A CA^+ Pair Adjacent to a Sheared GA or AA Pair Stabilizes Size-Symmetric RNA Internal Loops

(Supporting Information)

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Table S1. Measured thermodynamic parameters for several individual single strand homoduplexes or hairpins. Most values were done at pH 7 with those shown in bold at pH 5.5.

Sequence	–ΔH° (kcal/mol)	–ΔS° (eu)	$-\Delta G°_{37}$ (kcal/mol)	T_m (°C)	(C_T) (M)
GGCGAAGGCU[a]	60.2	170.0	7.47	44.7	5.76×10^{-5}
	66.5	189.6	7.68	42.2	2.25×10^{-5}
GGUGGAGGCU[a]	42.5	113.0	7.43	52.4	1.51×10^{-4}
	38.0	98.0	7.61	51.2	6.47×10^{-5}
GAGCAAGCGAC[a]	56.2	161.2	6.22	38.6	6.61×10^{-5}
GCUGAAGGCU	No apparent transition				
GCGAGACCCG	No apparent transition				
CGGAGGAUCGC	No apparent transition				
GGC**GAG**GCU[b]	46.2±3.9	133.7±11.6	4.73±0.38	72.4	
GGU**AGAG**GCU[b]	55.8±2.3	164.2±6.6	4.89±0.24	66.8	
GC**CAAUGA**GCCP[b]	23.4±2.5	70.9±7.4	1.45±0.22	57.5	
	25.0±1.8	**75.9±5.5**	**1.46±0.10**	**56.2**	
GC_CG**A**GCCP[c]	(51.3±3.1)	(148.7±10.1)	(5.18±0.08)	(34.0)	
PCCG**A**GC CG	47.1±7.3	135.0±23.8	5.22±0.09	34.0	
	(51.0±3.8)	**(147.0±12.3)**	**(5.45±0.08)**	**(35.7)**	
	48.5±5.5	**138.7±17.5**	**5.45±0.14**	**35.5**	

[a] Parameters of homoduplex formation were derived by fitting to a two-state melting transition model. [b] Hairpin formation is indicated by concentration independent T_m. The parameters were the average of two-state melt curve fits. [c] The homoduplex formation is indicated by concentration dependent T_m. The parameters were the average of six to nine two-state melt curve fits with those in parentheses derived by linear fit of T_M^{-1} vs $\ln(C_T)$.

Table S2. Multiple linear regression analysis of free energies of medium-size RNA internal loops measured here and previously (*1-3*). Inin for $\Delta G^\circ_{\text{loop initiation}}(n)$; asym for $\Delta G^\circ_{\text{asym}}$; 3ga for $\Delta G^\circ_{\text{3GA bonus}}$; 2ga for $\Delta G^\circ_{\text{2GA bonus}}$; ug/ga for $\Delta G^\circ_{\text{5'UG/3'GA bonus}}$; AU for $\Delta G^\circ_{\text{AU/GU penalty}}$; GA for $\Delta G^\circ_{\text{GA bonus}}$; yAG for $\Delta G^\circ_{\text{5'YA/3'RG bonus}}$; 2gc/ag for $\Delta G^\circ_{2\times\text{(5'GA/3'CG) bonus } (3\times 3\text{ loop})}$; UU for $\Delta G^\circ_{\text{UU bonus}}$; mb for $\Delta G^\circ_{\text{middle GA bonus } (3\times 3\text{ loop})}$; uw for $\Delta G^\circ_{\text{5'GU/3'AN penalty } (3\times 3\text{ loop})}$; CA for $\Delta G^\circ_{\text{5'CR/3'AA bonus}}$. See Discussion section, Table 3, and refs (*1, 2*) for details of the free energy bonus and penalty terms. The highlighted values in the last column were used to derive the pH stabilization bonus, $\Delta G^\circ_{\text{5'CR/3'AA, pH bonus}}$.

n1	n2	5'-3'	3'-5'	$\Delta G^\circ_{37,\text{loop}}$	ini6	ini7	ini8	ini9	ini10	asym	3ga	2ga	ug/ga	AU	GA	yAG	2gc/ag	UU	mb	uw	**CA**	$\Delta G^\circ_{\text{predicted}}$	$\Delta\Delta G^\circ_{37,\text{ pH}}$
3	3	gAAAc	cAAAg	2.76	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.15	
3	3	cAAAg	gACCc	2.65	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	2.15	-0.82
3	3	gAAAc	cAAAg	2.47	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.15	
3	3	cAUAg	gAUGc	2.46	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1.95	
3	3	cAAAc	gAAAg	2.41	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.15	
3	3	cAUAg	gAAAc	2.37	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.15	
3	3	uGAAu	aAGAa	2.30	1	0	0	0	0	0	0	1	0	2	1	0	0	0	0	0	0	1.27	
3	3	cAGAc	gAGAg	2.29	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.15	
3	3	cAGAg	gAUGc	2.25	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1.95	
3	3	cGUAc	gAUAg	2.15	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1.95	
3	3	cAAAg	gAUGc	1.98	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1.95	
3	3	cAGAc	gAUAg	1.94	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.15	
3	3	cCAAc	gAAAg	1.94	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.15	
3	3	cAAAc	gAACg	1.91	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.15	
3	3	cAGAc	gAUGg	1.90	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1.95	
3	3	gAAAc	cAAAg	1.84	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.15	
3	3	gCACc	cCACg	1.82	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.15	
3	3	cGGAc	gAUAg	1.77	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1.21	
3	3	cAAAg	gAAAc	1.75	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.15	

3	3	aGAAu	uAAGa	1.66	1	0	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	1.49	
3	3	cUCUg	gUCUc	1.64	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0.93	
3	3	cAAAc	gAUGg	1.57	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1.95	
3	3	cAAAg	gAACc	1.56	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1.08	-1.02
3	3	cCGAc	gAGAg	1.52	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.15	-0.31
3	3	cAAAc	gAGAg	1.50	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1.26	
3	3	cGCAg	gAAAc	1.50	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1.21	0.09
3	3	gACAc	cACAg	1.44	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.15	
3	3	cUGCc	gUAUg	1.44	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1.54	0.17
3	3	cCGAc	gAUAg	1.43	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.15	-0.09
3	3	cGAAc	gAAAg	1.39	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1.21	
3	3	uGAAa	aAAGu	1.38	1	0	0	0	0	0	0	0	0	2	2	0	0	0	0	0	0	1.49	
3	3	cGAAc	gAUAg	1.33	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1.21	
3	3	cGAGc	gAUAg	1.28	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1.21	
3	3	cCGAc	gUAAg	1.26	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1.26	0.41
3	3	cGAAg	gAAGc	1.25	1	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0.27	
3	3	gAAGc	cGUAg	1.21	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1.04	
3	3	cGAGc	gAAAg	1.08	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1.21	
3	3	cGAGc	gAAGg	1.07	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1.21	
3	3	cGAAg	gACCc	1.00	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	**0**	1.21	-0.69
3	3	cUGUc	gUAUg	0.99	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0.93	
3	3	cAGAc	gAAAg	0.91	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1.26	
3	3	cGAAg	gAAAc	0.89	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1.21	
3	3	gAAGc	cGAAg	0.87	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1.04	
3	3	cUGAc	gUAAg	0.79	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0.65	
3	3	gUUUc	cUUUg	0.79	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0.93	
3	3	cUCUg	gUUUc	0.78	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0.93	
3	3	cGCAg	gAAGc	0.73	1	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0.27	0.06
3	3	cUUUg	gUCUc	0.72	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0.93	
3	3	cAAGg	gGAAc	0.71	1	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0.71	
3	3	gGAAc	cAUGg	0.70	1	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0.27	

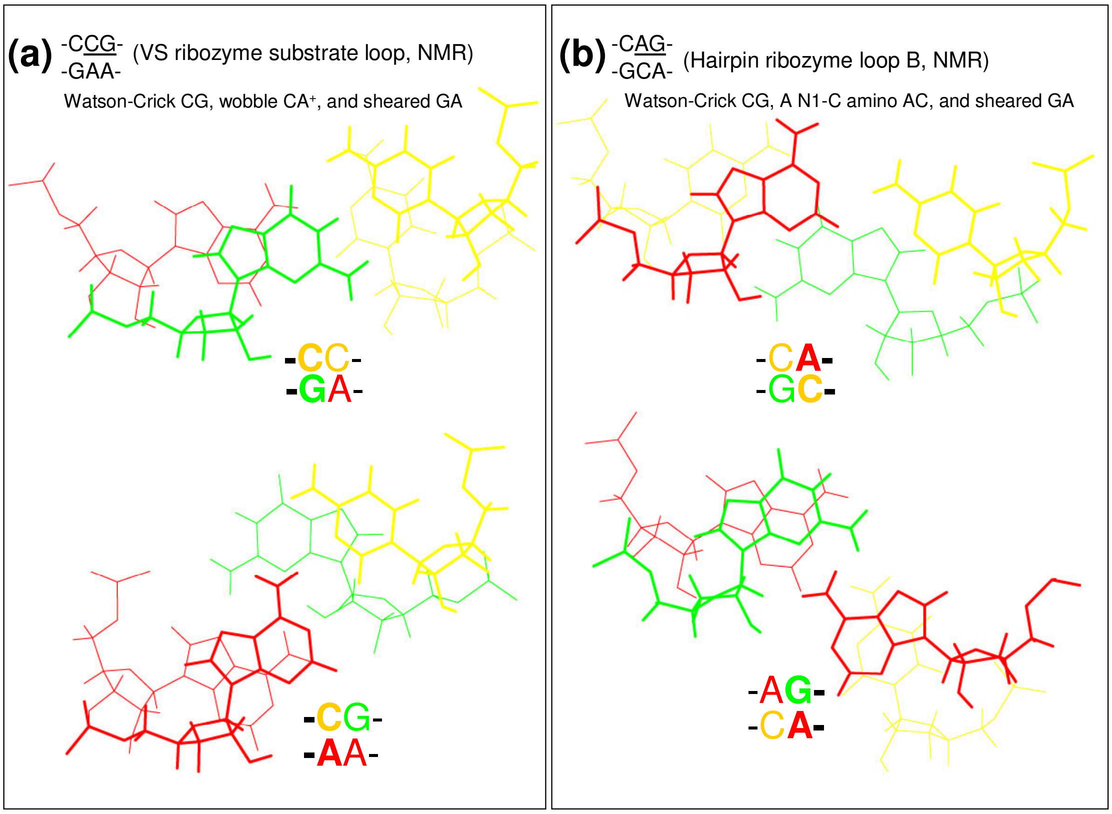
3	3	cUUUg	gUUUc	0.69	1	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0.93	
3	3	cCGAc	gAAAg	0.69	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0.19	-1.14
3	3	cUGCc	gUAAg	0.63	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0.65	0.13
3	3	cAGAg	gAAAc	0.58	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1.26	
3	3	cGAAg	gAUGc	0.57	1	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0.27	
3	3	cGAGc	gAGAg	0.54	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0.05	
3	3	cAUAg	gAAGc	0.51	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1.21	
3	3	gGAAc	cAAGg	0.49	1	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0.27	
3	3	gGUAc	cAUGg	0.29	1	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0.27	
3	3	cAAAg	gAAGc	0.26	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1.21	
3	3	cGAAc	gAGAg	0.16	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0.05	
3	3	cGAAg	gAAGc	0.15	1	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0.27	
3	3	cCGAg	gAAAc	0.05	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0.19	-0.63
3	3	cGAAc	gAAGg	-0.07	1	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0.27	
3	3	cGAAg	gAACc	-0.13	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0.14	-0.84
3	3	cAGAc	gAAGg	-0.24	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0.05	
3	3	cCGAc	gAAGg	-0.36	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	-1.02	-0.97
3	3	cAGAg	gAAGc	-0.45	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0.05	
3	3	cCGAg	gAAGc	-0.60	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	-1.02	-1.12
3	3	cGGAc	gAAAg	-0.65	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0.05	
3	3	cGAAg	gAGGg	-1.73	1	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0	0	-2.09	
3	3	cGGAc	gAAGg	-1.80	1	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0	0	-2.09	
3	3	uAAAu	gAAAg	4.73	1	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	3.37	
3	3	uAAAu	gAAGg	3.59	1	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	2.43	
3	3	uGAAu	gAAAg	3.41	1	0	0	0	0	0	0	0	1	2	1	0	0	0	0	0	0	1.58	
3	3	uAAAg	gAAAu	2.79	1	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	3.37	
3	3	uGAAu	gAAGg	0.62	1	0	0	0	0	0	0	0	1	2	2	0	0	0	0	0	0	0.64	
3	3	cCAAg	gACGu	0.10	1	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0.97	
3	3	cGAAg	gAGGu	-3.42	1	0	0	0	0	0	1	0	1	1	2	0	0	0	0	0	0	-2.33	
3	3	uGGAg	gAAGc	-2.62	1	0	0	0	0	0	1	0	1	1	2	0	0	0	0	0	0	-2.33	
3	3	uGGAg	gAAGu	-2.53	1	0	0	0	0	0	1	0	2	2	2	0	0	0	0	0	0	-2.57	

3	3	uGGAg	aAAGc	-2.27	1	0	0	0	0	0	1	0	0	1	2	0	0	0	0	0	0	-1.48	
3	3	cGGAg	gAAGc	-2.20	1	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0	0	-2.09	
3	3	cGGAg	gAAGu	-2.00	1	0	0	0	0	0	1	0	1	1	2	0	0	0	0	0	0	-2.33	
3	3	uGUAg	gAAGc	-0.77	1	0	0	0	0	0	0	0	1	1	2	0	0	0	0	0	0	0.03	
3	3	uGAAg	aAAGc	-0.51	1	0	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0.88	
3	3	uGAAg	gAAGC	-0.48	1	0	0	0	0	0	0	0	1	1	2	0	0	0	0	0	0	0.03	
3	3	uGAAg	gAUGc	-0.43	1	0	0	0	0	0	0	0	1	1	2	0	0	0	0	0	0	0.03	
3	3	cGAAg	gAAGc	-0.37	1	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0.27	
3	3	uAGAg	gAAGc	-0.26	1	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0.66	
3	3	uGAAg	gAAGu	-0.16	1	0	0	0	0	0	0	0	2	2	2	0	0	0	0	0	0	-0.21	
3	3	cGAAg	gAUGc	-0.11	1	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0.27	
3	3	cGAAg	gAAGu	0.43	1	0	0	0	0	0	0	0	1	1	2	0	0	0	0	0	0	0.03	
2	4	uGAg	gAAGGc	1.28	1	0	0	0	0	2	0	0	1	1	2	0	0	0	0	0	0	0.95	
2	4	uGGAAg	gAGc	1.32	1	0	0	0	0	2	0	0	1	1	2	0	0	0	0	0	0	0.95	
2	4	cGAAAg	gAGc	1.73	1	0	0	0	0	2	0	0	0	0	2	0	0	0	0	0	0	1.19	
2	4	cAAc	gAAAAg	3.01	1	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	3.07	
2	4	gAAg	cAAAAc	3.08	1	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	3.07	
3	4	uGGAAg	gAAGc	-1.18	0	1	0	0	0	1	1	0	1	1	1	0	0	0	0	0	0	-0.86	
3	4	cGGAg	gAAGGc	-1.00	0	1	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	-0.62	
3	4	uGGAg	gAAGGc	-0.92	0	1	0	0	0	1	1	0	1	1	1	0	0	0	0	0	0	-0.86	
3	4	cGAAg	gAAGGc	-0.67	0	1	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	-0.62	
3	4	uGAAg	gAAGGc	-0.52	0	1	0	0	0	1	1	0	0	1	1	0	0	0	0	0	0	-0.01	
3	4	cAAAg	gAAGGc	0.59	0	1	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0.58	
3	4	cGGAc	gAAGAg	0.07	0	1	0	0	0	1	1	0	0	0	1	0	0	0	0	0	0	-0.62	
3	4	cAGAc	gAAAGg	0.88	0	1	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0.58	
3	4	cGAAAg	gAAGc	1.10	0	1	0	0	0	1	0	0	0	0	2	0	0	0	0	0	0	0.80	
3	4	cAAGAc	gAAGg	1.24	0	1	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0.58	
3	4	cAGGAc	gAAGg	1.26	0	1	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0	0.58	
3	4	uAGAg	gAAGGc	1.63	0	1	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	2.35	
3	4	cAGGAc	gAUGg	1.72	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1.74	
3	4	cAGAGc	gAGAg	2.74	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2.68	

2	5	uGAg	gAAGGAc	2.50	0	1	0	0	0	3	0	0	1	1	1	0	0	0	0	0	0	2.42	
2	5	cGAg	gAGUAAc	3.00	0	1	0	0	0	3	0	0	0	0	1	0	0	0	0	0	0	2.66	
4	4	uGGAAg	gAAGGc	-4.27	0	0	1	0	0	0	1	1	1	1	2	0	0	0	0	0	0	-3.50	
4	4	cGAAAg	gAAGGc	-0.90	0	0	1	0	0	0	0	1	0	0	2	0	0	0	0	0	0	-0.90	
4	4	cAGGAc	gAAAGg	0.03	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0.04	
4	4	cAAGAc	gAAAGg	0.85	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0.04	
4	4	cGAAAg	gAAAGc	0.96	0	0	1	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0.26	
4	4	cAGAGc	gAAGAg	2.07	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.14	
4	4	cAAAAg	gAAAAc	2.23	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.14	
4	4	cAAAGc	gAAGAg	2.88	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.14	
4	4	gAAAAc	cAAAAg	3.01	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.14	
3	5	cGGAg	gAAGGAc	0.80	0	0	1	0	0	2	1	0	0	0	1	0	0	0	0	0	0	-0.24	
3	5	uGGAg	gAAGGAc	1.07	0	0	1	0	0	2	1	0	1	1	1	0	0	0	0	0	0	-0.48	
3	5	cGAAg	gAAGGAc	1.76	0	0	1	0	0	2	0	0	0	0	1	0	0	0	0	0	0	2.12	
3	5	uGAAg	gAAGGAc	1.78	0	0	1	0	0	2	0	0	1	1	1	0	0	0	0	0	0	1.88	
3	5	cAAAg	gAAGGAc	2.45	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	3.06	
3	5	uAGAg	gAAGGAc	2.68	0	0	1	0	0	2	0	0	0	1	0	0	0	0	0	0	0	3.67	
2	6	cGAg	gAAAAAAc	2.43	0	0	1	0	0	4	0	0	0	0	1	0	0	0	0	0	0	3.04	
4	5	uGGAAg	gAAGGAc	-0.29	0	0	0	1	0	1	1	0	1	1	1	0	0	0	0	0	0	-0.73	
4	5	cGAAAg	gAAGGAc	1.66	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	1.87	
3	6	uGGAg	gAAGUUUc	-0.33	0	0	0	1	0	3	1	0	1	1	1	0	0	0	0	0	0	0.19	
3	6	cGGAg	gAAGUUUc	-0.22	0	0	0	1	0	3	1	0	0	0	1	0	0	0	0	0	0	0.43	
3	6	uGGAg	gAAGAAAc	0.17	0	0	0	1	0	3	1	0	1	1	1	0	0	0	0	0	0	0.19	
3	6	cGGAg	gAAGAAAc	1.46	0	0	0	1	0	3	1	0	0	0	1	0	0	0	0	0	0	0.43	
3	6	cGAAg	gAAGAAAc	2.49	0	0	0	1	0	3	0	0	0	0	1	0	0	0	0	0	0	2.79	
3	6	cGGAg	gAAAAAAc	2.79	0	0	0	1	0	3	0	0	0	0	1	0	0	0	0	0	0	2.79	
3	6	cGAAg	gAAAAAAc	2.84	0	0	0	1	0	3	0	0	0	0	1	0	0	0	0	0	0	2.79	
3	6	cGGAg	cAGAAAAc	2.99	0	0	0	1	0	3	0	0	0	0	1	0	0	0	0	0	0	2.79	
3	6	cAAAg	gAAAAAAc	3.08	0	0	0	1	0	3	0	0	0	0	0	0	0	0	0	0	0	3.73	
3	6	uGUAg	gAAAAAAc	3.25	0	0	0	1	0	3	0	0	1	1	1	0	0	0	0	0	0	2.55	
4	6	uGGAAg	gAAGAAAc	0.76	0	0	0	0	1	2	1	0	1	1	1	0	0	0	0	0	0	0.33	

4	6	uGGAAg	gAAAAAAc	2.18	0	0	0	0	1	2	0	0	1	1	1	0	0	0	0	0	0	2.69	
4	6	cGAAAg	gAAAAAAc	2.83	0	0	0	0	1	2	0	0	0	0	1	0	0	0	0	0	0	2.93	
4	6	cGAAAg	gAGAAAAc	3.06	0	0	0	0	1	2	0	0	0	0	1	0	0	0	0	0	0	2.93	
4	4	gCGAAg	cAAAGc	-0.07	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0.13	-1.73
4	4	gCGGAg	cAAAGc	-2.00	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	1	-2.23	-1.72
4	4	cCGAAg	gAAGCc	-1.76	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	2	-1.16	-2.38
3	3	gAAAc	cAACg	2.03	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1.08	-1.19
4	4	cCGAAg	gAAGCc	-1.16	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	2	-1.16	-2.14
3	4	gCGAg	cAAAGc	-0.99	0	1	0	0	0	1	0	1	0	0	1	0	0	0	0	0	1	-0.49	-0.89
3	3	uGAAg	gAACc	-0.04	1	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	1	-0.10	-0.80
5	5	gCGAACg	cAAGAAc	0.79	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0.72	-1.56
4	4	aAGAAg	uCAGCc	-0.09	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0.52	-0.73
2	4	cCGAGc	gAGg	2.60	1	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	3.07	-0.73
3	3	cAUAg	gAACc	2.40	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.15	-0.64
3	3	uCAAg	aAAGc	0.84	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	0.75	-0.64
4	4	aAGAAg	uCUGCc	2.13	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	1	2.42	-0.73
3	3	uGUAg	gAACc	1.41	1	0	0	0	0	0	0	0	1	1	1	0	0	0	0	1	0	1.71	-0.30
3	3	uGGAg	gAACc	-0.09	1	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	-0.19	-0.29
3	3	uAGAg	gAACc	1.66	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1.87	-0.25
3	3	uCAAg	gAAGc	0.47	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	**1**	0.75	-0.19
3	3	cAGAg	gAACc	1.77	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1.26	-0.04
3	4	aGACc	uAGGAg	0.69	0	1	0	0	0	1	0	1	0	1	1	0	0	0	0	0	0	1.19	0.00
3	3	cAAAc	gCAAg	2.27	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.15	0.01
3	3	cAAGc	gCAAg	2.48	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.15	0.08
4	4	aAGAAg	uCAGGc	-1.25	0	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0	-0.55	0.19
				#	110	18	23	12	5	90	25	24	31	58	144	2	2	15	10	8	20		

Figure S1. Base stacking and base pairing involving CA. Base pairs shown in thicker lines are closer to viewer. Hydrogen atoms are shown in NMR structures but not in crystal structures. (**a**) The Watson-Crick CG, wobble CA^+ (the proton from protonation is not shown), and sheared GA pairs in $\frac{\text{C CG}}{\text{G AA}}$ segment of the NMR structure of substrate loop of VS ribozyme (*4*). (**b**) The Watson-Crick CG, A N1-C amino pair AC, and sheared GA pairs in $\frac{\text{C AG}}{\text{G CA}}$ segment of the NMR structure of loop B of a hairpin ribozyme (*5, 6*). (**c**) The Watson-Crick GC, wobble CA^+ (the proton from protonation is not shown), and sheared AA pairs in the J4/5 loop of a crystal structure of group I intron (*7*). (**d**) The wobble CA^+ (the proton from protonation is not shown) flanked by two Watson-Crick AU pairs from a crystal structure (*8*). (**e**) The Watson-Crick CG and wobble CA^+ (the proton from protonation is not shown) pairs in a crystal structure (*9*). The stacking figures are generated by the 3DNA program (*10*).



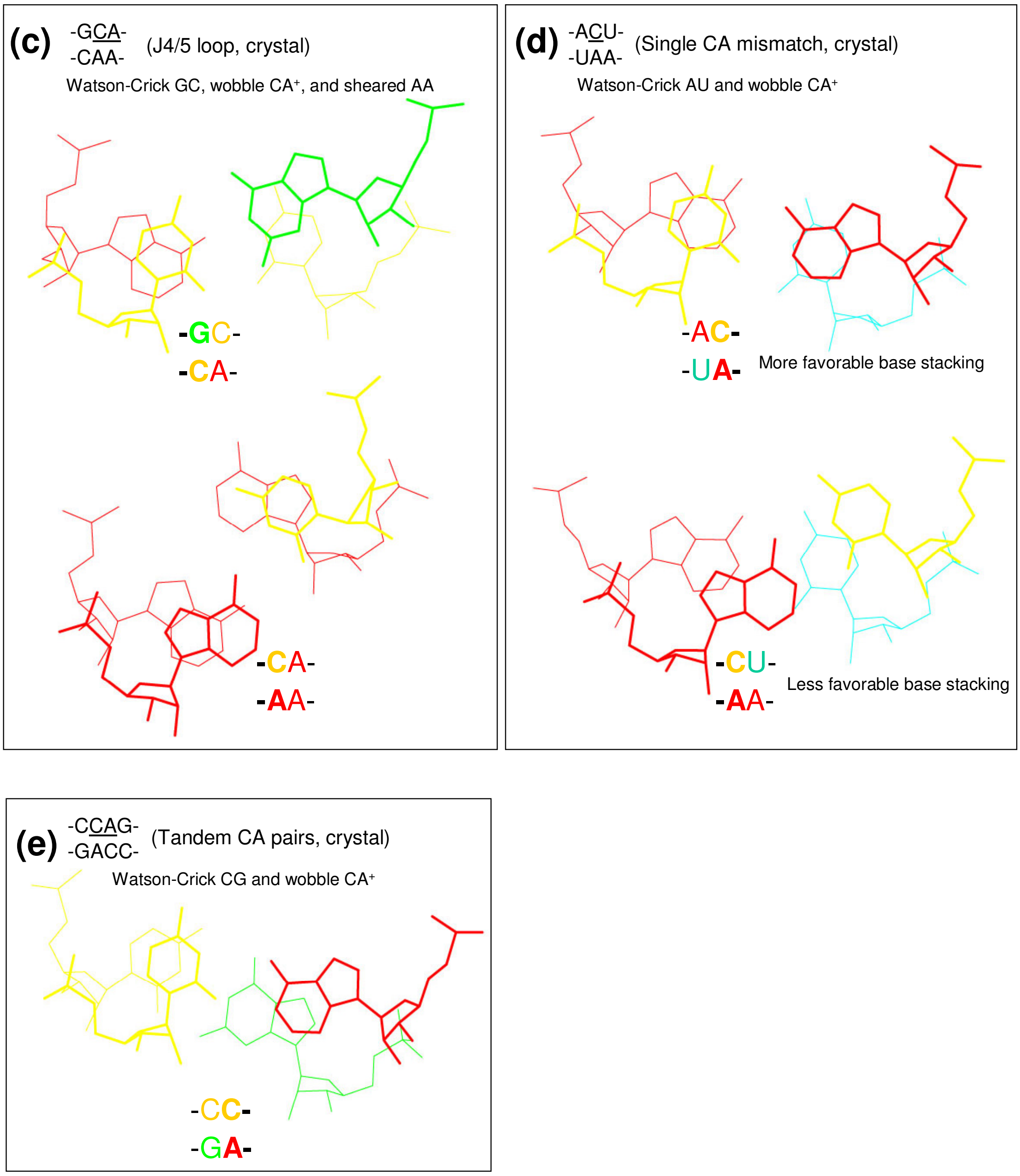
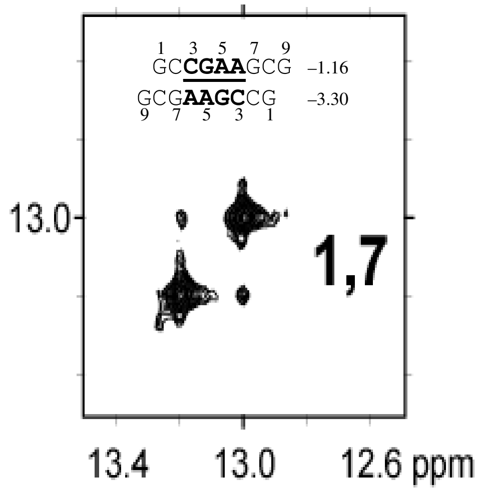

(c)
-GCA-
-CAA-
(J4/5 loop, crystal)
Watson-Crick GC, wobble CA+, and sheared AA
-GC-
-CA-
-CA-
-AA-
(d)
-ACU-
-UAA-
(Single CA mismatch, crystal)
Watson-Crick AU and wobble CA+
-AC-
-UA-
More favorable base stacking
-CU-
-AA-
Less favorable base stacking
(e)
-CCAG-
-GACC-
(Tandem CA pairs, crystal)
Watson-Crick CG and wobble CA+
-CC-
-GA-

Figure S2. Two-dimensional exchangeable proton SNOESY spectrum of $\frac{\text{GC CGAA GCG}}{\text{GCG AAGC CG}}$ (200 ms mixing time, in 80 mM NaCl, 10 mM sodium phosphate, 0.5 mM sodium EDTA, pH 5.9 at 0 ˚C). The NOE cross-peak of G1H1-G7H1 is observed. Values beside the sequence are $\Delta G^{\circ}_{37,loop}$ in kcal/mol measured in 1 M NaCl, at pH 5.5 (bottom) and pH 7 (top). The 1D spectrum is shown in Figure 2e.



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