--Supporting Information--

Pairwise coupling analysis of tertiary structural hydrogen bonding interactions in luteoviral RNA pseudoknots

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TABLE S1: Melting transition temperatures ($t_{m,i}$) and thermodynamic parameters (ΔG_i and ΔH_i) for the folding of the wild-type ScYLV RNA pseudoknot acquired over a range of pH values. The resolved parameters reflect the mean and standard error derived from 3-5 replicate melting profiles as described in Materials and Methods. A two-transition unfolding model was used to fit individual melting profiles was applied (see Scheme 1) with the derived parameters shown only for transition 1 (*1*). See Fig. 3, panels A, C, E for deconvolutions of representative melting profiles.

TABLE S2: Melting transition temperatures ($t_{m,i}$) and thermodynamic parameters (ΔG_i and ΔH_i) for the folding of the dC14 ScYLV RNA pseudoknot acquired over a range of pH values. The resolved parameters reflect the mean and standard error derived from at least three replicate melting profiles. A three-transition fit was judged satisfactory to fit these melting profiles over the entire pH range (see Scheme 2). Derived parameters are shown for transitions 1 and 2 only. See Fig. 3, panels B, D, F for representative melting profile deconvolutions.

TABLE S3. Melting transition temperatures ($t_{m,i}$) and thermodynamic parameters (ΔG_i and ΔH_i) for the folding of the C27A ScYLV RNA pseudoknot acquired over a range of pH values. The resolved parameters reflect the mean and standard error derived from 3-5 replicate melting profiles. A two transition unfolding model was used to fit individual melting profiles (*1*). See Fig. 4, panels A, C, E for deconvolutions of representative melting profiles.

TABLE S4. Melting transition temperatures ($t_{m,i}$) and thermodynamic parameters (ΔG_i and ΔH_i) for the folding of the dC14/C27A ScYLV RNA pseudoknot acquired over a range of pH values. The resolved parameters reflect the mean and standard error derived from at least three replicate melting profiles as described in Materials and Methods. A three-transition fit was judged satisfactory to fit these melting profiles from pH 5.0 to 7.0 (see Scheme 2), with the first unfolding transition apparently lost at higher pH (>7.0; $\Delta H_1 \leq 5$ kcal mol^{-1}, a lower limit of detection) and was therefore fit to Scheme 1. See Fig. 4, panels B, D, F for deconvolutions of representative melting profiles.

TABLE S1: WT ScYLV thermodynamic parameters

pH	t_{m1} (ºC)	ΔH_1 (kcal mol^{-1})	ΔG_1 (kcal mol^{-1})
5.0	77.5 ± 0.3	-55.2 ± 0.6	-6.4 ± 0.1
5.5	77.9 ± 0.3	-55.2 ± 0.7	-6.5 ± 0.1
6.0	76.4 ± 0.4	-56.9 ± 0.7	-6.4 ± 0.1
6.5	75.5 ± 0.6	-55.8 ± 1.4	-6.2 ± 0.1
7.0	72.6 ± 0.7	-55.3 ± 0.8	-5.7 ± 0.1
7.5	71.1 ± 0.3	-51.1 ± 0.3	-5.1 ± 0.1
8.0	67.4 ± 0.3	-46.5 ± 1.0	-4.2 ± 0.1
8.5	64.6 ± 0.4	-45.1 ± 0.4	-3.7 ± 0.1
9.0	64.6 ± 0.3	-44.0 ± 0.5	-3.6 ± 0.1

TABLE S2: dC14 ScYLV thermodynamic parameters

pH	t_{m1} (ºC)	ΔH_1 (kcal mol^{-1})	ΔG_1 (kcal mol^{-1})	t_{m2} (ºC)	ΔH_2 (kcal mol^{-1})	ΔG_2 (kcal mol^{-1})
5.0	58.2 ± 0.4	-33.4 ± 0.7	-2.2 ± 0.1	67.6 ± 0.5	-43.1 ± 0.5	-3.9 ± 0.1
5.5	58.5 ± 0.1	-31.7 ± 0.3	-2.1 ± 0.1	68.3 ± 0.1	-43.1 ± 0.1	-4.0 ± 0.1
6.0	57.0 ± 0.1	-29.2 ± 1.4	-1.8 ± 0.1	65.3 ± 0.4	-45.3 ± 0.4	-3.8 ± 0.1
6.5	56.3 ± 0.6	-31.0 ± 1.8	-1.8 ± 0.2	65.4 ± 0.8	-42.1 ± 0.5	-3.6 ± 0.1
7.0	55.7 ± 0.3	-31.9 ± 1.3	-1.8 ± 0.1	64.0 ± 0.6	-40.7 ± 1.6	-3.3 ± 0.1
7.5	53.8 ± 1.3	-35.9 ± 3.5	-1.9 ± 0.2	61.9 ± 0.4	-36.1 ± 1.3	-2.7 ± 0.1
8.0	53.6 ± 0.4	-32.3 ± 2.0	-1.7 ± 0.1	61.8 ± 1.9	-29.1 ± 3.3	-2.2 ± 0.1
8.5	50.6 ± 0.3	-35.1 ± 0.4	-1.5 ± 0.1	62.6 ± 0.7	-30.7 ± 2.9	-2.4 ± 0.3
9.0	47.8 ± 0.3	-34.1 ± 0.8	-1.2 ± 0.1	61.7 ± 0.6	-32.4 ± 1.5	-2.4 ± 0.1

TABLE S3: C27A ScYLV thermodynamic parameters

pH	t_{m1} (ºC)	ΔH_1 (kcal mol^{-1})	ΔG_1 (kcal mol^{-1})
5.0	73.0 ± 0.5	-60.2 ±1.0	-6.3 ± 0.1
5.5	73.0 ± 0.3	-58.2 ± 0.2	-6.1 ± 0.1
6.0	71.6 ± 0.5	-54.7 ± 0.4	-5.5 ± 0.1
6.5	70.1 ± 0.3	-56.6 ± 1.0	-5.5 ± 0.1
7.0	69.3 ± 0.9	-43.3 ± 1.8	-4.1 ± 0.1
7.5	66.0 ± 0.2	-46.6 ± 1.2	-4.0 ± 0.1
8.0	62.9 ± 0.3	-41.4 ± 1.2	-3.2 ± 0.1
8.5	60.7 ± 0.4	-39.6 ± 0.8	-2.8 ± 0.1
9.0	60.2 ± 0.9	-32.9 ± 1.1	-2.3 ± 0.1

Table S4: dC14/C27A ScYLV thermodynamic parameters

pH	dC14/C27A ScYLV					
	t_{m1} (ºC)	ΔH_1 (kcal mol^{-1})	ΔG_1 (kcal mol^{-1})	t_{m2} (ºC)	ΔH_2 (kcal mol^{-1})	ΔG_2 (kcal mol^{-1})
5.0	56.0 ± 0.1	-29.5 ± 2.8	-1.7 ± 0.2	68.2 ± 2.6	-43.5 ± 5.0	-4.0 ± 0.8
5.5	56.0 ± 0.1	-35.4 ± 4.2	-2.1 ± 0.2	69.3 ± 1.1	-48.0 ± 1.7	-4.6 ± 0.2
6.0	55.0 ± 0.1	-27.9 ± 2.7	-1.5 ± 0.2	68.4 ± 0.5	-41.9 ± 3.2	-3.9 ± 0.3
6.5	54.0 ± 0.1	-32.8 ± 4.1	-1.7 ± 0.2	66.9 ± 0.1	-44.7 ± 1.8	-4.0 ± 0.1
7.0	53.3 ± 1.2	-31.7 ± 1.6	-1.6 ± 0.2	64.9 ± 0.2	-40.6 ± 5.3	-3.4 ± 0.4
7.5	–	–	–	62.8 ± 0.4	-38.0 ± 1.0	-2.9 ± 0.1
8.0	–	–	–	59.6 ± 0.2	-36.9 ± 1.0	-2.5 ± 0.1
8.5	–	–	–	58.1 ± 0.3	-34.7 ± 1.9	-2.2 ± 0.1
9.0	–	–	–	56.5 ± 0.9	-34.4 ± 3.2	-2.0 ± 0.1

References:

1. Cornish, P. V., Hennig, M., and Giedroc, D. P. (2005) A loop 2 cytidine-stem 1 minor groove interaction as a positive determinant for pseudoknot-stimulated -1 ribosomal frameshifting. *Proc Natl Acad Sci U S A 102*, 12694-12699.