Supplementary Information

MeltR: A Software package for Facile Determination of RNA Folding Energies from Absorbance Data

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Keywords

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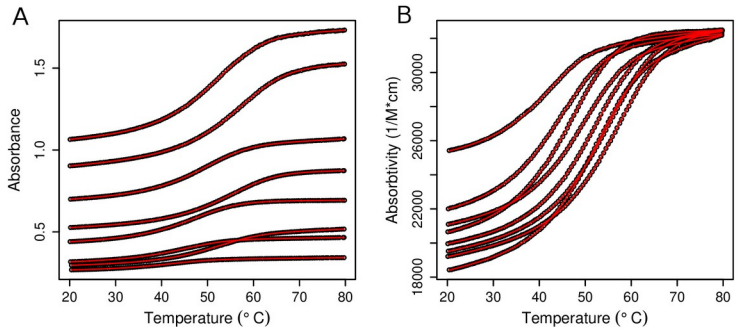
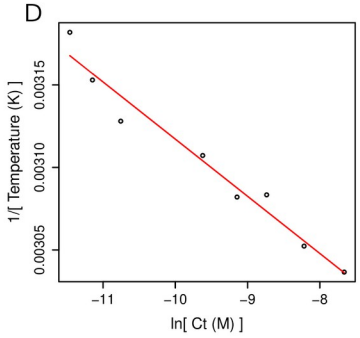
Table of Contents

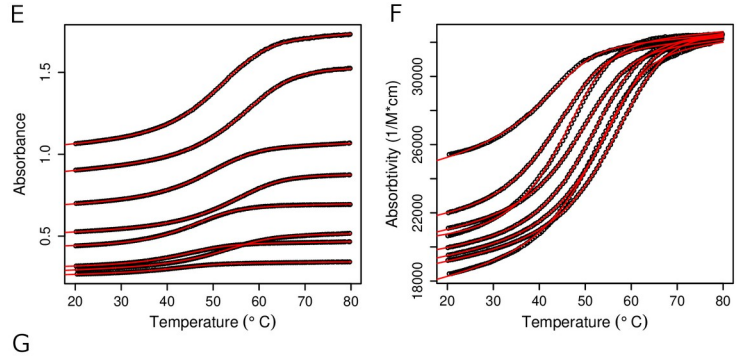
Supplementary information figures



C

Sample	Ct	H	S	G	Tm
2	4.70E-04	-56.73	-0.157	-8.02	56.17
3	1.07E-04	-55.04	-0.1515	-8.06	51.31
4	2.13E-05	-55.14	-0.1511	-8.27	46.54
6	2.70E-04	-56.5	-0.1561	-8.08	54.47
7	6.63E-05	-56.33	-0.1559	-7.97	48.68
8	1.44E-05	-54.41	-0.1494	-8.07	44.01
10	1.61E-04	-57.33	-0.1594	-7.89	51.17
12	1.05E-05	-57.75	-0.161	-7.82	41.13

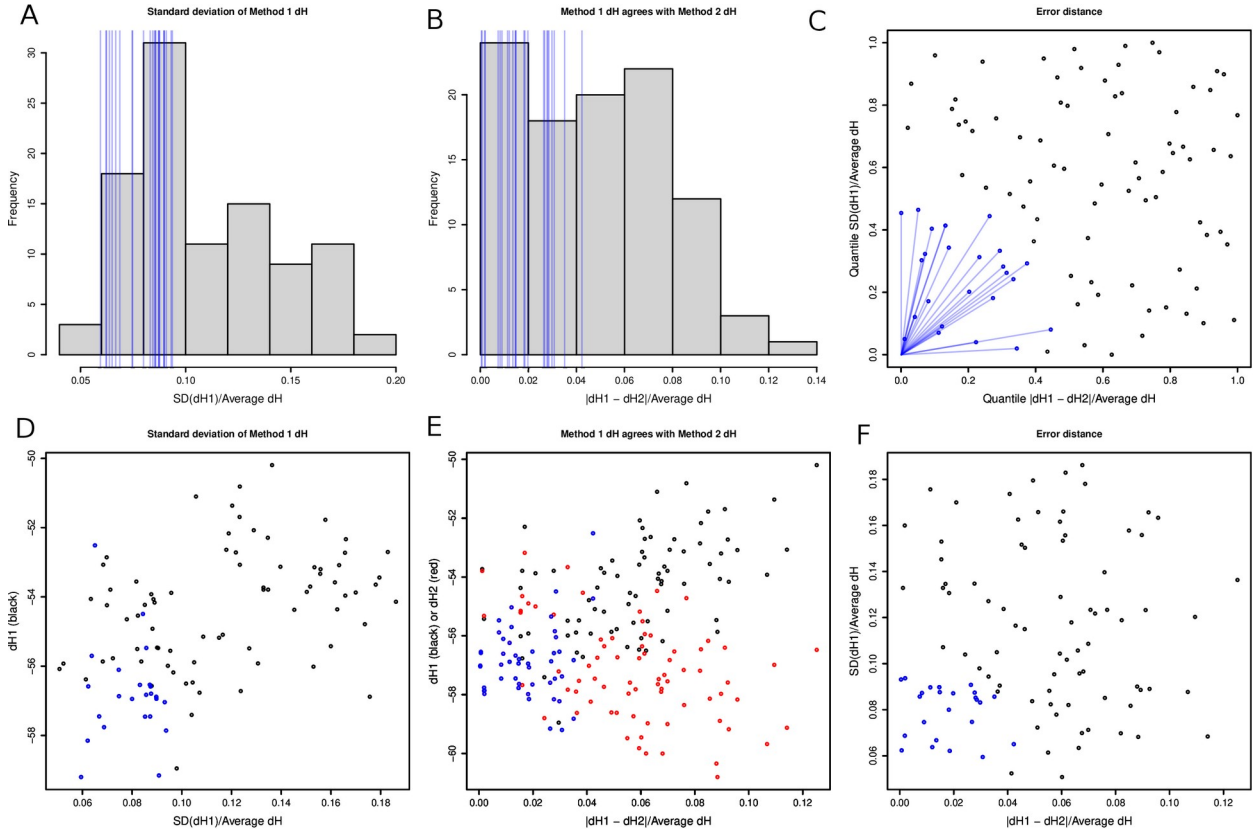




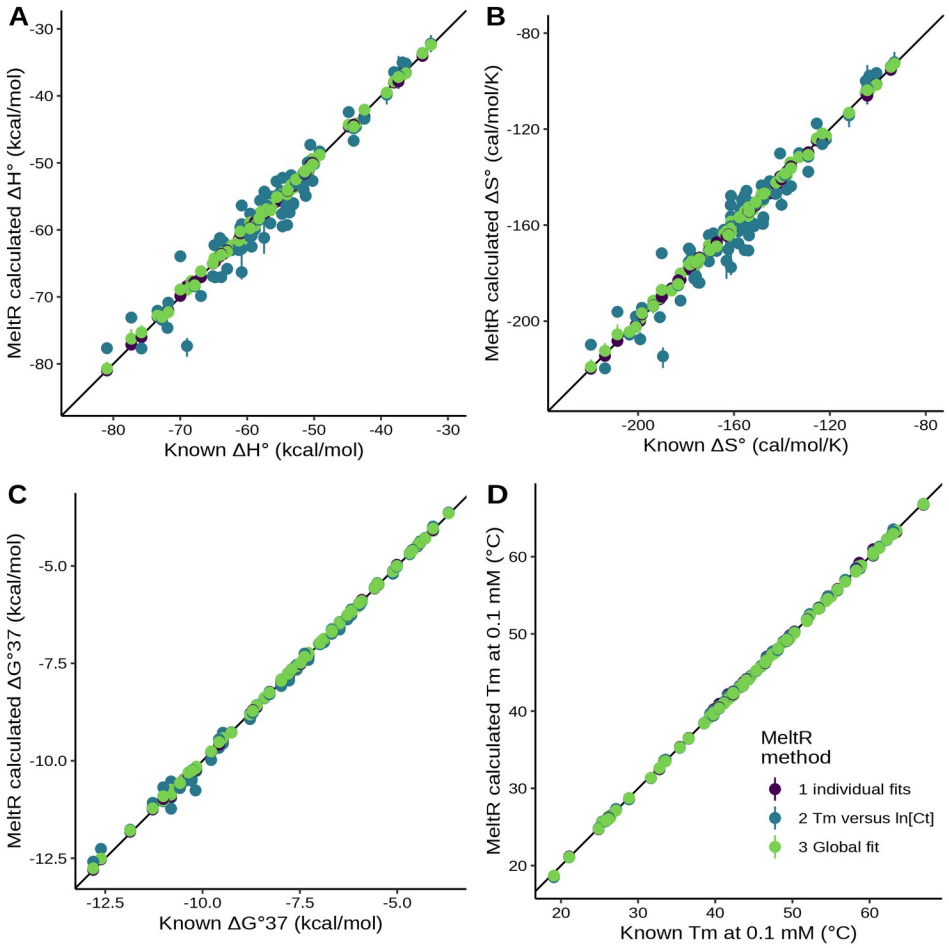
G

Method	H	SE.H	S	SE.S	G	SE.G	Tm_at_0.1mM	SE.Tm_at_0.1mM
1 individual fits	-56.15	1.18	-155.18	4.14	-8.02	0.14	50.51	1.72
2 Tm versus ln[Ct]	-57.35	4.46	-158.89	13.83	-8.07	0.18	50.51	5.9
3 Global fit	-56.33	0.2	-155.56	0.63	-8.08	0.01	50.84	0.27

SI Figure 1 Precanned meltR.A figures and tables for assessing the quality of the results are written as PDF and comma separated value files, respectively. Black circles represent data points and red lines represent fits. **(A)** Raw data fit using method 1, fitting the melting curve for each sample individually. **(B)** Normalized data fit using method 1. Data were normalized to calculate the Absorbtivity at each temperature by dividing the raw absorbance by the strand concentration and the pathlength of the cuvette. **(C)** Table summarizing the individual fits for method 1. Sample is the sample identifier in the data. Ct is the total RNA strand concentration, C_t. H is the helix association enthalpy, ΔH°, in kcal/mol. S is the helix association entropy, ΔS°, in cal/mol/K. G is the free energy at 37 °C of helix association entropy, $\Delta G°_{37}$, in kcal/mol. Tm is the melting temperature, T_m, of the curve in °C. **(D)** Plot of $1/T_m$ versus the natural log of the C_t, used to determine folding energies for method 2. **(E)** Raw data fit using method 3, fitting melting curves from each sample to a single regression model. **(F)** Normalized data fit using method 3. Data were normalized as described in A. **(G)** Table summarizing results from the three methods. Parameters are summarized in A. SE stands for the standard error, which is calculated from the regression models, and represent the precision of the parameter given the data.



SI Figure 2 Precanned BLtrimmer outputs for assessing the quality of the results are written as a PDF file. Blue lines and points represent the 25% of baseline combinations that produce parameters that exhibit the best internal consistency across the data set. Each point comes from fitting a combination of baselines with method 1 or 2. **(A)** Distribution of the standard deviation of enthalpy (ΔH°) values, determined by fitting combinations of baselines options. Low standard deviation indicate that the baseline combination exhibits high agreement in enthalpies calculated using method 1, fitting melting curves for each sample individually. **(B)** Distribution of the ΔH° agreement between method 1 and method 2, fitting melting curves for each sample individually and fitting the relationship between $1/T_m$ versus $\ln C_t$, respectively, determined by fitting combinations of baselines options. Low difference indicates that the baseline combination exhibits high agreement in ΔH° values calculated using method 1 and method 2. **(C)** Quantalized (ranked from smallest to largest and assigned a quantile between 0 and 1) standard deviation of ΔH° values in the method 1 fit versus the difference between ΔH° values in the method 1 and method 2 fit, for each baseline combination. Error distance, which is used to identify the 25% of baseline combinations that produce parameters that exhibit the best internal consistency, is the Pythagorean distance between a point and the origin of the plot. **(D)** The average enthalpy produced by method 1 versus the standard deviation of ΔH° values produced by method 1 for each baseline combination. **(E)** The average enthalpy produced by method 1 (Black) or the enthalpy produced by method 2 versus the difference in ΔH° between methods 1 and 2, for each baseline combination. **(F)** The standard deviation of ΔH° values produced by method 1 versus difference in ΔH° between methods 1 and 2, for each baseline combination.



SI Figure 3 The BLtrimmer accurately fits modeled data. **(A)** Helix association ΔH° determined using meltR.A followed by the BLtrimmer to fit modeled data, versus the known ΔH°. Vertical error bars represent 95% confidence intervals calculated using the BLtrimmer. **(B)** Helix association ΔS° determined using meltR.A followed by the BLtrimmer to fit modeled data, versus the known ΔH°. Error bars are the same as A. **(C)** Helix association $\Delta G°_{37}$ determined using meltR.A followed by the BLtrimmer to fit modeled data, versus the known ΔH°. Error bars are the same as A. **(D)** T_m at 0.1 mM determined using meltR.A followed by the BLtrimmer to fit modeled data, versus the known ΔH°. Error bars are the same as A.

Supplementary information tables

SI Table 1 Precanned BLtrimmer results table are written as a comma separated value file.

Method	ΔH° (kcal/mol)	ΔH° 95% confidence interval (kcal/mol)	ΔS° (cal/mol/K)	ΔS° 95% confidence interval (cal/mol/K)	$\Delta G°_{37}$ (kcal/mol)	$\Delta G°_{37}$ 95% confidence interval (kcal/mol)	T_m[a] (°C)	T_m[a] 95% confidence interval (°C)
1 individual fits	-57.03	-58.66 to -55.08	-161.73	-166.82 to -155.52	-6.87	-6.91 to -6.84	43.63	43.34 to 43.84
2 Tm versus ln[Ct]	-57.2	-58.75 to -54.69	-162.27	-167.23 to -154.31	-6.87	-6.9 to -6.83	43.63	43.45 to 43.86
3 Global fit	-56.89	-60.26 to -54.15	-161.08	-171.78 to -152.38	-6.93	-7.05 to -6.84	43.99	43.37 to 44.52

[a]Expectation maximized T_m at a C_t of 0.1 mM.

SI Table 2 BLtrimmer results comparing MeltR methods 1, 2, and 3.

Helix	ΔH° Method 1[a] (kcal/mol)	ΔH° Method 2[b] (kcal/mol)	ΔH° Method 3[c] (kcal/mol)	ΔS° Method 1[a] (cal/mol/K)	ΔS° Method 2[b] (cal/mol/K)	ΔS° Method 3[c] (cal/mol/K)	$\Delta G°_{37}$ Method 1[a] (kcal/mol)	$\Delta G°_{37}$ Method 2[b] (kcal/mol)	$\Delta G°_{37}$ Method 3[c] (kcal/mol)	T_m[d] Method 1[a] (°C)	T_m[d] Method 2[b] (°C)	T_m[d] Method 3[c] (°C)	%error ΔH°	%error ΔS°	%error $\Delta G°_{37}$	%error T_m[d]
5'-CGCGCG-3'[e] '3'-GCGCGC-3 '	-55.69 (-56.46 to -54.89)	-55.78 (-56.71 to -54.76)	-56.76 (-57.94 to -55.81)	-153.72 (-156.09 to -151.27)	-154.04 (-156.9 to -150.91)	-156.93 (-160.59 to -153.94)	-8.01 (-8.05 to -7.98)	-8.00 (-8.16 to -8.01)	-8.09 (-8.16 to -8.01)	50.57 (50.46 to 50.66)	50.49 (50.37 to 50.61)	50.76 (50.35 to 51.04)	1.9	2.1	1.1	0.5
5'-ACCGGU-3'[e] 3'-UGGCCA-3'	-56.26 (-58.73 to -54.06)	-58.57 (-60.33 to -56.49)	-56.53 (-60.25 to -53.16)	-159.24 (-167.15 to -152.29)	-166.59 (-172.23 to -159.94)	-159.96 (-172.21 to -149.33)	-6.87 (-6.93 to -6.8)	-6.9 (-6.95 to -6.87)	-6.91 (-7.07 to -6.79)	43.71 (43.42 to 44.02)	43.64 (43.43 to 43.87)	43.94 (43.08 to 44.54)	4.0	4.5	0.6	0.7
5'-CCAUGG-3'[e] 3'-GGUACC-5'	-60.25 (-60.95 to -59.42)	-59.82 (-60.51 to -59.15)	-59.86 (-61.28 to -58.83)	-172.7 (-174.95 to -170.05)	-171.31 (-173.56 to -169.19)	-171.4 (-175.85 to -168.12)	-6.69 (-6.71 to -6.67)	-6.69 (-6.7 to -6.67)	-6.7 (-6.75 to -6.67)	42.29 (42.19 to 42.4)	42.33 (42.25 to 42.41)	42.37 (42.28 to 42.51)	0.7	0.8	0.1	0.2
5'-GAUAUAUC-3'[e] 3'-CUAUAUAG-5'	-73.53 (-74.25 to -72.58)	-63.12 (-64.49 to -62.15)	-74.15 (-78.59 to -71.74)	-220.06 (-222.4 to -217.04)	-186.19 (-190.72 to -182.98)	-221.96 (-236.28 to -214.21)	-5.28 (-5.3 to -5.24)	-5.37 (-5.4 to -5.33)	-5.31 (-5.34 to -5.27)	35.33 (35.19 to 35.45)	35.52 (35.35 to 35.63)	35.47 (35.26 to 35.59)	15.7	17.1	1.7	0.5
5'-GCAAUUGC-3'[e] 3'-CGUUAACG-5'	-77.7 (-79.22 to -76.53)	-75.39 (-77.18 to -73.68)	-81.24 (-84.35 to -79.04)	-223.08 (-227.87 to -219.42)	-215.93 (-221.55 to -210.59)	-234.11 (-243.79 to -227.28)	-8.52 (-8.56 to -8.46)	-8.42 (-8.47 to -8.35)	-8.63 (-8.73 to -8.54)	48.76 (48.57 to 48.88)	48.7 (48.59 to 48.82)	48.71 (48.64 to 48.78)	7.5	8.1	2.5	0.1
5'-CGAAAGGU-3'[f] 3'-GCUUUCCA-5'	-68.64 (-70.07 to -67.32)	-68.03 (-69.92 to -66.25)	-67.98 (-69.89 to -66.16)	-188.69 (-193.12 to -184.54)	-186.82 (-192.62 to -181.35)	-186.59 (-192.46 to -181.12)	-10.12 (-10.18 to -10.06)	-10.09 (-10.19 to -10)	-10.11 (-10.2 to -10)	54.12 (53.87 to 54.38)	54.13 (53.9 to 54.33)	54.21 (53.95 to 54.54)	1.0	1.1	0.3	0.2
5'-CUGAGUC-3'[f] 3'-GACUCAG-5'	-64.09 (-65.15 to -63.2)	-64.14 (-65.4 to -63.08)	-63.31 (-64 to -62.69)	-176.35 (-179.73 to -173.53)	-176.51 (-180.45 to -173.21)	-173.83 (-175.92 to -171.93)	-9.4 (-9.42 to -9.38)	-9.4 (-9.44 to -9.36)	-9.39 (-9.44 to -9.35)	51.52 (51.37 to 51.68)	51.5 (51.39 to 51.62)	51.69 (51.43 to 51.88)	1.3	1.5	0.1	0.4
5'-CGUUGC-3'[f] 3'-GCAACG-5'	-51.69 (-53.13 to -50.18)	-47.58 (-49.77 to -45.95)	-51.92 (-54.53 to -50.37)	-142.99 (-147.72 to -138.15)	-129.92 (-137.09 to -124.62)	-143.61 (-151.88 to -138.78)	-7.34 (-7.39 to -7.27)	-7.28 (-7.32 to -7.22)	-7.38 (-7.45 to -7.28)	41.92 (41.43 to 42.35)	41.98 (41.49 to 42.36)	42.13 (41.56 to 42.39)	8.6	9.9	1.4	0.5
FAM-CGAAAGGU-3'[f] BHQ1- GCUUUCCA-5'	-83.25 (-85.73 to -80.95)	-81.76 (-85.14 to -79.11)	-80.92 (-83.27 to -79.05)	-223.16 (-230.67 to -216.32)	-218.73 (-228.81 to -210.85)	-216.39 (-223.4 to -210.93)	-14.03 (-14.22 to -13.86)	-13.92 (-14.18 to -13.72)	-13.81 (-14 to -13.65)	67.73 (67.44 to 67.96)	67.81 (67.54 to 68.01)	67.66 (67.13 to 68.11)	2.8	3.1	1.6	0.2
FAM-CUGAGUC-3' [f] BHQ1-GACUCAG-5'	-74.69 (-76.00 to -73.40)	-74.94 (-76.63 to -72.43)	-75.38 (-77.21 to -73.3)	-197.57 (-201.48 to -193.77)	-198.28 (-203.34 to -190.84)	-199.36 (-204.73 to -193.23)	-13.42 (-13.53 to -13.3)	-13.44 (-13.57 to -13.24)	-13.54 (-13.71 to -13.34)	68.51 (68.32 to 68.64)	68.51 (68.36 to 68.67)	68.82 (68.47 to 69.33)	0.9	0.9	0.9	0.5
FAM-CGUUGC-3'[f] BHQ1-GCAACG-5'	-58.64 (-61.37 to -56.25)	-63.94 (-69.16 to -56.71)	-60.32 (-62.02 to -58.19)	-155.58 (-163.9 to -148.39)	-171.81 (-187.7 to -149.35)	-160.72 (-165.71 to -154.29)	-10.39 (-10.55 to -10.23)	-10.66 (-10.97 to -10.3)	-10.48 (-10.61 to -10.33)	58.83 (58.56 to 59.39)	58.4 (58.11 to 59.6)	58.7 (58.37 to 58.92)	8.7	10.0	2.6	0.7
												Average % error between method 1, 2, & 3	4.8	5.4	1.2	0.4

[a]Determined with method 1, fitting curves individually and averaging the results.
[b]Determined with method 2, $1/T_m$ versus $\ln C_t$ analysis.
[c]Determined with method 3, globally fitting all curves with the same non-linear model.
[d]Expectation maximized T_m at a C_t of 0.1 mM.
[e]Data compiled from a published source.[1]
[f]Data compiled in this work.

SI Table 3 BLtrimmer results comparing MeltR method 1, fitting curves individually and averaging the results, to Meltwin method 1. MeltR data are reused from SI table 2 for convenience.

Helix	ΔH° MeltR (kcal/mol)	ΔH° Meltwin (kcal/mol)	ΔS° MeltR (cal/mol/K)	ΔS° Meltwin (cal/mol/K)	$\Delta G°_{37}$ MeltR (kcal/mol)	$\Delta G°_{37}$ Meltwin (kcal/mol)	T_m[a] Meltwin (°C)	T_m[a] Meltwin (°C)	%error ΔH°	%error ΔS°	%error $\Delta G°_{37}$	%error T_m[a]
5'-CGCGCG-3'[a] '3'-GCGCGC-3'	-159.24 (-58.73 to -54.06)	-54.1 (±4)	-159.24 (-167.15 to -152.29)	-151.5 (±12.4)	-6.87 (-6.93 to -6.8)	-7.11 (±0.2)	43.71 (43.42 to 44.02)	45.4	3.9	5.0	3.4	3.8
5'-ACCGGU-3'[a] 3'-UGGCCA-3'	-172.7 (-60.95 to -59.42)	-60.4 (±2)	-172.7 (-174.95 to -170.05)	-173.6 (±6.4)	-6.69 (-6.71 to -6.67)	-6.52 (±0.05)	42.29 (42.19 to 42.4)	41.4	0.2	0.5	2.6	2.1
5'-CCAUGG-3'[a] 3'-GGUACC-5'	-188.69 (-70.07 to -67.32)	-67.0 (±3.025)	-188.69 (-193.12 to -184.54)	-184.51 (±9.497)	-10.12 (-10.18 to -10.06)	-9.8 (±0.087)	54.12 (53.87 to 54.38)	52.9	2.4	2.2	3.2	2.3
5'-GAUAUAUC-3'[a] 3'-CUAUAUAG-5'	-153.72 (-56.46 to -54.89)	-55.6 (±1.3)	-153.72 (-156.09 to -151.27)	-153.9 (±4.3)	-8.01 (-8.05 to -7.98)	-7.85 (±0.17)	50.57 (50.46 to 50.66)	49.6	0.2	0.1	2.0	1.9
5'-GCAAUUGC-3'[a] 3'-CGUUAACG-5'	-142.99 (-53.13 to -50.18)	-51.06 (±3.567)	-142.99 (-147.72 to -138.15)	-141.39 (±11.476)	-7.34 (-7.39 to -7.27)	-7.21 (±0.087)	41.92 (41.43 to 42.35)	41.2	1.2	1.1	1.8	1.7
5'-CGAAAGGU-3'[b] 3'-GCUUUCCA-5'	-176.35 (-65.15 to -63.2)	-63.32 (±1.928)	-176.35 (-179.73 to -173.53)	-174.65 (±6.191)	-9.4 (-9.42 to -9.38)	-9.15 (±0.03)	51.52 (51.37 to 51.68)	50.4	1.2	1.0	2.7	2.2
5'-CUGAGUC-3'[b] 3'-GACUCAG-5'	-223.16 (-85.73 to -80.95)	-81.12 (±1.583)	-223.16 (-230.67 to -216.32)	-217.61 (±5.076)	-14.03 (-14.22 to -13.86)	-13.63 (±0.092)	67.73 (67.44 to 67.96)	66.7	2.6	2.5	2.9	1.5
5'-CGUUGC-3'[b] 3'-GCAACG-5'	-155.58 (-61.37 to -56.25)	-61.47 (±3.4)	-155.58 (-163.9 to -148.39)	-164.74 (±10.499)	-10.39 (-10.55 to -10.23)	-10.38 (±0.163)	58.83 (58.56 to 59.39)	57.7	4.7	5.7	0.1	1.9
FAM-CGAAAGGU-3'[b] BHQ1-GCUUUCCA-5'	-197.57 (-76 to -73.4)	-74.43 (±2.316)	-197.57 (-201.48 to -193.77)	-197.46 (±6.709)	-13.42 (-13.53 to -13.3)	-13.19 (±0.275)	68.51 (68.32 to 68.64)	67.5	0.3	0.1	1.7	1.5
FAM-CUGAGUC-3'[b] BHQ1-GACUCAG-5'	-220.06 (-74.25 to -72.58)	-74.2 (±4.4)	-220.06 (-222.4 to -217.04)	-221.7 (±14.2)	-5.28 (-5.3 to -5.24)	-5.41 (±0.06)	35.33 (35.19 to 35.45)	35.9	0.9	0.7	2.4	1.6
FAM-CGUUGC-3'[b] BHQ1-GCAACG-5'	-223.08 (-79.22 to -76.53)	-79.4 (±3.8)	-223.08 (-227.87 to -219.42)	-229.8 (±11.9)	-8.52 (-8.56 to -8.46)	-8.15 (±0.12)	48.76 (48.57 to 48.88)	47.0	2.2	3.0	4.4	3.7
							Average % error between MeltR method 1 and Meltwin method 1		1.8	2.0	2.5	2.2

[a]Expectation maximized T_m at a C_t of 0.1 mM.
[b]Data compiled from a published source.[1]
[c]Data compiled in this work.

SI Table 4 BLtrimmer results comparing MeltR method 2, fitting the relationship between $1/T_m$ versus $\ln C_t$, to Meltwin method 2. MeltR data are reused from SI table 2 for convenience.

Helix	ΔH° MeltR (kcal/mol)	ΔH° Meltwin (kcal/mol)	ΔS° MeltR (cal/mol/K)	ΔS° Meltwin (cal/mol/K)	$\Delta G°_{37}$ MeltR (kcal/mol)	$\Delta G°_{37}$ Meltwin (kcal/mol)	T_m[a] Meltwin (°C)	T_m[a] Meltwin (°C)	%error ΔH°	%error ΔS°	%error $\Delta G°_{37}$	%error T_m[a]
5'-CGCGCG-3'[a] '3'-GCGCGC-3'	-166.59 (-60.33 to -56.49)	-53.8 (±2.9)	-166.59 (-172.23 to -159.94)	-150.5 (±9.1)	-6.9 (-6.95 to -6.87)	-7.12 (±0.06)	43.64 (43.43 to 43.87)	45.6	8.5	10.1	3.1	4.4
5'-ACCGGU-3'[a] 3'-UGGCCA-3'	-171.31 (-60.51 to -59.15)	-59.8 (±1)	-171.31 (-173.56 to -169.19)	-171.9 (±3.3)	-6.69 (-6.7 to -6.67)	-6.52 (±0.01)	42.33 (42.25 to 42.41)	41.4	0.0	0.3	2.6	2.2
5'-CCAUGG-3'[a] 3'-GGUACC-5'	-186.82 (-69.92 to -66.25)	-65.85 (±1.448)	-186.82 (-192.62 to -181.35)	-180.82 (±4.495)	-10.09 (-10.19 to -10)	-9.77 (±0.055)	54.13 (53.9 to 54.33)	53	3.3	3.3	3.2	2.1
5'-GAUAUAUC-3'[a] 3'-CUAUAUAG-5'	-154.04 (-56.71 to -54.76)	-52 (±3.8)	-154.04 (-156.9 to -150.91)	-142.6 (±11.9)	-8 (-8.05 to -7.97)	-7.72 (±0.16)	50.49 (50.37 to 50.61)	49.7	7.0	7.7	3.6	1.6
5'-GCAAUUGC-3'[a] 3'-CGUUAACG-5'	-129.92 (-49.77 to -45.95)	-48.17 (±1.779)	-129.92 (-137.09 to -124.62)	-132.17 (±5.73)	-7.28 (-7.32 to -7.22)	-7.18 (±0.024)	41.98 (41.49 to 42.36)	41.2	1.2	1.7	1.4	1.9
5'-CGAAAGGU-3'[b] 3'-GCUUUCCA-5'	-176.51 (-65.4 to -63.08)	-63.61 (±1.507)	-176.51 (-180.45 to -173.21)	-175.57 (±4.712)	-9.4 (-9.44 to -9.36)	-9.16 (±0.047)	51.5 (51.39 to 51.62)	50.4	0.8	0.5	2.6	2.2
5'-CUGAGUC-3'[b] 3'-GACUCAG-5'	-218.73 (-85.14 to -79.11)	-81.05 (±6.734)	-218.73 (-228.81 to -210.85)	-217.41 (±20.017)	-13.92 (-14.18 to -13.72)	-13.62 (±0.538)	67.81 (67.54 to 68.01)	66.7	0.9	0.6	2.2	1.7
5'-CGUUGC-3'[b] 3'-GCAACG-5'	-171.81 (-69.16 to -56.71)	-62.23 (±3.751)	-171.81 (-187.7 to -149.35)	-167.11 (±11.45)	-10.66 (-10.97 to -10.3)	-10.4 (±0.207)	58.4 (58.11 to 59.6)	57.6	2.7	2.8	2.5	1.4
FAM-CGAAAGGU-3'[b] BHQ1-GCUUUCCA-5'	-198.28 (-76.63 to -72.43)	-74.63 (±6.17)	-198.28 (-203.34 to -190.84)	-198.05 (±18.308)	-13.44 (-13.57 to -13.24)	-13.21 (±0.508)	68.51 (68.36 to 68.67)	67.5	0.4	0.1	1.7	1.5
FAM-CUGAGUC-3'[b] BHQ1-GACUCAG-5'	-186.19 (-64.49 to -62.15)	-66 (±2.4)	-186.19 (-190.72 to -182.98)	-195 (±7.9)	-5.37 (-5.4 to -5.33)	-5.48 (±0.04)	35.52 (35.35 to 35.63)	36.1	4.5	4.6	2.0	1.6
FAM-CGUUGC-3'[b] BHQ1-GCAACG-5'	-215.93 (-77.18 to -73.68)	-78.1 (±1.7)	-215.93 (-221.55 to -210.59)	-225.7 (±5.3)	-8.42 (-8.47 to -8.35)	-8.09 (±0.05)	48.7 (48.59 to 48.82)	46.9	3.5	4.4	4.0	3.8
							Average % error between MeltR method 2 and Meltwin method 2		3.0	3.3	2.6	2.2

[a]Expectation maximized T_m at a C_t of 0.1 mM.
[b]Data compiled from a published source.[1]
[c]Data compiled in this work.

SI Table 5 BLtrimmer results comparing MeltR method 3 to Meltwin method 1. MeltR data are reused from SI table 2 and Meltwin data are reused from SI Table 2 and 3 for convenience.

Helix	ΔH° MeltR (kcal/mol)	ΔH° Meltwin (kcal/mol)	ΔS° MeltR (cal/mol/K)	ΔS° Meltwin (cal/mol/K)	$\Delta G°_{37}$ MeltR (kcal/mol)	$\Delta G°_{37}$ Meltwin (kcal/mol)	T_m[a] Meltwin (°C)	T_m[a] Meltwin (°C)	%error ΔH°	%error ΔS°	%error $\Delta G°_{37}$	%error T_m[a]
5'-CGCGCG-3'[a] '3'-GCGCGC-3'	-56.53 (-60.25 to -53.16)	-54.1 (±4)	-159.96 (-172.21 to -149.33)	-151.5 (±12.4)	-6.91 (-7.07 to -6.79)	-7.11 (±0.2)	43.94 (43.08 to 44.54)	45.4	4.4	5.4	2.9	3.3
5'-ACCGGU-3'[a] 3'-UGGCCA-3'	-59.86 (-61.28 to -58.83)	-60.4 (±2)	-171.4 (-175.85 to -168.12)	-173.6 (±6.4)	-6.7 (-6.75 to -6.67)	-6.52 (±0.05)	42.37 (42.28 to 42.51)	41.4	0.9	1.3	2.7	2.3
5'-CCAUGG-3'[a] 3'-GGUACC-5'	-67.98 (-69.89 to -66.16)	-67.02 (±3.025)	-186.59 (-192.46 to -181.12)	-184.51 (±9.497)	-10.11 (-10.2 to -10)	-9.8 (±0.087)	54.21 (53.95 to 54.54)	52.9	1.4	1.1	3.1	2.4
5'-GAUAUAUC-3'[a] 3'-CUAUAUAG-5'	-56.76 (-57.94 to -55.81)	-55.6 (±1.3)	-156.93 (-160.59 to -153.94)	-153.9 (±4.3)	-8.09 (-8.16 to -8.01)	-7.85 (±0.17)	50.76 (50.35 to 51.04)	49.6	2.1	1.9	3.0	2.3
5'-GCAAUUGC-3'[a] 3'-CGUUAACG-5'	-51.92 (-54.53 to -50.37)	-51.06 (±3.567)	-143.61 (-151.88 to -138.78)	-141.39 (±11.476)	-7.38 (-7.45 to -7.28)	-7.21 (±0.087)	42.13 (41.56 to 42.39)	41.2	1.7	1.6	2.3	2.2
5'-CGAAAGGU-3'[b] 3'-GCUUUCCA-5'	-63.31 (-64 to -62.69)	-63.32 (±1.928)	-173.83 (-175.92 to -171.93)	-174.65 (±6.191)	-9.39 (-9.44 to -9.35)	-9.15 (±0.03)	51.69 (51.43 to 51.88)	50.4	0.0	0.5	2.6	2.5
5'-CUGAGUC-3'[b] 3'-GACUCAG-5'	-80.92 (-83.27 to -79.05)	-81.12 (±1.583)	-216.39 (-223.4 to -210.93)	-217.61 (±5.076)	-13.81 (-14 to -13.65)	-13.63 (±0.092)	67.66 (67.13 to 68.11)	66.7	0.2	0.6	1.3	1.4
5'-CGUUGC-3'[b] 3'-GCAACG-5'	-60.32 (-62.02 to -58.19)	-61.47 (±3.4)	-160.72 (-165.71 to -154.29)	-164.74 (±10.499)	-10.48 (-10.61 to -10.33)	-10.38 (±0.163)	58.7 (58.37 to 58.92)	57.7	1.9	2.5	1.0	1.7
FAM-CGAAAGGU-3'[b] BHQ1-GCUUUCCA-5'	-75.38 (-77.21 to -73.3)	-74.43 (±2.316)	-199.36 (-204.73 to -193.23)	-197.46 (±6.709)	-13.54 (-13.71 to -13.34)	-13.19 (±0.275)	68.82 (68.47 to 69.33)	67.5	1.3	1.0	2.6	1.9
FAM-CUGAGUC-3'[b] BHQ1-GACUCAG-5'	-74.15 (-78.59 to -71.74)	-74.2 (±4.4)	-221.96 (-236.28 to -214.21)	-221.7 (±14.2)	-5.31 (-5.34 to -5.27)	-5.41 (±0.06)	35.47 (35.26 to 35.59)	35.9	0.1	0.1	1.9	1.2
FAM-CGUUGC-3'[b] BHQ1-GCAACG-5'	-81.24 (-84.35 to -79.04)	-79.4 (±3.8)	-234.11 (-243.79 to -227.28)	-229.8 (±11.9)	-8.63 (-8.73 to -8.54)	-8.15 (±0.12)	48.71 (48.64 to 48.78)	47	2.3	1.9	5.7	3.6
							Average % error between MeltR method 2 and Meltwin method 2		1.5	1.6	2.6	2.3

[a]Expectation maximized T_m at a C_t of 0.1 mM.
[b]Data compiled from a published source.[1]
[c]Data compiled in this work.

SI Table 6 BLtrimmer results comparing MeltR method 3 to Meltwin method 1 and 2.MeltR data are reused from SI table 2 and Meltwin data are reused from SI Table 2 and 4 for convenience.

Helix	ΔH° MeltR (kcal/mol)	ΔH° Meltwin (kcal/mol)	ΔS° MeltR (cal/mol/K)	ΔS° Meltwin (cal/mol/K)	$\Delta G°_{37}$ MeltR (kcal/mol)	$\Delta G°_{37}$ Meltwin (kcal/mol)	T_m[a] Meltwin (°C)	T_m[a] Meltwin (°C)	%error ΔH°	%error ΔS°	%error $\Delta G°_{37}$	%error T_m[a]
5'-CGCGCG-3'[a] '3'-GCGCGC-3'	-56.53 (-60.25 to -53.16)	-53.8 (±2.9)	-159.96 (-172.21 to -149.33)	-150.5 (±9.1)	-6.91 (-7.07 to -6.79)	-7.12 (±0.06)	43.94 (43.08 to 44.54)	45.6	2.1	2.6	1.2	1.6
5'-ACCGGU-3'[a] 3'-UGGCCA-3'	-59.86 (-61.28 to -58.83)	-59.8 (±1)	-171.4 (-175.85 to -168.12)	-171.9 (±3.3)	-6.7 (-6.75 to -6.67)	-6.52 (±0.01)	42.37 (42.28 to 42.51)	41.4	0.0	0.1	1.0	1.0
5'-CCAUGG-3'[a] 3'-GGUACC-5'	-67.98 (-69.89 to -66.16)	-65.85 (±1.448)	-186.59 (-192.46 to -181.12)	-180.82 (±4.495)	-10.11 (-10.2 to -10)	-9.77 (±0.055)	54.21 (53.95 to 54.54)	53	1.6	1.6	1.9	1.2
5'-GAUAUAUC-3'[a] 3'-CUAUAUAG-5'	-56.76 (-57.94 to -55.81)	-52 (±3.8)	-156.93 (-160.59 to -153.94)	-142.6 (±11.9)	-8.09 (-8.16 to -8.01)	-7.72 (±0.16)	50.76 (50.35 to 51.04)	49.7	3.7	4.0	2.0	1.0
5'-GCAAUUGC-3'[a] 3'-CGUUAACG-5'	-51.92 (-54.53 to -50.37)	-48.17 (±1.779)	-143.61 (-151.88 to -138.78)	-132.17 (±5.73)	-7.38 (-7.45 to -7.28)	-7.18 (±0.024)	42.13 (41.56 to 42.39)	41.2	2.9	3.2	1.1	0.9
5'-CGAAAGGU-3'[b] 3'-GCUUUCCA-5'	-63.31 (-64 to -62.69)	-63.61 (±1.507)	-173.83 (-175.92 to -171.93)	-175.57 (±4.712)	-9.39 (-9.44 to -9.35)	-9.16 (±0.047)	51.69 (51.43 to 51.88)	50.4	0.2	0.5	1.3	1.3
5'-CUGAGUC-3'[b] 3'-GACUCAG-5'	-80.92 (-83.27 to -79.05)	-81.05 (±6.734)	-216.39 (-223.4 to -210.93)	-217.41 (±20.017)	-13.81 (-14 to -13.65)	-13.62 (±0.538)	67.66 (67.13 to 68.11)	66.7	0.1	0.3	1.1	0.9
5'-CGUUGC-3'[b] 3'-GCAACG-5'	-60.32 (-62.02 to -58.19)	-62.23 (±3.751)	-160.72 (-165.71 to -154.29)	-167.11 (±11.45)	-10.48 (-10.61 to -10.33)	-10.4 (±0.207)	58.7 (58.37 to 58.92)	57.6	1.5	1.8	0.4	1.1
FAM-CGAAAGGU-3'[b] BHQ1-GCUUUCCA-5'	-75.38 (-77.21 to -73.3)	-74.63 (±6.17)	-199.36 (-204.73 to -193.23)	-198.05 (±18.308)	-13.54 (-13.71 to -13.34)	-13.21 (±0.508)	68.82 (68.47 to 69.33)	67.5	0.6	0.4	1.8	1.3
FAM-CUGAGUC-3'[b] BHQ1-GACUCAG-5'	-74.15 (-78.59 to -71.74)	-66 (±2.4)	-221.96 (-236.28 to -214.21)	-195 (±7.9)	-5.31 (-5.34 to -5.27)	-5.48 (±0.04)	35.47 (35.26 to 35.59)	36.1	6.3	7.4	0.9	0.6
FAM-CGUUGC-3'[b] BHQ1-GCAACG-5'	-81.24 (-84.35 to -79.04)	-78.1 (±1.7)	-234.11 (-243.79 to -227.28)	-225.7 (±5.3)	-8.63 (-8.73 to -8.54)	-8.09 (±0.05)	48.71 (48.64 to 48.78)	46.9	2.4	2.3	3.0	1.8
							Average % error between MeltR method 2 and Meltwin method 2		1.9	2.2	1.4	1.2

[a]Expectation maximized T_m at a C_t of 0.1 mM.
[b]Data compiled from a published source.[1]
[c]Data compiled in this work.

Supplementary information references

(1) Adams, M. S.; Znosko, B. M. Thermodynamic Characterization and Nearest Neighbor Parameters for RNA Duplexes under Molecular Crowding Conditions. *Nucleic Acids Research* **2019**, *47* (7), 3658–3666. https://doi.org/10.1093/nar/gkz019.