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Supplemental information

Thermodynamic determination of RNA duplex stability in magnesium solutions

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Supplemental Data

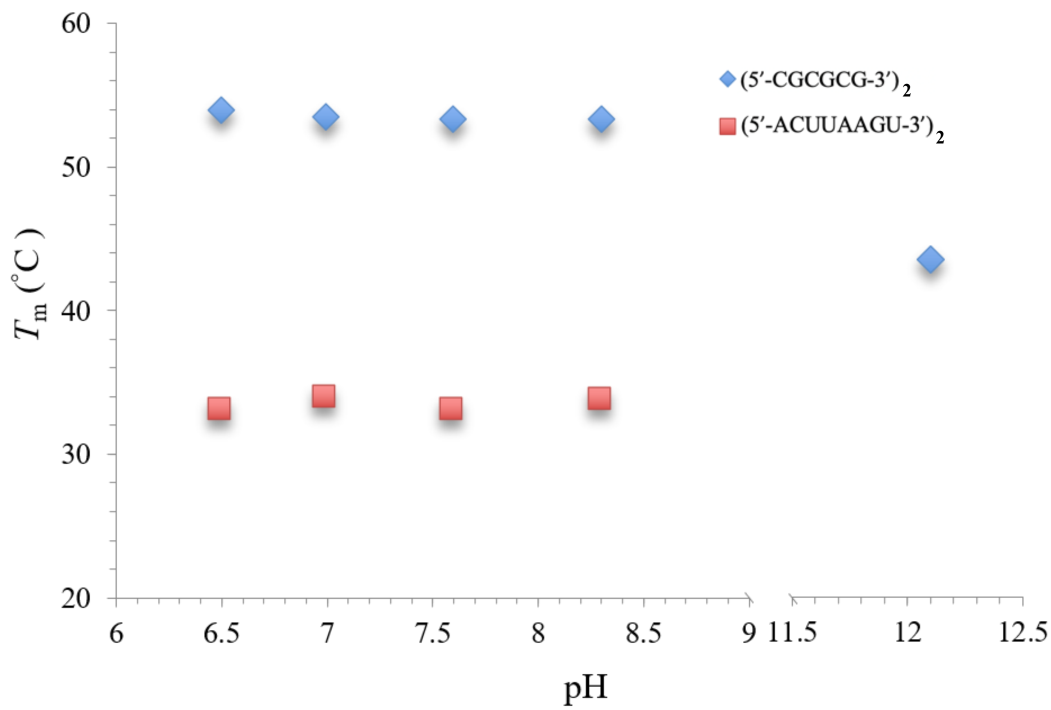

FIGURE S1. Melting temperatures (T_m) for (5'-CGCGCG-3')$_2$ and (5'-ACUUAAGU-3')$_2$ in buffered solutions at pH 6.5, 7.0, 7.6, 8.3, and 12.1. Data was acquired following the same melt scheme outlined in the Optical Melting Studies section of the Materials and Methods in the main text. *MeltWin v3.5* (15) was used to fit the data from the melt curves to an assumed two-state model and construct a T_m^{-1} vs ln C_T plots. Data from the T_m^{-1} vs ln C_T plots were used to determine the T_m of each duplex at 0.1 mM.

Supplemental Data

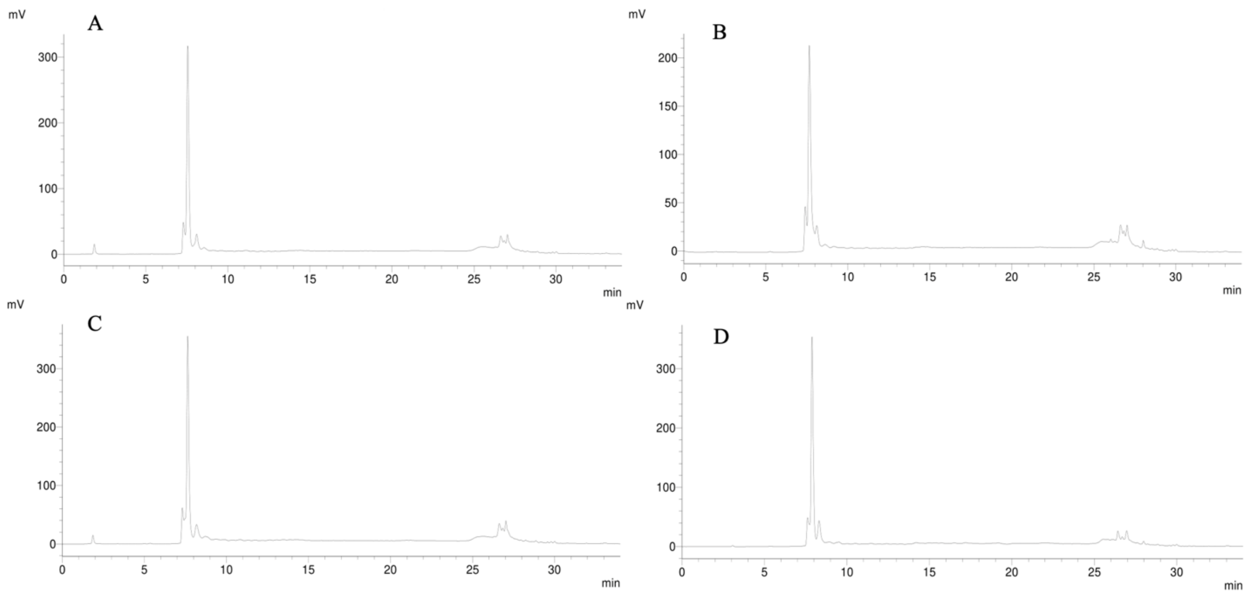

FIGURE S2. Liquid chromatography chromatograms for (5'-CGCGCG-3')$_2$ before and after melting experiments. The before melting in (A) 10.0 mM $MgCl_2$ with 2 mM Tris and (B) in standard 1.0 M NaCl buffer chromatograms. Additionally, after melting in (C) 10.0 mM $MgCl_2$ with 2 mM Tris and (D) in 1.0 M NaCl standard buffer chromatograms.

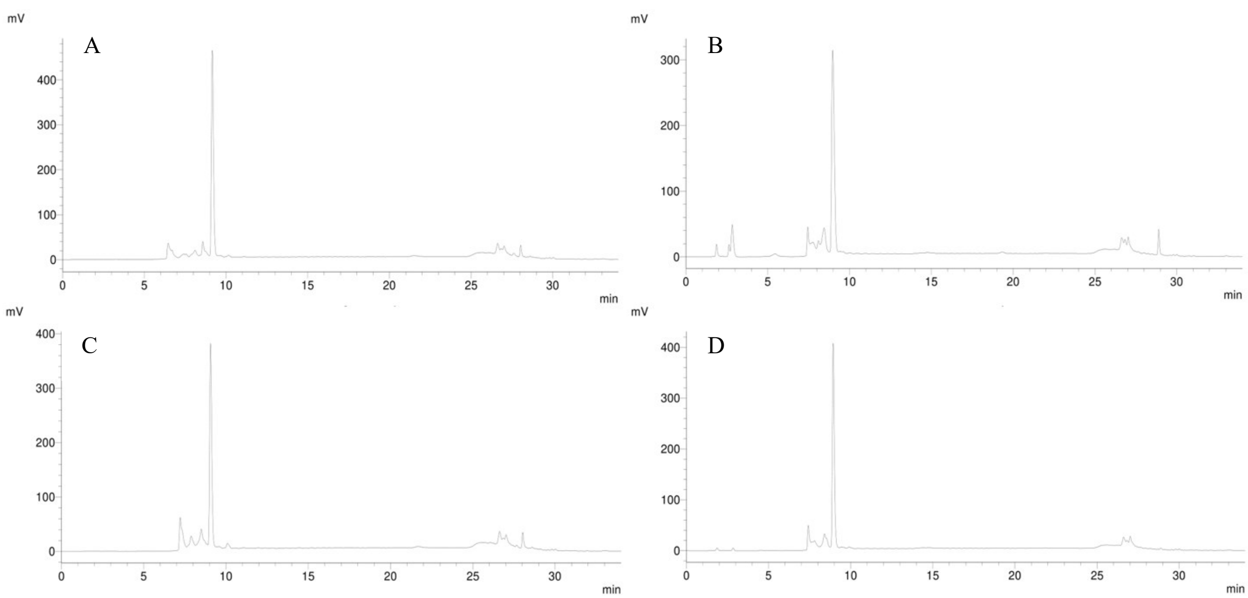

FIGURE S3. Liquid chromatography chromatograms for (5'-ACUUAAGU-3')$_2$ before and after melting experiments. The before melting in (A) 10.0 mM $MgCl_2$ with 2 mM Tris and (B) in standard 1.0 M NaCl buffer chromatograms. Additionally, after melting in (C) 10.0 mM $MgCl_2$ with 2 mM Tris and (D) in 1.0 M NaCl standard buffer chromatograms.

Supplemental Data

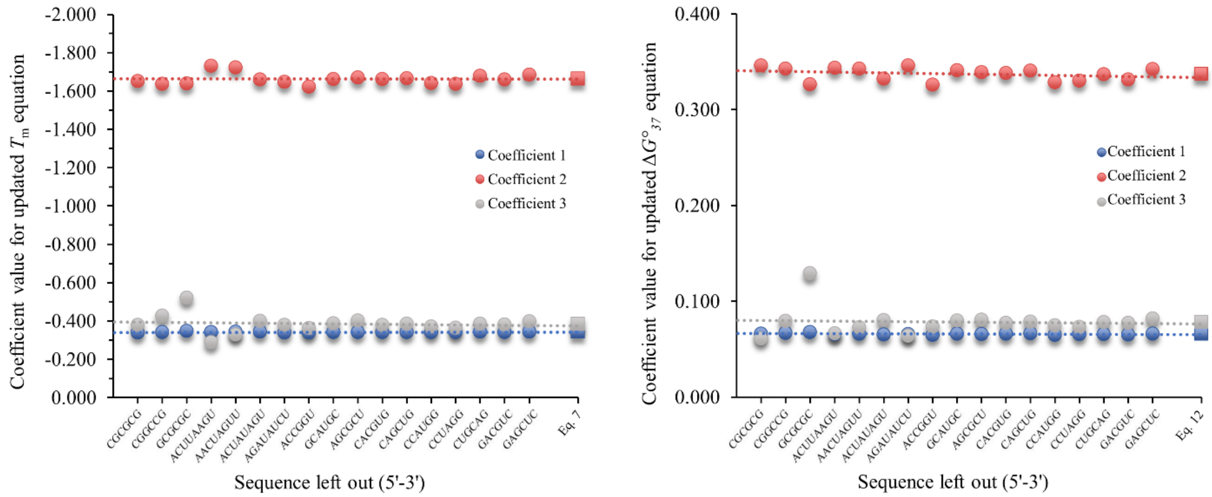

FIGURE S4. Leave-one-out analysis with updated coefficients for Eq. 7 and Eq. 12. The sequences excluded in each of the leave-one-out analyses are shown on the horizontal axis. The updated coefficients used in the Eq. 7 (left) and Eq. 12 (right) leave-one-out analysis are shown as circles with the actual, proposed coefficients shown as squares. Coefficients are numbered in the order they appear in the equations.

Supplemental Data

TABLE S1. Experimental RNA Thermodynamic Parameters for Duplexes Subjected to Forward and Reverse Optical Melting Experiments in Buffers Containing Magnesium and Standard Conditions.

RNA Sequence (5' to 3')[b]	Exper.[c]	[Salt]	Analysis of curve fits ΔH° (kcal/mol)	ΔS° (cal/K·mol)	ΔG°_{37} (kcal/mol)	T_m[a] (°C)	Analysis of T_m^{-1} vs. ln C_T Plot ΔH° (kcal/mol)	ΔS° (cal/K·mol)	ΔG°_{37} (kcal/mol)	T_m[a] (°C)
CGCGCG	Forward	10.0 mM $MgCl_2$	-57.2 ± 2.7	-153.6 ± 8.4	-9.59 ± 0.16	59.8	-59.9 ± 1.8	-161.6 ± 5.4	-9.75 ± 0.12	59.6
	Reverse	10.0 mM $MgCl_2$	-58.6 ± 2.2	-158.0 ± 7.0	-9.62 ± 0.08	59.4	-60.8 ± 2.9	-164.5 ± 8.7	-9.74 ± 0.19	59.2
	Forward	1.0 M NaCl	-56.5 ± 4.2	-152.5 ± 12.9	-9.17 ± 0.20	57.4	-59.9 ± 1.5	-163.1 ± 4.6	-9.31 ± 0.08	57.0
	Reverse	1.0 M NaCl	-57.1 ± 2.4	-154.1 ± 7.3	-9.26 ± 0.16	57.8	-56.7 ± 1.4	-153.2 ± 4.2	-9.21 ± 0.08	57.6
ACUUAAGU	Forward	10.0 mM $MgCl_2$	-61.5 ± 8.4	-177.8 ± 26.9	-6.36 ± 0.07	40.5	-58.3 ± 6.5	-167.8 ± 21.0	-6.26 ± 0.08	40.2
	Reverse	10.0 mM $MgCl_2$	-59.4 ± 8.1	-171.1 ± 25.5	-6.32 ± 0.20	40.4	-52.2 ± 1.5	-148.2 ± 4.7	-6.21 ± 0.00	40.2
	Forward	1.0 M NaCl	-63.3 ± 7.8	-183.9 ± 24.9	-6.30 ± 0.14	40.1	-52.4 ± 3.3	-148.8 ± 10.6	-6.24 ± 0.03	40.4
	Reverse	1.0 M NaCl	-59.2 ± 6.9	-170.6 ± 22.2	-6.33 ± 0.04	40.5	-53.9 ± 4.2	-153.5 ± 13.8	-6.25 ± 0.05	40.4

[a]Calculated for 0.1 mM oligonucleotide concentrations based on the T_m^{-1} vs ln C_T plots.
[b]All oligonucleotides are forming self-complementary duplexes in solution.
[c]Forward and reverse optical melting experiments followed the same heating rate as mentioned in the optical melting studies section of the Materials and Methods.

TABLE S2. Experimental RNA Thermodynamic Parameters for Reused or Fresh Duplexes in the Melt Scheme.

RNA Sequence (5' to 3')[b]	Oligo.[c]	[Salt]	Analysis of curve fits ΔH° (kcal/mol)	ΔS° (cal/K·mol)	ΔG°_{37} (kcal/mol)	T_m[a] (°C)	Analysis of T_m^{-1} vs. ln C_T Plot ΔH° (kcal/mol)	ΔS° (cal/K·mol)	ΔG°_{37} (kcal/mol)	T_m[a] (°C)
CGCGCG	Reused	10.0 mM $MgCl_2$	-58.5 ± 6.9	-156.9 ± 20.9	-9.80 ± 0.48	60.5	-59.9 ± 5.0	-161.3 ± 15.2	-9.86 ± 0.33	60.3
	Fresh	10.0 mM $MgCl_2$	-57.3 ± 2.7	-154.1 ± 8.3	-9.54 ± 0.12	59.4	-62.7 ± 2.4	-170.4 ± 7.3	-9.86 ± 0.16	59.2
ACUUAAGU	Reused	10.0 mM $MgCl_2$	-69.9 ± 8.7	-204.6 ± 28.0	-6.42 ± 0.08	40.4	-69.9 ± 1.8	-204.8 ± 5.6	-6.34 ± 0.01	40.0
	Fresh	10.0 mM $MgCl_2$	-59.6 ± 6.4	-171.9 ± 20.7	-6.32 ± 0.06	40.4	-56.1 ± 2.1	-160.8 ± 6.9	-6.25 ± 0.02	40.2

[a]Calculated for 0.1 mM oligonucleotide concentrations based on the T_m^{-1} vs ln C_T plots.
[b]All oligonucleotides are forming self-complementary duplexes in solution.
[c]Reused oligonucleotide in the melt scheme corresponds to performing an optical melting experiment where previously melted oligonucleotide samples were diluted and melted again at a different concentration. Fresh oligonucleotide in the melt scheme corresponds to performing an optical melting experiment where fresh, never melted oligonucleotide samples were used at each concentration.

Supplemental Data

TABLE S3. Experimental RNA Thermodynamic Parameters for Duplex Formation.

		Analysis of curve fits				Analysis of T_m^{-1} vs. ln C_T Plot			
Sequence[a]	[Mg^{2+}]	ΔH° (kcal/mol)	ΔS° (cal/K·mol)	ΔG°_{37} (kcal/mol)	T_m[c] (˚C)	ΔH° (kcal/mol)	ΔS° (cal/K·mol)	ΔG°_{37} (kcal/mol)	T_m[c] (˚C)
CGCGCG	0.5 mM	-56.8±7.4	-155.4±22.8	-8.59±0.31	53.8	-62.2±4.1	-172.1±12.7	-8.78±0.18	53.3
	1.5 mM	-59.4±8.0	-161.6±24.5	-9.31±0.38	57.2	-60.3±2.3	-164.3±6.9	-9.35±0.11	57.1
	3.0 mM	-55.3±2.2	-148.4±7.1	-9.25±0.08	58.4	-58.1±5.1	-156.8±15.7	-9.40±0.32	58.3
	10.0 mM	-58.5±6.9	-156.9±20.9	-9.80±0.48	60.5	-59.9±5.0	-161.3±15.2	-9.86±0.33	60.3
	1 M NaCl[b]	-53.8	-144.2	-9.08	57.9	-54.5	-146.4	-9.12	57.8
CGGCCG	0.5 mM	-56.1±9.2	-150.8±28.1	-9.37±0.51	58.9	-57.6±5.2	-155.5±15.9	-9.37±0.32	58.2
	1.5 mM	-54.3±2.7	-143.9±8.1	-9.64±0.15	61.4	-56.5±0.9	-150.7±2.6	-9.77±0.06	61.2
	3.0 mM	-55.5±7.7	-146.5±22.6	-10.12±0.69	63.9	-55.6±3.9	-146.7±11.6	-10.07±0.30	63.6
	10.0 mM	-55.2±4.1	-145.0±12.1	-10.25±0.32	65.0	-55.5±3.6	-145.9±10.8	-10.25±0.26	64.9
	1 M NaCl[b]	-56.6	-150.0	-10.11	63.3	-54.1	-142.6	-9.90	63.2
GCGCGC	0.5 mM	-59.6±7.5	-162.3±22.6	-9.29±0.46	57.0	-60.9±5.9	-166.1±17.9	-9.34±0.34	56.9
	1.5 mM	-60.8±8.6	-164.6±25.7	-9.78±0.70	59.5	-59.8±14.9	-161.4±44.4	-9.70±1.40	59.4
	3.0 mM	-60.6±4.0	-163.1±12.1	-10.04±0.25	61.0	-62.0±2.8	-167.4±8.4	-10.13±0.17	61.0
	10.0 mM	-59.3±6.1	-158.3±18.3	-10.19±0.47	62.6	-60.2±7.5	-161.2±22.2	-10.22±0.59	62.3
	1 M NaCl[b]	-61.2	-164.0	-10.38	62.8	-64.3	-173.4	-10.56	62.5
ACCGGU	0.5 mM	-55.0±5.0	-150.5±15.4	-8.36±0.25	52.9	-60.1±3.2	-166.3±9.9	-8.50±0.12	52.3
	1.5 mM	-54.5±2.7	-147.4±8.5	-8.79±0.12	55.8	-55.8±2.4	-151.4±7.5	-8.83±0.10	55.6
	3.0 mM	-59.6±9.1	-162.1±27.6	-9.30±0.53	57.1	-58.7±7.8	-159.5±23.9	-9.21±0.46	56.8
	10.0 mM	-59.6±9.2	-161.0±28.2	-9.64±0.50	59.1	-63.0±2.7	-171.8±8.2	-9.74±0.13	58.4
	1 M NaCl[b]	-53.2	-144.9	-8.26	52.8	-59.8	-164.5	-8.51	53.9
AGCGCU	0.5 mM	-49.9±7.0	-139.2±22.2	-6.70±0.15	43.5	-48.6±3.6	-135.1±11.6	-6.65±0.10	43.3
	1.5 mM	-46.9±3.2	-127.8±10.0	-7.28±0.15	48.0	-52.8±4.7	-146.7±14.9	-7.33±0.15	47.0
	3.0 mM	-48.5±4.8	-132.1±14.9	-7.55±0.26	49.5	-51.3±2.9	-140.7±9.3	-7.61±0.10	49.2
	10.0 mM	-52.0±6.2	-141.6±18.9	-8.10±0.34	52.1	-54.3±3.0	-148.9±9.4	-8.13±0.12	51.7
	1 M NaCl[b]	-50.9	-137.9	-8.13	52.7	-50.1	-135.7	-7.99	52.0
	0.5 mM	-54.2±2.4	-155.3±7.8	-5.99±0.06	38.8	-53.3±1.5	-152.5±4.9	-5.96±0.02	38.7

Supplemental Data

CACGUG	1.5 mM	-56.1±4.2	-159.8±13.3	-6.52±0.08	41.7	-52.7±2.0	-149.2±6.4	-6.46±0.03	41.7
	3.0 mM	-56.5±2.8	-160.3±8.8	-6.81±0.08	43.3	-56.5±1.4	-160.2±4.5	-6.78±0.02	43.2
	10.0 mM	-56.8±5.7	-160.3±17.8	-7.09±0.19	44.9	-55.3±3.5	-155.5±11.2	-7.05±0.09	44.9
	1 M NaCl[b]	-55.3	-156.8	-6.68	42.8	-50.3	-141.0	-6.59	42.7
CAGCUG	0.5 mM	-52.4±3.5	-150.3±11.1	-5.81±0.19	37.8	-51.8±4.4	-148.3±14.1	-5.83±0.12	37.9
	1.5 mM	-54.6±7.9	-155.3±25.3	-6.41±0.11	41.2	-53.8±2.0	-153.0±6.3	-6.32±0.02	40.8
	3.0 mM	-54.4±3.6	-153.9±11.6	-6.65±0.06	42.6	-54.7±2.0	-155.1±6.3	-6.61±0.02	42.4
	10.0 mM	-54.9±4.0	-154.4±12.6	-7.04±0.10	44.9	-54.7±1.5	-153.9±4.8	-6.99±0.02	44.7
	1 M NaCl[b]	-50.2	-140.0	-6.78	44.0	-51.6	-144.7	-6.68	43.1
CCAUGG	0.5 mM	-58.8±5.4	-166.8±17.2	-7.04±0.13	44.3	-62.4±2.5	-178.4±8.1	-7.02±0.04	43.9
	1.5 mM	-59.6±4.6	-167.9±14.7	-7.48±0.14	46.7	-62.0±4.9	-175.8±15.5	-7.48±0.14	46.3
	3.0 mM	-60.7±4.2	-170.3±13.5	-7.83±0.08	48.4	-64.8±2.4	-183.5±7.6	-7.88±0.06	47.9
	10.0 mM	-60.5±6.9	-168.6±21.8	-8.23±0.17	50.7	-64.0±2.8	-179.6±8.9	-8.26±0.08	50.1
	1 M NaCl[b]	-61.0	-172.5	-7.49	46.6	-56.9	-159.9	-7.30	46.3
CCUAGG	0.5 mM	-56.3±3.0	-156.3±9.6	-7.77±0.11	49.0	-61.4±3.2	-172.7±10.2	-7.86±0.08	48.4
	1.5 mM	-56.6±6.0	-156.0±18.2	-8.20±0.35	51.5	-59.5±3.6	-165.1±11.1	-8.31±0.12	51.4
	3.0 mM	-60.9±5.6	-168.5±17.6	-8.61±0.16	52.7	-62.3±3.4	-173.1±10.6	-8.61±0.13	52.3
	10.0 mM	-56.6±9.2	-154.3±28.7	-8.78±0.29	55.0	-60.3±6.1	-165.8±19.1	-8.84±0.26	54.2
	1 M NaCl[b]	-59.7	-166.5	-8.06	49.9	-54.1	-149.1	-7.80	50.0
CUGCAG	0.5 mM	-54.4±3.8	-155.2±12.6	-6.29±0.27	40.5	-53.4±6.9	-152.0±22.2	-6.27±0.27	40.5
	1.5 mM	-54.1±4.7	-152.6±14.8	-6.76±0.14	43.3	-59.0±3.2	-168.6±10.4	-6.76±0.05	42.8
	3.0 mM	-58.1±7.4	-164.5±24.1	-7.13±0.09	44.9	-66.1±3.0	-189.8±9.7	-7.18±0.05	44.2
	10.0 mM	-57.9±4.1	-162.5±12.8	-7.52±0.13	47.2	-59.7±2.4	-168.2±7.7	-7.53±0.06	46.9
	1 M NaCl[b]	-54.5	-153.0	-7.05	45.0	-55.4	-155.7	-7.11	45.3
GACGUC	0.5 mM	-57.4±4.1	-164.1±13.2	-6.56±0.10	41.8	-56.6±2.8	-161.4±8.9	-6.51±0.04	41.6
	1.5 mM	-57.9±3.5	-163.3±11.2	-7.22±0.05	45.5	-60.4±2.4	-171.5±7.6	-7.24±0.04	45.2
	3.0 mM	-60.5±3.5	-171.2±11.6	-7.43±0.10	46.2	-65.2±3.9	-186.1±12.4	-7.48±0.09	45.8
	10.0 mM	-60.6±4.2	-169.8±13.3	-7.90±0.09	48.8	-64.6±2.8	-182.6±8.8	-7.96±0.08	48.4
	1 M NaCl[b]	-57.3	-161.0	-7.37	46.4	-58.1	-163.5	-7.35	46.2
GAGCUC	0.5 mM	-55.8±2.7	-159.2±8.6	-6.48±0.10	41.5	-54.7±2.3	-155.4±7.5	-6.45±0.03	41.4

Supplemental Data

	1.5 mM	-59.7±6.5	-169.2±20.8	-7.17±0.10	45.0	-61.7±2.9	-176.0±9.2	-7.14±0.05	44.5
	3.0 mM	-57.4±4.3	-161.1±13.5	-7.49±0.18	47.1	-61.2±2.5	-173.1±8.1	-7.53±0.05	46.7
	10.0 mM	-57.0±3.6	-158.7±11.5	-7.79±0.09	48.9	-61.3±2.4	-172.2±7.6	-7.87±0.07	48.5
	1 M NaCl[b]	-58.3	-163.0	-7.75	48.4	-62.3	-175.3	-7.98	48.6
GCAUGC	0.5 mM	-55.9±4.7	-160.0±14.8	-6.28±0.16	40.4	-56.1±4.5	-160.7±14.4	-6.23±0.12	40.1
	1.5 mM	-58.1±5.7	-165.2±17.9	-6.90±0.15	43.7	-58.9±3.9	-167.7±12.3	-6.85±0.08	43.3
	3.0 mM	-56.6±9.0	-159.4±28.4	-7.20±0.20	45.6	-58.6±2.3	-165.8±7.2	-7.15±0.04	45.0
	10.0 mM	-58.1±5.6	-162.9±17.6	-7.59±0.19	47.6	-58.3±3.5	-163.7±11.1	-7.52±0.10	47.2
	1 M NaCl[b]	-59.6	-168.4	-7.41	46.3	-62.3	-177.2	-7.38	45.7
AACUAGUU	0.5 mM	-58.6±4.2	-169.8±13.6	-5.99±0.14	38.7	-55.6±3.3	-160.1±10.9	-6.00±0.08	38.8
	1.5 mM	-60.9±7.1	-174.8±23.0	-6.73±0.14	42.5	-64.0±3.2	-184.6±10.4	-6.69±0.04	42.0
	3.0 mM	-61.7±6.2	-176.9±19.9	-6.88±0.11	43.2	-58.7±1.6	-167.0±5.1	-6.86±0.02	43.4
	10.0 mM	-61.5±5.7	-174.4±18.0	-7.43±0.12	46.1	-61.1±2.8	-173.3±8.9	-7.36±0.05	45.8
	1 M NaCl[b]	-55.2	-155.0	-7.13	45.4	-54.6	-153.0	-7.16	45.7
ACUAUAGU	0.5 mM	-60.7±3.5	-176.8±11.3	-5.90±0.05	38.2	-60.2±2.0	-175.3±6.6	-5.89±0.03	38.1
	1.5 mM	-63.9±4.3	-184.9±13.8	-6.54±0.10	41.2	-64.8±3.0	-188.0±9.7	-6.51±0.04	41.0
	3.0 mM	-65.7±4.4	-189.3±14.1	-6.94±0.07	43.1	-63.2±2.4	-181.6±7.7	-6.89±0.03	43.1
	10.0 mM	-69.4±4.0	-199.8±12.9	-7.39±0.09	44.9	-68.7±2.8	-197.7±9.1	-7.35±0.05	44.7
	1 M NaCl[b]	-61.4	-175.0	-7.12	44.5	-59.2	-168.4	-6.98	44.0
ACUUAAGU	0.5 mM	-55.5±8.9	-163.5±29.3	-4.76±0.30	32.0	-51.1±5.9	-149.2±19.4	-4.85±0.22	32.1
	1.5 mM	-54.1±7.8	-156.6±25.4	-5.55±0.15	36.3	-57.3±4.4	-167.0±14.2	-5.47±0.09	35.9
	3.0 mM	-65.1±6.4	-190.4±20.7	-6.01±0.13	38.6	-59.4±3.7	-172.1±11.9	-5.97±0.06	38.5
	10.0 mM	-69.9±8.7	-204.6±28.0	-6.42±0.08	40.4	-69.9±1.8	-204.8±5.6	-6.34±0.01	40.0
	1 M NaCl[b]	-49.4	-139.0	-6.29	40.9	-47.2	-132.4	-6.16	40.3
AGAUAUCU	0.5 mM	-59.6±2.8	-175.2±9.1	-5.28±0.03	35.0	-61.1±1.4	-180.0±4.8	-5.27±0.04	34.9
	1.5 mM	-62.1±4.9	-181.4±15.8	-5.82±0.08	37.7	-61.4±2.2	-179.2±7.2	-5.83±0.03	37.8
	3.0 mM	-62.9±3.5	-182.3±11.2	-6.33±0.09	40.2	-63.6±2.3	-184.9±7.5	-6.29±0.02	40.0
	10.0 mM	-62.1±4.0	-178.1±12.8	-6.83±0.07	42.9	-57.1±1.2	-162.2±3.9	-6.78±0.01	43.1
	1 M NaCl[b]	-63.4	-183.0	-6.64	41.8	-64.5	-186.8	-6.58	41.4

[a]Sequences are written 5’ to 3’ and form self-complementary duplexes in solution.
[b]All 1 M NaCl data is in 1 M NaCl with no magnesium and are from Xia et al. (1) except for GCGCGC which is from Chen and Znosko (9).

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[c]Calculated at 0.1 mM oligomer concentration based on the T_m^{-1} vs ln C_T plots.

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TABLE S4. Previously Published DNA/RNA Correction Factors

Eq no.	Name	Ref no.	Equation	Average Deviation[b]
DNA/RNA T_m Correction Factors[a]				
S1	Schildkraut-Lifson T_m equation[c,h]	2	$T_m(2)=T_m(1)+16.6\log\frac{[Na^+]_2}{[Na^+]_1}$	42.8 °C
S2	Wetmur T_m equation[c,h]	3	$T_m(2)=T_m(1)+16.6\log\frac{[Na^+]_2\left(1+0.7[Na^+]_1\right)}{[Na^+]_1\left(1+0.7[Na^+]_2\right)}$	38.9 °C
S3	Frank-Kamenetskii T_m equation[c,h]	4	$T_m(2)=T_m(1)+\left(7.95-3.057fGC\right)\ln\frac{[Na^+]_2}{[Na^+]_1}$	35.5 °C
S4	Marmur-Schildkraut-Doty T_m equation[c,h]	5 & 6	$T_m(2)=T_m(1)+\left(8.75-2.83fGC\right)\ln\frac{[Na^+]_2}{[Na^+]_1}$	41.3 °C
S5	T_m and 12.5log[Na^+] equation[c,h]	7	$T_m(2)=T_m(1)+12.5\log\frac{[Na^+]_2}{[Na^+]_1}$	31.8 °C
S6	SantaLucia T_m equation[c,g,h]	8	$\frac{1}{T_m(2)}=\frac{1}{T_m(1)}+\frac{0.368\left(N_{bp}-1\right)}{\Delta H^\circ}\ln\frac{[Na^+]_2}{[Na^+]_1}$	18.8 °C
S7	Chen T_m quadratic equation[d,h]	9	$T_m(2)=T_m(1)+\left(2.675-1.842fGC\right)\ln\frac{[Na^+]_2}{[Na^+]_1}-0.7348\left(\ln^2[Na^+]_2-\ln^2[Na^+]_1\right)$	36.3 °C
S8	Chen 1/T_m quadratic equation[d,g,h]	9	$\frac{1}{T_m(2)}=\frac{1}{T_m(1)}+\left(2.297fGC-2.886\right)\times10^{-5}\ln\frac{[Na^+]_2}{[Na^+]_1}+7.575\times10^{-6}\left(\ln^2[Na^+]_2-\ln^2[Na^+]_1\right)$	33.3 °C
S9	Peyret-SantaLucia equation[e,g,i]	10	$\frac{1}{T_m(Mg^{2+})}=\frac{1}{T_m(1\ M\ Na^+)}+\frac{0.368(N_{bp}-1)}{\Delta H^\circ}\times\ln\left(3.3\sqrt{[Mg^{2+}]}+[Mon^+]\right)$	5.0 °C
S10	Ahsen-Wittwer-Schütz equation[e,g,i]	11	$\frac{1}{T_m(Mg^{2+})}=\frac{1}{T_m(1\ M\ Na^+)}+\frac{0.368(N_{bp}-1)}{\Delta H^\circ}\times\ln\left(3.79\sqrt{[Mg^{2+}]}+[Mon^+]\right)$	4.5 °C
S11	Tan-Chen equation[f,j]	12	$\frac{1}{T_m(Mg^{2+})}=\frac{1}{T_m(1\ M\ Na^+)}-\frac{0.00322(N_{bp}-1)}{\Delta H^\circ}\times\left(-\frac{0.6}{N}+0.025\ln[Mg^{2+}]+0.0068\ln^2[Mg^{2+}]+\frac{\left(\ln[Mg^{2+}]+0.38\ln^2[Mg^{2+}]\right)}{N^2}\right)$	2.6 °C
S12	Mitsuhashi equation[e,i]	13	$T_m(Mg^{2+})=T_m(1\ M\ Na^+)+16.6\log\left(4\sqrt{[Mg^{2+}]}+[Mon^+]\right)$	10.6 °C

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S13	Owczarzy magnesium T_m correction factor equation[e,g]	14	$\frac{1}{T_m(Mg^{2+})} = \frac{1}{T_m(1\ M\ Na^+)} + 3.92 \times 10^{-5} - 9.11 \times 10^{-6} \ln\left[Mg^{2+}\right] + fGC\left(6.26 \times 10^{-5} + 1.42 \times 10^{-5} \ln\left[\right.\right.$ $\frac{1}{2\left(N_{bp}-1\right)}\left[-4.82 \times 10^{-4} + 5.25 \times 10^{-4} \ln\left[Mg^{2+}\right] + 8.31 \times 10^{-5}\left(\ln^2\left[Mg^{2+}\right]\right)\right]$	2.8 °C
DNA/RNA ΔG°_{37} Correction Factors[a]				
S14	SantaLucia ΔG°_{37} equation[c,h]	8	$\Delta G^\circ_{37}(2) = \Delta G^\circ_{37}(1) - 0.114 \times \left(N_{bp}-1\right)\ln\frac{\left[Na^+\right]_2}{\left[Na^+\right]_1}$	3.66 kcal/mol
S15	Chen ΔG°_{37} derived equation[d,h]	9	$\Delta G^\circ_{37}(2) = \Delta G^\circ_{37}(1) - 310.15 \times \Delta H^\circ \times$ $\left[\left(2.297 fGC - 2.886\right) \times 10^{-5} \times \ln\frac{\left[Na^+\right]_2}{\left[Na^+\right]_1} + 7.575 \times 10^{-6} \times \left(\ln^2\left[Na^+\right]_2 - \ln^2\left[Na^+\right]_1\right)\right]$	6.78 kcal/mol
S16	Chen ΔG°_{37} linear equation[d,h]	9	$\Delta G^\circ_{37}(2) = \Delta G^\circ_{37}(1) + \left(0.324 fGC - 0.765\right)\ln\frac{\left[Na^+\right]_2}{\left[Na^+\right]_1}$	3.28 kcal/mol
S17	Chen ΔG°_{37} quadratic equation[d,h]	9	$\Delta G^\circ_{37}(2) = \Delta G^\circ_{37}(1) + \left(0.324 fGC - 0.468\right)\ln\frac{\left[Na^+\right]_2}{\left[Na^+\right]_1} + 0.133\left(\ln^2\left[Na^+\right]_2 - \ln^2\left[Na^+\right]_1\right)$	6.60 kcal/mol
S18	Chen $1/\Delta G^\circ_{37}$ quadratic equation[d,h]	9	$\frac{1}{\Delta G^\circ_{37}(2)} = \frac{1}{\Delta G^\circ_{37}(1)} + \left(0.016 - 0.0213 fGC\right) \times 10^{-5} \ln\frac{\left[Na^+\right]_2}{\left[Na^+\right]_1} -$ $0.0045\left(\ln^2\left[Na^+\right]_2 - \ln^2\left[Na^+\right]_1\right)$	4.13 kcal/mol
S19	Owczarzy magnesium ΔG°_{37} correction factor equation[e]	14	$\Delta G^\circ_{37}(Mg^{2+}) = \Delta G^\circ_{37}(1\ M\ Na^+) - 310.15 \Delta H^\circ \times$ $\left\{3.92 \times 10^{-5} - 9.11 \times 10^{-6} \ln\left[Mg^{2+}\right] + fGC\left(6.26 \times 10^{-5} + 1.42 \times 10^{-5} \ln\left[Mg^{2+}\right]\right) + \frac{1}{2\left(N_{bp}-1\right)}\left[-4.82 \times 10^{-4} + 5.25 \times 10^{-4} \ln\left[Mg^{2+}\right] + 8.31 \times 10^{-5}\left(\ln^2\left[Mg^{2+}\right]\right)\right]\right\}$	0.59 kcal/mol

[a]1 M NaCl melting temperatures and free energy values were used as the starting point for all correction factors tested.
[b]As stated in Materials and Methods, $|\Delta T_m|_{ave}$ is used to evaluate the average deviation of the T_m correction factors, and $|\Delta\Delta G^\circ_{37}|_{ave}$ is used to evaluate the average deviation of the ΔG°_{37} correction factors.
[c]Correction factors for DNA in Na^+.
[d]Correction factors for RNA in Na^+.
[e]Correction factors for DNA in Mg^{2+}.
[f]Correction factors for RNA in Mg^{2+}.
[g]In this equation, T_m should be in units of K.
[h]$[Na^+]_2$ is replaced with $[Mg^{2+}]$ to test for average deviation.
[i]Original equation includes a contribution from the monovalent ion concentration. Here, these values were irrelevant.
[j]ΔH° in this equation is in kcal/mol. In every other equation, it is in cal/mol.

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TABLE S5. Leave-one-out T_m predictions compared to Eq. 7 T_m predictions.

Sequences (5'-3')[a]	T_m (°C)											
	0.5 mM Mg^{2+}			1.5 mM Mg^{2+}			3.0 mM Mg^{2+}			10.0 mM Mg^{2+}		
	LOO Pred.[b]	Eq. 7 Pred.[c]	$\|\Delta T_m\|$	LOO Pred.[b]	Eq. 7 Pred.[c]	$\|\Delta T_m\|$	LOO Pred.[b]	Eq. 7 Pred.[c]	$\|\Delta T_m\|$	LOO Pred.[b]	Eq. 7 Pred.[c]	$\|\Delta T_m\|$
CGCGCG	53.6	53.6	0.0	56.7	56.7	0.0	58.2	58.2	0.0	60.0	60.0	0.0
CGGCCG	59.2	59.0	0.2	62.2	62.1	0.1	63.7	63.6	0.1	65.5	65.4	0.1
GCGCGC	58.8	58.3	0.5	61.9	61.4	0.5	63.3	62.9	0.4	65.1	64.7	0.4
ACUUAAGU	34.3	33.9	0.4	37.6	37.3	0.3	39.3	39.0	0.3	41.4	41.2	0.2
AACUAGUU	39.5	39.3	0.2	42.9	42.7	0.2	44.6	44.4	0.2	46.7	46.6	0.1
ACUAUAGU	37.6	37.6	0.0	41.0	41.0	0.0	42.7	42.7	0.0	44.9	44.9	0.0
AGAUAUCU	35.1	35.0	0.1	38.4	38.4	0.0	40.1	40.1	0.0	42.3	42.3	0.0
ACCGGU	48.5	48.8	0.3	51.7	52.0	0.3	53.3	53.5	0.2	55.3	55.5	0.2
GCAUGC	40.6	40.6	0.0	43.8	43.8	0.0	45.4	45.3	0.1	47.3	47.3	0.0
AGCGCU	47.1	46.9	0.2	50.2	50.1	0.1	51.8	51.6	0.2	53.8	53.6	0.2
CACGUG	37.6	37.7	0.1	40.8	40.9	0.1	42.4	42.4	0.0	44.4	44.4	0.0
CAGCUG	38.0	38.0	0.0	41.2	41.2	0.0	42.8	42.7	0.1	44.7	44.7	0.0
CCAUGG	41.1	41.3	0.2	44.3	44.5	0.2	45.9	46.0	0.1	47.9	48.0	0.1
CCUAGG	44.6	44.9	0.3	47.9	48.1	0.2	49.5	49.6	0.1	51.5	51.6	0.1
CUGCAG	40.2	40.2	0.0	43.4	43.4	0.0	45.0	44.9	0.1	47.0	46.9	0.1
GACGUC	41.0	41.1	0.1	44.2	44.3	0.1	45.8	45.8	0.0	47.8	47.8	0.0
GAGCUC	43.7	43.6	0.1	46.9	46.8	0.1	48.5	48.3	0.2	50.4	50.3	0.1
Average			0.2			0.1			0.1			0.1
Overall Average							0.1					

[a]All sequences form self-complementary duplexes in solution.
[b]Predicted T_m using coefficients derived from leave-one-out (LOO) analysis.
[c]Predicted T_m using coefficients in Eq. 7.

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TABLE S6. Leave-one-out $\Delta G°_{37}$ predictions compared to Eq. 12 $\Delta G°_{37}$ predictions.

Sequences (5'-3')[a]	$\Delta G°_{37}$ (kcal/mol)											
	0.5 mM Mg^{2+}			1.5 mM Mg^{2+}			3.0 mM Mg^{2+}			10.0 mM Mg^{2+}		
	LOO Pred.[b]	Eq. 12 Pred.[c]	$\lvert\Delta\Delta G°_{37}\rvert$	LOO Pred.[b]	Eq. 12 Pred.[c]	$\lvert\Delta\Delta G°_{37}\rvert$	LOO Pred.[b]	Eq. 12 Pred.[c]	$\lvert\Delta\Delta G°_{37}\rvert$	LOO Pred.[b]	Eq. 12 Pred.[c]	$\lvert\Delta\Delta G°_{37}\rvert$
CGCGCG	-8.41	-8.48	0.07	-8.98	-9.04	0.06	-9.27	-9.32	0.05	-9.60	-9.64	0.04
CGGCCG	-9.26	-9.26	0.00	-9.83	-9.82	0.00	-10.11	-10.10	0.01	-10.44	-10.42	0.01
GCGCGC	-10.11	-9.92	0.19	-10.66	-10.48	0.18	-10.92	-10.76	0.17	-11.23	-11.08	0.14
ACUUAAGU	-5.12	-5.07	0.05	-5.74	-5.70	0.04	-6.05	-6.02	0.03	-6.44	-6.41	0.02
AACUAGUU	-6.09	-6.07	0.02	-6.72	-6.70	0.02	-7.03	-7.02	0.02	-7.43	-7.41	0.01
ACUAUAGU	-5.89	-5.89	0.01	-6.51	-6.52	0.01	-6.83	-6.84	0.01	-7.22	-7.23	0.01
AGAUAUCU	-5.55	-5.49	0.06	-6.17	-6.12	0.05	-6.48	-6.44	0.04	-6.87	-6.83	0.03
ACCGGU	-7.62	-7.67	0.05	-8.22	-8.26	0.05	-8.51	-8.56	0.05	-8.87	-8.91	0.04
GCAUGC	-6.56	-6.54	0.02	-7.15	-7.13	0.02	-7.44	-7.43	0.02	-7.80	-7.78	0.02
AGCGCU	-7.18	-7.15	0.03	-7.77	-7.74	0.03	-8.06	-8.04	0.02	-8.41	-8.39	0.02
CACGUG	-5.74	-5.75	0.01	-6.33	-6.34	0.01	-6.63	-6.64	0.01	-6.99	-6.99	0.00
CAGCUG	-5.84	-5.84	0.00	-6.44	-6.43	0.00	-6.73	-6.73	0.01	-7.09	-7.08	0.01
CCAUGG	-6.43	-6.46	0.03	-7.02	-7.05	0.03	-7.31	-7.35	0.03	-7.67	-7.70	0.03
CCUAGG	-6.90	-6.96	0.06	-7.50	-7.55	0.05	-7.80	-7.85	0.05	-8.16	-8.20	0.04
CUGCAG	-6.27	-6.27	0.00	-6.86	-6.86	0.00	-7.16	-7.16	0.00	-7.51	-7.51	0.00
GACGUC	-6.51	-6.51	0.00	-7.10	-7.10	0.01	-7.39	-7.40	0.01	-7.74	-7.75	0.01
GAGCUC	-7.18	-7.14	0.04	-7.77	-7.73	0.04	-8.06	-8.03	0.04	-8.41	-8.38	0.03
Average			0.04			0.04			0.03			0.03
Overall Average						0.04						

[a]All sequences form self-complementary duplexes in solution.
[b]Predicted $\Delta G°_{37}$ using coefficients derived from leave-one-out (LOO) analysis.
[c]Predicted $\Delta G°_{37}$ using coefficients in Eq. 12.

Supplemental Data

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