Supporting Information

Molecular Mechanism for Folding Cooperativity of Functional RNAs in Living Organisms

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Table S1. Thermodynamic parameters for tRNA folding 2.0 mM free Mg^{2+}.

RNA Construct	T_M (°C)	ΔH (kcal/mol)	$T_{M(M)}$ - $T_{M(WT)}$ (°C)	$\Delta H_M/\Delta H_{WT}$
Buffer				
M1	64.0	-80.4	-2.2	1.1
WT	66.2	-72.2	0.0	1.0
M2	66.1	-81.5	-0.1	1.1
M3	69.2	-66.1	3.0	0.9
M4	72.6	-51.7	6.4	0.7
M5	75.4	-40.4	9.2	0.6
M6	72.8	-38.0	6.6	0.5
20% PEG8000				
M1	66.1	-77.7	-1.9	1.0
WT	68.0	-80.4	0.0	1.0
M2	68.8	-91.4	0.8	1.1
M3	71.1	-66.8	3.1	0.8
M4	67.2	-74.3	-0.8	0.9
M5	70.2	-62.7	2.2	0.8
M6	61.1	-21.4	-6.9	0.3
Mg^{2+} Chelated Amino Acids				
M1	70.2	-149.3	-0.7	1.0
WT	70.9	-145.9	0.0	1.0
M2	72.0	-141.6	1.1	1.0
M3	74.7	-94.7	3.8	0.6
M4	75.5	-86	4.6	0.6
M5	74.1	-84.7	3.2	0.6
M6	73.5	-88.1	2.6	0.6
20% PEG8000 & Mg^{2+} Chelated Amino Acids				
M1	71.9	-152.3	0.2	1.0
WT	71.8	-148.0	0.0	1.0
M2	73.3	-155.5	1.5	1.1
M3	75.9	-130.2	3.9	0.9
M4	77.3	-130.3	3.9	0.9
M5	76.7	-119.8	4.9	0.8
M6	78.7	-81.3	6.9	0.5

Constructs are ordered in the table according to the stability of the accepter stem.
Thermodynamic parameters are derived from global fits of thermal denaturation data from 250 nm to 290 nm according to a two-state model.

Table S2. Thermodynamic parameters for tRNA folding in 0.5 mM free Mg^{2+}.

RNA Construct	T_M (°C)	ΔH (kcal/mol)	$T_{M(M)} - T_{M(WT)}$ (°C)	$\Delta H_M/\Delta H_{WT}$
Buffer				
M1	35.3	-24.5	-25.9	0.5
WT	61.2	-44.9	0.0	1.0
M2	59.9	-46.6	-1.3	1.0
M3	65.8	-38.8	4.6	0.5
M4	42.4	-14.7	-18.8	1.0
M5	64.9	-26.6	3.7	0.5
M6	9.43	-18.0	-51.8	1.0
20% PEG8000				
M1	60.0	-74.9	-1.8	1.1
WT	61.8	-70.7	0.0	1.0
M2	65.2	-43.7	3.4	0.6
M3	65.3	-52.8	3.5	0.7
M4	63.4	-48.9	1.6	0.7
M5	59.5	-35.1	-2.3	0.5
M6	53.7	-47.4	-8.1	0.7
Mg^{2+} Chelated Amino Acids				
M1	64.4	-78.5	-1.7	0.6
WT	66.1	-125.9	0.0	1.0
M2	66.7	-107.1	0.6	0.6
M3	71.9	-59.6	5.8	0.9
M4	73.6	-45.9	7.5	0.5
M5	69.6	-25.9	3.5	0.4
M6	69.6	-25.9	3.5	0.2
20% PEG8000 & Mg^{2+} Chelated Amino Acids				
M1	68.6	-79.4	-0.1	1.0
WT	68.7	-83	0.0	1.0
M2	69.1	-103.9	0.4	1.3
M3	71.9	-77	3.2	0.9
M4	67.8	-139.7	-0.8	1.7
M5	71.4	-63.9	2.7	0.8
M6	67.9	-139.7	-0.8	1.7

Constructs are ordered in the table according to the stability of the accepter stem.
Thermodynamic parameters are derived from global fits of thermal denaturation data from 250 nm to 290 nm according to a two-state model

Table S3. Quality of the global fits of thermal denaturation data in 0.5 and 2.0 mM Mg^{2+}.

RNA Construct	0.5 mM Mg^{2+} χ^2	2.0 mM Mg^{2+} χ^2
	Buffer	
M1	8.94E-02	1.77E-02
WT	2.24E-01	8.46E-03
M2	2.13E-03	1.16E-02
M3	7.88E-03	2.27E-03
M4	2.25E-02	1.92E-03
M5	7.42E-03	3.05E-03
M6	4.60E-02	2.61E-03
	20% PEG8000	
M1	3.18E-03	1.46E-02
WT	5.64E-03	1.61E-02
M2	1.11E-02	6.88E-03
M3	1.17E-02	6.36E-03
M4	2.29E-02	1.09E-02
M5	4.03E-03	4.68E-03
M6	6.95E-03	1.09E-02
	Mg^{2+} Chelated Amino Acids	
M1	3.17E-04	2.57E-04
WT	3.17E-04	1.56E-04
M2	3.55E-04	1.87E-04
M3	1.28E-03	
M4	1.28E-03	3.05E-04
M5	1.02E-03	6.95E-04
M6	1.02E-03	5.29E-04
	PEG8000 & Mg^{2+} Chelated Amino Acids	
M1	7.86E-04	6.57E-04
WT	8.15E-03	9.01E-04
M2	2.37E-03	8.45E-04
M3	1.85E-03	4.58E-04
M4	7.93E-03	4.58E-04
M5	1.24E-03	2.43E-03
M6	7.93E-03	8.63E-04

Table S4. Structural parameters for WT and MT tRNAs obtained by SAXS in 2.0 mM Mg^{2+}.

RNA Construct	MW[a] (kDa)	R_g[a] (Å)	R_g[b] (Å)	D_{max}[b] (Å)	Excluded Volume[c] (Å^3)	RMSD[c] (Å)	FoXS[d] χ^2
WT	28.5	24.2	25.1	82	47,700	3.30	1.03
M1	35.2	23.6	25.7	83	50,400	3.35	1.02
M2	28.7	25.6	26.4	85	53,000	3.90	1.21
M3	32.2	25.5	26.9	85	56,100	3.84	1.14
M4	31.9	26.1	26.6	87	50,800	3.80	1.25
M5	25.2	26.3	27.4	87	51,400	3.94	1.10

Solutions contain 25 mM HEPES (pH 7.5), 140 mM KCl, and 2.0 mM $MgCl_2$. [a]Parameters were obtained by analysis of the experimental scattering curves using BioXTAS RAW software. [b]Values were obtained using the pairwise distribution function in GNOM using the ATSAS software package. [c]Parameter was found using the alignments of the DAMAVER bead models with the tRNA crystal structure in SUPCOMB. [d]Parameter found from the alignments of experimental scattering curves to the predicted scatting curve of tRNA crystal structure (PDB ID: 1ehz)

Table S5. Composition of samples containing Mg^{2+}-chelated amino acids

Concentration	0.5 mM free Mg^{2+}	2.0 mM free Mg^{2+}
Amino acids[a]	106.6 mM	106.6 mM
Total Mg^{2+}	4.6 mM	16.0 mM
Free Mg^{2+}	0.5 mM	2.0 mM
Amino acid chelated Mg^{2+}	4.1 mM	14.0 mM
KCl	140 mM	140 mM
Sodium cacodylate	10 mM	10 mM

[a]Composed of 96.0 mM glutamate, 4.2 mM aspartate, 3.8 mM glutamine, and 2.6 mM alanine at pH 7.0.

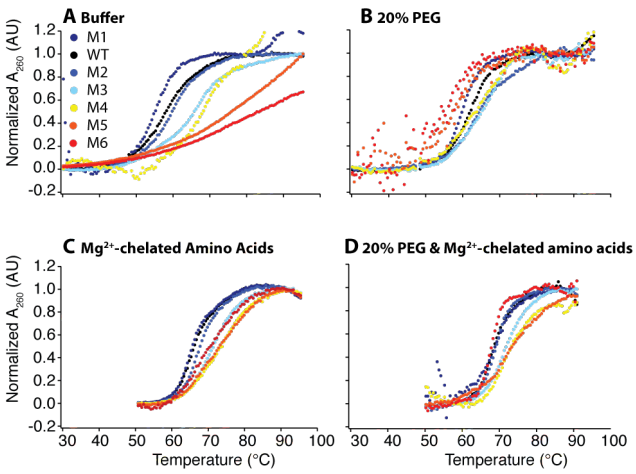

Figure S1. WT and mutant thermal denaturation under *in vivo*-like solutions in the background of 0.5 mM free Mg^{2+}. Each construct was globally fit every 2 nm between 250 and 290 nm. Thermal denaturation scans at 260 nm normalized using global fitting parameters in **(A)** buffer, **(B)** 20% PEG8000, **(C)** Mg^{2+}-chelated amino acids, and **(D)** 20% PEG8000 and Mg^{2+}-chelated amino acids. All four panels are in the background of 0.5 mM Mg^{2+} and 140 mM K^{+}. Low temperature data was truncated in the fitting to avoid excess baselines, as is plotted above.

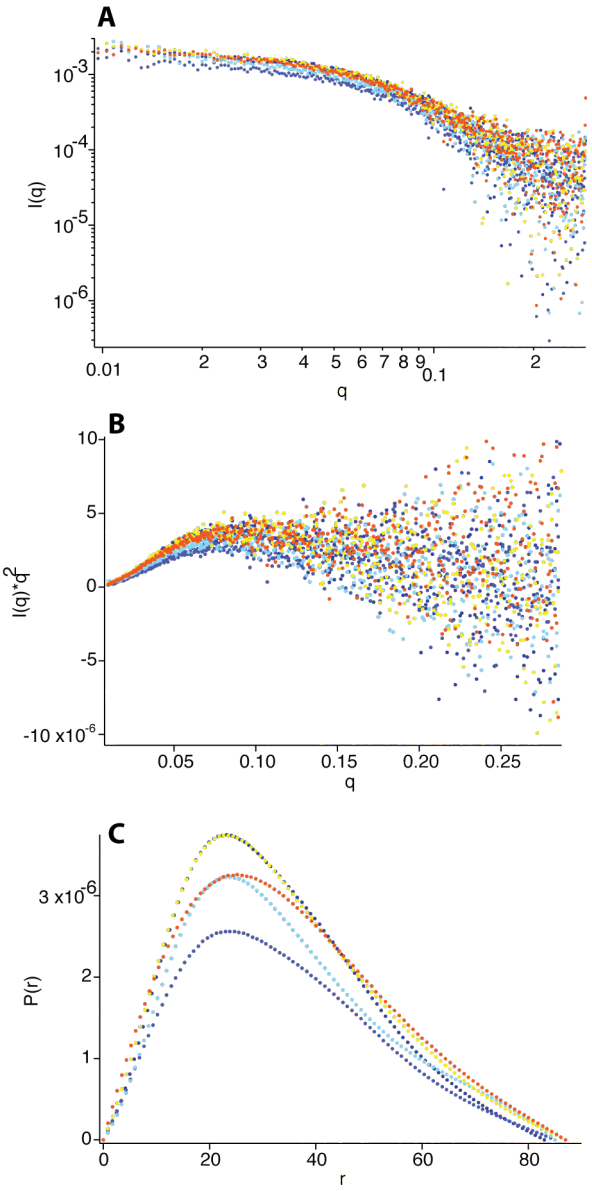

Figure S2. Small angle X-ray scattering data in buffer with 2.0 mM free Mg^{2+}. The experimental **(A)** scattering curves, **(B)** Kratky plots, and **(C)** Porod plots of tRNA mutants. Colors are as in Figure 1.

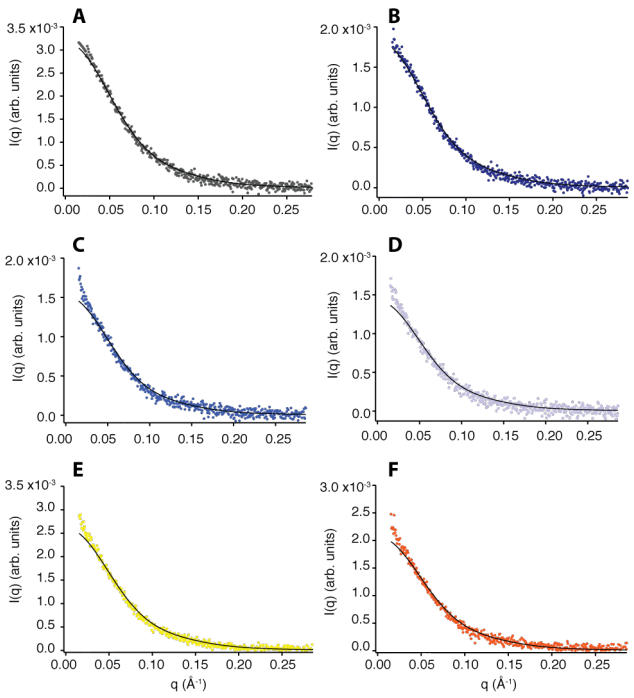

Figure S3. Comparison of experimental scattering curves of WT and mutant tRNAs (data points) and the theoretical scattering curve (smooth curve) of tRNA crystal structure, PDB: 1ehz, generated with FoXS. Experimental scattering curves of **(A)** WT, **(B)** M1, **(C)** M2, **(D)** M3, **(E)** M4, and **(F)** M5.

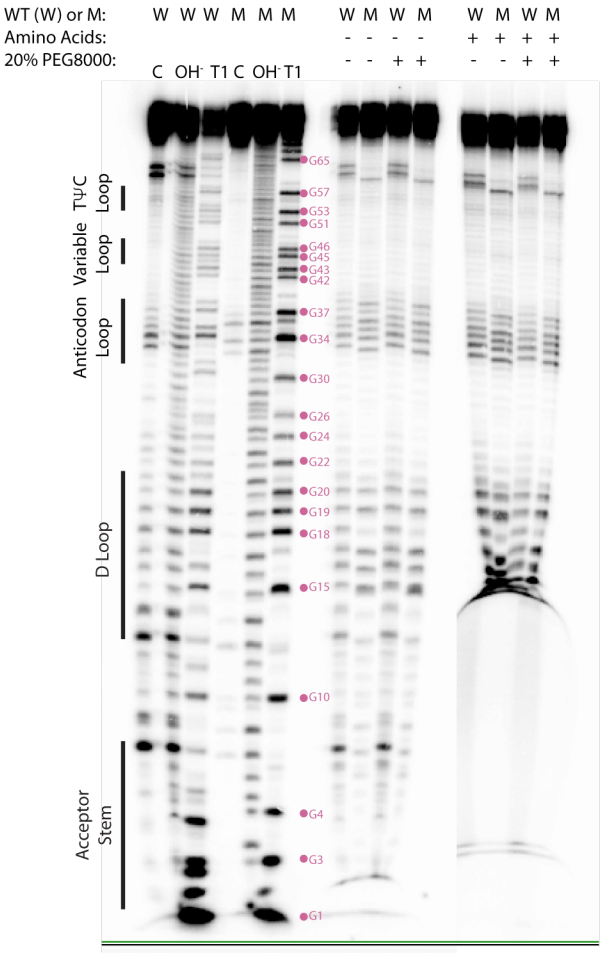

Figure S4. In-line probing PAGE of WT and M5 in buffer, 20% PEG800, Mg^{2+}-chelated amino acids, and 20% PEG8000 with Mg^{2+}-chelated amino acids. Control lanes are unreacted RNA, a hydrolysis ladder, and a denaturing T1 ladder.

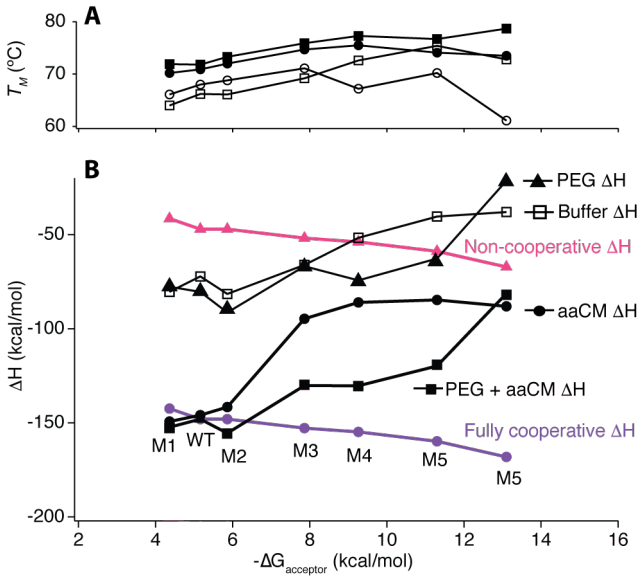

Figure S5. Melting temperature and enthalpy of unfolding of WT and mutant tRNAs in 2.0 mM free Mg^{2+}. **(Top)** Melting temperature and **(Bottom)** $\Delta H_{folding}$ of tRNA and mutants in buffer (open squares), 20% PEG8000 (closed squares), aaCM (circles), and 20% PEG8000 and additional aaCM (triangles). The non-cooperative ΔH was calculated using nearest neighbor parameters for the WT and mutant acceptor stems. See Materials and Methods for the calculation of non-cooperative ΔH and fully cooperative ΔH limits.

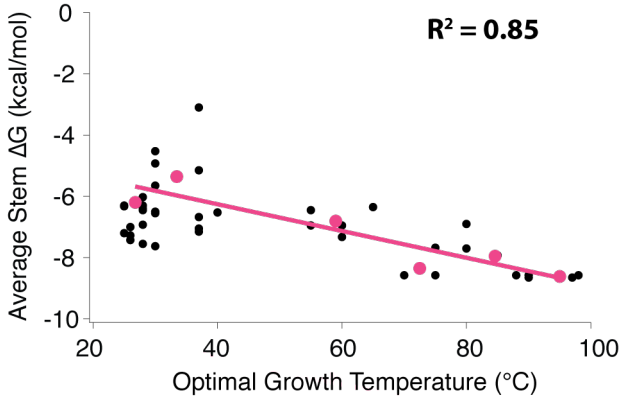

Figure S6. Average tRNA stem $\Delta G_{average}$ from organisms with a large range of optimal growing temperatures. The threshold $\Delta G_{folding}$ for stem stability is in pink. See Materials and Methods for further details.