+} data collected on a different trip than the 0 and 0.5 mM Mg^{2+} data. The data in the presence of 20% PEG8000 were collected without SEC.

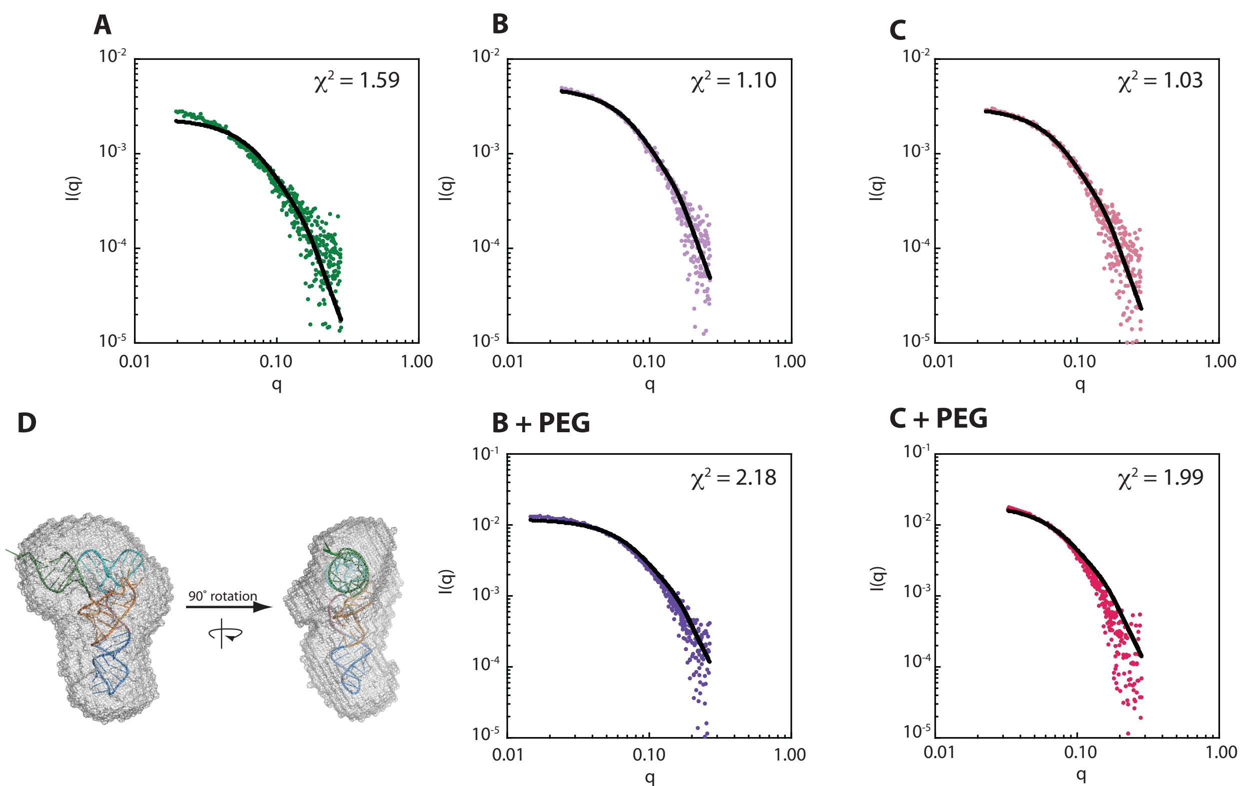

Figure S9. Comparison of experimental SAXS scattering curves and the theoretical scattering curve of a $tRNA^{phe}$ crystal structure (PDB ID: 1ehz) generated with FoXS. *(A-C)* Overlay of experimental scattering curves with the theoretical $tRNA^{phe}$ scattering curve generated with FoXS in *(A)* 0, *(B)* 0.5, and *(C)* 2.0 mM Mg^{2+} with and without PEG8000. The χ^2 of the FoXS fits are provided on each plot. *(D)* DAMAVER bead model generated from the $tRNA^{phe}$ theoretical scatting curve overlaid with the crystal structure.