

# Supporting Information

## Molecular Mechanism for Folding Cooperativity of Functional RNAs in Living Organisms

Kathleen A. Leamy<sup>1,2</sup>, Neela H. Yennawar<sup>3</sup>, and Philip C. Bevilacqua<sup>1,2,4</sup>

<sup>1</sup>Department of Chemistry, Pennsylvania State University, University Park, PA, 16802, U.S.A

<sup>2</sup>Center for RNA Molecular Biology, Pennsylvania State University, University Park, PA, 16802, U.S.A

<sup>3</sup>Huck Institutes of the Life Sciences, Pennsylvania State University, University Park, PA, 16802, U.S.A

<sup>4</sup>Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA, 16802, U.S.A

**P.C.B.:** email: [pcb5@psu.edu](mailto:pcb5@psu.edu),

address: 104 Chemistry Building, Pennsylvania State University, University Park, PA,  
16802, U. S. A

phone: 814-863-3812

**Table S1.** Thermodynamic parameters for tRNA folding 2.0 mM free Mg<sup>2+</sup>.

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

Constructs are ordered in the table according to the stability of the acceptor 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<sup>2+</sup>.

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

Constructs are ordered in the table according to the stability of the acceptor 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<sup>2+</sup>.

| RNA Construct   | 0.5 mM Mg <sup>2+</sup> $\chi^2$ | 2.0 mM Mg <sup>2+</sup> $\chi^2$ |
|---|----------------------------------|----------------------------------|
| <b>Buffer</b>   |                                  |                                  |
| 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                         |
| <b>20% PEG8000</b>  |                                  |                                  |
| 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                         |
| <b>Mg<sup>2+</sup> Chelated Amino Acids</b>               |                                  |                                  |
| 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                         |
| <b>PEG8000 &amp; Mg<sup>2+</sup> Chelated Amino Acids</b> |                                  |                                  |
| 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<sup>2+</sup>.

| RNA Construct | MW <sup>a</sup> (kDa) | R <sub>g</sub> <sup>a</sup> (Å) | R <sub>g</sub> <sup>b</sup> (Å) | D <sub>max</sub> <sup>b</sup> (Å) | Excluded Volume <sup>c</sup> (Å <sup>3</sup> ) | RMSD <sup>c</sup> (Å) | FoXS <sup>d</sup> χ <sup>2</sup> |
|---------------|-----------------------|---------------------------------|---------------------------------|-----------------------------------|--|-----------------------|----------------------------------|
| 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                             |

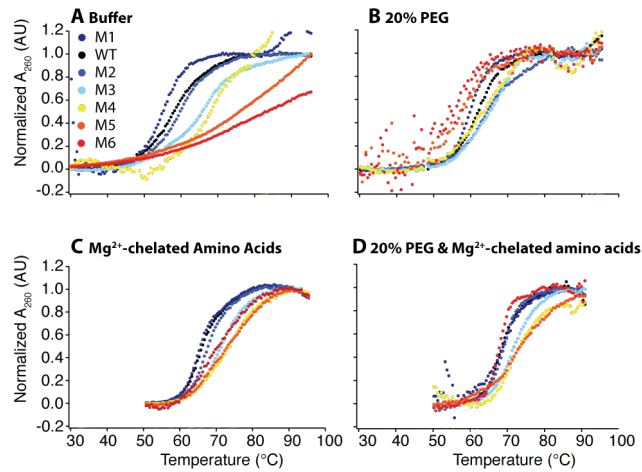
Solutions contain 25 mM HEPES (pH 7.5), 140 mM KCl, and 2.0 mM MgCl<sub>2</sub>. <sup>a</sup>Parameters were obtained by analysis of the experimental scattering curves using BioXTAS RAW software.

<sup>b</sup>Values were obtained using the pairwise distribution function in GNOM using the ATSAS software package. <sup>c</sup>Parameter was found using the alignments of the DAMAVER bead models with the tRNA crystal structure in SUPCOMB. <sup>d</sup>Parameter found from the alignments of experimental scattering curves to the predicted scattering curve of tRNA crystal structure (PDB ID: 1ehz)

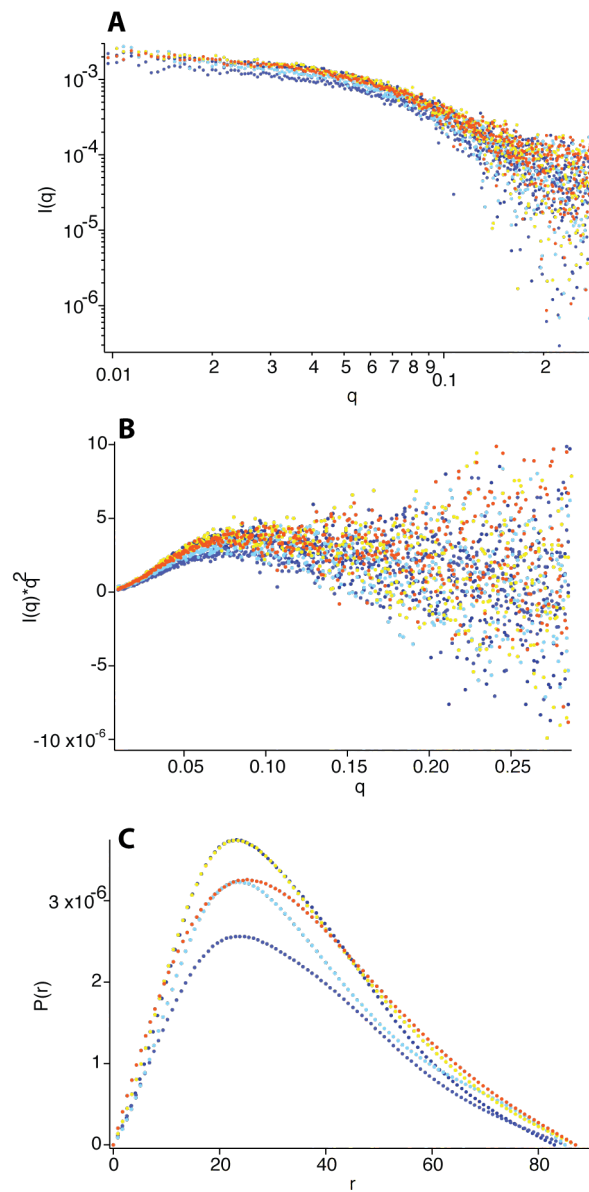
**Table S5.** Composition of samples containing Mg<sup>2+</sup>-chelated amino acids

| Concentration                        | 0.5 mM free Mg <sup>2+</sup> | 2.0 mM free Mg <sup>2+</sup> |
|--------------------------------------|------------------------------|------------------------------|
| Amino acids <sup>a</sup>             | 106.6 mM                     | 106.6 mM                     |
| Total Mg <sup>2+</sup>               | 4.6 mM                       | 16.0 mM                      |
| Free Mg <sup>2+</sup>                | 0.5 mM                       | 2.0 mM                       |
| Amino acid chelated Mg <sup>2+</sup> | 4.1 mM                       | 14.0 mM                      |
| KCl                                  | 140 mM                       | 140 mM                       |
| Sodium cacodylate                    | 10 mM                        | 10 mM                        |

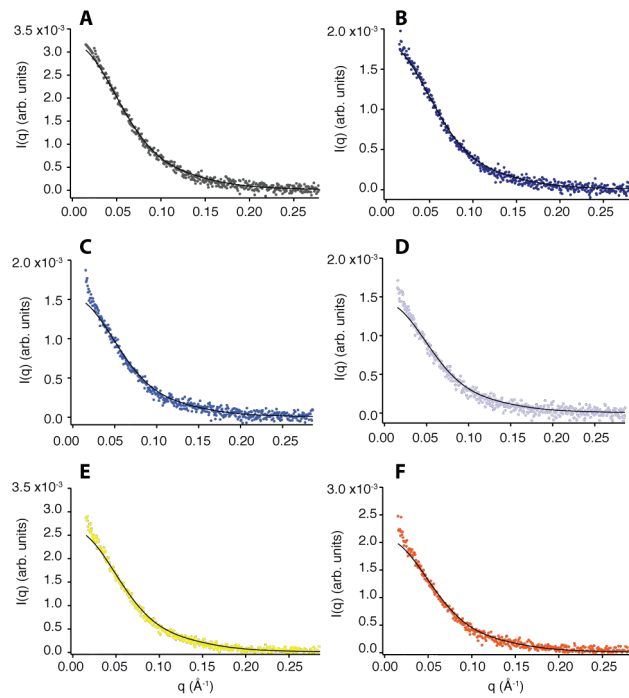
<sup>a</sup>Composed of 96.0 mM glutamate, 4.2 mM aspartate, 3.8 mM glutamine, and 2.6 mM alanine at pH 7.0.



**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.

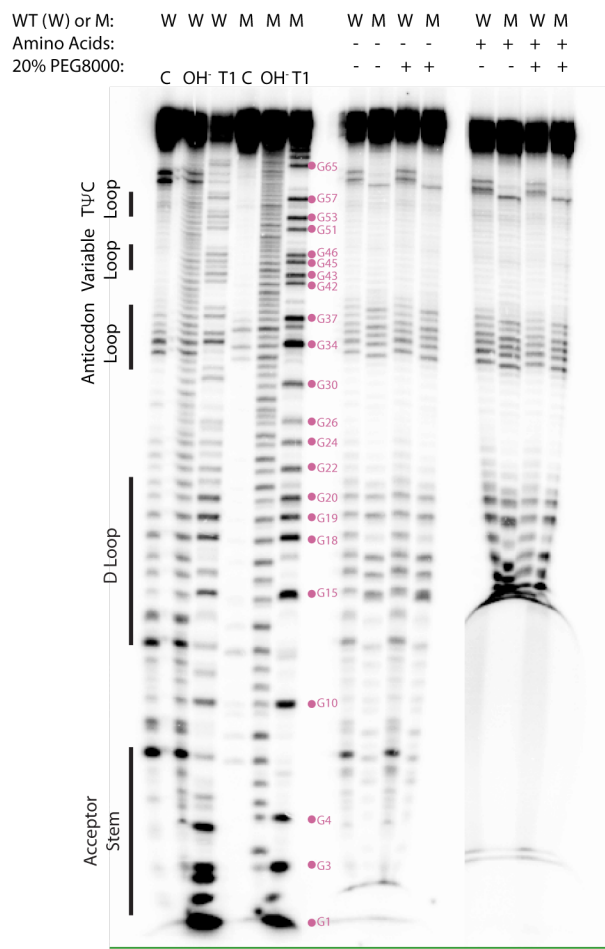


**Figure S2.** Small angle X-ray scattering data in buffer with 2.0 mM free  $\text{Mg}^{2+}$ . The experimental **(A)** scattering curves, **(B)** Kratky plots, and **(C)** Porod plots of tRNA mutants. Colors are as in Figure 1.

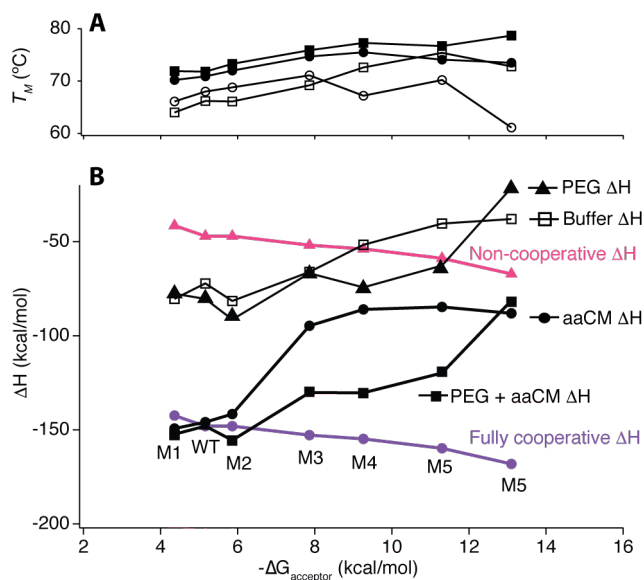


**Figure S3.** Comparison of experimental scattering curves of WT and mutant tRNAs (data points) and the theoretical scattering curve (smooth curve) of tRNA crystal structure, PDB: 1ehz, generated with FoXS. Experimental scattering curves of **(A)** WT, **(B)** M1, **(C)** M2, **(D)** M3, **(E)** M4,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

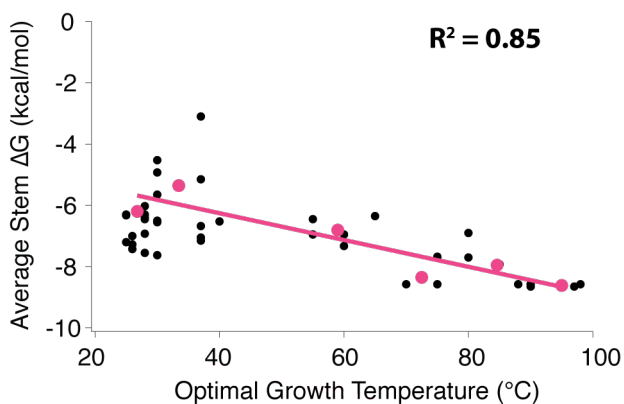




**Figure S4.** In-line probing PAGE of WT and M5 in buffer, 20% PEG800,  $Mg^{2+}$ -chelated amino acids, and 20% PEG8000 with  $Mg^{2+}$ -chelated amino acids. Control lanes are unreacted RNA, a hydrolysis ladder, and a denaturing T1 ladder.



**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)**  $\Delta H_{\text{folding}}$  of tRNA and mutants in buffer (open squares), 20% PEG8000 (closed squares), aaCM (circles), and 20% PEG8000 and additional aaCM (triangles). The non-cooperative  $\Delta H$  was calculated using nearest neighbor parameters for the WT and mutant acceptor stems. See Materials and Methods for the calculation of non-cooperative  $\Delta H$  and fully cooperative  $\Delta H$  limits.



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