Supporting information for:

Direct Measurement of Tertiary Contact Cooperativity in RNA Folding by Single Molecule FRET

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Supporting information

Methods

Molecule Construction

The three dye-labeled RNAs were created with a Moore/Sharp splint ligation[1] of three *in vitro*-transcribed pieces (ggA104-A148, G163-G234, and G250-A261+26 nucleotide extension[2]) and two purchased synthetic oligonucleotides (G149-U162, and A235-U249, Dharmacon, Inc., Lafayette,CO). The synthetic oligonucleotides (with internal U155 and U241 C5-amino-allyl modifications) were dye-labeled with Cy3 and Cy5 NHS esters (Amersham Biosciences, Piscatawy, NJ), respectively. The homogeneity of the 3′-ends of the transcribed pieces was ensured using a DNAzyme strategy[3]. All pieces were gel purified before and after the ligation reaction.

Single molecule FRET

Single molecule experiments were conducted on a home built prism total internal reflection fluorescence microscope equipped with a Cascade 512B CCD camera (Roper Scientific, Inc., Tucson, AZ) for imaging, as described previously[2]. Briefly, clean quartz slides were first coated with biotinylated BSA (Sigma Aldrich, St. Louis, MO) then coated with streptavidin (Sigma Aldrich, St. Louis, MO). The dye-labeled RNA constructs were annealed to a 3'-biotinylated DNA 26mer and incubated with the modified quartz surface. RNA-coated surfaces were incubated under imaging buffer containing 10 mM $MgCl_2$, 200 mM NaCl, 50 mM NaMOPS, pH 7.0, an oxygen scavenging system (0.125 µg/ml Glucose Oxidase, 0.025 µg/ml Catalase, 0.025% (w/v) glucose) to slow the dye photobleaching time, and a triplet state quencher (1 mM chloramphenicol and 1 mM 2-mercaptoethanol) to minimize photophysical effects. Molecules were excited with laser light at 532 nm. Cy3 and Cy5 fluorescence were collected on opposite halves of the same CCD camera by filtering the collected light through HQ580/60 m and HQ700/75 m band-pass filters (Chroma Technology Corp., Rockingham, VT), respectively. The FRET traces and cumulative distributions displayed in Fig. 2 & S1 are corrected for crosstalk between the red and green channels, thereby producing some apparent negative FRET values. The reported folding equilibrium constants are the ratio of total time spent in the high FRET and low FRET states and are reported for >1000 molecules. Error estimates were produced by bootstrap analysis of the data[4] and are at most ±0.01 in the fraction folded.

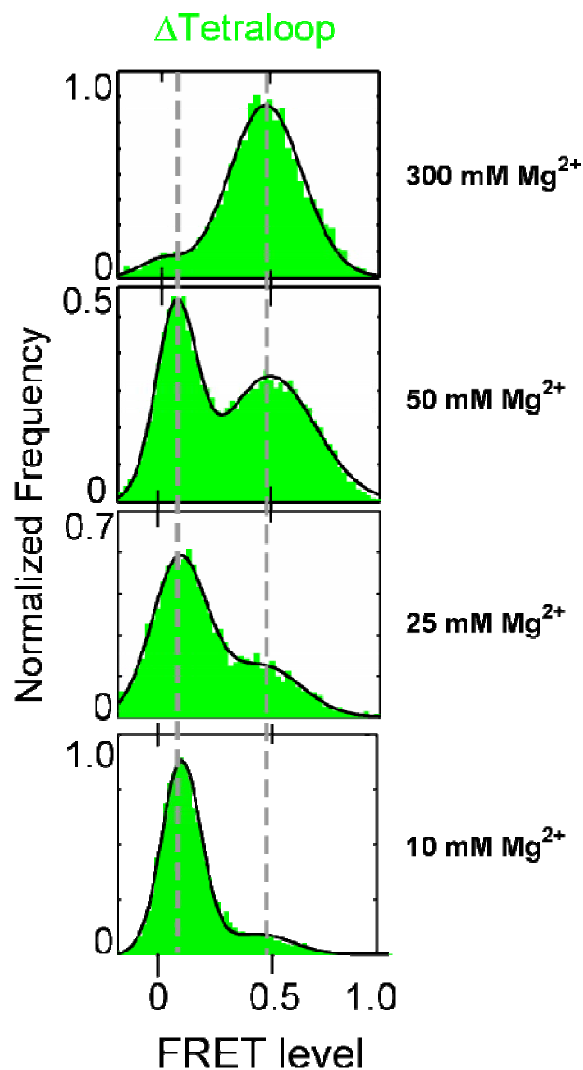

Figure S1. The FRET level of the high FRET state of the ΔTL (tetraloop) mutant P4-P6 is independent of Mg^{2+} concentration. Cumulative distribution histograms of the tetraloop ablation construct are shown for a series of Mg^{2+} concentrations (top to bottom): 300, 50, 25, and 10 mM Mg^{2+} (in a buffer containing 200 mM NaCl, 50 mM NaMOPS, pH 7.0, 22 °C). The black lines represent unrestricted fits to a two-peak Gaussian model. In each case the value for the high FRET state is the same within error (values of 0.47, 0.51, 0.46, 0.48 from top to bottom). The observation that this FRET value does not change as a function of Mg^{2+} and changes in the folding equilibrium constant indicates that the value is not significantly affected by temporal averaging of higher and lower FRET values from rapidly converting unfolded and folded states.

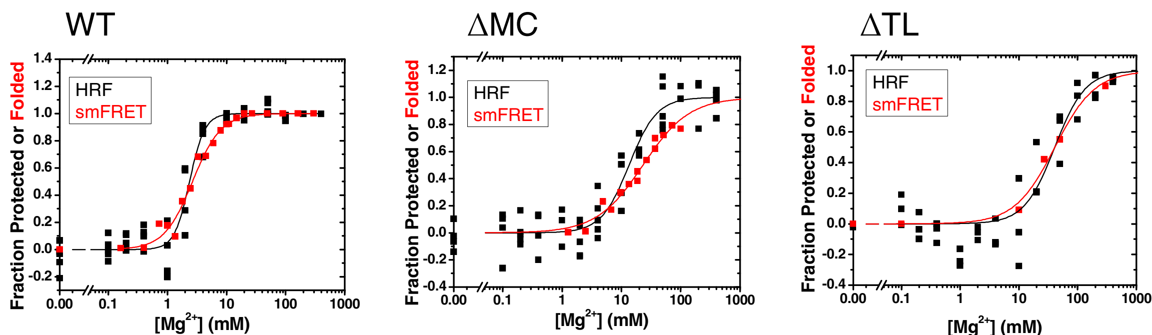

Figure S2. Comparison of Mg^{2+}-dependent folding transition as measured by hydroxyl radical footprinting (HRF, black), or by single molecule FRET (smFRET, red) of the WT and 2 tertiary contact ablation mutants. All experiments were performed in a buffer comprised of 200 mM NaCl, 50 mM NaMOPS, pH 7, at 22 °C. The HRF was performed and data was normalized as previously described[5]. The lines represent two parameter Hill fits of the HRF (black) or smFRET (red) data. (WT: $[Mg^{2+}]_{1/2}$ = 2.6 ± 0.2 and 2.4 ± 0.1 mM (smFRET & footprinting, respectively); ΔMetal Core: $[Mg^{2+}]_{1/2}$ = 18 ± 4 and 13.5 ± 1.4 mM; ΔTetraloop: $[Mg^{2+}]_{1/2}$ = 41 ± 4 and 41 ± 7 mM;):

$$\text{Fraction Protected or Folded} = \frac{[\mathrm{Mg}^{2+}]^n}{([\mathrm{Mg}^{2+}]_{1/2}^{n} + [\mathrm{Mg}^{2+}]^n)}$$

(The Hill equation is used solely as an empirical descriptor of the data.)

Table S1. Extrapolated HRF Hill fits to calculate tertiary contact cooperativity at 10 mM Mg^{2+} (200 mM NaCl, 50 mM NaMOPS, pH 7, 22 °C).

	$[Mg^{2+}]_{1/2}$ (mM)	n_{Hill}	Frac folded (10 mM Mg)	K (10 mM Mg)[a]	K' (10 mM Mg)[a,b]	K'/K	ΔG_{coop} = RTln(K'/K) (kcal/mol)
WT	2.4 ± 0.1	3.4 ± 0.6	0.992	128	n/a	n/a	n/a
ΔMetal Core	13.5 ± 1.4	1.9 ± 0.3	0.361	0.57	1406	2466	4.6
ΔTetraloop	41 ± 7	1.7 ± 0.3	0.083	0.091	224	2466	4.6

[a] Equilibrium constants are defined in Figure 1c of the main text. K_{TL} and K_{MC} were determined using the ΔMetal Core and ΔTetraloop mutants, respectively, and are extrapolated to 10 mM Mg using the Hill Equation above.
[b] K'_{TL} and K'_{MC} were calculated from the measured equilibrium constants as in equation 1 of the main text.

References:

(1) Moore, M. J.; Sharp, P. A. *Science* **1992**, *256*, 992-997.

(2) Zhuang, X. W.; Bartley, L. E.; Babcock, H. P.; Russell, R.; Ha, T. J.; Herschlag, D.; Chu, S. *Science* **2000**, *288*, 2048-2051.

(3) Pyle, A. M.; Chu, V. T.; Jankowsky, E.; Boudvillain, H. *Methods Enzymol.* **2000**, *317*, 140-146.

(4) Efron, B.; Tibshirani, R. *An introduction to the bootstrap*; Chapman & Hall: New York, 1993.

(5) Takamoto, K.; Das, R.; He, Q.; Doniach, S.; Brenowitz, M.; Herschlag, D.; Chance, M. R. *J. Mol. Biol.* **2004**, *343*, 1195-1206.