Supporting Information

Nearest-neighbor parameters for the prediction of RNA duplex stability in diverse *in vitro* and cellular-like crowding conditions

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Contents:

Supporting Method

Energetic estimation of nearest-neighbor (NN) parameters for G-U base pair and loop contribution

For G-U wobble base pairs, we estimated the stability of NN parameters including G-U pair according to the rules of the changes in NN pairs containing A-U pair because both base pairs have two hydrogen bonds, that are approximated to have same energy contribution of terminal A-U pair (Table S19) (1). For example, $\Delta G^{\circ}_{37,\ \mathrm{NN,\ no\ cosolute}}$ value for r(GA/UU) in 100 mM NaCl was determined to be -0.96 kcal mol^{-1} from its value of -1.27 kcal mol^{-1} in 1 M NaCl, applying the same extent of reduction in the stability observed for r(AA/UU) in 100 mM NaCl from its value in 1 M NaCl. The m_{cs} values for NN pairs containing G-U base pairs in different cosolutes were considered to be same as determined for NN pairs having A-U pair (Table S19). Empirical relationships were proposed for the stability of different types of loop structures in varying concentrations of Na^{+} and Mg^{2+}, although negligible cation dependency was observed for shorter loops (2). A previous study suggested that the destabilization effect of the hairpin tetraloop was reduced by 58% in 40 wt% PEG200 (Table S20) (3). Based on this information and as the stability of the loop changes with changing the water activity of the solution, we formulated an empirical relation for loop stability in the cosolute solutions ($\Delta G^{\circ}_{37,\ \mathrm{loop,\ cosolute}}$) from their stability in the absence of cosolute ($\Delta G^{\circ}_{37,\ \mathrm{loop,\ no\ cosolute}}$) and change in the water activity of cosolute solution (Δa_{w}) as follows:

$$\Delta G^{\circ}_{37,\ \mathrm{loop,\ cosolute}} = \Delta G^{\circ}_{37,\ \mathrm{loop,\ no\ cosolute}} \bullet (1 - 11.8 \bullet \Delta a_{\mathrm{w}})$$

Δa_{w} is 0.049 for 40 wt% PEG200. The factor 11.8 was determined to match the extent of the reduction in the measured stability of a tetraloop in 40 wt% PEG200 (Table S20).

Table S1. RNA sequences with nearest-neighbor frequencies[a]

		Nearest-neighbor combinations present in duplex									
No.	Sequence[b]	AA UU	AU UA	UA AU	CU GA	CA GU	GU CA	GA CU	CG GC	GC CG	GG CC
NS1	CAUGCC		1			2				1	1
NS2	CUAGGC			1	2					1	1
NS3	CAGCGG				1	1			1	1	1
NS4	CUACGC			1	1		1		1	1	
NS5	GAACUCC	1			1		1	2			1
NS6	GGCUGUCC										
NS7a	GGCUGUUC	1			1	1	1	1		1	1
NS7b	GGUUCUGC	1			1	1	1	1		1	1
NS8a	AGCUGUCU				3	1	1	1		1	
NS8b	AGUCUGCU				3	1	1	1		1	
NS9a	GGCAGUUC	1			1	1	1	1		1	1
NS9b	GGUUCAGC	1			1	1	1	1		1	1
NS10a	CGCUGUCG				1	1	1	1	2	1	
NS10b	CGUCUGCG				1	1	1	1	2	1	
NS11a	UGCUGUCA				1	3	1	1		1	
NS11b	UGUCUGCA				1	3	1	1		1	
NS12a	UAUGAGGA		1	1	1	1		2			1
NS12b	UAGAUGGA		1	1	1	1		2			1
NS13a	GUGCCGAG				1	1	1	1	1	1	1
NS13b	GCCGAGUG				1	1	1	1	1	1	1
NS14a	CGCUGUAG			1	2	1	1		1	1	
NS14b	CGUGCUAG			1	2	1	1		1	1	
NS15a	AUUGGAUACAAA	3	2	1		2	1	1			1
NS15b	AUACAUUGGAAA	3	2	1		2	1	1			1
NS16	GGCUCAAUUGAC	2	1		1	2	1	2		1	1
S1	UACAUGUA		1	2		2	2				
S2	GGUAUACC		1	2			2				2
S3	GGUUAACC	2		1			2				2
S4	CGAAUUCG	2	1					2	2		
S5	GCUUAAGC	2	1		2					2	
S6	CGUAUACG		1	2			2		2		
S7	CGCAUGCG		1			2			2	2	
S8	CGGUACCG			1			2		2		2
S9a	CAAGCUUG	2			2	2				1	
S9b	CUUGCAAG	2			2	2				1	
S10a	GAACGUUC	2					2	2	1		
S10b	GUUCGAAC	2					2	2	1		
S11a	GAUCCGGAUC		2					4	1		2
S11b	GGAUCGAUCC		2					4	1		2
S12a	AUGAGCUCAU		2		2		2	2		1	

S12b	AUCAGCUGAU		2		2		2	2		1	
S13	UUACGCGUAA	2		2	2		2		2	1	
S14	AUCGCUAGCGAU		2	1				2	2	2	
S15a	CAUAGGCCUAUG		2	2	2		2			1	2
S15b	CUAUGGCCAUAG		2	2	2		2			1	2

[a]Total frequencies of the NN parameters are as follows: r(AA/UU) = 29, r(AU/UA) = 27, r(UA/AU) = 24, r(CA/GU) = 46, r(GU/CA) = 36, r(CU/GA) = 45, r(GA/CU) = 43, r(CG/GC) = 26, r(GC/CG) = 31, and r(GG/CC) = 33. [b]RNA duplex consists of denoted RNA strand (5' → 3') and complementary RNA strand.

Table S2. Thermodynamic parameters for RNA duplexes measured in the crowding condition and predicted in the absence of cosolute

		Measured in the presence of 40 wt% PEG200[b]				Predicted in the absence of cosolute[c]		Difference between with and without cosolute	
No.	Sequence[a]	$\Delta H°$ (kcal mol^{-1})	$T\Delta S°$ (kcal mol^{-1})	$\Delta G°_{37}$ (kcal mol^{-1})	T_m[d] (°C)	$\Delta G°_{37}$ (kcal mol^{-1})	T_m[d] (°C)	$\Delta\Delta G°_{37}$ (kcal mol^{-1})	ΔT_m (°C)
NS1	CAUGCC	-56.5 ± 1.9	-50.9 ± 1.7	-5.6 ± 0.3	31.0	-6.4	35.2	0.8	-4.2
NS2	CUAGGC	-57.5 ± 1.9	-51.4 ± 1.7	-6.1 ± 0.3	34.3	-7.2	39.8	1.1	-5.5
NS3	CAGCGG	-65.6 ± 3.0	-59.2 ± 2.7	-6.4 ± 0.4	36.4	-8.1	44.4	1.7	-8.0
NS4	CUACGC	-62.8 ± 4.7	-58.1 ± 4.4	-4.7 ± 0.6	27.8	-6.6	37.2	1.9	-9.4
NS5	GAACUCC	-71.8 ± 2.3	-65.9 ± 2.1	-5.9 ± 0.3	34.1	-8.1	43.3	2.2	-9.2
NS6	GGCUGUCC	-92.0 ± 2.0	-80.6 ± 3.0	-11.4 ± 0.6	54.3	-13.4	63.2	2.0	-8.9
NS7a	GGCUGUUC	-79.8 ± 1.9	-71.1 ± 1.7	-8.7 ± 0.7	45.6	-11.0	54.0	2.3	-8.4
NS7b	GGUUCUGC	-76.9 ± 2.1	-68.5 ± 1.9	-8.4 ± 0.8	44.9	-11.0	54.0	2.6	-9.1
NS8a	AGCUGUCU	-72.6 ± 2.1	-64.8 ± 1.9	-7.8 ± 0.9	42.4	-9.9	50.8	2.1	-8.4
NS8b	AGUCUGCU	-61.6 ± 0.6	-53.9 ± 0.6	-7.7 ± 0.3	42.6	-9.9	50.8	2.2	-8.2
NS9a	GGCAGUUC	-75.2 ± 2.3	-66.5 ± 2.0	-8.7 ± 0.9	46.2	-11.0	54.0	2.3	-7.8
NS9b	GGUUCAGC	-74.9 ± 2.2	-66.4 ± 2.0	-8.5 ± 0.9	45.1	-11.0	54.0	2.5	-8.9
NS10a	CGCUGUCG	-78.4 ± 2.4	-68.6 ± 2.1	-9.8 ± 1.0	49.8	-11.5	56.2	1.7	-6.4
NS10b	CGUCUGCG	-75.4 ± 2.4	-65.7 ± 2.1	-9.7 ± 1.0	50.3	-11.5	56.2	1.8	-5.9
NS11a	UGCUGUCA	-66.2 ± 1.8	-58.4 ± 1.6	-7.8 ± 0.7	43.0	-9.8	49.6	2.0	-6.6
NS11b	UGUCUGCA	-65.3 ± 0.9	-57.5 ± 0.8	-7.8 ± 0.4	42.9	-9.8	49.6	2.0	-6.7
NS12a	UAUGAGGA	-71.4 ± 1.4	-65.4 ± 1.3	-6.0 ± 0.6	34.7	-8.0	41.4	2.0	-6.7
NS12b	UAGAUGGA	-62.7 ± 0.8	-56.6 ± 0.7	-6.1 ± 0.3	35.2	-8.0	41.4	1.9	-6.2
NS13a	GUGCCGAG	-81.3 ± 1.3	-70.5 ± 1.1	-10.8 ± 0.5	54.3	-12.4	59.3	1.6	-5.0
NS13b	GCCGAGUG	-71.6 ± 2.1	-61.2 ± 1.8	-10.4 ± 0.8	54.1	-12.4	59.3	2.0	-5.2
NS14a	CGCUGUAG	-72.2 ± 0.9	-64.0 ± 0.8	-8.2 ± 0.4	44.1	-10.2	51.7	2.0	-7.6
NS14b	CGUGCUAG	-72.7 ± 1.9	-64.2 ± 1.7	-8.5 ± 0.8	45.8	-10.2	51.7	1.7	-5.9
NS15a	AUUGGAUACAAA	-105.8 ± 1.4	-97.4 ± 1.3	-8.4 ± 0.5	42.3	-10.5	48.4	2.1	-6.1
NS15b	AUACAUUGGAAA	-107.5 ± 1.2	-99.4 ± 1.1	-8.1 ± 0.5	41.6	-10.5	48.4	2.4	-6.8
NS16	GGCUCAAUUGAC	-118.5 ± 1.5	-102.8 ± 1.2	-15.7 ± 0.5	56.3	-16.2	61.7	0.5	-5.4
S1	UACAUGUA	-62.7 ± 2.9	-59.1 ± 2.8	-3.6 ± 0.3	28.2	-5.6	35.9	2.0	-7.7
S2	GGUAUACC	-71.9 ± 3.9	-65.7 ± 3.6	-6.2 ± 0.5	39.2	-9.1	51.8	2.9	-12.6
S3	GGUUAACC	-73.4 ± 2.8	-67.7 ± 2.6	-5.7 ±0.4	36.5	-8.7	50.3	3.0	-13.8
S4	CGAAUUCG	-70.3 ± 2.9	-65.8 ± 2.7	-4.5 ± 0.4	31.4	-7.0	38.5	2.5	-7.1
S5	GCUUAAGC	-70.4 ± 3.2	-64.3 ±2.9	-6.1 ± 0.4	38.6	-8.4	48.1	2.3	-9.5

S6	CGUAUACG	-73.8 ± 3.5	-68.3 ± 3.3	-5.5 ± 0.5	35.7	-7.3	43.9	1.8	-8.2
S7	CGCAUGCG	-79.3 ±3.7	-69.7 ± 3.2	-9.6 ± 0.6	52.7	-10.6	55.5	1.0	-2.8
S8	CGGUACCG	-73.4 ±6.2	-64.4 ± 5.5	-9.0 ± 1.0	51.0	-11.7	62.3	2.7	-11.3
S9a	CAAGCUUG	-78.3 ± 2.4	-73.0 ± 2.3	-5.3 ± 1.0	35.8	-7.4	45.3	2.1	-9.5
S9b	CUUGCAAG	-77.5 ± 2.6	-71.5 ± 2.4	-6.0 ± 1.1	38.3	-7.4	45.3	1.4	-7.0
S10a	GAACGUUC	-73.9 ± 1.2	-68.2 ± 1.1	-5.7 ± 0.5	36.8	-7.8	45.4	2.1	-8.6
S10b	GUUCGAAC	-83.1 ± 3.4	-77.9 ± 3.2	-5.2 ± 1.3	35.4	-7.8	45.4	2.6	-10.0
S11a	GAUCCGGAUC	-103.9 ± 1.7	-91.6 ± 1.5	-12.3 ± 0.7	58.4	-13.8	60.6	1.5	-2.2
S11b	GGAUCGAUCC	-100.1 ± 1.8	-88.7 ± 1.6	-11.4 ± 0.7	56.0	-13.8	60.6	2.4	-4.6
S12a	AUGAGCUCAU	-80.7 ± 2.3	-72.1 ± 2.0	-8.6 ± 0.3	48.8	-11.3	56.7	2.7	-7.9
S12b	AUCAGCUGAU	-83.3 ± 1.3	-75.1 ± 1.1	-8.2 ± 0.5	46.7	-11.3	56.7	3.1	-10.0
S13	UUACGCGUAA	-78.9 ±4.7	-71.7 ± 4.2	-7.2 ±0.6	42.2	-10.5	54.6	3.3	-12.4
S14	AUCGCUAGCGAU	-105.3 ± 4.7	-92.3 ± 4.8	-13.0 ± 0.8	60.0	-16.0	65.5	3.0	-5.5
S15a	CAUAGGCCUAUG	-130.9 ± 2.1	-116.5 ± 1.9	-14.4 ± 0.7	59.1	-16.5	68.2	2.1	-9.1
S15b	CUAUGGCCAUAG	-131.7 ± 2.2	-117.2 ± 2.2	-14.5 ± 0.7	59.4	-16.5	68.2	2.0	-8.8

[a]RNA duplex consists of a denoted RNA strand (5' → 3') and complementary RNA strand. The pair of RNA oligonucleotides with identical nearest neighbors are shown by the numbers a and b. [b]All experiments were performed in a buffer containing 100 mM NaCl, 10 mM Na_2HPO_4 (pH 7.0), and 1 mM Na_2EDTA. [c]The values in the absence of cosolute were calculated from the parameters of 121 mM Na^+ reported by Weber *et al.*(4) (Table S3) [d]Melting temperatures were calculated for a total strand concentration of 100 μM.

Table S3. NN parameters for RNA duplex formation in the absence of cosolute in 100 mM NaCl at 37 °C[a]

Sequence	ΔH°_{NN} (kcal mol^{-1})	ΔS°_{NN} (cal mol^{-1} K^{-1})	$\Delta G^{\circ}_{37, NN, no\ cosolute}$ (kcal mol^{-1})
r(AA/UU)	-10.7	-32.2	-0.70
r(AU/UA)	-7.6	-22.8	-0.52
r(UA/AU)	-11.5	-32.9	-1.30
r(CA/GU)	-11.7	-32.0	-1.78
r(GU/CA)	-11.2	-29.2	-2.14
r(CU/GA)	-9.6	-25.1	-1.80
r(GA/CU)	-14.8	-40.6	-2.22
r(CG/GC)	-10.9	-28.1	-2.18
r(GC/CG)	-15.1	-38.0	-3.30
r(GG/CC)	-12.9	-31.6	-3.10
initiation	3.6	-1.5	4.09
per terminal AU	3.7	10.5	0.45
self-complementary[b]	0	-1.4	0.43
non-self-complementary	0	0	0

[a]Parameters are collected from the report by Weber et al.(4) [b]Symmetry factors for self-complementary sequences are independent of the solution conditions.

Table S4. Stability contribution of 40 wt% PEG200 ($\Delta\Delta G°_{37,\ [40\ wt\%\ PEG200]} = \Delta G°_{37,\ [40\ wt\%\ PEG200,\ 100\ mM\ NaCl]} - \Delta G°_{37,\ [no\ cosolute,\ 100\ mM\ NaCl]}$) on the individual nearest-neighbor base pairs for RNA duplex formation at 100 mM NaCl

Sequence	$\Delta\Delta G°_{37,\ [40\ wt\%\ PEG200]}$[a] (kcal mol^{-1})
r(AA/UU)	0.13
r(AU/UA)	-0.03
r(UA/AU)	-0.03
r(CA/GU)	-0.36
r(GU/CA)	0.34
r(CU/GA)	0.03
r(GA/CU)	-0.02
r(CG/GC)	0.02
r(GC/CG)	0.23
r(GG/CC)	0.21
initiation	1.41
per terminal AU	0.40

[a]$\Delta G°_{37,\ [40\ wt\%\ PEG200,\ 100\ mM\ NaCl]}$ were taken from Table 1 and $\Delta G°_{37,\ [no\ cosolute,\ 100\ mM\ NaCl]}$ were collected from Table S3.

Table S5. Measured and predicted thermodynamic parameters for RNA duplex formation with 40 wt% PEG200 and 100 mM NaCl in 10 mM phosphate buffer (pH 7.0)

		Measured[b]				Predicted			
No.	Sequence[a]	$\Delta H°$ (kcal mol^{-1})	$\Delta S°$ (cal mol^{-1} K^{-1})	$\Delta G°_{37}$ (kcal mol^{-1})	T_m[c] (°C)	$\Delta H°$ (kcal mol^{-1})	$\Delta S°$ (cal mol^{-1}K^{-1})	$\Delta G°_{37}$ (kcal mol^{-1})	T_m[c] (°C)
NS1	CAUGCC	-56.5	-164.1	-5.6	31.0	-58.3	-170.9	-5.3	30.6
NS2	CUAGGC	-57.5	-165.7	-6.1	34.3	-57.5	-168.2	-5.3	30.7
NS3	CAGCGG	-65.6	-190.9	-6.4	36.4	-58.4	-167.2	-6.5	37.1
NS4	CUACGC	-62.8	-187.3	-4.7	27.8	-53.3	-156.9	-4.6	26.4
NS5	GAACUCC	-71.8	-212.5	-5.9	34.1	-65.5	-191.8	-6.0	34.6
NS6	GGCUGUCC	-92.0	-259.9	-11.4	54.3	-84.5	-236.0	-11.3	55.6
NS7a	GGCUGUUC	-79.8	-229.2	-8.7	45.6	-79.7	-228.0	-9.0	46.9
NS7b	GGUUCUGC	-76.9	-220.9	-8.4	44.9	-79.7	-228.0	-9.0	46.9
NS8a	AGCUGUCU	-72.6	-208.9	-7.8	42.4	-64.3	-183.6	-7.4	41.0
NS8b	AGUCUGCU	-61.6	-173.8	-7.7	42.6	-64.3	-183.6	-7.4	41.0
NS9a	GGCAGUUC	-75.2	-214.4	-8.7	46.2	-79.7	-228.0	-9.0	46.9
NS9b	GGUUCAGC	-74.9	-214.1	-8.5	45.1	-79.7	-228.0	-9.0	46.9
NS10a	CGCUGUCG	-78.4	-221.2	-9.8	49.8	-77.1	-216.8	-9.8	51.0
NS10b	CGUCUGCG	-75.4	-211.8	-9.7	50.3	-77.1	-216.8	-9.8	51.0
NS11a	UGCUGUCA	-66.2	-188.3	-7.8	43.0	-66.1	-187.0	-8.1	44.6
NS11b	UGUCUGCA	-65.3	-185.4	-7.8	42.9	-66.1	-187.0	-8.1	44.6
NS12a	UAUGAGGA	-71.4	-210.9	-6.0	34.7	-65.1	-190.7	-6.0	34.3
NS12b	UAGAUGGA	-62.7	-182.5	-6.1	35.2	-65.1	-190.7	-6.0	34.3
NS13a	GUGCCGAG	-81.3	-227.3	-10.8	54.3	-80.8	-226.4	-10.6	53.4
NS13b	GCCGAGUG	-71.6	-197.3	-10.4	54.1	-80.8	-226.4	-10.6	53.4
NS14a	CGCUGUAG	-72.2	-206.4	-8.2	44.1	-76.6	-219.4	-8.5	45.4
NS14b	CGUGCUAG	-72.7	-207.0	-8.5	45.8	-76.6	-219.4	-8.5	45.4
NS15a	AUUGGAUACAAA	-105.8	-314.0	-8.4	42.3	-105.1	-312.6	-8.2	41.8
NS15b	AUACAUUGGAAA	-107.5	-320.5	-8.1	41.6	-105.1	-312.6	-8.2	41.8
NS16	GGCUCAAUUGAC	-118.5	-331.5	-15.7	56.3	-123.6	-351.8	-14.5	58.3
S1	UACAUGUA	-62.7	-190.6	-3.6	28.2	-60.3	-183.3	-3.5	26.0
S2	GGUAUACC	-71.9	-211.8	-6.2	39.2	-78.7	-232.3	-6.7	40.9
S3	GGUUAACC	-73.4	-218.3	-5.7	36.5	-77.5	-230.8	-5.9	38.0
S4	CGAAUUCG	-70.3	-212.2	-4.5	31.4	-71.1	-214.5	-4.6	32.3
S5	GCUUAAGC	-70.4	-207.3	-6.1	38.6	-76.5	-226.6	-6.2	39.2
S6	CGUAUACG	-73.8	-220.2	-5.5	35.7	-71.3	-213.1	-5.2	35.0
S7	CGCAUGCG	-79.3	-224.7	-9.6	52.7	-79.5	-226.1	-9.4	52.1

S8	CGGUACCG	-73.4	-207.6	-9.0	51.0	-79.7	-227.6	-9.1	51.0
S9a	CAAGCUUG	-78.3	-235.4	-5.3	35.8	-75.8	-224.7	-6.1	38.8
S9b	CUUGCAAG	-77.5	-230.5	-6.0	38.3	-75.8	-224.7	-6.1	38.8
S10a	GAACGUUC	-73.9	-219.9	-5.7	36.8	-71.3	-212.3	-5.5	36.0
S10b	GUUCGAAC	-83.1	-251.2	-5.2	35.4	-71.3	-212.3	-5.5	36.0
S11a	GAUCCGGAUC	-103.9	-295.3	-12.3	58.4	-103.1	-293.5	-12.1	57.5
S11b	GGAUCGAUCC	-100.1	-286.0	-11.4	56.0	-103.1	-293.5	-12.1	57.5
S12a	AUGAGCUCAU	-80.7	-232.5	-8.6	48.8	-86.4	-250.1	-8.8	48.8
S12b	AUCAGCUGAU	-83.3	-242.1	-8.2	46.7	-86.4	-250.1	-8.8	48.8
S13	UUACGCGUAA	-78.9	-231.2	-7.2	42.2	-82.0	-241.3	-7.2	42.7
S14	AUCGCUAGCGAU	-105.3	-297.6	-13.0	60.0	-109.3	-309.6	-13.3	60.2
S15a	CAUAGGCCUAUG	-130.9	-375.6	-14.4	59.1	-127.8	-365.3	-14.5	60.0
S15b	CUAUGGCCAUAG	-131.7	-377.9	-14.5	59.4	-127.8	-365.3	-14.5	60.0

[a]RNA duplex consists of denoted RNA strand (5' → 3') and complementary RNA strand. [b]Errors associated with the measured values were mentioned in the Table S2. [c]Melting temperatures were calculated for total strand concentration of 100 µM.

Table S6. Parameters for RNA-PEG excluded volume calculations

Sequence	R [a] (Å)	L [b] (Å)	P [c]	$C(p)$ [d]	k [e] (Å)	V/N [f] (L mol^{-1})
6-bp duplex	10.0	20.4	2.04	1.20	8.88	0.76
6-nt strand	5.0	20.4	4.08	1.76	6.09	0.52
12-bp duplex	10.0	40.8	4.08	1.76	12.17	1.04
12-nt strand	5.0	40.8	8.16	2.37	9.01	0.77
24-bp duplex	10.0	81.6	8.16	2.37	18.03	1.54
24-nt strand	5.0	81.6	16.32	3.03	14.12	1.20
36-bp duplex	10.0	122.4	12.24	2.75	23.29	1.99
36-nt strand	5.0	122.4	24.48	3.42	18.75	1.60
34-nt hairpin	10.0	57.8	5.78	2.06	14.71	1.25
34-nt strand	5.0	115.6	23.12	3.36	18.01	1.54

[a] Radius of equivalent cylinder. [b]Length of equivalent cylinder. [a, b] Values are collected from the report of Knowles et al. (5) following both DNA and RNA oligomeric duplexes has almost same radius and length.(6) [c]$p = L/R$. [d]$C(p) = (1+p^2)^{3/2}/3p - p^2/3 + 2/3p - (1+p^2)^{1/2}/p + \ln [p+(1+p^2)^{1/2}]$. [e]$k = \pi pR/6C(p)$. [f]Obtained by using equation 10 and Kuhn length (l) of PEG was taken as 11.9 Å.(5)

Table S7. Excluded volume and energy contribution of excluded volume for different lengths of RNA duplexes with PEG

Sequence	$\Delta V/N$ [a] (L mol^{-1})	$\Delta G°_{37,\ ev,\ dx}/C{\bullet}N$ [b] (kcal mol^{-2} kg)
6-bp duplex	-0.28	-0.17
12-bp duplex	-0.50	-0.31
24-bp duplex	-0.86	-0.52
36-bp duplex	-1.21	-0.74

[a]Calculated from the values in Table S6 using equation 9. [b]Calculated by using equation 8 and taking density of water 0.99 kg L^{-1} at 37 °C.(5)

Table S8. Thermodynamic parameters of GAUUACGCCUG in different cosolute solutions and the water activity values of the solutions

Solution	$\Delta H°$ (kcal mol^{-1})	$T\Delta S°$ (cal mol^{-1} K^{-1})	$\Delta G°_{37}$ (kcal mol^{-1})	$\Delta G°_{37,\text{ ev, dup}}$[a] (kcal mol^{-1})	$\Delta G°_{37,\text{ wa, dup}}$[b] (kcal mol^{-1})	Water activity[c]
In 100 mM NaCl						
No cosolute	-97.8 ± 1.6	-83.7 ± 1.5	-14.1 ± 0.1			0.996
10 wt% EG	-97.9 ± 4.0	-84.3 ± 3.8	-13.6 ± 0.3	-0.45	0.95	0.970
15 wt% EG	-101.1 ± 1.0	-87.7 ± 0.9	-13.4 ± 0.1	-0.68	1.38	0.959
5 wt% Gly	-95.4 ± 2.6	-81.5 ± 2.3	-13.9 ± 0.3	-0.15	0.35	0.987
10 wt% Gly	-98.4 ± 1.6	-84.4 ± 1.5	-13.6 ± 0.1	-0.30	0.80	0.975
15 wt% Gly	-98.6 ± 1.0	-85.2 ± 0.8	-13.4 ± 0.1	-0.45	1.15	0.965
20 wt% Gly	-97.3 ± 1.7	-84.2 ± 1.6	-13.1 ± 0.1	-0.61	1.61	0.951
5 wt% 1,3 PDO	-100.1 ± 1.2	-86.2 ± 1.1	-13.9 ± 0.1	-0.18	0.38	0.985
10 wt% 1,3 PDO	-100.4 ± 3.1	-86.7 ± 2.9	-13.7 ± 0.3	-0.37	0.77	0.971
20 wt% 1,3 PDO	-103.5 ± 0.5	-90.0 ± 0.5	-13.5 ± 0.1	-0.73	1.33	0.947
40 wt% 1,3 PDO	-105.8 ± 2.2	-92.9 ± 2.1	-12.9 ± 0.1	-1.47	2.67	0.907
10 wt% 2-ME	-100.8 ± 1.6	-87.7 ± 1.6	-13.1 ± 0.1	-0.37	1.37	0.975
40 wt% 1,2-DME	-94.7 ± 5.5	-81.5 ± 5.1	-13.2 ± 0.3	-1.24	2.14	0.965
10 wt% PEG200	-102.2 ± 1.8	-88.3 ± 1.6	-13.9 ± 0.2	-0.56	0.76	0.985
20 wt% PEG200	-100.7 ± 1.3	-87.3 ± 1.2	-13.4 ± 0.1	-1.12	1.82	0.971
30 wt% PEG200	-101.4 ± 1.7	-88.4 ± 1.6	-13.0 ± 0.2	-1.67	2.77	0.956
40 wt% PEG200	-96.2 ± 1.9	-84.2 ± 1.8	-12.0 ± 0.2	-2.23	4.33	0.947
50 wt% PEG200	-90.6 ± 1.0	-79.6 ± 1.0	-11.0 ± 0.1	-2.79	5.89	0.931
20 wt% PEG400	-96.0 ± 5.4	-82.5 ± 5.1	-13.5 ± 0.3	-1.12	1.72	0.980
20 wt% PEG600	-103.5 ± 2.9	-88.9 ± 2.7	-14.6 ± 0.2	-1.21	0.71	0.983
In 1 M NaCl						
No cosolute	-98.4 ± 1.2	-81.9 ± 1.2	-16.5 ± 0.1			0.967
20 wt% EG	-99.8 ± 1.9	-84.8 ± 1.8	-15.0 ± 0.1	-0.90	2.40	0.907
15 wt% 1,3 PDO	-103.8 ± 2.1	-88.0 ± 2.0	-15.8 ± 0.2	-0.55	1.25	0.933
15 wt% 2-ME	-99.5 ± 0.6	-84.9 ± 0.5	-14.6 ± 0.1	-0.55	2.45	0.927
20 wt% 1,2-DME	-99.0 ± 2.7	-83.8 ± 2.7	-15.2 ± 0.2	-0.62	1.92	0.934
10 wt% PEG200	-98.5 ± 1.8	-82.4 ± 1.6	-16.1 ± 0.1	-0.56	0.96	0.953
20 wt% PEG200	-100.5 ± 2.4	-85.1 ± 2.2	-15.4 ± 0.2	-1.12	2.22	0.939
40 wt% PEG200	-92.3 ± 2.3	-78.9 ± 2.7	-13.4 ± 0.7	-2.23	5.33	0.911

[a]Calculated by using equation 11. Excluded volume for Gly, 1,3 PDO, 2-ME and 1,2-DME were considered to be same as EG due to similar molecular weights. [b]$\Delta G°_{37,\text{ wa, dup}} = \Delta G°_{37} - \Delta G°_{37,\text{ no cosolute}} - \Delta G°_{37,\text{ ev, dup}}$. [c]Water activities were calculated at 37 °C from corresponding osmolality values of respective solutions calculated as described in Methods section. Error limit was ± 0.002.

Table S9. Thermodynamic parameters for RNA duplexes measured in the presence of NaCl and KCl with 40 wt% PEG200

		NaCl[b]				KCl[c]			
No.	Sequence[a]	$\Delta H°$ (kcal mol^{-1})	$T\Delta S°$ (cal mol^{-1} K^{-1})	$\Delta G°_{37}$ (kcal mol^{-1})	T_m[d] (°C)	$\Delta H°$ (kcal mol^{-1})	$T\Delta S°$ (cal mol^{-1}K^{-1})	$\Delta G°_{37}$ (kcal mol^{-1})	T_m[d] (°C)
NS3	CAGCGG	-65.6 ± 3.0	-59.2 ± 2.7	-6.4 ± 0.4	36.4	-55.2 ± 2.7	-49.1 ± 2.4	-6.1 ± 0.4	34.3
NS5	GAACUCC	-71.8 ± 2.3	-65.9 ± 2.1	-5.9 ± 0.3	34.1	-63.2 ± 1.9	-57.8 ± 1.8	-5.4 ± 0.3	31.2
NS10a	CGCUGUCG	-78.4 ± 2.4	-68.6 ± 2.1	-9.8 ± 1.0	49.8	-71.9 ± 0.7	-63.1 ± 0.6	-8.8 ± 0.1	47.1
NS11a	UGCUGUCA	-66.2 ± 1.8	-58.4 ± 1.6	-7.8 ± 0.7	43.0	-62.6 ± 1.6	-55.6 ± 1.4	-7.0 ± 0.2	39.6
NS13a	GUGCCGAG	-81.3 ± 1.3	-70.5 ± 1.1	-10.8 ± 0.5	54.3	-72.5 ± 3.7	-62.8 ± 3.2	-9.7 ± 0.6	51.1
NS13b	GCCGAGUG	-71.6 ± 2.1	-61.2 ± 1.8	-10.4 ± 0.8	54.1	-71.8 ± 5.4	-62.0 ± 4.7	-9.8 ± 0.9	51.6
S2	GGUAUACC	-71.9 ± 3.9	-65.7 ± 3.6	-6.2 ± 0.5	39.2	-72.3 ± 2.0	-66.8 ± 1.8	-5.5 ± 0.3	36.1
S3	GGUUAACC	-73.4 ± 2.8	-67.7 ± 2.6	-5.7 ± 0.4	36.5	-66.7 ± 3.3	-61.7 ± 3.1	-5.0 ± 0.5	33.6
S5	GCUUAAGC	-70.4 ± 3.2	-64.3 ± 2.9	-6.1 ± 0.4	38.6	-64.2 ± 1.1	-59.1 ± 1.0	-5.1 ± 0.1	34.1
S7	CGCAUGCG	-79.3 ± 3.7	-69.7 ± 3.2	-9.6 ± 0.6	52.7	-75.1 ± 2.9	-66.6 ± 2.6	-8.5 ± 0.5	48.6
S8	CGGUACCG	-73.4 ± 6.2	-64.4 ± 5.5	-9.0 ± 1.0	52.7	-69.9 ± 3.5	-61.0 ± 3.1	-8.6 ± 0.6	50.4
S11a	GAUCCGGAUC	-103.9 ± 1.7	-91.6 ± 1.5	-12.3 ± 0.7	58.4	-105.3 ± 4.1	-93.9 ± 3.7	-11.4 ± 0.6	55.0
S12a	AUGAGCUCAU	-80.7 ± 2.3	-72.1 ± 2.0	-8.6 ± 0.3	48.8	-79.6 ± 3.5	-72.2 ± 3.2	-7.4 ± 0.5	44.1
S12b	AUCAGCUGAU	-83.3 ± 1.3	-75.1 ± 1.1	-8.2 ± 0.5	46.7	-79.8 ± 2.7	-72.8 ± 2.4	-7.0 ± 0.4	42.1
S13	UUACGCGUAA	-78.9 ± 4.7	-71.7 ± 4.2	-7.2 ± 0.6	42.2	-77.7 ± 1.7	-71.5 ± 1.5	-6.2 ± 0.2	38.8
S14	AUCGCUAGCGAU	-105.3 ± 4.7	-92.3 ± 4.8	-13.0 ± 0.8	60.0	-100.7 ± 1.9	-89.4 ± 1.6	-11.3 ± 0.3	55.4

[a]RNA duplex consists of denoted RNA strand (5′ → 3′) and complementary RNA strand. [b]Experiments were performed in a buffer containing 100 mM NaCl, 10 mM Na_2HPO_4, and 1 mM Na_2EDTA in 40 wt% PEG200 at pH 7.0. Values were collected from Table S2. [c]Experiments were performed in a buffer containing 100 mM KCl, 10 mM K_2HPO_4, and 1 mM K_2EDTA in 40 wt% PEG200 at pH 7.0. [d]Melting temperatures were calculated for total strand concentration of 100 μM.

Table S10. Dielectric constants (ε_r) of the NaCl and KCl solutions in the presence and absence of cosolutes at 37 °C

Solutions	ε_r
In 100 mM NaCl with 10 mM Na_2HPO_4, and 1 mM Na_2EDTA	
absence of cosolute	76.8 ± 0.6
40 wt% PEG200	39.4 ± 0.4
40 wt% EG	62.0 ± 0.5
In 100 mM KCl with 10 mM K_2HPO_4, and 1 mM K_2EDTA	
absence of cosolute	74.5 ± 0.6
40 wt% PEG200	42.5 ± 0.4
40 wt% EG	63.2 ± 0.5

Table S11. Thermodynamic parameters for RNA duplexes measured in the presence of NaCl and KCl without any cosolute

		NaCl[b]				KCl[c]			
No.	Sequence[a]	$\Delta H°$ (kcal mol^{-1})	$T\Delta S°$ (cal mol^{-1} K^{-1})	$\Delta G°_{37}$ (kcal mol^{-1})	T_m[d] (°C)	$\Delta H°$ (kcal mol^{-1})	$T\Delta S°$ (cal mol^{-1}K^{-1})	$\Delta G°_{37}$ (kcal mol^{-1})	T_m[d] (°C)
NS1	CAUGCC	-62.0 ± 3.2	-55.0 ± 2.9	-7.0 ± 0.5	39.2	-57.6 ± 5.4	-50.7 ± 4.8	-6.9 ± 0.8	38.5
NS2	CUAGGC	-54.7 ± 5.5	-47.1 ± 4.9	-7.6 ± 0.9	42.5	-69.4 ± 7.3	-62.0 ± 6.6	-7.4 ± 1.0	40.7
NS5	GAACUCC	-67.3 ± 3.5	-59.4 ± 3.2	-7.9 ± 0.5	43.1	-71.1 ± 4.1	-63.5 ± 3.7	-7.6 ± 0.6	41.6
NS11a	UGCUGUCA	-71.8 ± 1.7	-61.6 ± 1.5	-10.2 ± 0.3	54.1	-69.4 ± 1.8	-59.5 ± 1.5	-9.9 ± 0.3	53.1
S6	CGUAUACG	-74.2 ± 0.4	-67.0 ± 0.3	-7.2 ± 0.1	43.3	-77.3 ± 3.0	-70.4 ± 2.8	-6.9 ± 0.4	41.6
S8	CGGUACCG	-85.3 ± 1.9	-73.8 ± 1.6	-11.5 ± 0.3	59.9	-82.0 ± 2.6	-70.6 ± 2.3	-11.4 ± 0.4	60.0
S12a	AUGAGCUCAU	-83.2 ± 8.0	-71.6 ± 6.9	-11.6 ± 1.3	60.9	-77.0 ± 3.8	-66.0 ± 3.3	-11.0 ± 0.7	59.8
S13	UUACGCGUAA	-85.2 ± 3.1	-75.1 ± 2.8	-10.1 ± 0.5	53.6	-84.8 ± 3.7	-75.1 ± 3.3	-9.7 ± 0.6	52.1

[a]RNA duplex consists of denoted RNA strand (5' → 3') and complementary RNA strand. [b]Experiments were performed in a buffer containing 100 mM NaCl, 10 mM Na_2HPO_4, and 1 mM Na_2EDTA at pH 7.0. [c]Experiments were performed in a buffer containing 100 mM KCl, 10 mM K_2HPO_4, and 1 mM K_2EDTA at pH 7.0. [d]Melting temperatures were calculated for total strand concentration of 100 µM.

Table S12. Thermodynamic parameters for RNA duplexes measured in the presence of NaCl and KCl with 40 wt% EG

		NaCl[b]				KCl[c]			
No.	Sequence[a]	$\Delta H°$ (kcal mol^{-1})	$T\Delta S°$ (cal mol^{-1} K^{-1})	$\Delta G°_{37}$ (kcal mol^{-1})	T_m[d] (°C)	$\Delta H°$ (kcal mol^{-1})	$T\Delta S°$ (cal mol^{-1}K^{-1})	$\Delta G°_{37}$ (kcal mol^{-1})	T_m[d] (°C)
NS2	CUAGGC	-58.4 ± 6.1	-52.3 ± 5.8	-6.1 ± 0.8	33.8	-55.5 ± 3.2	-49.7 ± 2.9	-5.8 ± 0.4	33.2
NS5	GAACUCC	-65.3 ± 1.4	-59.2 ± 1.2	-6.1 ± 0.2	35.2	-65.5 ± 5.6	-59.7 ± 5.2	-5.8 ± 0.7	33.9
S2	GGUAUACC	-69.3 ± 4.7	-63.5 ± 4.3	-5.8 ± 0.7	36.0	-72.0 ± 3.2	-66.4 ± 3.0	-5.6 ± 0.4	36.5
S3	GGUUAACC	-71.4 ± 2.3	-66.1 ± 2.1	-5.3 ± 0.3	35.1	-67.2 ± 2.2	-62.0 ± 2.0	-5.2 ± 0.3	34.7
S5	GCUUAAGC	-68.3 ± 2.7	-62.6 ± 2.5	-5.7 ± 0.4	36.5	-64.1 ± 1.2	-58.8 ± 1.1	-5.3 ± 0.2	35.0
S8	CGGUACCG	-77.2 ± 4.2	-68.3 ± 3.7	-8.9 ± 0.7	50.1	-74.8 ± 0.9	-66.0 ± 0.8	-8.8 ± 0.1	50.2
S12a	AUGAGCUCAU	-80.4 ± 2.0	-71.9 ± 1.8	-8.5 ± 0.3	48.1	-75.1 ± 6.4	-67.1 ± 5.7	-8.0 ± 0.9	46.8
S13	UUACGCGUAA	-88.0 ± 2.8	-81.3 ± 2.6	-6.7 ± 0.4	40.8	-89.4 ± 5.8	-83.0 ± 5.4	-6.4 ± 0.7	39.8

[a]RNA duplex consists of denoted RNA strand (5' → 3') and complementary RNA strand. [b]Experiments were performed in a buffer containing 100 mM NaCl, 10 mM Na_2HPO_4, and 1 mM Na_2EDTA in 40 wt% EG at pH 7.0. [c]Experiments were performed in a buffer containing 100 mM KCl, 10 mM K_2HPO_4, and 1 mM K_2EDTA in 40 wt% EG at pH 7.0. [d]Melting temperatures were calculated for total strand concentration of 100 µM.

Table S13. Measured and calculated $\Delta G°_{37}$ for RNA duplexes under the molecular crowding environment of 40 wt% PEG200 with 100 mM KCl

No.	Sequence[a]	Measured $\Delta G°_{37}$ [b] (kcal mol^{-1})	Calculated $\Delta G°_{37}$ [c] (kcal mol^{-1})
NS3	CAGCGG	-6.1	-5.6
NS5	GAACUCC	-5.4	-5.1
NS10a	CGCUGUCG	-8.8	-8.7
NS11a	UGCUGUCA	-7.0	-7.1
NS13a	GUGCCGAG	-9.7	-9.4
NS13b	GCCGAGUG	-9.8	-9.4
S2	GGUAUACC	-5.5	-5.8
S3	GGUUAACC	-5.0	-5.0
S5	GCUUAAGC	-5.1	-5.3
S7	CGCAUGCG	-8.5	-8.3
S8	CGGUACCG	-8.6	-8.0
S11a	GAUCCGGAUC	-11.4	-10.8
S12a	AUGAGCUCAU	-7.4	-7.7
S12b	AUCAGCUGAU	-7.0	-7.7
S13	UUACGCGUAA	-6.2	-6.4
S14	AUCGCUAGCGAU	-11.3	-11.9

[a]RNA duplex consists of denoted RNA strand (5′ → 3′) and complementary RNA strand. [b]Errors associated with the measured values were mentioned in the Table S9. [c]Calculated using equation 15 and the parameters of Table 1.

Table S14. Measured and predicted $\Delta G°_{37}$ of RNA duplexes in different cosolutes and NaCl concentrations

Solution/Sequences[a]	Measured $\Delta G°_{37}$ (kcal mol^{-1})	Predicted $\Delta G°_{37}$ (kcal mol^{-1})
GGCUCAAUUGAC in 100 mM NaCl		
10 wt% PEG200	-15.1 ± 0.8	-15.4
20 wt% PEG200	-14.8 ± 0.6	-14.7
30 wt% PEG200	-14.0 ± 0.7	-14.0
40 wt% PEG200	-13.8 ± 0.6	-13.7
In 20 wt% EG at 100 mM NaCl		
GGAUCGAUCC	-12.7 ± 0.7	-13.1
AUCAGCUGAU	-9.9 ± 0.6	-9.9
GGCUCAAUUGAC	-14.4 ± 0.6	-15.0
In 20 wt% 1,3 PDO at 100 mM NaCl		
GAUCCGGAUC	-14.5 ± 0.7	-12.9
GGCUCAAUUGAC	-14.7 ± 0.6	-14.7
In 20 wt% PEG2000 at 100 mM NaCl[b]		
GGCUCAAUUGAC	-16.9 ± 0.8	-16.3
GAUUACGCCUG	-15.5 ± 0.5	-15.4
In 20 wt% PEG8000 at 100 mM NaCl[c]		
GGCUCAAUUGAC	-16.7 ± 0.4	-16.5
GGCUCAAUUGAC in 1 M NaCl		
10 wt% PEG200	-18.9 ± 0.8	-17.9
20 wt% PEG200	-18.3 ± 0.6	-17.2
In 20 vol% PEG200 at 1 M NaCl[d]		
UCAUGA[e]	-3.0 ± 0.1	-3.8
ACUGCG[e]	-6.7 ± 0.0	-7.2
AUGGAC[e]	-5.9 ± 0.1	-6.1
GCGAUA[e]	-5.3 ± 0.2	-5.6
GCUAUG[e]	-5.2 ± 0.2	-5.2
ACCGGU[e]	-7.1 ± 0.1	-6.0
AGCGCU[e]	-6.8 ± 0.1	-6.4
CACGUG[e]	-5.8 ± 0.0	-5.7
CAGCUG[e]	-5.9 ± 0.0	-6.7
CCAUGG[e]	-6.5 ± 0.0	-6.7
CCUAGG[e]	-6.9 ± 0.0	-6.4
CUGCAG[e]	-6.2 ± 0.1	-6.7
GACGUC[e]	-6.6 ± 0.0	-5.8
GAGCUC[e]	-6.9 ± 0.1	-6.8
GCAUGC[e]	-6.9 ± 0.1	-7.0

CGCGCG[e]	-7.7 ± 0.2	-8.3
CGGCCG[e]	-8.5 ± 0.1	-8.9
GCCGGC[e]	-10.2 ± 0.5	-9.9
GCGCGC[e]	-9.0 ± 0.4	-9.2
UAUAUAUA[e]	-2.3 ± 0.2	-1.9
UAAUAUUA[e]	-1.9 ± 0.4	-1.2
AACUAGUU[e]	-6.0 ± 0.0	-4.5
ACUAUAGU[e]	-5.8 ± 0.0	-5.2
AGAUAUCU[e]	-5.7 ± 0.1	-5.6
GAUAUAUC[e]	-5.5 ± 0.0	-5.3
GAAUAUUC[e]	-4.7 ± 0.1	-4.6
AACCGGUU[e]	-9.0 ± 0.3	-7.6
ACUGCAGU[e]	-9.2 ± 0.1	-9.3
GCAAUUGC[e]	-8.1 ± 0.1	-8.6
GAACGUUC[e]	-7.8 ± 0.1	-7.4
AGCGCGCU[e]	-12.5 ± 0.5	-11.9
AGCCGGCU[e]	-13.9 ± 0.2	-12.5
GCGAUCGC[e]	-12.4 ± 0.4	-11.7
GACCGGUC[e]	-12.9 ± 0.2	-12.0
UUAUCGAUAA[e]	-6.9 ± 0.0	-6.8
UAUCGAUA[e]	-5.8 ± 0.0	-5.2
AGCGCU[e]	-6.6 ± 0.1	-6.4
CGCGCG[e]	-8.6 ± 0.2	-8.3

[a]RNA duplex consists of denoted RNA strand (5' → 3') and complementary RNA strand. [b,c]Water activities were measured to be 0.987 and 0.990 for PEG2000 and PEG8000, respectively, at their 20 wt% solution in 10 mM Na-phosphate buffer with 100 mM NaCl and 0.1 M Na_2EDTA. [d]20 (v/v)% PEG200 is equivalent to 21.7 (w/w)% PEG200 at 37 °C. Since the contribution of water activity and excluded volume effect will be negligibly different between 21.7 wt% and 20 wt%, we calculated $\Delta G°_{37}$ in 20 vol% PEG200 using the parameters obtained for 20 wt% PEG200. [e]Measured values were collected from the report by Adams and Znosko.(7)

Table S15. Thermodynamic parameters for RNA duplexes measured in the presence of NaCl and exact intracellular cation condition with 40 wt% PEG200

		122 mM Na^{+b}				Intracellular cation condition[c]			
No.	Sequence[a]	$\Delta H°$ (kcal mol^{-1})	$T\Delta S°$ (cal mol^{-1} K^{-1})	$\Delta G°_{37}$ (kcal mol^{-1})	T_m[d] (°C)	$\Delta H°$ (kcal mol^{-1})	$T\Delta S°$ (cal $mol^{-1}K^{-1}$)	$\Delta G°_{37}$ (kcal mol^{-1})	T_m[d] (°C)
NS3	CAGCGG	-65.6 ± 3.0	-59.2 ± 2.7	-6.4 ± 0.4	36.4	-56.5 ± 1.4	-50.2 ± 1.3	-6.3 ± 0.2	36.0
NS5	GAACUCC	-71.8 ± 2.3	-65.9 ± 2.1	-5.9 ± 0.3	34.1	-76.9 ± 2.8	-70.9 ± 2.6	-6.0 ± 0.4	35.1
NS10a	CGCUGUCG	-78.4 ± 2.4	-68.6 ± 2.1	-9.8 ± 1.0	49.8	-74.7 ± 1.8	-65.4 ± 1.5	-9.3 ± 0.3	48.7
NS11a	UGCUGUCA	-66.2 ± 1.8	-58.4 ± 1.6	-7.8 ± 0.7	43.0	-61.0 ± 2.3	-53.6 ± 2.0	-7.4 ± 0.4	41.6
NS13a	GUGCCGAG	-81.3 ± 1.3	-70.5 ± 1.1	-10.8 ± 0.5	54.3	-73.1 ± 4.6	-62.9 ± 4.0	-10.2 ± 0.8	53.0
NS13b	GCCGAGUG	-71.6 ± 2.1	-61.2 ± 1.8	-10.4 ± 0.8	54.1	-74.1 ± 3.2	-63.8 ± 2.7	-10.3 ± 0.5	53.6
S2	GGUAUACC	-71.9 ± 3.9	-65.7 ± 3.6	-6.2 ± 0.5	39.2	-73.4 ± 1.4	-67.5 ± 1.3	-5.9 ± 0.2	37.9
S3	GGUUAACC	-73.4 ± 2.8	-67.7 ± 2.6	-5.7 ± 0.4	36.5	-68.5 ± 2.9	-63.1 ± 2.5	-5.4 ± 0.4	35.4
S5	GCUUAAGC	-70.4 ± 3.2	-64.3 ± 2.9	-6.1 ± 0.4	38.6	-74.0 ± 5.1	-68.7 ± 4.8	-5.3 ± 0.7	35.4
S7	CGCAUGCG	-79.3 ± 3.7	-69.7 ± 3.2	-9.6 ± 0.6	52.7	-78.1 ± 5.2	-69.3 ± 4.6	-8.8 ± 0.9	50.3
S8	CGGUACCG	-73.4 ± 6.2	-64.4 ± 5.5	-9.0 ± 1.0	52.7	-75.0 ± 1.7	-65.9 ± 1.5	-9.1 ± 0.3	52.0
S11a	GAUCCGGAUC	-103.9 ± 1.7	-91.6 ± 1.5	-12.3 ± 0.7	58.4	-94.8 ± 3.3	-83.3 ± 2.9	-11.5 ± 0.5	57.0
S12a	AUGAGCUCAU	-80.7 ± 2.3	-72.1 ± 2.0	-8.6 ± 0.3	48.8	-80.9 ± 5.8	-72.8 ± 5.2	-8.1 ± 0.7	46.8
S12b	AUCAGCUGAU	-83.3 ± 1.3	-75.1 ± 1.1	-8.2 ± 0.5	46.7	-81.9 ± 1.9	-74.2 ± 1.8	-7.7 ± 0.3	44.7
S13	UUACGCGUAA	-78.9 ± 4.7	-71.7 ± 4.2	-7.2 ± 0.6	42.2	-75.7 ± 2.2	-68.8 ± 2.0	-6.9 ± 0.3	41.7
S14	AUCGCUAGCGAU	-105.3 ± 4.7	-92.3 ± 4.8	-13.0 ± 0.8	60.0	-95.0 ± 8.3	-83.4 ± 7.3	-11.6 ± 1.5	57.1

[a]RNA duplex consists of denoted RNA strand (5′ → 3′) and complementary RNA strand. [b]Experiments were performed in a buffer containing 100 mM NaCl, 10 mM Na_2HPO_4, and 1 mM Na_2EDTA in 40 wt% PEG200 at pH 7.0. Values were collected from Table S2. [c]Experiments were performed in a buffer containing 120 mM KCl, 10 mM NaCl, 0.5 mM $MgCl_2$, 0.0001 mM $CaCl_2$ and 10 mM K_2HPO_4 in 40 wt% PEG200 at pH 7.2. [d]Melting temperatures were calculated for total strand concentration of 100 μM.

Table S16. Measured thermodynamic parameters for RNA duplexes in exact intracellular cation condition and the predicted values using parameters in 40 wt% PEG200 with 100 mM NaCl

		Measured[b]				Predicted[c]			
No.	Sequence[a]	$\Delta H°$ (kcal mol^{-1})	$\Delta S°$ (cal mol^{-1} K^{-1})	$\Delta G°_{37}$ (kcal mol^{-1})	T_m[d] (°C)	$\Delta H°$ (kcal mol^{-1})	$\Delta S°$ (cal mol^{-1}K^{-1})	$\Delta G°_{37}$ (kcal mol^{-1})	T_m[d] (°C)
NS3	CAGCGG	-56.5	-161.9	-6.3	36.0	-58.4	-167.2	-6.5	37.1
NS5	GAACUCC	-76.9	-228.6	-6.0	35.1	-65.5	-191.8	-6.0	34.6
NS10a	CGCUGUCG	-74.7	-210.9	-9.3	48.7	-77.1	-216.8	-9.8	51.0
NS11a	UGCUGUCA	-61.0	-172.8	-7.4	41.6	-66.1	-187.0	-8.1	44.6
NS13a	GUGCCGAG	-73.1	-202.8	-10.2	53.0	-80.8	-226.4	-10.6	53.4
NS13b	GCCGAGUG	-74.1	-205.7	-10.3	53.6	-80.8	-226.4	-10.6	53.4
S2	GGUAUACC	-73.4	-217.6	-5.9	37.9	-78.7	-232.3	-6.7	40.9
S3	GGUUAACC	-68.5	-203.4	-5.4	35.4	-77.5	-230.8	-5.9	38.0
S5	GCUUAAGC	-74.0	-221.5	-5.3	35.4	-76.5	-226.6	-6.2	39.2
S7	CGCAUGCG	-78.1	-233.4	-8.8	50.3	-79.5	-226.1	-9.4	52.1
S8	CGGUACCG	-75.0	-212.5	-9.1	52.0	-79.7	-227.6	-9.1	51.0
S11a	GAUCCGGAUC	-94.8	-268.6	-11.5	57.0	-103.1	-293.5	-12.1	57.5
S12a	AUGAGCUCAU	-80.9	-234.7	-8.1	46.8	-86.4	-250.1	-8.8	48.8
S12b	AUCAGCUGAU	-81.9	-239.2	-7.7	44.7	-86.4	-250.1	-8.8	48.8
S13	UUACGCGUAA	-75.7	-221.8	-6.9	41.7	-82.0	-241.3	-7.2	42.7
S14	AUCGCUAGCGAU	-95.0	-268.9	-11.6	57.1	-109.3	-309.6	-13.3	60.2

[a]RNA duplex consists of denoted RNA strand (5′ → 3′) and complementary RNA strand. [b]Errors associated with the measured values were mentioned in the Table S15. [c]Predicted using the parameters of Table 1. [d]Melting temperatures were calculated for total strand concentration of 100 µM.

Table S17. Parameters for $\Delta G^\circ_{\text{NN, [no cosolute, 122 mM Na}^+]}$, $\Delta G^\circ_{\text{NN, ev}}$, $\Delta G^\circ_{\text{NN, wa}}$ and prefactor of PEG (m_{PEG}) at 25 °C

Sequence	$\Delta G^\circ_{\text{25, NN, [no cosolute, 122 mM Na}^+]}$[a] (kcal mol^{-1})	$\Delta G^\circ_{\text{25, NN, ev [40 wt\% PEG200]}}$[b] (kcal mol^{-1})	$\Delta G^\circ_{\text{25, NN, wa [40 wt\% PEG200]}}$[c] (kcal mol^{-1})	m_{PEG}[d] (kcal mol^{-1})
r(AA/UU)	-1.10	-0.21	0.37	7.6
r(AU/UA)	-0.81	-0.21	0.09	1.9
r(UA/AU)	-1.70	-0.21	0.19	3.9
r(CA/GU)	-2.16	-0.21	-0.16	-3.3
r(GU/CA)	-2.50	-0.21	0.56	11.4
r(CU/GA)	-2.12	-0.21	0.19	3.9
r(GA/CU)	-2.70	-0.21	0.30	6.1
r(CG/GC)	-2.53	-0.21	0.22	4.5
r(GC/CG)	-3.78	-0.21	0.50	10.1
r(GG/CC)	-3.48	-0.21	0.34	6.9
initiation	4.05	-0.21	1.63	33.2
per terminal AU	0.57	NA	0.51	10.4

[a]Calculated from the ΔH° and ΔS° values in Table S3. [b]$\Delta G^\circ_{\text{25, NN, ev [40 wt\% PEG200]}}$ were calculated similarly as done in Table 2. [c]$\Delta G^\circ_{\text{25, NN, wa [40 wt\% PEG200]}} = \Delta G^\circ_{\text{25, NN, [40 wt\% PEG200, 122 mM Na}^+]} - \Delta G^\circ_{\text{25, NN, [no cosolute, 122 mM Na}^+]} - \Delta G^\circ_{\text{25, NN, ev [40 wt\% PEG200]}}$. $\Delta G^\circ_{\text{25, NN, [40 wt\% PEG200, 122 mM Na}^+]}$ were calculated from the ΔH° and ΔS° values in Table 1. [d]Determined by using equation 13.

Table S18. Calculated $\Delta G°_{25,\ [crowder]}$ for $(ACUG)_3$ and $(ACUG)_6$ in various PEG200 concentrations at 25 °C in 100 mM NaCl

Solution	$(ACUG)_3$			$(ACUG)_6$		
	$\Delta G°_{25,\ wa}$[a] (kcal mol^{-1})	$\Delta G°_{25,\ ev}$[b] (kcal mol^{-1})	$\Delta G°_{25,\ [crowder]}$[c] (kcal mol^{-1})	$\Delta G°_{25,\ wa}$[a] (kcal mol^{-1})	$\Delta G°_{25,\ ev}$[b] (kcal mol^{-1})	$\Delta G°_{25,\ [crowder]}$[c] (kcal mol^{-1})
10 wt% PEG200	1.0	-0.6	0.4	1.6	-1.0	0.6
20 wt% PEG200	2.3	-1.2	1.1	3.7	-2.0	1.7
30 wt% PEG200	3.7	-1.7	2.0	5.8	-3.0	2.8
40 wt% PEG200	4.5	-2.3	2.2	7.2	-4.0	3.2
50 wt% PEG200	6.0	-2.9	3.1	9.5	-5.1	4.4

[a]$\Delta G°_{25,\ wa}$ were calculated from the m_{PEG} values in Table S17 and Δa_w values using equation 13 at 298 K. [b]$\Delta G°_{25,\ ev}$ were calculated using equation 11 at 298 K.[c]$\Delta G°_{25,[crowder]} = \Delta G°_{25,\ wa} + \Delta G°_{25,\ ev}$.

Table S19. Estimated NN parameters including G-U wobble base pairs present in Im-4U

Sequence[a]	$\Delta G°_{37\ NN,\ [no,\ cosolute,\ 122\ mM\ Na^{+}]}$ (kcal mol^{-1})	$m_{PEG/2\text{-}ME/1,2\ DME}$ (kcal mol^{-1})	$m_{EG/Gly/1,3\ PDO}$ (kcal mol^{-1})
r(GA/UU)	-0.96	7.1	2.9
r(GG/UU)	-0.38	7.1	2.9
r(AG/UU)	-0.41	7.1	2.9
r(AU/UG)	-0.64	3.9	1.6
r(UG/AU)	-0.98	3.9	1.6

[a]$\Delta G°_{37}$ in 1 M NaCl solution were collected from collected from the report by Turner et al. (1)

Table S20. ΔG°_{37} of GAAA tetraloop in the absence and presence of 40 wt% PEG200

Sequence[a]	In the absence of PEG200[b] (kcal mol^{-1})	In the presence of 40 wt% PEG200[b] (kcal mol^{-1})
5′GGAGGAAACUCC 3′	1.47 ± 0.06	0.63 ± 0.09
5′GAAGGAAACUUC 3′	1.36 ± 0.06	0.57 ± 0.09

[a]Underlined region is the loop sequence. [b]Values were collected from the report by Leonard and Blose.(3)

Table S21. Predicted $\Delta G°_{37}$ of RNA hairpin Im-4U in different cosolute concentrations at 100 mM NaCl along with the Δa_w values in those cosolute solutions

Solution	Δa_w	$\Delta G°_{37}$[a] (kcal mol^{-1})
10 wt% EG	0.026	-0.5
20 wt% EG	0.047	-1.3
30 wt% EG	0.072	-2.2
40 wt% EG	0.087	-2.8
10 wt% Gly	0.021	-0.2
20 wt% Gly	0.045	-1.0
30 wt% Gly	0.076	-2.0
40 wt% Gly	0.101	-2.8
10 wt% 1,3 PDO	0.025	-0.4
20 wt% 1,3 PDO	0.049	-1.2
30 wt% 1,3 PDO	0.066	-1.9
40 wt% 1,3 PDO	0.089	-2.7

[a]$\Delta G°_{37} = \Delta G°_{37,\text{ no cosolute, 100 mM NaCl}} + \Delta G°_{37,\text{ wa}} + \Delta G°_{37,\text{ ev}}$, as shown in Figure S6. $\Delta G°_{37,\text{ no cosolute, 100 mM NaCl}}$ were calculated from the data in Table 2 and Table S19. $\Delta G°_{37,\text{ wa}}$ were determined from the m_{cs} values in Table S19 and Δa_w values in this Table and equation 13. $\Delta G°_{37,\text{ loop, [1M NaCl]}}$ values were collected from the report of Turner et al.(1) $\Delta G°_{37,\text{ ev}}$ was determined from the data in Table S6 and equation 8. For hairpin, $\Delta V = V_{\text{hairpin}} – V_{\text{single strand}}$.

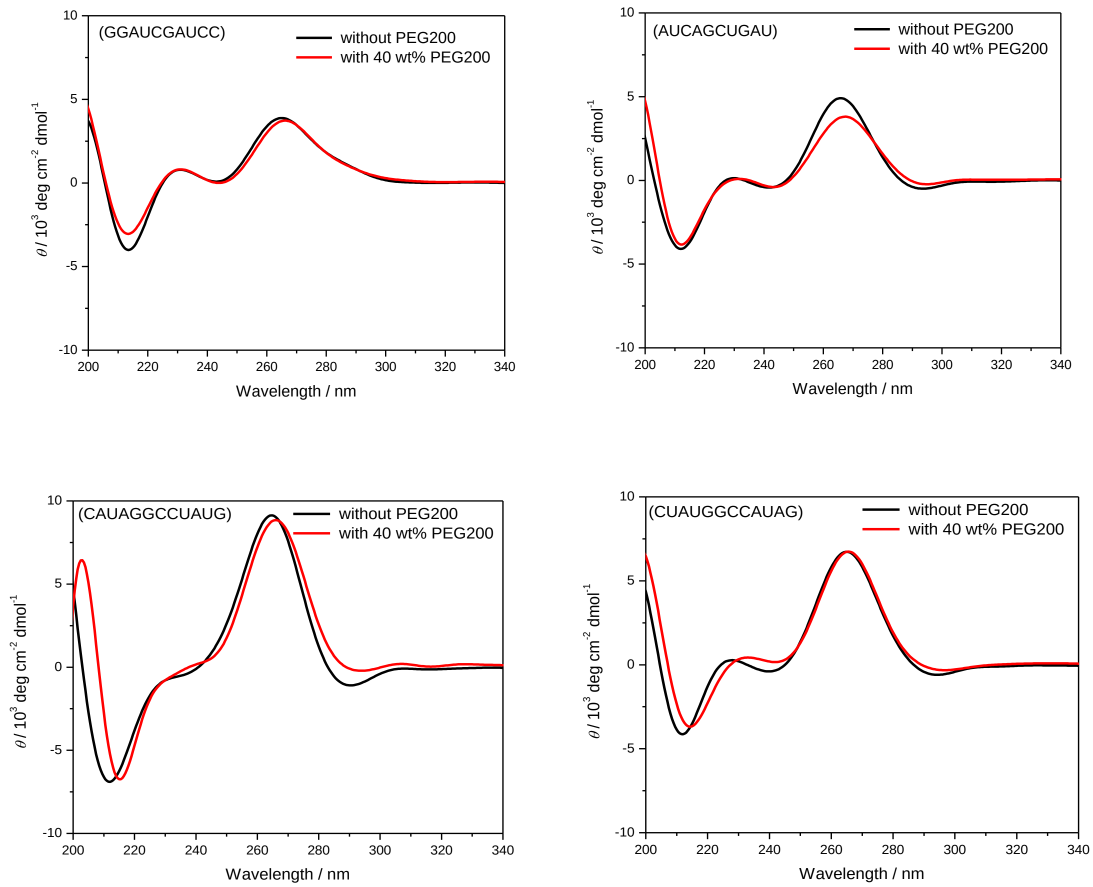


Figure S1. Representative CD spectra of 20 μM RNA oligonucleotides at 4 °C in a buffer containing 0.1 M NaCl, 10 mM Na_2HPO_4 (pH 7.0) and 1 mM Na_2EDTA in the absence (black) and presence (red) of 40 wt% PEG200.

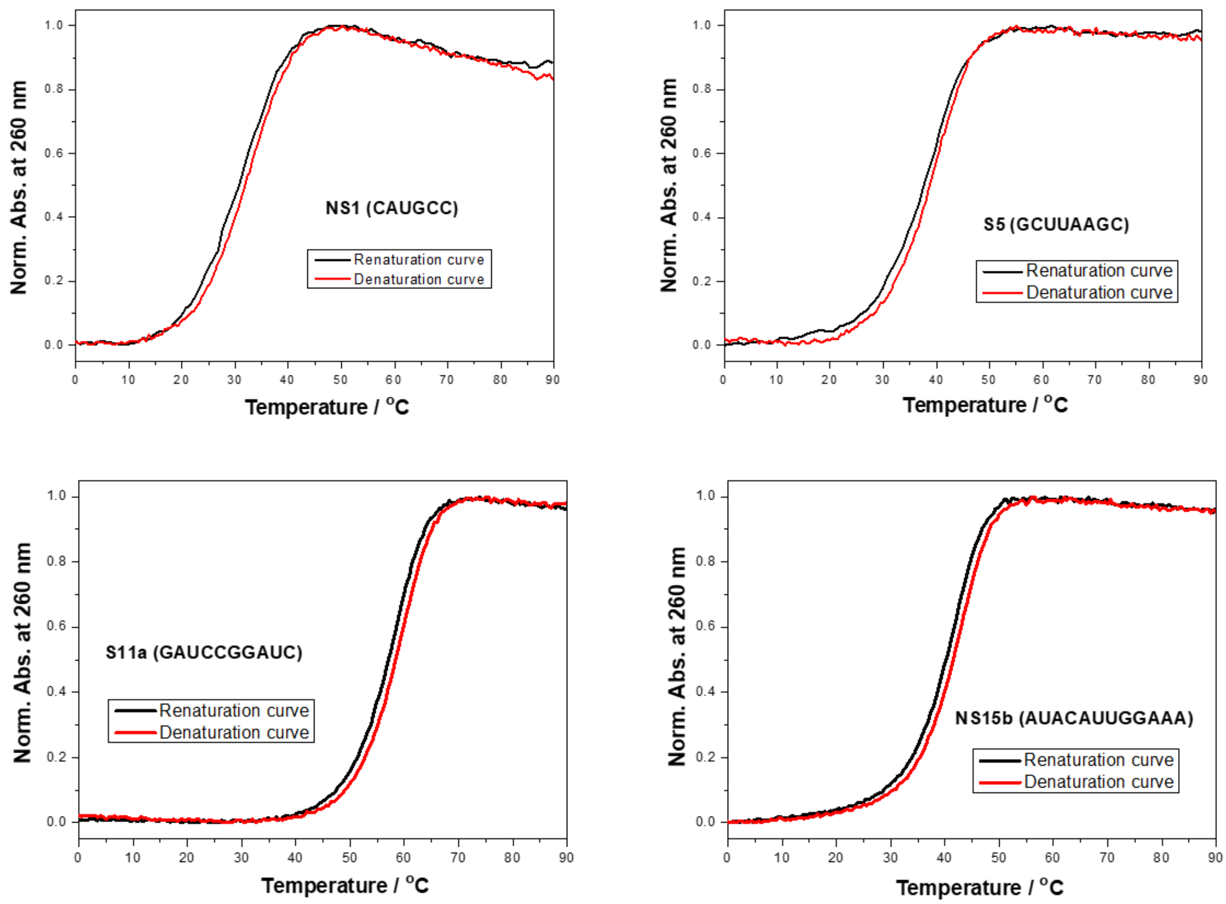
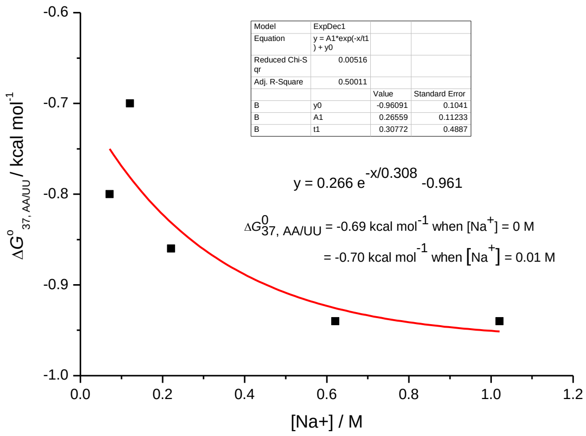
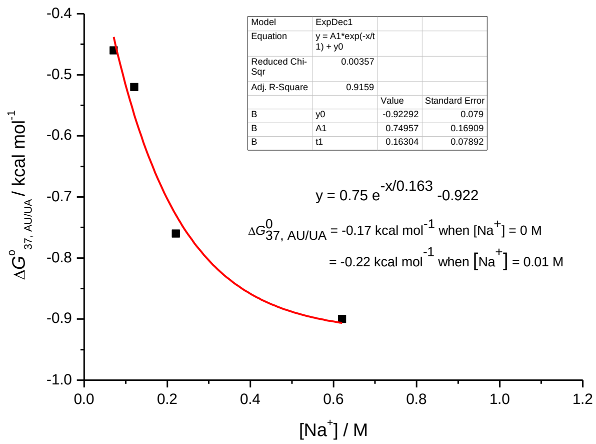


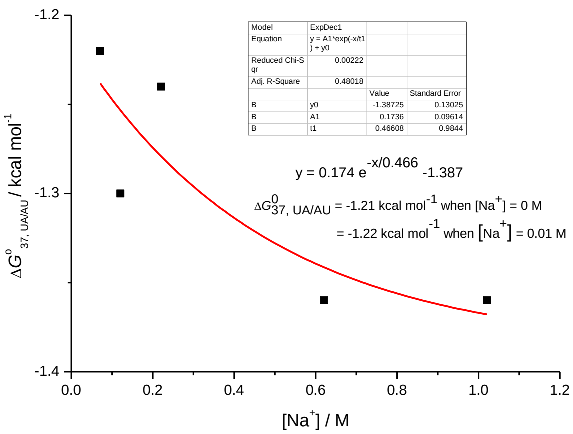
Figure S2. Renaturation and denaturation curves for some representative RNA oligonucleotides at 100 µM concentration in a buffer containing 0.1 M NaCl, 10 mM Na_2HPO_4 (pH 7.0), 1 mM Na_2EDTA and 40 wt% PEG200.



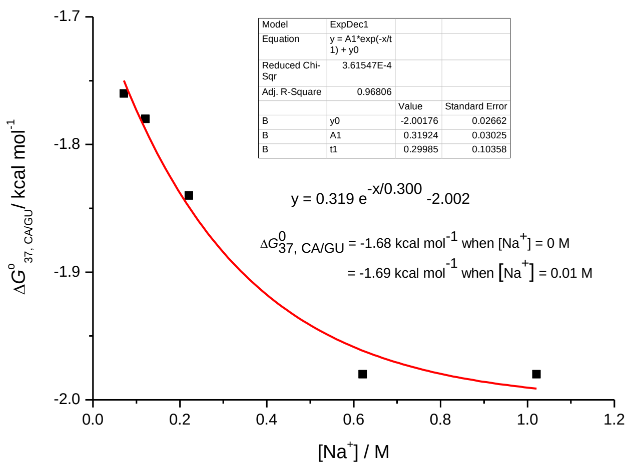
Model | ExpDec1
Equation | y = A1*exp(-x/t1) + y0
Reduced Chi-Sqr | 0.00516
Adj. R-Square | 0.50011
Value | Standard Error
B | y0 | -0.96091 | 0.1041
B | A1 | 0.26559 | 0.11233
B | t1 | 0.30772 | 0.4887
y = 0.266 e^-x/0.308 -0.961
ΔG0 37, AA/UU = -0.69 kcal mol-1 when [Na+] = 0 M
= -0.70 kcal mol-1 when [Na+] = 0.01 M
ΔGo 37, AA/UU / kcal mol-1
[Na+] / M



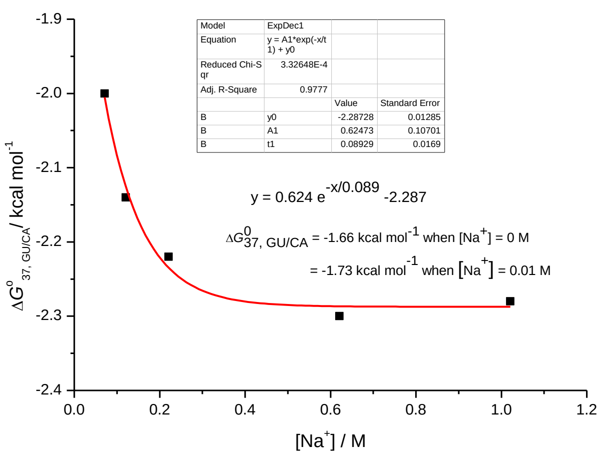
Model | ExpDec1
Equation | y = A1*exp(-x/t1) + y0
Reduced Chi-Sqr | 0.00357
Adj. R-Square | 0.9159
Value | Standard Error
B | y0 | -0.92292 | 0.079
B | A1 | 0.74957 | 0.16909
B | t1 | 0.16304 | 0.07892
y = 0.75 e^-x/0.163 -0.922
ΔG0 37, AU/UA = -0.17 kcal mol-1 when [Na+] = 0 M
= -0.22 kcal mol-1 when [Na+] = 0.01 M
ΔGo 37, AU/UA / kcal mol-1
[Na+] / M



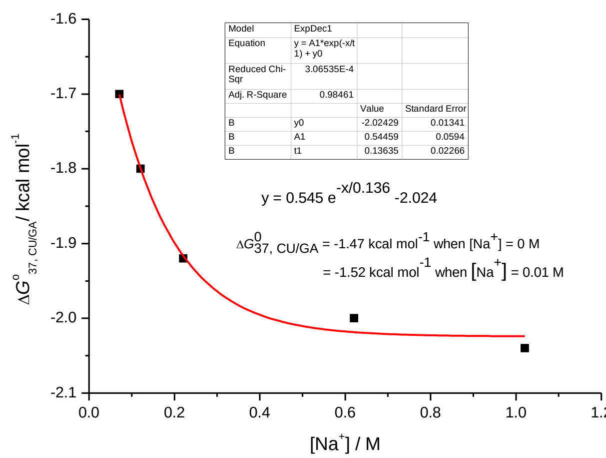
Model | ExpDec1
Equation | y = A1*exp(-x/t1) + y0
Reduced Chi-Sqr | 0.00222
Adj. R-Square | 0.48018
Value | Standard Error
B | y0 | -1.38725 | 0.13025
B | A1 | 0.1736 | 0.09614
B | t1 | 0.46608 | 0.9844
y = 0.174 e^-x/0.466 -1.387
ΔG0 37, UA/AU = -1.21 kcal mol-1 when [Na+] = 0 M
= -1.22 kcal mol-1 when [Na+] = 0.01 M
ΔGo 37, UA/AU / kcal mol-1
[Na+] / M



Model | ExpDec1
Equation | y = A1*exp(-x/t1) + y0
Reduced Chi-Sqr | 3.61547E-4
Adj. R-Square | 0.96806
Value | Standard Error
B | y0 | -2.00176 | 0.02662
B | A1 | 0.31924 | 0.03025
B | t1 | 0.29985 | 0.10358
y = 0.319 e^-x/0.300 -2.002
ΔG0 37, CA/GU = -1.68 kcal mol-1 when [Na+] = 0 M
= -1.69 kcal mol-1 when [Na+] = 0.01 M
ΔGo 37, CA/GU / kcal mol-1
[Na+] / M



Model | ExpDec1
Equation | y = A1*exp(-x/t1) + y0
Reduced Chi-Sqr | 3.32648E-4
Adj. R-Square | 0.9777
Value | Standard Error
B | y0 | -2.28728 | 0.01285
B | A1 | 0.62473 | 0.10701
B | t1 | 0.08929 | 0.0169
y = 0.624 e^-x/0.089 -2.287
ΔG0 37, GU/CA = -1.66 kcal mol-1 when [Na+] = 0 M
= -1.73 kcal mol-1 when [Na+] = 0.01 M
ΔGo 37, GU/CA / kcal mol-1
[Na+] / M



Model | ExpDec1
Equation | y = A1*exp(-x/t1) + y0
Reduced Chi-Sqr | 3.06535E-4
Adj. R-Square | 0.98461
Value | Standard Error
B | y0 | -2.02429 | 0.01341
B | A1 | 0.54459 | 0.0594
B | t1 | 0.13635 | 0.02266
y = 0.545 e^-x/0.136 -2.024
ΔG0 37, CU/GA = -1.47 kcal mol-1 when [Na+] = 0 M
= -1.52 kcal mol-1 when [Na+] = 0.01 M
ΔGo 37, CU/GA / kcal mol-1
[Na+] / M

Continued

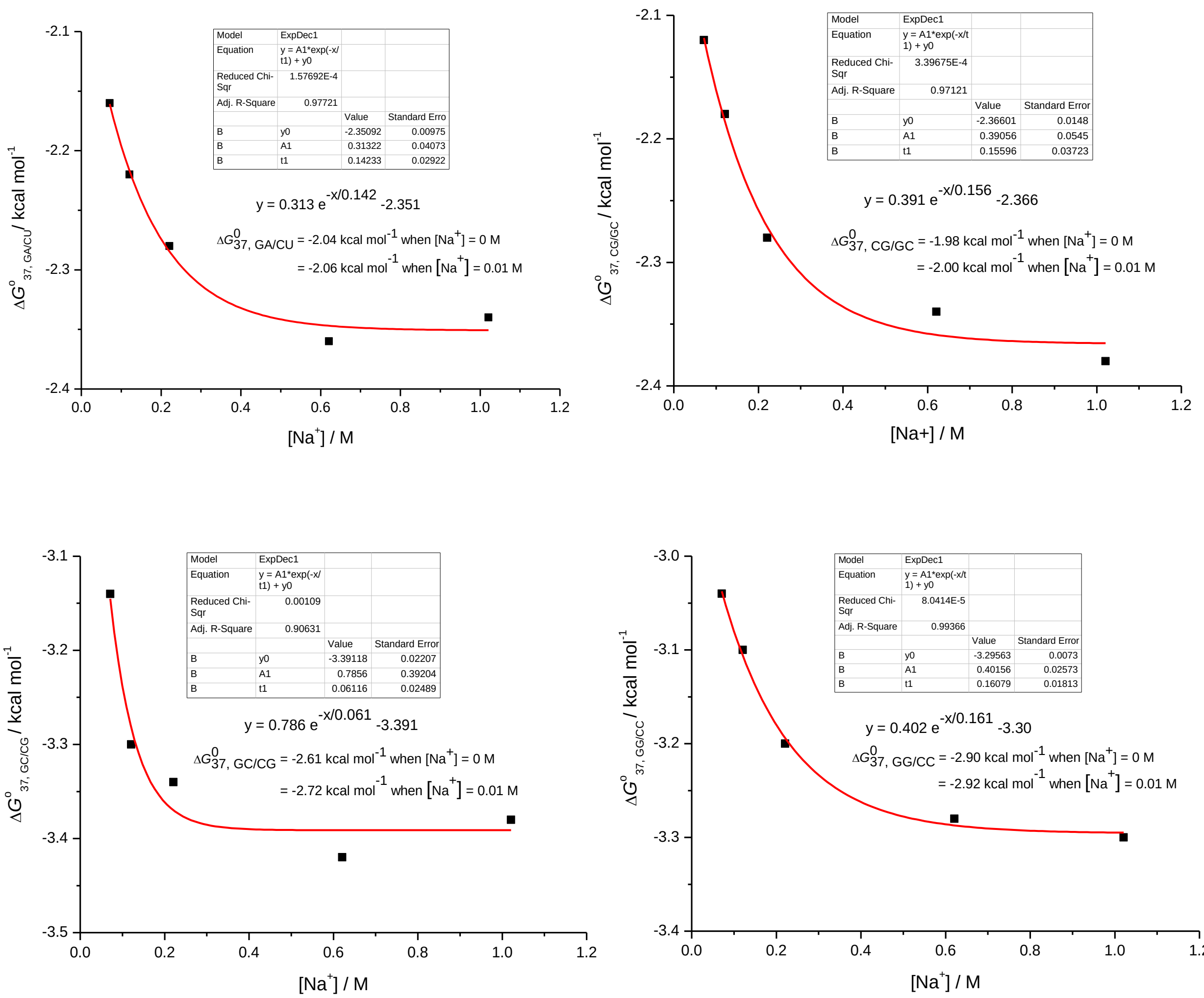


Figure S3. Plot of individual $\Delta G°_{37,\ NN}$ against the concentration of Na^+. Values are collected from the report by Weber et al.(4)

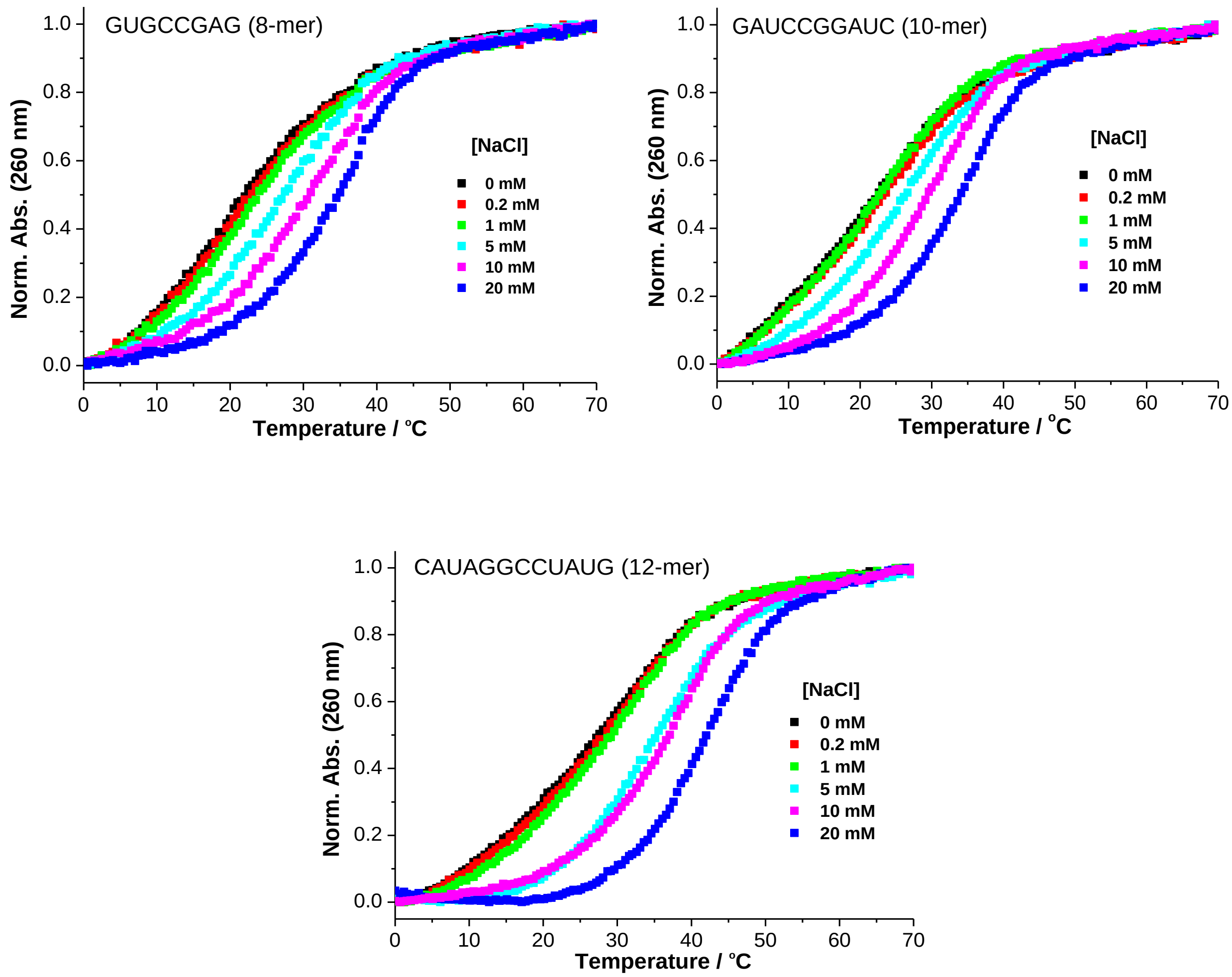
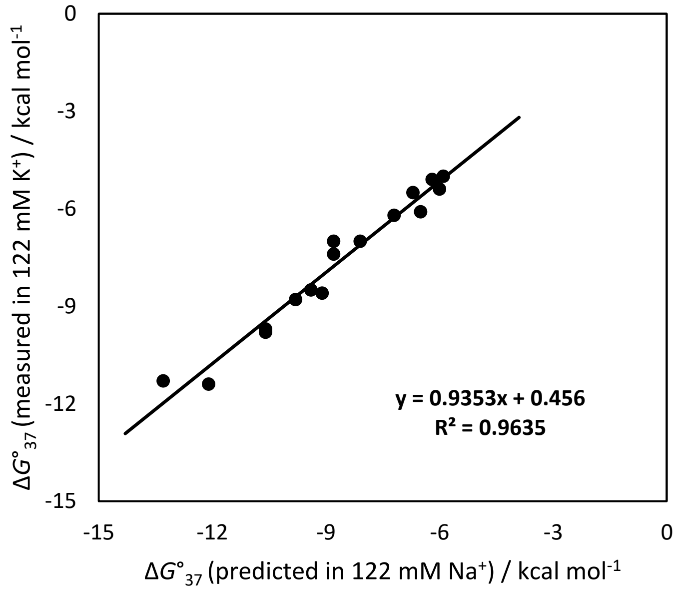


Figure S4. UV melting profiles of 10 μM RNA duplexes with the varying concentrations of NaCl. Samples were dialyzed overnight in Milli-Q water before setting the cation concentrations.

(A)



(B)

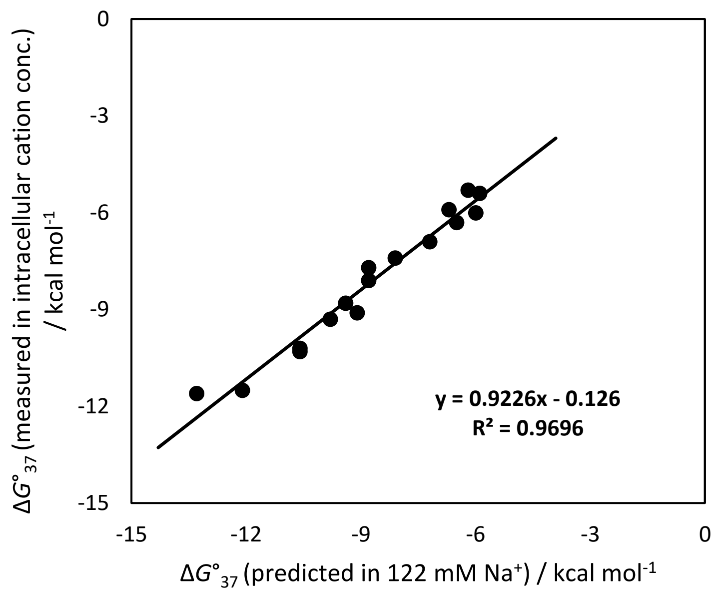
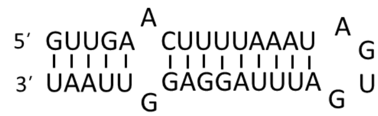


Figure S5. Relationship between measured ΔG°_{37} of RNA duplexes in **(A)** 122 mM K^+ solution or **(B)** intracellular cation concentration and predicted ΔG°_{37} in 122 mM Na^+ solution under the crowding condition of 40 wt% PEG200. Measured values are provided in Table S9 and Table S15, respectively and predicted values collected from Table S5.



Im-4U

$$\Delta G^\circ_{37,\,\text{hairpin}} = 3\Delta G^\circ_{37}(\text{AA/UU}) + \Delta G^\circ_{37}(\text{CU/GA}) + \Delta G^\circ_{37}(\text{AU/UA}) + \Delta G^\circ_{37}(\text{UA/AU}) + 2\Delta G^\circ_{37}(\text{GA/UU}) + \Delta G^\circ_{37}(\text{GG/UU}) + \Delta G^\circ_{37}(\text{AG/UU}) + \Delta G^\circ_{37}(\text{AU/UG}) + \Delta G^\circ_{37}(\text{UG/AU}) + \Delta G^\circ_{37,\,\text{initiation}} + \Delta G^\circ_{37,\,\text{terminal GU}} + \Delta G^\circ_{37,\,\text{hairpin loop}} + \Delta G^\circ_{37,\,\text{internal loop}}$$

$$\Delta G^\circ_{37}(\text{pred.}) = \Delta G^\circ_{37,\,\text{hairpin, no cosolute}} + \Delta G^\circ_{37,\,\text{hairpin, wa}} + \Delta G^\circ_{37,\,\text{hairpin, ev}}$$

$\Delta G^\circ_{37}(\text{AA/UU}) = \{ -0.70 + (7.1 \times 0.049)\}$ $\Delta G^\circ_{37}(\text{CU/GA}) = \{ -1.80 + (5.1 \times 0.049)\}$

$\Delta G^\circ_{37}(\text{AU/UA}) = \{ -0.52 + (3.9 \times 0.049)\}$ $\Delta G^\circ_{37}(\text{UA/AU}) = \{ -1.30 + (3.9 \times 0.049)\}$

$\Delta G^\circ_{37}(\text{GA/UU}) = \{ -0.96 + (7.1 \times 0.049)\}$ $\Delta G^\circ_{37}(\text{GG/UU}) = \{ -0.38 + (7.1 \times 0.049)\}$

$\Delta G^\circ_{37}(\text{AG/UU}) = \{ -0.41 + (7.1 \times 0.049)\}$ $\Delta G^\circ_{37}(\text{AU/UG}) = \{ -0.64 + (3.9 \times 0.049)\}$

$\Delta G^\circ_{37}(\text{UG/AU}) = \{ -0.98 + (3.9 \times 0.049)\}$ $\Delta G^\circ_{37,\,\text{initiation}} = \{ 4.09 + (33.3 \times 0.049)\}$

$\Delta G^\circ_{37,\,\text{terminal GU}} = \{ 0.45 + (8.2 \times 0.049)\}$ $\Delta G^\circ_{37,\,\text{hairpin loop}} = \{4.95 - (11.8 \times 5.25 \times 0.049)\}$

$\Delta G^\circ_{37,\,\text{internal loop}} = \{1.05 - (11.8 \times 1.05 \times 0.049)\}$ $\Delta G^\circ_{37,\,\text{hairpin, ev}} = -1.42$

$$\Delta G^\circ_{37}(\text{pred.}) = 1.1 \text{ kcal mol}^{-1}$$

$$\Delta G^\circ_{37}(\text{exp.}) = 0.4 \text{ kcal mol}^{-1}$$

Figure S6. Secondary structure of RNA hairpin Im-4U and prediction of its stability in 40 wt% PEG200 at 100 mM NaCl.

References

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