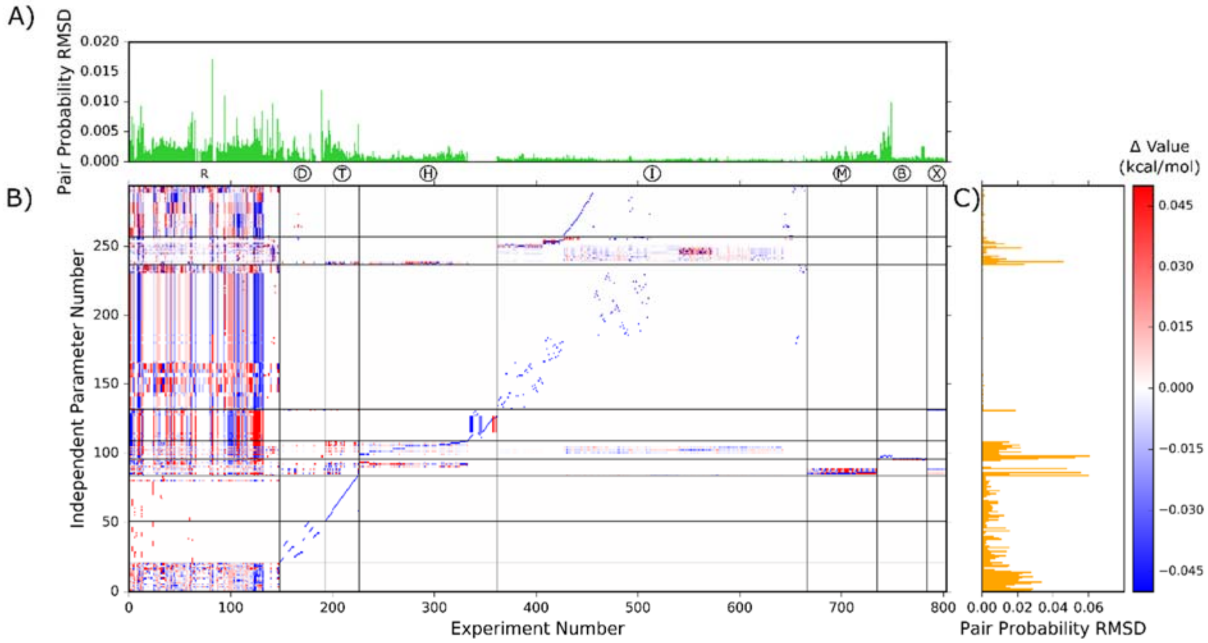
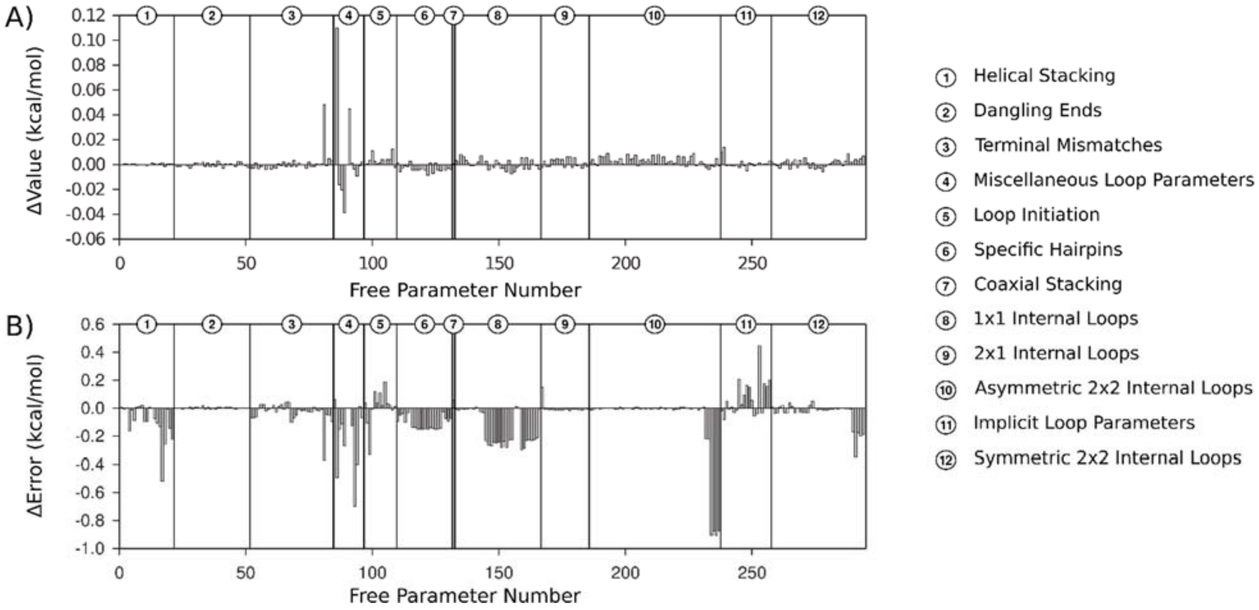
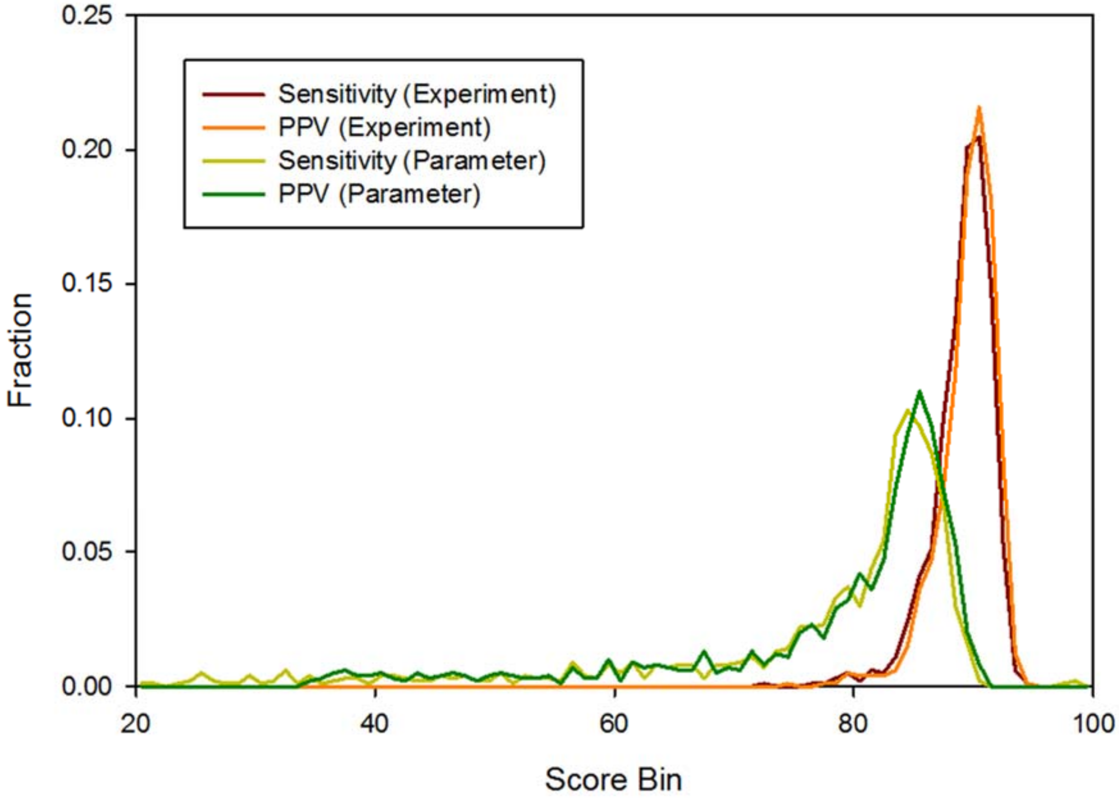
Supplemental Figures



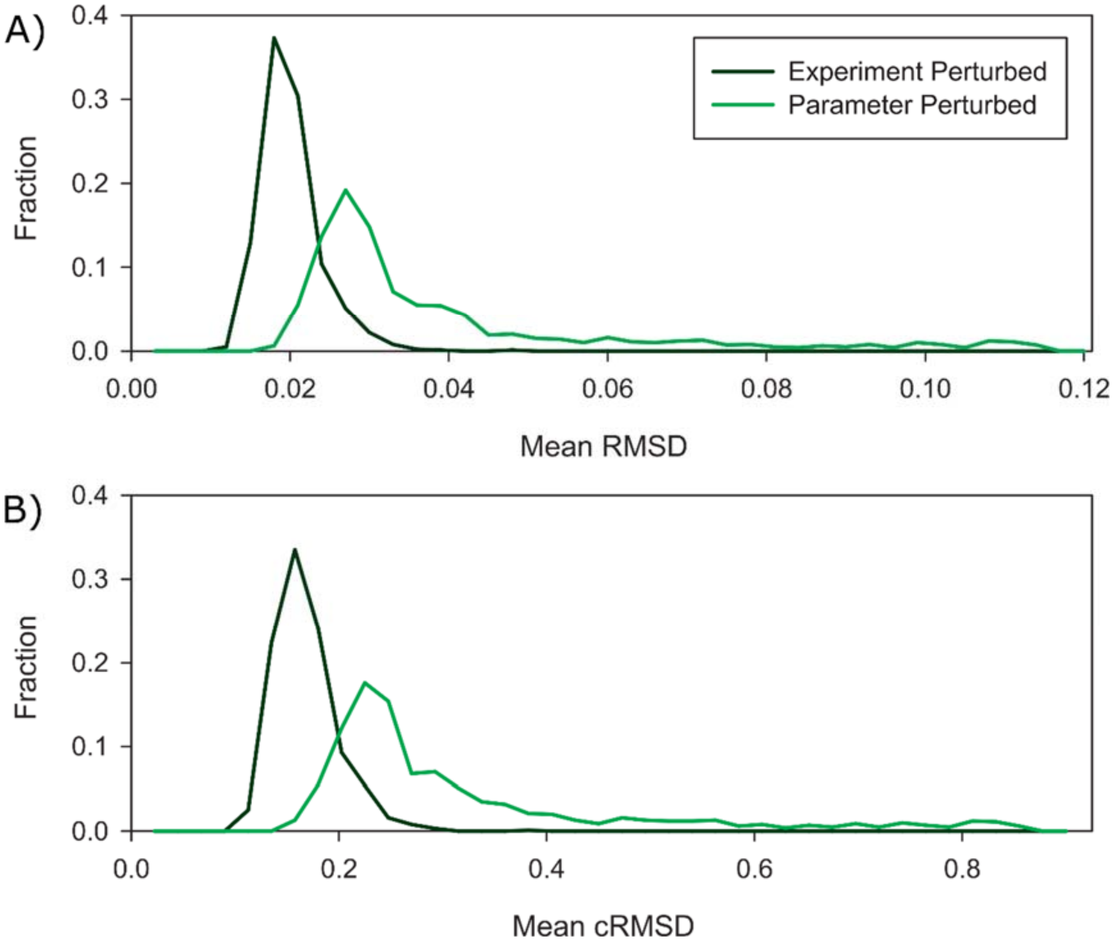
Supplemental Figure S1: Experiment Mapping. This figure shows the effect of perturbing each single experiment based on the experimental error. A) Mean base pair probability RMSD, averaged between +/- 3 σ perturbations of the experimental values. The x-axis, which is shared with subplot B, shows the experiment number. This shows how perturbing a single experiment and then propagating the perturbation through the nearest neighbor parameters values alters the predicted base pairing probabilities. B) The impact of changing an individual experimental value by - 3 σ on the parameter values. Independent parameters are along the y-axis, organized by motif type, and the experiment number is shown on the x-axis. This shows how perturbing each experiment perturbs the nearest neighbor parameters. C) Mean base pair probability RMSD, averaged between +/- 3 σ perturbations of the parameter values. Parameter indices are along the y-axis, grouped by parameter type and shared with subplot B. An interactive version of this figure is available at http://rna.urmc.rochester.edu/publications.html.



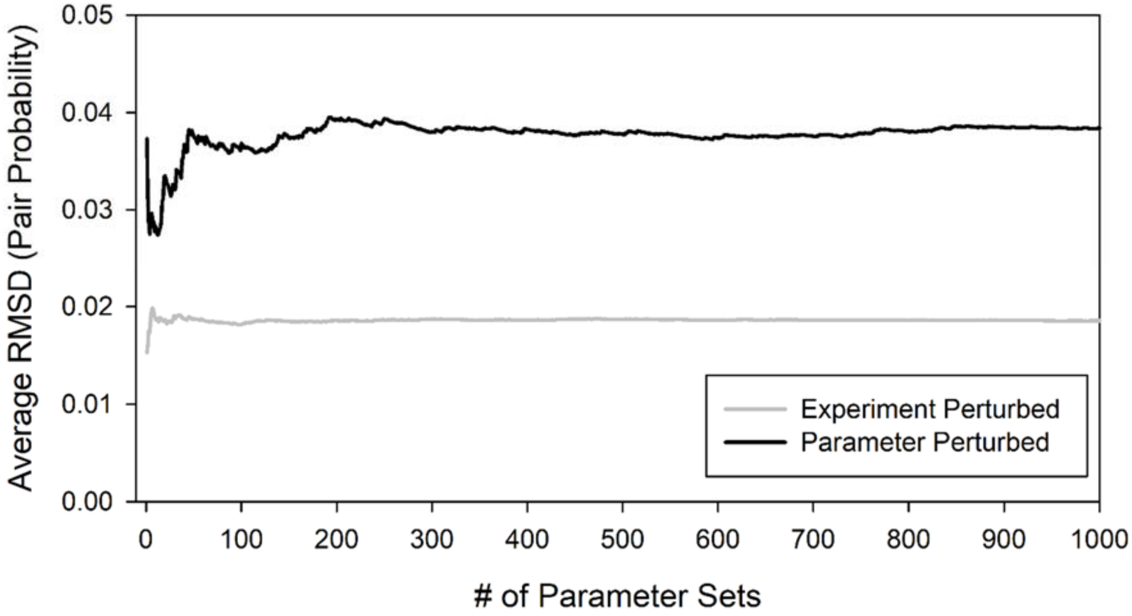
Supplemental Figure S2: Average parameter values and observed parameter standard deviations. Randomly perturbed experiment values were used to generate 100,000 parameter sets. A) The difference between the average values of the parameters across all 100,000 parameter sets and the values calculated using unmodified experiment values is plotted against parameter index. B) The difference between the errors calculated by the propagation of uncertainties and the observed standard deviations for each free parameter is plotted against parameter index.



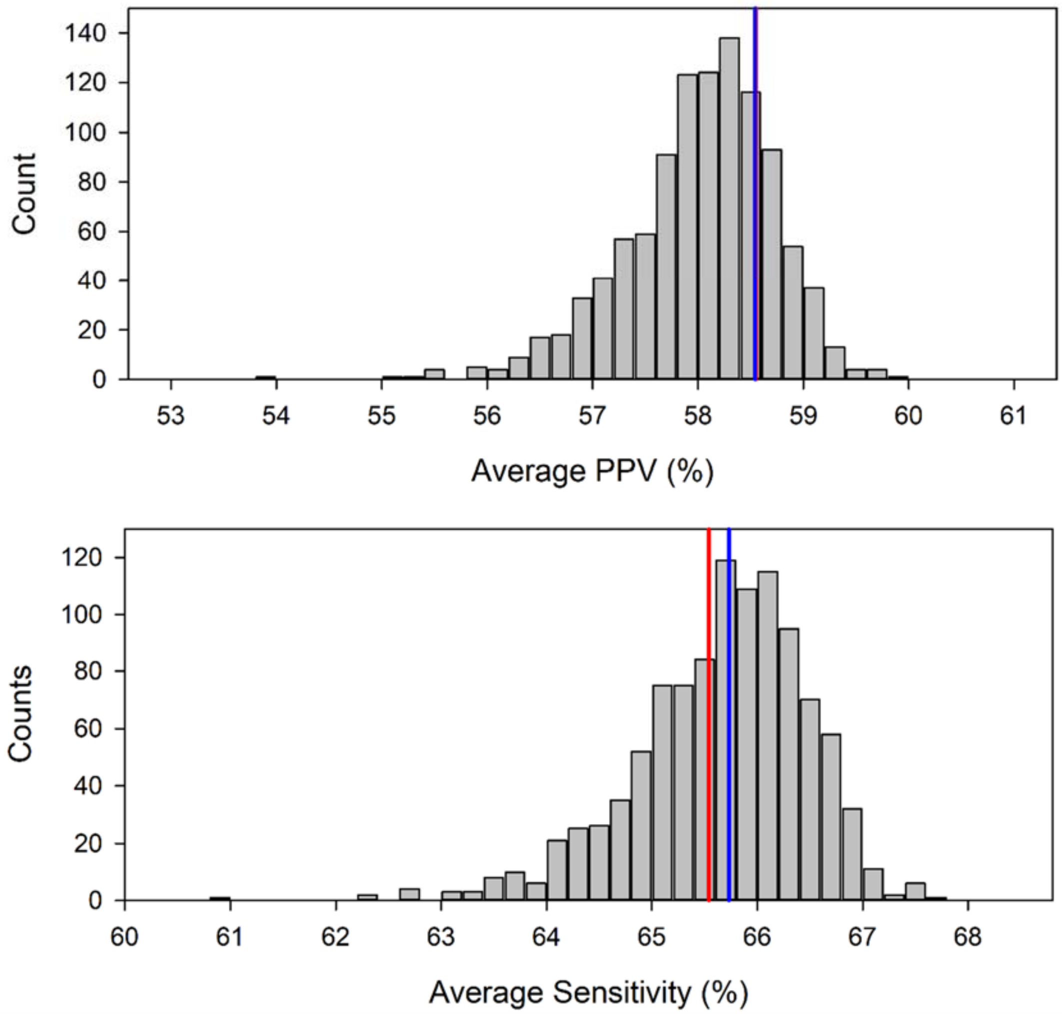
Supplemental Figure S3: Score distributions for perturbed parameter sets. The average sensitivity and positive predictive value was calculated for each perturbed parameter set compared against predictions made with an unperturbed parameter set. Parameter sets were generated by either randomly perturbing every free parameter value independently or by randomly perturbing every experiment value within experimental uncertainty. 1,000 parameter sets were evaluated for both classes.



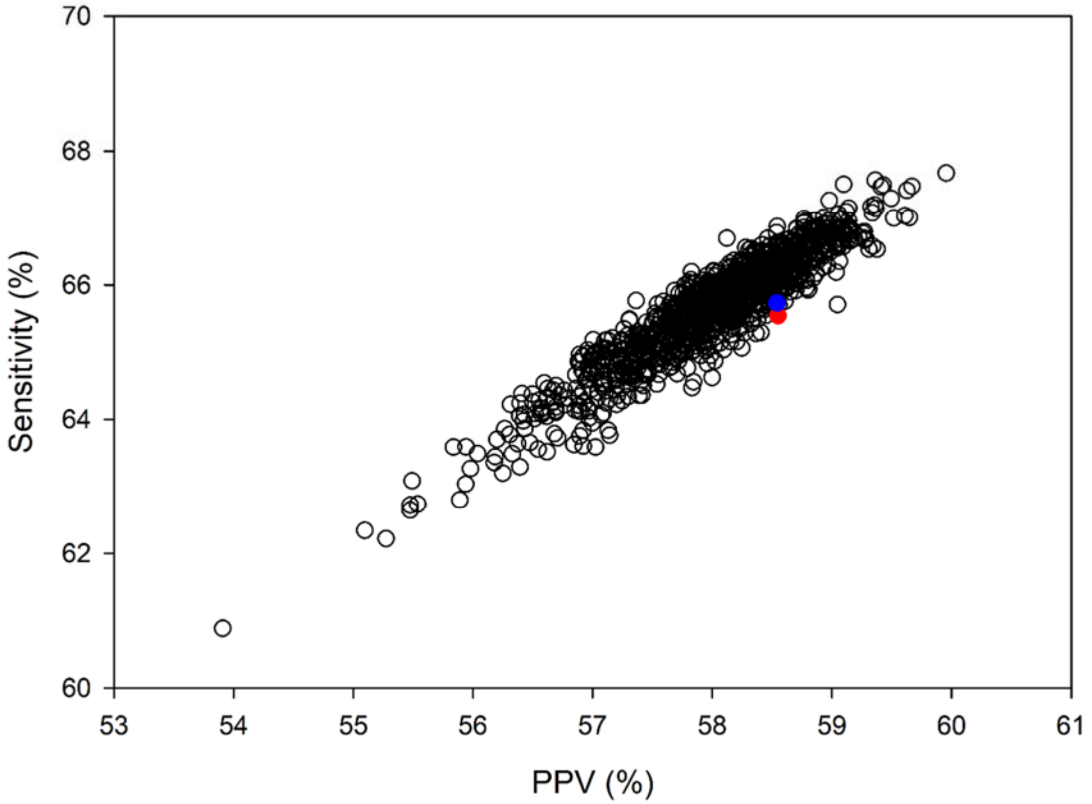
Supplemental Figure S4: Base pair probability RMSD distributions for perturbed parameter sets. Parameter sets were generated by either randomly perturbing every free parameter value independently or by randomly perturbing every experimental value within experimental uncertainty. A) The mean base pair probability RMSD across 1650 sequences was calculated for each parameter set compared against predictions made with an unperturbed parameter set. B) The mean base pair probability length-corrected RMSD across 1650 sequences was calculated for each parameter set compared against predictions made with an unperturbed parameter set. 1,000 parameter sets were evaluated for both classes.



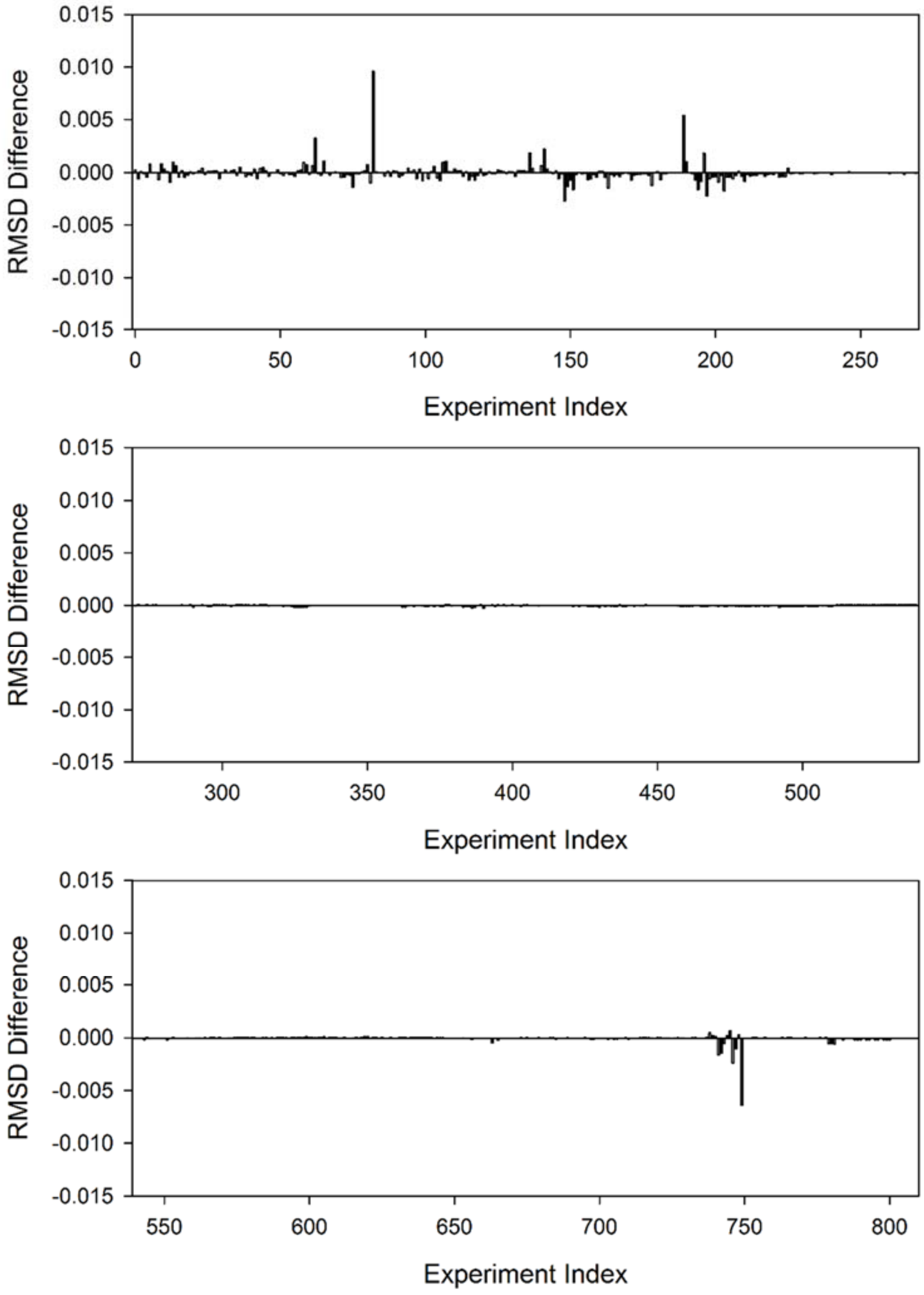
Supplemental Figure S5: Convergence of mean RMSD calculations. The cumulative mean average RMSD is plotted as the number of parameter sets is increased. The convergence of the values for both data tables generated by simultaneously perturbing all free nearest neighbor parameter values and data tables generated by perturbing all experiment values within experimental uncertainty demonstrates that sufficient numbers of data table sets have been sampled for both types.



Supplemental Figure S6: Accuracy distributions for randomly perturbed parameter sets. 1000 parameter sets were generated by randomly perturbing optical melting values within experimental uncertainty. The parameter sets were then benchmarked by predicting secondary structures for 1,450 sequences of known structure. The average positive predictive value (PPV) and sensitivity scores across the RNA families are plotted (A and B respectively). The red line indicates the score for the unperturbed parameter set and the blue line indicates the score of the "average" parameter set.



Supplemental Figure S7: Accuracy scores for randomly perturbed parameter sets. 1000 parameter sets were generated by randomly perturbing optical melting values within experimental uncertainty. The parameter sets were then benchmarked by predicting secondary structures for 1,450 sequences of known structure. The sensitivities are plotted against PPVs, where the predicted structures are scored against the known structures. Each data point represents the scores for a single parameter set, with the red data point illustrating the performance of the unperturbed parameter set and the blue dot representing the scores for the “average” parameter set.



Supplemental Figure S8: RMSD difference between positive and negative ΔG perturbations. Experiment indices are along the x-axis, organized by structure type. Difference in Mean base pair probability RMSD for the entire sequence archive except randomized sequences for +3 and -3 σ perturbations. The details of each experiment are available in the Supplemental Tables S1-S8.

Supplementary Table 1: Helical Duplex Experiment List

Note: The top strand is shown 5' to 3'. The bottom strand is shown 3' to 5'.

Index	ID#	Duplex	Folding Free Energy Change (kcal/mol)	Reference
1	R1	UCAUGA AGUACU	-4.30	(1)
2	R3	CAAAAAAG GUUUUUUC	-4.10	(2)
3	R4	CAAAAAAAG GUUUUUUUC	-5.00	(2)
4	R5	CCGG GGCC	-4.55	(3)
5	R6	ACCGGU UGGCCA	-8.48	(3)
6	R8	GGCC CCGG	-5.37	(4)
7	R9	GGCC CCGG	-5.90	(4)
8	R12	GGCC CCGG	-5.32	(5)
9	R14	AGGCCU UCCGGA	-8.50	(5)
10	R15	CGGCCG GCCGGC	-9.90	(5)
11	R18	GCCGGC CGGCCG	-11.22	(5)
12	R19	UCCGGA AGGCCU	-7.86	(5)
13	R20	GCGC CGCG	-4.63	(6)
14	R21	GCGCGC CGCGCG	-10.60	(6)
15	R22	CGCGCG GCGCGC	-9.06	(6)
16	R24	AUGCGU UGCGUA	-4.22	(7)
17	R25	AUGCGCGU UGCGCGUA	-9.31	(7)

18	R26	AUGCGUAU UAUGCGUA	-5.27	(7)
19	R27	AUGUGCAU UACGUGUA	-6.17	(7)
20	R28	GCUGGC CGGUCG	-6.47	(7)
21	R29	GGCGUC CUGCGG	-4.67	(7)
22	R30	CUGCGG GGCGUC	-4.31	(7)
23	R31	CGGCUG GUCGGC	-5.55	(7)
24	R32	AUGCAU UACGUA	-4.73	(7)
25	R33	AUGCGCAU UACGCGUA	-10.19	(7)
26	R34	AUACGUAU UAUGCAUA	-6.57	(7)
27	R35	AUGUACAU UACAUGUA	-6.49	(7)
28	R36	GCUAGC CGAUCG	-7.89	(7)
29	R37	GACGUC CUGCAG	-7.24	(7)
30	R38	CUGCAG GACGUC	-7.11	(7)
31	R39	CAGCUG GUCGAC	-6.72	(7)
32	R40	UGGCCG GCCGGU	-8.56	(8)
33	R41	UCCGGG GGGCCU	-7.44	(8)
34	R42	GCCGGU UGGCCG	-9.17	(8)
35	R43	GGCGCU UCGCGG	-8.42	(8)
36	R44	GCAUGC CGUACG	-7.34	(9)
37	R45	GUGCAC CACGUG	-7.65	(9)
38	R46	GUCUAGAC CAGAUCUG	-10.09	(9)

39	R47	GAUAUAUC CUAUAUAG	-6.05	(9)
40	R48	GUAUAUAC CAUAUAUG	-5.99	(9)
41	R49	GAGAGA CUCUCU	-6.86	(9)
42	R50	AGAGAGAG UCUCUCUC	-11.14	(9)
43	R51	AAUGCAUU UUACGUAA	-7.17	(9)
44	R52	UAUGCAUA AUACGUAU	-7.22	(9)
45	R53	GAUGCAUC CUACGUAG	-10.18	(9)
46	R54	CAUGCAUG GUACGUAC	-9.72	(9)
47	R55	AGAUAUCU UCUAUAGA	-6.50	(10)
48	R56	AUCUAGAU UAGAUCUA	-7.17	(10)
49	R57	AACUAGUU UUGAUCAA	-7.15	(10)
50	R58	AGUUAACU UCAAUUGA	-6.19	(10)
51	R59	ACUUAAGU UGAAUUCA	-6.26	(10)
52	R60	GAACGUUC CUUGCAAG	-9.39	(10)
53	R61	GUUCGAAC CAAGCUUG	-8.76	(10)
54	R62	UCUAUAGA AGAUAUCU	-6.89	(10)
55	R63	UAGAUCUA AUCUAGAU	-7.06	(10)
56	R64	GUCGAC CAGCUG	-7.08	(10)
57	R65	ACUAUAGU UGAUAUCA	-7.09	(10)
58	R67	UGAUCA ACUAGU	-5.00	(10)
59	R68	UCAUGA AGUACU	-3.82	(10)

60	R70	UGCGCA ACGCGU	-8.17	(11)
61	R71	AGCGCU UCGCGA	-8.01	(11)
62	R72	GGCGCC CCGCGG	-11.31	(11)
63	R73	UGCGCA ACGCGU	-8.12	(12)
64	R74	GAUGCAUU UUACGUAG	-6.82	(13)
65	R75	UAUGCAUG GUACGUAU	-6.44	(13)
66	R76	GCGGCG CGCCGC	-10.43	(14)
67	R78	AUGGUCAU UACUGGUA	-5.42	(15)
68	R79	CCUGUAGG GGAUGUCC	-6.81	(15)
69	R80	CGGGUCCG GCCUGGGC	-11.18	(15)
70	R81	CGUUGACG GCAGUUGC	-6.94	(15)
71	R82	CUGGUCAG GACUGGUC	-7.11	(15)
72	R83	GGAGUUCC CCUUGAGG	-6.45	(15)
73	R84	GGAUGUCC CCUGUAGG	-8.36	(15)
74	R85	GGCGUGCC CCGUGCGG	-9.23	(15)
75	R86	GUCGUGAC CAGUGCUG	-6.05	(15)
76	R87	GAGUUGAG CUCGGCUC	-8.21	(15)
77	R88	AGGCUU UUCGGA	-4.04	(15)
78	R89	AGUCGAUU UUAGCUGA	-5.97	(15)
79	R90	CGGAUUCG GCUUAGGC	-6.54	(15)
80	R91	CCUAGG GGAUCC	-7.86	(15)

81	R93	GGAUCC CCUAGG	-7.46	(15)
82	R94	CACAG GUGUC	-4.41	(16)
83	R95	UGACCUCA ACUGGAGU	-12.34	(17)
84	R96	GAGCUC CUCGAG	-7.73	(18)
85	R97	GCGUGC CGUGCG	-5.11	(18)
86	R98	GAGGUCUC CUCUGGAG	-8.77	(19)
87	R99	GAGCUC CUCGAG	-7.93	(19)
88	R100	GGUACC CCAUGG	-7.32	(19)
89	R101	GAGUGCUC CUCGUGAG	-9.17	(20)
90	R102	GGUUGACC CCAGUUGG	-8.30	(20)
91	R103	GGCUGGCC CCGGUCGG	-13.26	(20)
92	R106	CCAUGUGG GGUGUACC	-7.82	(21)
93	R107	CCAGUUGG GGUUGACC	-5.71	(21)
94	R108	CCAUGG GGUACC	-7.39	(21)
95	R109	GAGGAG CUCCUC	-8.50	(22)
96	R110	GAGUGGAG CUCGUCUC	-9.69	(22)
97	R112	GAGGUGAG CUCUGCUC	-7.62	(22)
98	R113	CGCG GCGC	-3.66	(1)
99	R114	ACGCA UGCGU	-4.96	(1)
100	R115	AGCGA UCGCU	-5.06	(1)
101	R116	GCACG CGUGC	-6.17	(1)

102	R117	GCUCG CGAGC	-6.13	(1)
103	R118	CACGUG GUGCAC	-6.58	(1)
104	R119	CCGCGG GGCGCC	-9.83	(1)
105	R120	GCAACG CGUUGC	-6.99	(1)
106	R121	GCAUCG CGUAGC	-7.26	(1)
107	R122	GCCGCG CGGCGC	-10.87	(1)
108	R123	GCGCCG CGCGGC	-10.92	(1)
109	R124	GCGCGG CGCGCC	-11.39	(1)
110	R125	GCGUCG CGCAGC	-8.77	(1)
111	R126	GCUACG CGAUGC	-7.56	(1)
112	R127	GGCGCG CCGCGC	-10.77	(1)
113	R128	GUGGUG CACCAC	-7.68	(1)
114	R129	GUGUCG CACAGC	-7.18	(1)
115	R130	UCGCGA AGCGCU	-6.85	(1)
116	R131	UCUAGA AGAUCU	-4.96	(1)
117	R132	AAGGAGG UUCCUCC	-9.53	(1)
118	R133	ACUGUCA UGACAGU	-7.92	(1)
119	R134	AGUCUGA UCAGACU	-7.50	(1)
120	R135	GACUCAG CUGAGUC	-9.06	(1)
121	R136	GAGUGAG CUCACUC	-9.70	(1)
122	R137	GUCACUG CAGUGAC	-8.62	(1)

123	R138	ACCUUUGC UGGAAACG	-10.64	(1)
124	R139	CGACGCAG GCUGCGUC	-12.32	(1)
125	R140	CUCGCACA GAGCGUGU	-12.10	(1)
126	R141	GGCUUCAA CCGAAGUU	-10.20	(1)
127	R142	UCCUUGCA AGGAACGU	-11.09	(1)
128	R143	UUCCGGAA AAGGCCUU	-10.80	(1)
129	R144	UUGCGCAA AACGCGUU	-10.19	(1)
130	R145	UUGGCCAA AACCGGUU	-11.02	(1)
131	R146	UUGUACAA AACAUGUU	-6.71	(1)
132	R147	AAGGUUGGAA UUCCAACCUU	-12.69	(1)
133	R148	CAUGCG GUACGC	-7.01	(1)
134	R150	GCUGAG CGACUC	-7.71	(1)
135	R151	GUGCAG CACGUC	-7.68	(1)
136	R156	UCCGCGCA AGGCGCGU	-14.59	(1)
137	R157	GCGGCG CGCCGC	-10.91	(23)
138	R158	GCGCGC CGCGCG	-10.82	(23)
139	R159	AAGGCCGGAA UUCCGGCCUU	-18.15	(24)
140	R160	CCUCUGGUGA GGAGACCGCU	-15.31	(24)
141	R161	AGGCCGGA UCCGGCCU	-15.26	(24)
142	R162	GAGCCGAC CUCGGCUG	-13.76	(24)
143	R163	UCACCUGA AGUGGACU	-10.84	(24)

144	R166	CUGGUC GACCAG	-8.05	(25)
145	R168	GAGUUGAC CUCAACUG	-10.60	(26)
146	R169	GAGUGAG CUCGCUC	-7.81	(23)
147	R170	AUCUAGGU UGGAUCUA	-5.90	Unpublished
148	R171	GUCUAGAU UAGAUCUG	-7.66	Unpublished

Supplementary Table 2: Dangling End Duplex Experiment List

Note: The top strand is shown 5’ to 3’. The bottom strand is shown 3’ to 5’. Unpaired nucleotides are in lower case.

Index	ID#	Duplex	Folding Free Energy Change (kcal/mol)	Reference
149	D1	UGCGCAa aACGCGU	-9.60	(12)
150	D2	UGCGCAc cACGCGU	-9.11	(12)
151	D3	UGCGCAg gACGCGU	-9.79	(12)
152	D4	UGCGCAu uACGCGU	-9.27	(12)
153	D5	AUGCAUa aUACGUA	-6.06	(12)
154	D6	AUGCAUc cUACGUA	-4.98	(12)
155	D7	AUGCAUg gUACGUA	-6.06	(12)
156	D8	AUGCAUu uUACGUA	-4.91	(12)
157	D9	CCGGa aGGCC	-6.85	(3)
158	D10	CCGGa aGGCC	-6.84	(3)
159	D11	CCGGc cGGCC	-5.25	(4)
160	D12	CCGGg gGGCC	-7.05	(4)
161	D13	CCGGu uGGCC	-5.81	(4)
162	D14	CCGGu uGGCC	-5.76	(3)
163	D15	GCGCa aCGCG	-7.92	(11)
164	D16	GGCCa aCCGG	-9.01	(4)
165	D17	GCGCc cCGCG	-6.16	(11)
166	D18	GGCCc cCCGG	-6.96	(4)

167	D19	GCGCg gCGCG	-7.70	(11)
168	D20	GGCCg gCCGG	-8.88	(4)
169	D21	GGCCu uCCGG	-8.16	(5)
170	D22	GCGCu uCGCG	-6.93	(11)
171	D23	GGCCu uCCGG	-7.98	(4)
172	D24	aUGCGCA ACGCGUa	-8.72	(12)
173	D25	cUCAUGA AGUACUc	-4.09	Unpublished
174	D26	aAUGCAU UACGUAa	-5.38	(12)
175	D27	cAUGCAU UACGUAc	-5.23	(12)
176	D28	gAUGCAU UACGUAg	-5.44	(12)
177	D29	uAUGCAU UACGUAu	-5.11	(12)
178	D30	aCCGG GGCCa	-5.60	(3)
179	D31	cCCGG GGCCc	-5.27	Unpublished
180	D32	gCCGG GGCCg	-4.90	(5)
181	D33	uCCGG GGCCu	-4.84	(5)
182	D34	aGGCC CCGGa	-5.81	(5)
183	D35	cGGCC CCGGc	-5.79	(5)
184	D36	cGCGC CGCGc	-5.27	(11)
185	D37	gGCGC CGCGg	-4.55	(11)
186	D38	uGGCC CCGGu	-5.17	(5)
187	D39	uGCGC CGCGu	-5.08	(11)

188	D40	GGCCc cCCGG	-7.22	(4)
189	D41	GCCGGUa aUGGCCG	-11.37	(8)
190	D42	GCGGCGa aCGCCGC	-14.63	(14)
191	D43	GACCGCa aCUGGCG	-12.17	(14)
192	D44	AGCGCGp pGCGCGA	-8.73	(27)
193	D45	GCCGGAg gAGGCCG	-8.44	(27)

Supplementary Table 3: Terminal Mismatch Duplex Experiment List

Note: The top strand is shown 5’ to 3’. The bottom strand is shown 3’ to 5’. Unpaired nucleotides are in lower case.

Index	ID#	Duplex	Folding Free Energy Change (kcal/mol)	Reference
194	T1	aUGCGCAa aACGCGUa	-9.68	(13)
195	T2	aUGCGCAc cACGCGUa	-9.42	(13)
196	T3	aUGCGCAg gACGCGUa	-9.77	(13)
197	T4	aGCGCa aCGCGa	-7.64	(28)
198	T5	cGGCCa aCCGGc	-8.30	(28)
199	T6	gGCGCa aCGCGg	-7.52	(28)
200	T7	uGGCCc cCCGGu	-6.90	(28)
201	T8	gGCGCg gCGCGg	-7.71	(28)
202	T9	cGGCCu uCCGGc	-8.09	(28)
203	T10	uGCGCu uCGCGu	-7.01	(8)
204	T11	aCCGGa aGGCCa	-6.73	(29)
205	T12	gCCGGa aGGCCg	-7.03	(8)
206	T13	aCCGGc cGGCCa	-6.72	(29)
207	T14	aCCGGg gGGCCa	-7.71	(29)
208	T15	gCCGGg gGGCCg	-7.48	(8)
209	T16	aUCCGGGa aGGGCCUa	-7.94	(30)
210	T17	aUGGCCGg gGCCGGUa	-9.72	(30)
211	T18	uUGGCCGu uGCCGGUu	-9.70	(31)

212	T19	aAUGCAUa aUACGUAa	-6.61	(13)
213	T20	cAUGCAUa aUACGUAc	-6.25	(13)
214	T21	gAUGCAUa aUACGUAg	-6.93	(13)
215	T22	aAUGCAUc cUACGUAa	-6.17	(13)
216	T23	cAUGCAUc cUACGUAc	-5.90	(13)
217	T24	uAUGCAUc cUACGUAu	-5.66	(13)
218	T25	aAUGCAUg gUACGUAa	-6.96	(13)
219	T26	gAUGCAUg gUACGUAg	-7.02	(13)
220	T27	cAUGCAUu uUACGUAc	-5.91	(13)
221	T28	uAUGCAUu uUACGUAu	-5.75	(13)
222	T29	aGCGUa aUGCGa	-3.97	(30)
223	T30	aGGCGCUg gUCGCGGa	-9.38	(30)
224	T31	gGCCGGUg gUGGCCGg	-10.79	(30)
225	T32	uGCCGGUu uUGGCCGu	-9.97	(31)
226	T33	aGCGCg gCGCGa	-7.47	(27)

Supplementary Table 4: Hairpin Loop Experiment List

Note: The sequence is shown 5' to 3'. Unpaired nucleotides are in lower case.

Index	ID#	Duplex	Folding Free Energy Change (kcal/mol)	Reference
227	H1	GGAauaUCC	0.64	(32)
228	H2	GGAGaaaUUCC	-0.93	(30)
229	H3	GGCauaGCC	-0.96	(32)
230	H4	GGGaaaUCC	0.33	(30)
231	H5	GGGauaCCC	-0.21	(32)
232	H6	GGGAUACaaaGUAUCCA	-6.30	(33)
233	H7	GGGAUACcccGUAUCCA	-4.64	(33)
234	H8	GGGAUACuuuGUAUCCA	-7.03	(33)
235	H9	GGUauaACC	0.58	(32)
236	H10	GGUauaGCC	0.87	(30)
237	H11	GCGauuaUGC	-0.32	(30)
238	H12	GCGGauuaUCGC	-1.41	(30)
239	H13	GGAauuaUCC	-0.06	(32)
240	H14	GGCauuaGCC	-2.52	(32)
241	H15	GGGaccaUCC	-1.81	(30)
242	H16	GGGauuaCCC	-1.28	(32)
243	H17	GGGAUACaaaaGUAUCCA	-7.60	(33)
244	H18	GGGAUACccccGUAUCCA	-4.30	(33)
245	H19	GGGAUACuuuuGUAUCCA	-8.16	(33)
246	H20	GGUauuaACC	-1.79	(32)
247	H21	GGUauuaGCC	0.17	(30)
248	H22	GGUgcaaGCC	-1.44	(30)
249	H23	GGCgagaGCC	-3.31	(31)
250	H24	GGCgcgaGCC	-3.50	(31)
251	H25	GGCgggaGCC	-3.23	(31)

252	H26	GGCgugaGCC	-3.16	(31)
253	H27	GGCgaaaGCC	-3.19	(31)
254	H28	GGCgcaaGCC	-3.35	(31)
255	H29	GGCggaaGCC	-3.23	(31)
256	H30	GGCguaaGCC	-3.53	(31)
257	H31	GGACuuuuGUCC	-4.20	(34)
258	H32	GGAGuucgCUCC	-4.05	(34)
259	H33	GGACgcuuGUCC	-4.43	(34)
260	H34	GCGgaagaUGC	-0.28	(30)
261	H35	GGAauuuaUCC	0.42	(32)
262	H36	GGCauauaGCC	-2.38	(32)
263	H37	GGCauuuaGCC	-2.66	(32)
264	H38	GGGauauaCCC	-1.38	(32)
265	H39	GGGauuuaCCC	-1.70	(32)
266	H40	GGGauuuaUCC	-0.70	(30)
267	H41	GGGAUACaaaaaGUAUCCA	-7.72	(33)
268	H42	GGGAUACcccccGUAUCCA	-4.10	(33)
269	H43	GGGAUACuuuuuGUAUCCA	-8.13	(33)
270	H44	GGUauuuaACC	-0.25	(32)
271	H45	GGUauuuaGCC	-0.57	(30)
272	H46	ACCgacacaGGU	-1.60	(28)
273	H47	AGGAauaauaUCCU	-2.38	(35)
274	H48	AGGUauaauaGCCU	-2.21	(35)
275	H49	CGGuuaauuCCG	-1.95	(28)
276	H50	CUCUacaccaAGAG	-1.78	(28)
277	H51	GCGgugaaaUGC	-0.69	(30)
278	H52	GCGuuaauuUGC	0.35	(30)
279	H53	GGAauaauaUCC	-0.73	(35)
280	H54	GGAguaauaUCC	-1.72	(28)

281	H55	GGCauaauaGCC	-2.76	(35)
282	H56	GGCauaaucGCC	-2.56	(28)
283	H57	GGCauaaugGCC	-3.11	(28)
284	H58	GGCcuaauaGCC	-2.15	(28)
285	H59	GGCcuaaucGCC	-2.38	(28)
286	H60	GGCcuaauuGCC	-1.82	(28)
287	H61	GGCguaauaGCC	-3.47	(35)
288	H62	GGCguaaugGCC	-3.85	(28)
289	H63	GGCuuaaucGCC	-2.89	(28)
290	H64	GGCuuaauuGCC	-6.23	(28)
291	H65	GGGauaauaUCC	-1.42	(35)
292	H66	GGUauaauaACC	-0.34	(35)
293	H67	GGUauaauaGCC	-0.47	(35)
294	H68	GGUguaauaACC	-1.54	(28)
295	H69	GGUguaauaGCC	-1.85	(28)
296	H70	GGUguaaugACC	-1.04	(28)
297	H71	GGUguaaugGCC	-1.29	(30)
298	H72	GUGguaauaCAC	-1.08	(28)
299	H73	GUGguaauaUAC	0.94	(28)
300	H74	GGAuuaauuUCC	-1.29	(31)
301	H75	GGUuuaauuACC	-0.83	(31)
302	H76	GGUuuaauuGCC	-0.53	(31)
303	H77	GCGguaaugCGC	-2.54	(31)
304	H78	GGAguaaugUCC	-1.16	(31)
305	H80	GCGguaaugUGC	-0.80	(31)
306	H81	GCGAauaaaauaUCGC	-2.41	(32)
307	H82	GGCauaaauaGCC	-2.11	(32)
308	H83	GGGacggacaUCC	-1.03	(30)
309	H84	GGGauaaauaCCC	-2.12	(32)

310	H85	GGGauaaauaUCC	-0.22	(30)
311	H86	GGGAUACaaaaaaaGUAUCCA	-7.19	(33)
312	H87	GGGAUACcccccccGUAUCCA	-2.91	(33)
313	H88	GGGAUACuuuuuuuGUAUCCA	-7.38	(33)
314	H89	GGUauaaauaACC	-0.41	(32)
315	H90	GGUauaaauaGCC	0.56	(30)
316	H91	GGUguaaaaaGCC	-0.66	(30)
317	H92	GCGaauucauaUGC	-0.65	(30)
318	H93	GCUgaauggaaGGC	-1.28	(30)
319	H94	GGAauaaaauaUCC	-2.19	(32)
320	H95	GGCauaaaauaGCC	-2.16	(32)
321	H96	GGGauaaaauaCCC	-2.35	(32)
322	H97	GGGauaaaauaUCC	-1.65	(30)
323	H98	GGUaauucauaGCC	-0.08	(30)
324	H99	GGUauaaaauaACC	0.51	(32)
325	H100	GGUauaaaauaGCC	0.03	(30)
326	H101	GCGAauaaaaauaUCGC	-2.23	(32)
327	H102	GCGUauaaaaauaACGC	-1.08	(32)
328	H103	GGAauaaaaauaUCC	0.25	(32)
329	H104	GGCauaaaaauaGCC	-2.14	(32)
330	H105	GGGauaaaaauaCCC	-2.09	(32)
331	H106	GGGAUACaaaaaaaaaGUAUCCA	-5.55	(33)
332	H107	GGGAUACcccccccccGUAUCCA	-2.10	(33)
333	H108	GGGAUACuuuuuuuuuGUAUCCA	-8.05	(33)
334	H126	GGACaacGUCC	-1.32	(36)
335	H127	GGAGuuaCUCC	-0.92	(36)
336	H132	GGCuacgGCC	-3.22	(31)
337	H133	GGCuccgGCC	-3.95	(31)
338	H134	GGCuucgGCC	-4.09	(31)

339	H135	GAAGAcaguguUCUUC	-5.52	(37)
340	H136	GAAGAcagugcUCUUC	-5.44	(37)
341	H137	GAAGAcaguacUCUUC	-4.44	(37)
342	H138	GGAcagugcUCC	-1.02	(31)
343	H139	GGAcagugaUCC	-1.59	(31)
344	H141	GGACuuugGUCC	-3.79	(38)
345	H148	GGACuucgGUCC	-6.31	(34)
346	H149	GGACuuugGUCC	-4.30	(34)
347	H150	GGACuacgGUCC	-5.68	(34)
348	H152	GGACcaagGUCC	-4.02	(39)
349	H153	GGACccagGUCC	-3.83	(39)
350	H154	GGACcgagGUCC	-3.67	(39)
351	H155	GGACcuagGUCC	-3.64	(39)
352	H156	GGACcacgGUCC	-3.56	(39)
353	H158	GGACcgcgGUCC	-3.67	(39)
354	H159	GGACcucgGUCC	-4.77	(39)
355	H160	GGACuaagGUCC	-3.61	(39)
356	H161	GGACucagGUCC	-3.61	(39)
357	H163	GGACuuagGUCC	-3.76	(39)
358	H164	GGACuacgGUCC	-4.88	(39)
359	H165	GGACuccgGUCC	-4.45	(39)
360	H166	GGACugcgGUCC	-4.42	(39)
361	H167	GGACuucgGUCC	-4.78	(39)
362	H170	GGACaacgGUCC	-1.77	(39)

Supplementary Table 5: Internal Loop Duplex Experiment List

Note: The top strand is shown 5’ to 3’. The bottom strand is shown 3’ to 5’. Unpaired nucleotides are in lower case. The underscore character (_) does not represent a nucleotide. It is used to maintain alignment between the top and bottom strands.

Index	ID#	Duplex	Folding Free Energy Change (kcal/mol)	Reference
363	I1	GAGcGAG CUCaCUC	-5.63	(23)
364	I2	GAGaGAG CUCgCUC	-5.32	(23)
365	I3	GAGcGAG CUCuCUC	-5.22	(23)
366	I4	GAGuGAG CUCuCUC	-5.24	(23)
367	I5	GAGaGAG CUCcCUC	-4.95	(23)
368	I6	GAGaGAG CUCaCUC	-4.85	(23)
369	I7	GAGcGAG CUCcCUC	-4.75	(23)
370	I8	GAGuGAG CUCcCUC	-4.75	(23)
371	I9	GCGuCCG CGCuGGC	-8.23	(23)
372	I10	GCGuCGC CGCuGCG	-7.90	(23)
373	I11	GCGuGCG CGCuCGC	-7.79	(23)
374	I12	GCCuGCG CGGuCGC	-8.15	(23)
375	I13	CGCuGCG GCGuCGC	-6.31	(23)
376	I14	GCGuUCG CGCuAGC	-5.99	(23)
377	I15	GUGuUCG CACuAGC	-3.80	(23)
378	I16	GCAuUCG CGUuAGC	-4.98	(23)
379	I17	GCAuACG CGUuUGC	-4.55	(23)

380	I18	GCUuACG CGAuUGC	-4.47	(23)
381	I19	CGCaGCG GCGaCGC	-6.08	(17)
382	I20	GCGaCCG CGCaGGC	-6.67	(23)
383	I21	GGCaGCC CCGaCGG	-7.83	(23)
384	I22	GgCUGAG CgGACUC	-6.87	(23)
385	I23	CGgCAUG GCgGUAC	-6.07	(23)
386	I24	GUGgCAG CACgGUC	-6.66	(23)
387	I25	GCGgCGC CGCgGCG	-9.28	(23)
388	I26	CGCgGCG GCGgCGC	-8.14	(23)
389	I27	UGACaCUCA ACUGaGAGU	-7.94	(17)
390	I28	GAGaGGAG CUCgUCUC	-5.83	(22)
391	I29	GAGuGGAG CUCuUCUC	-6.26	(22)
392	I30	GAGaGGAG CUCaUCUC	-5.27	(22)
393	I31	GAGgUGAG CUCaGCUC	-4.97	(22)
394	I32	GAGaUGAG CUCgGCUC	-4.67	(22)
395	I33	GAGuUGAG CUCuGCUC	-4.69	(22)
396	I34	GAGaUGAG CUCaGCUC	-4.36	(22)
397	I35	GAGUaGAG CUCGgCUC	-6.11	(22)
398	I36	GAGUgGAG CUCGaCUC	-6.09	(22)
399	I37	GAGUuGAG CUCGuCUC	-6.24	(22)
400	I38	GAGUcGAG CUCGcCUC	-5.56	(22)

401	I39	GAGUcGAG CUCGaCUC	-6.00	(22)
402	I40	GAGUaGAG CUCGaCUC	-5.15	(22)
403	I41	GAGGaGAG CUCUgCUC	-6.56	(22)
404	I42	GAGGuGAG CUCUuCUC	-5.21	(22)
405	I43	GAGGcGAG CUCUuCUC	-5.39	(22)
406	I44	GAGGcGAG CUCUaCUC	-5.83	(22)
407	I45	GAGGaGAG CUCUaCUC	-5.74	(22)
408	I46	UGACa_CUCA ACUGaaGAGU	-6.80	(40)
409	I47	UGACa_CUCA ACUGaaGAGU	-6.43	(17)
410	I48	UGAGa_GUCA ACUCaaCAGU	-6.60	(17)
411	I49	UGACa_CUCA ACUGagGAGU	-8.32	(40)
412	I50	UCAGa_GUGA AGUCagCACU	-6.44	(24)
413	I51	UGACa_CUCA ACUGccGAGU	-7.42	(40)
414	I52	UGACa_CUCA ACUGgaGAGU	-8.01	(40)
415	I53	UGAGa_GUCA ACUCgaCAGU	-6.98	(40)
416	I54	UGACc_CUCA ACUGaaGAGU	-6.80	(40)
417	I55	UGACc_CUCA ACUGauGAGU	-6.60	(40)
418	I56	UGACc_CUCA ACUGccGAGU	-6.62	(40)
419	I57	UGACc_CUCA ACUGcuGAGU	-7.16	(40)
420	I58	UGACg_CUCA ACUGaaGAGU	-7.35	(40)
421	I59	UGACg_CUCA ACUGagGAGU	-7.92	(40)

422	I60	UGACg_CUCA ACUGgaGAGU	-8.32	(40)
423	I61	UGACu_CUCA ACUGccGAGU	-6.85	(40)
424	I62	UCACu_CUGA AGUGcuGACU	-5.91	(24)
425	I63	UGACu_CUCA ACUGucGAGU	-7.54	(40)
426	I64	UGACu_CUCA ACUGuuGAGU	-7.90	(40)
427	I65	AGGCu_CGGA UCCGuuGCCU	-10.43	(24)
428	I66	GCGagCGC CGCgaGCG	-8.46	(18)
429	I67	GCGuuCGC CGCuuGCG	-7.66	(19)
430	I68	CGGcaCCG GCCacGGC	-5.49	(19)
431	I69	CUGcuCAG GACucGUC	-2.32	(19)
432	I70	CGGcuCCG GCCucGGC	-5.57	(19)
433	I71	GCGacCGC CGCcaGCG	-6.23	(19)
434	I72	GCGaaCGC CGCaaGCG	-5.66	(19)
435	I73	GGCagGCC CCGgaCGG	-9.44	Unpublished
436	I74	CGCagGCG GCGgaCGC	-7.76	(41)
437	I75	CGCuuGCG GCGuuCGC	-7.18	(42)
438	I76	UGCggGCA ACGggCGU	-5.02	(42)
439	I77	CGCcaGCG GCGacCGC	-5.69	(42)
440	I78	CGCcuGCG GCGucCGC	-5.45	(42)
441	I79	CGCucGCG GCGcuCGC	-5.38	(42)
442	I80	CGCccGCG GCGccCGC	-5.13	(42)

443	I81	GGCacGCC CCGcaCGG	-6.97	(42)
444	I82	CGCaaGCG GCGaaCGC	-5.44	(17)
445	I83	GCUgaAGC CGAagUCG	-5.88	(18)
446	I84	GCUuuAGC CGAuuUCG	-5.50	(19)
447	I85	GGUcaACC CCAacUGG	-4.14	(19)
448	I86	GGUcuACC CCAucUGG	-3.86	(19)
449	I87	GGUucACC CCAcuUGG	-3.21	(19)
450	I88	GCUaaAGC CGAaaUCG	-3.82	(19)
451	I89	GGAgaUCC CCUagAGG	-6.41	(18)
452	I90	GCAgaUGC CGUagACG	-5.61	(18)
453	I91	GCAuuUGC CGUuuACG	-5.61	(19)
454	I92	GGAcaUCC CCUacAGG	-4.01	(19)
455	I93	GGAacUCC CCUcaAGG	-3.89	(19)
456	I94	GCAaaUGC CGUaaACG	-3.52	(19)
457	I95	GGCgaGCC CCGagCGG	-9.69	(41)
458	I96	GAGgaGAG CUCagCUC	-6.91	(22)
459	I97	GAGaaGAG CUCggCUC	-5.74	(22)
460	I98	GAGuaGAG CUCugCUC	-4.26	(22)
461	I99	GAGaaGAG CUCcgCUC	-4.61	(22)
462	I100	UGAGaaGUCA ACUCaaCAGU	-7.04	(17)
463	I101	GAGaaGAG CUCagCUC	-5.39	(22)

464	I102	GAGagGAG CUCgaCUC	-5.92	(22)
465	I103	GAGugGAG CUCuaCUC	-4.37	(22)
466	I104	GAGcgGAG CUCaaCUC	-5.99	(22)
467	I105	GAGagGAG CUCcaCUC	-6.19	(22)
468	I106	GAGagGAG CUCaaCUC	-5.23	(22)
469	I107	GAGguGAG CUCauCUC	-4.77	(22)
470	I108	GAGauGAG CUCguCUC	-3.88	(22)
471	I109	GAGuuGAG CUCuuCUC	-5.88	(22)
472	I110	GAGauGAG CUCcuCUC	-4.96	(22)
473	I111	GAGauGAG CUCauCUC	-3.85	(22)
474	I112	GAGaaGAG CUCgcCUC	-4.49	(22)
475	I113	GAGuaGAG CUCucCUC	-5.41	(22)
476	I114	GAGuuGAG CUCucCUC	-5.10	(22)
477	I115	GAGacGAG CUCguCUC	-4.69	(22)
478	I116	GAGgcGAG CUCacCUC	-4.47	(22)
479	I117	GAGacGAG CUCgcCUC	-4.53	(22)
480	I118	GAGucGAG CUCucCUC	-5.31	(22)
481	I119	GAGacGAG CUCccCUC	-3.34	(22)
482	I120	GAGacGAG CUCacCUC	-3.03	(22)
483	I121	GAGgcGAG CUCaaCUC	-5.14	(22)
484	I122	GAGacGAG CUCgaCUC	-4.81	(22)

485	I123	GAGucGAG CUCuaCUC	-5.24	(22)
486	I124	GAGacGAG CUCcaCUC	-4.14	(22)
487	I125	GAGacGAG CUCaaCUC	-4.58	(22)
488	I126	GAGgaGAG CUCaaCUC	-5.55	(22)
489	I127	GAGaaGAG CUCgaCUC	-4.90	(22)
490	I128	GAGuaGAG CUCuaCUC	-3.77	(22)
491	I129	GAGaaGAG CUCaaCUC	-4.71	(22)
492	I130	GGAagUCC CCUgaAGG	-4.64	(43)
493	I131	GGUagACC CCAgaUGG	-5.13	(43)
494	I132	GCAggUGC CGUggACG	-4.43	(25)
495	I133	GCUggAGC CGAggUCG	-4.26	(25)
496	I134	GUGgcGUG CACguCAC	-5.30	(25)
497	I135	GAGcgGAG CUCugCUC	-5.69	(25)
498	I136	GAGcgGAG CUCagCUC	-5.70	(25)
499	I137	GAGgcGAG CUCguCUC	-5.66	(25)
500	I138	CUGgaGUC GACggCAG	-5.00	(25)
501	I139	GAGgcGAG CUCgaCUC	-5.57	(25)
502	I140	CUGagGUC GACggCAG	-5.11	(25)
503	I141	GUGgaGUG CACgaCAC	-4.63	(25)
504	I142	GUGagGUG CACagCAC	-4.49	(25)
505	I143	GAGguGAG CUCgcCUC	-5.02	(25)

506	I144	GAGugGAG CUCcgCUC	-4.98	(25)
507	I145	GAGguGAG CUCguCUC	-4.56	(25)
508	I146	GAGugGAG CUCugCUC	-3.86	(25)
509	I147	GAGuuGAG CUCccCUC	-3.54	(22)
510	I148	GAGcgGAG CUCcaCUC	-5.92	(22)
511	I149	GAGcaGAG CUCagCUC	-5.60	(22)
512	I150	CGCaaGCG GCGaaCGC	-5.44	(17)
513	I151	CCACg___CUCC GGUGaaaGAGG	-9.65	(24)
514	I152	CGACg___GCAG GCUGgaaCGUC	-8.34	(24)
515	I153	UCAGc___GUGA AGUCaauCACU	-5.73	(24)
516	I154	CGACa___GCAG GCUGgaaCGUC	-8.01	(24)
517	I155	CCACa___CUCC GGUGaaaGAGG	-9.18	(24)
518	I156	UCCGa___CGCA AGGCaagGCGU	-9.02	(24)
519	I157	GGCu___CGG CCGuuuGCC	-6.03	(24)
520	I158	UGACu___CUCA ACUGcuuGAGU	-6.77	(24)
521	I159	UCACu___CUGA AGUGcucGACU	-5.30	(24)
522	I160	UGACa___CUCA ACUGaacGAGU	-6.59	(24)
523	I161	UGAGa___GUCA ACUCcgaCAGU	-6.55	(24)
524	I162	UGACa___CUCA ACUGaaaGAGU	-6.55	(17)
525	I163	UGACc___CUCA ACUGcuuGAGU	-6.34	(24)
526	I164	AGGCu___CGGA UCCGuuuGCCU	-9.25	(24)

527	I165	UGAGa___GUCA ACUCaaaCAGU	-6.16	(17)
528	I166	UCCGa___CGCA AGGCggaGCGU	-8.13	(24)
529	I167	AAGGCu___CGGAA UUCCGuuuGCCUU	-11.60	(24)
530	I168	UCCUg___UGCA AGGAgagACGU	-6.99	(24)
531	I169	UCCUa___UGCA AGGAgagACGU	-6.63	(24)
532	I170	UCCUa___UGCA AGGAaagACGU	-6.56	(24)
533	I171	AAGGUc___UGGAA UUCCAuuuACCUU	-7.87	(24)
534	I172	UCCUg___UGCA AGGAaaaACGU	-6.17	(24)
535	I173	UCCUa___UGCA AGGAgaaACGU	-6.18	(24)
536	I174	UCCUa___UGCA AGGAagaACGU	-6.09	(24)
537	I175	UCCUa___UGCA AGGAaaaACGU	-6.09	(24)
538	I176	ACCUc___UUGC UGGAacaAACG	-5.02	(24)
539	I177	CCUCUc___GGUGA GGAGAaaaCCGCU	-9.86	(24)
540	I178	GAGUg___UGAC CUCGaagGCUG	-5.07	(43)
541	I179	GAGCga_CGAC CUCGaagGCUG	-10.51	(24)
542	I180	CCACgg_CUCC GGUGagaGAGG	-9.84	(24)
543	I181	GAGCaa_CGAC CUCGaagGCUG	-9.23	(24)
544	I182	CGACga_GCAG GCUGgaaCGUC	-8.44	(24)
545	I183	CCUCUgc_GGUGA GGAGAaaaCCGCU	-11.57	(24)
546	I184	UGACuu_CUCA ACUGuuuGAGU	-7.36	(24)
547	I185	GAGCag_CGAC CUCGgaaGCUG	-8.80	(24)

548	I186	GAGCga_CGAC CUCGaaaGCUG	-8.77	(24)
549	I187	CCACgg_CUCC GGUGaaaGAGG	-9.22	(24)
550	I188	GAGCaa_CGAC CUCGgaaGCUG	-8.61	(24)
551	I189	UGACuu_CUCA ACUGcuuGAGU	-6.99	(24)
552	I190	GAGCag_CGAC CUCGaagGCUG	-8.50	(24)
553	I191	UCACuu_CUGA AGUGcucGACU	-5.34	(24)
554	I192	ACCUgc_UUGC UGGAacaAACG	-7.33	(24)
555	I193	UCAGcc_GUGA AGUCaauCACU	-5.11	(24)
556	I194	UGAGaa_GUCA ACUCaaaCAGU	-6.59	(17)
557	I195	UGAGaa_GUCA ACUCcgaCAGU	-6.58	(24)
558	I196	GAGCag_CGAC CUCGaaaGCUG	-8.14	(24)
559	I197	CUGUgg_ACGA GACGagaUGCU	-5.45	(24)
560	I198	GAGCaa_CGAC CUCGaaaGCUG	-7.99	(24)
561	I199	GAGUaa_CGAC CUCGaagGCUG	-7.96	(43)
562	I200	GAGUga_UGAC CUCGaagGCUG	-6.99	(43)
563	I201	GAGCaa_UGAC CUCGaagGCUG	-7.48	(43)
564	I202	CUGUau_GACG GACGaauCUGC	-6.58	(43)
565	I203	GAGUaa_CGAC CUCGaaaGCUG	-6.62	(43)
566	I204	GAGUaa_UGAC CUCGaagGCUG	-5.68	(43)
567	I205	GAGUga_UGAC CUCGaaaGCUG	-5.52	(43)
568	I206	GAGCaa_UGAC CUCGaaaGCUG	-6.23	(43)

569	I207	GAGUag_UGAC CUCGaaaGCUG	-4.79	(43)
570	I208	GAGUaa_UGAC CUCGaaaGCUG	-4.70	(43)
571	I209	UGACuuCUCA ACUGccuGAGU	-6.31	(24)
572	I210	CGACga_GCAG GCUGaagCGUC	-9.77	(44)
573	I211	UGACa___CUCA ACUGaaaaGAGU	-6.12	(17)
574	I212	UGAGa___GUCA ACUCaaaaCAGU	-5.56	(17)
575	I213	UGAGaaaGUCA ACUCaaaCAGU	-6.67	(17)
576	I214	UGACaa__CUCA ACUGaaaaGAGU	-6.07	(17)
577	I215	UGAGaa__GUCA ACUCaaaaCAGU	-6.00	(17)
578	I216	UGACa____CUCA ACUGaaaaaGAGU	-5.71	(17)
579	I217	UGAGa____GUCA ACUCaaaaaCAGU	-5.30	(17)
580	I218	UGACcaaCUCA ACUGaaaGAGU	-7.14	(17)
581	I219	UGACaaaCUCA ACUGaacGAGU	-7.17	(17)
582	I220	CGCaaaGCG GCGaaaCGC	-4.88	(17)
583	I221	CGGaaaCCG GCCaaaGGC	-4.64	(17)
584	I222	GCGaaaCGC CGCaaaGCG	-4.27	(26)
585	I223	CGCaaaGGC GCGaccCCG	-5.85	(26)
586	I224	CGGaaaCGC GCCaaaGCG	-5.00	(26)
587	I225	CGCauaGGC GCGaugCCG	-6.05	(26)
588	I226	CGCauaGGC GCGaaaCCG	-6.14	(26)
589	I227	GAGUgaaUGAC CUCAagaACUG	-7.01	(26)

590	I228	GAGCagaCGAC CUCGagaGCUG	-8.36	(26)
591	I229	CGCagaGGC GCGaugCCG	-6.33	(26)
592	I230	GAGCguaCGAC CUCGauaGCUG	-8.48	(26)
593	I231	CGCaaaGGC GCGaugCCG	-6.56	(26)
594	I232	GAGCagaCGAC CUCGauaGCUG	-8.78	(26)
595	I233	GAGCagaCGAC CUCGaugGCUG	-8.73	(26)
596	I234	CGGcacCCG GCCcacGGC	-4.70	(26)
597	I235	GAGCggaCGAC CUCGauaGCUG	-8.89	(26)
598	I236	CGCaaaGGC GCGaaaCCG	-6.78	(26)
599	I237	GCAgaaUGC CGUaagACG	-4.58	(26)
600	I238	CGCucuGGC GCGucuCCG	-6.90	(26)
601	I239	GAGCaaaCGAC CUCGaugGCUG	-9.12	(26)
602	I240	CGCaaaGGC GCGaacCCG	-6.98	(26)
603	I241	GAGCcgaCGAC CUCGagaGCUG	-9.13	(26)
604	I242	CGACgcaGCAG GCUGaaaCGUC	-8.43	(26)
605	I243	CGGacaCCG GCCacaGGC	-5.07	(26)
606	I244	GAGCugcCGAC CUCGuauGCUG	-9.26	(26)
607	I245	GAGCcgaCGAC CUCGauaGCUG	-9.23	(26)
608	I246	GAGCgaaCGAC CUCGaaaGCUG	-9.32	(26)
609	I247	GCUgaaAGC CGAaagUCG	-5.13	(26)
610	I248	GAGCgaaCGAC CUCGauaGCUG	-9.34	(26)

611	I249	GAGCgagCGAC CUCGauaGCUG	-9.45	(26)
612	I250	GGCgaaGCC CCGaagCGG	-7.74	(26)
613	I251	CGGaagCGC GCCguaGCG	-6.33	(26)
614	I252	GAGCgagCGAC CUCGaaaGCUG	-9.60	(26)
615	I253	GAGCgagCGAC CUCGaagGCUG	-9.64	(26)
616	I254	CGCgaaGGC GCGaccCCG	-7.48	(26)
617	I255	GAGCuguCGAC CUCGuauGCUG	-9.72	(26)
618	I256	CGCgaaGGC GCGaaaCCG	-7.67	(26)
619	I257	CGGaagCGC GCCgaaGCG	-6.60	(26)
620	I258	GCGuuuCGC CGCuuuGCG	-6.46	(26)
621	I259	CGCucuGGC GCGuuuCCG	-7.83	(26)
622	I260	CGACgcaGCAG GCUGaagCGUC	-9.22	(26)
623	I261	CGCuuuGGC GCGucuCCG	-7.85	(26)
624	I262	CGCaagGCG GCGgaaCGC	-6.02	(26)
625	I263	GCGgaaCGG CGCaugGCC	-6.65	(26)
626	I264	CGCuuuGGC GCGuuuCCG	-7.84	(26)
627	I265	CGCgaaGGC GCGaugCCG	-7.97	(26)
628	I266	GAGCgagCGAC CUCGagaGCUG	-10.12	(26)
629	I267	CGCauaGGC GCGaagCCG	-7.98	(26)
630	I268	GCGgaaCGC CGCaagGCG	-6.75	(26)
631	I269	GCGguaCGG CGCaugGCC	-7.73	(26)

632	I270	CGCaaaGGC GCGaagCCG	-8.26	(26)
633	I271	GAGCgaaCGAC CUCGagaGCUG	-10.55	(26)
634	I272	CGCgaaGGC GCGaagCCG	-8.41	(26)
635	I273	GAGCgaaCGAC CUCGaagGCUG	-10.75	(26)
636	I274	CGCgaaGGC GCGaacCCG	-8.65	(26)
637	I275	GAGCagaCGAC CUCGaagGCUG	-10.98	(26)
638	I276	GAGCcgaCGAC CUCGaagGCUG	-11.02	(26)
639	I277	CGCagaGGC GCGaagCCG	-9.01	(26)
640	I278	CGACcgaGCAG GCUGaagCGUC	-10.55	(26)
641	I279	GAGCggaCGAC CUCGaaaGCUG	-11.32	(26)
642	I280	CGCgaaGGC GCGaggCCG	-10.28	(26)
643	I281	GAGCggaCGAC CUCGaagGCUG	-12.48	(26)
644	I282	GCgaGCG GCGagCG	-6.68	(41)
645	I283	GCaaGCG GCGaaCG	-4.20	(41)
646	I284	GCagGCG GCGgaCG	-6.33	(41)
647	I285	GCgaGC CGagCG	-4.01	(41)
648	I286	GCgaGCA ACGagCG	-6.78	(41)
649	I287	GCgaGCU UCGagCG	-5.84	(41)
650	I288	CAGgaCUG GUCagGAC	-6.13	(18)
651	I289	GAGgaCUC CUCagGAG	-7.00	(18)
652	I290	GCGgaCGC CGCagGCG	-9.66	(18)

653	I291	GCUgaGGC CGGagUCG	-6.70	(18)
654	I292	GCGgaUGC CGUagGCG	-4.63	(18)
655	I293	GCUaaGGC CGGaaUCG	-4.71	(19)
656	I295	UGACu_CUCA ACUGcuGAGU	-9.50	(40)
657	I296	UGAGa_GUCA ACUCagCAGU	-8.74	(40)
658	I297	UGAGa_GUCA ACUCggCAGU	-8.64	(40)
659	I301	CUGUgaUGAC GACGagGCUG	-8.45	(43)
660	I302	GAGUaaCGAC CUCGaaGCUG	-7.87	(43)
661	I303	GAGUgaUGAC CUCGaaGCUG	-6.72	(43)
662	I304	GCGagUGC CGUgaGCG	-3.84	(43)
663	I305	GAGCaaUGAC CUCGaaGCUG	-7.12	(43)
664	I306	CUGUagGCAG GACGgaUGUC	-5.50	(43)
665	I307	GAGUagUGAC CUCGaaGCUG	-5.18	(43)
666	I308	GAGUaaUGAC CUCGaaGCUG	-5.22	(43)

Supplementary Table 6: Multibranch Loop Duplex Experiment List

Note: The top strand is shown 5' to 3'. The bottom strand is shown 5' to 3'. Unpaired nucleotides are in lower case. The underscore character (_) is used to delimit parts of the sequence that form different helices without intervening unpaired nucleotides.

Index	ID#	Duplex	Folding Free Energy Change (kcal/mol)	Reference
667	M1	GGAG_CGGCuucgGCCG_GACG CGUCaaCUCC	-5.42	(45)
668	M2	GGAGaCGGCuucgGCCG_GACG CGUCauaCUCC	-4.05	(45)
669	M3	GGAGaCGGCuucgGCCG_GCAG CUGCauaCUCC	-5.85	(45)
670	M4	GGAGgCGGCuucgGCCGuGACG CGUCcauaCUCC	-6.01	(45)
671	M5	GGAGaCGGCuucgGCCGcGACG CGUCauaCUCC	-4.78	(45)
672	M6	GGAGgCGGCuucgGCCGuGACG CGUCauaCUCC	-6.17	(45)
673	M7	GGAG_CGGCuucgGCCG_GACG CGUC_CUCC	-5.71	(45)
674	M8	GGAGaCGGCuucgGCCG_GACG CGUCcauaCUCC	-4.59	(45)
675	M9	GGAG_CGGCuucgGCCG_GACG CGUCauaCUCC	-5.43	(45)
676	M10	GGAG_CGGCuucgGCCG_GACG CGUCcauaCUCC	-5.92	(45)
677	M11	GGAGaCGGCuucgGCCGcGACG CGUCcauaCUCC	-5.25	(45)
678	M12	GGAGaCGGCuucgGCCGaGACG CGUCcauaCUCC	-5.91	(45)
679	M13	GGCAG_GCGCuucgGCGC_GGAGG CCUCC_CUGCC	-8.38	(46)
680	M14	GGCAG_GCGCuucgGCGC_GGAGG CCUCCaCUGCC	-10.41	(46)
681	M15	GGCAG_GCGCuucgGCGC_GGAGG CCUCCaaCUGCC	-11.51	(46)
682	M16	GGCAG_GCGCuucgGCGC_GGAGG CCUCCaaaCUGCC	-11.48	(46)
683	M17	GGCAG_GCGCuucgGCGC_GGAGG CCUCCauaCUGCC	-12.88	(46)
684	M18	GGCAG_GCGCuucgGCGC_GGAGG CCUCCaaaaCUGCC	-12.47	(46)
685	M19	GGCAGaGCGCuucgGCGC_GGAGG CCUCC_CUGCC	-8.80	(46)
686	M20	GGCAGaGCGCuucgGCGC_GGAGG CCUCCaCUGCC	-10.54	(46)
687	M21	GGCAGaGCGCuucgGCGC_GGAGG CCUCCaaCUGCC	-11.13	(46)

688	M22	GGCAGaGCGCuucgGCGC_GGAGG CCUCCaaaCUGCC	-10.69	(46)
689	M23	GGCAGaGCGCuucgGCGC_GGAGG CCUCCauaCUGCC	-11.35	(46)
690	M24	GGCAGaGCGCuucgGCGC_GGAGG CCUCCaaaaCUGCC	-10.98	(46)
691	M25	GGCAGaaGCGCuucgGCGC_GGAGG CCUCC_CUGCC	-9.72	(46)
692	M26	GGCAGaaGCGCuucgGCGC_GGAGG CCUCCaCUGCC	-11.59	(46)
693	M27	GGCAGaaGCGCuucgGCGC_GGAGG CCUCCaaCUGCC	-11.86	(46)
694	M28	GGCAGaaGCGCuucgGCGC_GGAGG CCUCCaaaCUGCC	-11.26	(46)
695	M29	GGCAGaaGCGCuucgGCGC_GGAGG CCUCCauaCUGCC	-11.48	(46)
696	M30	GGCAGaaGCGCuucgGCGC_GGAGG CCUCCaaaaCUGCC	-10.97	(46)
697	M31	GGCAGaaGCGCuucgGCGCaGGAGG CCUCC_CUGCC	-11.66	(46)
698	M32	GGCAGaaGCGCuucgGCGCaGGAGG CCUCCaCUGCC	-12.89	(46)
699	M33	GGCAGaaGCGCuucgGCGCaGGAGG CCUCCaaCUGCC	-13.05	(46)
700	M34	GGCAGaaGCGCuucgGCGCaGGAGG CCUCCaaaCUGCC	-13.15	(46)
701	M35	GGCAGaaGCGCuucgGCGCaGGAGG CCUCCauaCUGCC	-12.93	(46)
702	M36	GGCAGaaGCGCuucgGCGCaGGAGG CCUCCaaaaCUGCC	-12.37	(46)
703	M37	GGCAGaaGCGCuucgGCGCaaGGAGG CCUCC_CUGCC	-12.50	(46)
704	M38	GGCAGaaGCGCuucgGCGCaaGGAGG CCUCCaCUGCC	-13.15	(46)
705	M39	GGCAGaaGCGCuucgGCGCaaGGAGG CCUCCaaCUGCC	-12.88	(46)
706	M40	GGCAGaaGCGCuucgGCGCaaGGAGG CCUCCaaaCUGCC	-12.64	(46)
707	M41	GGCAGaaGCGCuucgGCGCaaGGAGG CCUCCauaCUGCC	-13.23	(46)
708	M42	GGCAGaaGCGCuucgGCGCaaGGAGG CCUCCaaaaCUGCC	-12.95	(46)
709	M43	GGCAG_UCGCuucgGCGA_GGAGG CCUCC_CUGCC	-9.15	(46)
710	M49	GGCAGaUCGCuucgGCGA_GGAGG CCUCC_CUGCC	-9.84	(46)
711	M50	GGCAGaUCGCuucgGCGA_GGAGG CCUCCaCUGCC	-11.79	(46)
712	M51	GGCAGaUCGCuucgGCGA_GGAGG CCUCCaaCUGCC	-12.38	(46)
713	M52	GGCAGaUCGCuucgGCGA_GGAGG	-12.00	(46)

		CCUCCaaaCUGCC		
714	M53	GGCAGaUCGCuucgGCGA_GGAGG CCUCCauaCUGCC	-12.06	(46)
715	M54	GGCAGaUCGCuucgGCGA_GGAGG CCUCCaaaaCUGCC	-11.91	(46)
716	M55	GGCAG_CGGCuucgGCCG_GCGCgcaaGCGC_GGAGG CCUCC_CUGCC	-16.48	(46)
717	M56	GGCAG_CGGCuucgGCCG_GCGCgcaaGCGC_GGAGG CCUCCaCUGCC	-13.90	(46)
718	M57	GGCAG_CGGCuucgGCCG_GCGCgcaaGCGC_GGAGG CCUCCaaCUGCC	-12.40	(46)
719	M58	GGCAG_CGGCuucgGCCG_GCGCgcaaGCGC_GGAGG CCUCCaaaCUGCC	-11.19	(46)
720	M59	GGCAG_CGGCuucgGCCG_GCGCgcaaGCGC_GGAGG CCUCCaaaaCUGCC	-10.46	(46)
721	M60	GGCAGaCGGCuucgGCCG_GCGCgcaaGCGC_GGAGG CCUCC_CUGCC	-13.12	(46)
722	M61	GGCAGaCGGCuucgGCCG_GCGCgcaaGCGC_GGAGG CCUCCaCUGCC	-15.40	(46)
723	M62	GGCAGaCGGCuucgGCCG_GCGCgcaaGCGC_GGAGG CCUCCaaCUGCC	-13.52	(46)
724	M63	GGCAGaCGGCuucgGCCG_GCGCgcaaGCGC_GGAGG CCUCCaaaCUGCC	-11.86	(46)
725	M64	GGCAGaCGGCuucgGCCG_GCGCgcaaGCGC_GGAGG CCUCCauaCUGCC	-12.31	(46)
726	M65	GGCAGaCGGCuucgGCCG_GCGCgcaaGCGC_GGAGG CCUCCaaaaCUGCC	-11.18	(46)
727	M66	GGCAGaCGGCuucgGCCG_GCGCgcaaGCGCaGGAGG CCUCC_CUGCC	-13.58	(46)
728	M67	GGCAGaCGGCuucgGCCG_GCGCgcaaGCGCaGGAGG CCUCCaCUGCC	-13.36	(46)
729	M68	GGCAGaCGGCuucgGCCG_GCGCgcaaGCGCaGGAGG CCUCCaaCUGCC	-14.76	(46)
730	M69	GGCAGaCGGCuucgGCCG_GCGCgcaaGCGCaGGAGG CCUCCaaaCUGCC	-13.01	(46)
731	M70	GGCAGaCGGCuucgGCCG_GCGCgcaaGCGCaGGAGG CCUCCauaCUGCC	-12.59	(46)
732	M71	GGCAGaCGGCuucgGCCG_GCGCgcaaGCGCaGGAGG CCUCCaaaaCUGCC	-12.66	(46)
733	M72	GGCAGaaCGGCuucgGCCGaaGCGCgcaaGCGCaaGGAGG CCUCC_CUGCC	-11.77	(46)
734	M73	GGCAGaaCGGCuucgGCCGaaGCGCgcaaGCGCaaGGAGG CCUCCaCUGCC	-12.65	(46)
735	M74	GGCAGaaCGGCuucgGCCGaaGCGCgcaaGCGCaaGGAGG CCUCCaaCUGCC	-12.77	(46)

Supplementary Table 7: Bulge Loop Duplex Experiment List

Note: The top strand is shown 5' to 3'. The bottom strand is shown 3' to 5'. Lower case letters in the top strand indicate unpaired nucleotides. Lower case letters in the bottom strand indicate nucleotides that can base pair with multiple possible nucleotides in the top strand. The underscore character (_) is used to maintain alignment between the top and bottom strands.

Index	ID#	Duplex	Folding Free Energy Change (kcal/mol)	Reference
736	B1	GCGaGCG CGC_CGC	-6.76	(14)
737	B2	GCGuGCG CGC_CGC	-6.51	(14)
738	B3	CGCaCGC GCG_GCG	-6.85	(14)
739	B4	GCGaaGCG CGC__CGC	-5.17	(14)
740	B5	GCGuuGCG CGC__CGC	-5.01	(14)
741	B6	CGCaaCGC GCG__GCG	-5.33	(14)
742	B7	GCGaaaGCG CGC___CGC	-4.76	(14)
743	B8	GCGuuuGCG CGC___CGC	-4.85	(14)
744	B9	CGCaaaCGC GCG___GCG	-6.63	(14)
745	B12	GCGaaGCGa aCGC__CGC	-6.64	(14)
746	B13	CGCaaCGCa aGCG__GCG	-7.16	(14)
747	B14	GCGaaaGCGa aCGC___CGC	-5.39	(14)
748	B15	CGCaaaCGCa aGCG___GCG	-7.38	(14)
749	B18	GCGaaGUCa aCGC__CAG	-6.60	(14)
750	B19	GCGaaaGUCa aCGC___CAG	-5.07	(14)
751	B20	CCAUUaCUACC GGUAA_GAUGG	-9.76	(47)
752	B21	GCACaGAGG CGUG_CUCC	-9.07	(47)

753	B22	GACCaUGUC CUGG_ACAG	-8.16	(47)
754	B23	UGAGaGUCA ACUC_CAGU	-7.82	(47)
755	B24	UGACaCUCA ACUG_GAGU	-7.17	(47)
756	B25	GACUaUGUC CUGA_ACAG	-6.06	(47)
757	B26	CAUGUgACUAC GUACA_UGAUG	-8.89	(47)
758	B27	UGACgCUCA ACUG_GAGU	-7.57	(47)
759	B28	GACUgUGUC CUGA_ACAG	-7.40	(47)
760	B29	GACAgAGUC CUGU_UCAG	-6.63	(47)
761	B30	GCACuGAGG CGUG_CUCC	-9.63	(47)
762	B31	ACUGuGAGU UGAC_CUCA	-8.06	(47)
763	B32	UGACuCUCA ACUG_GAGU	-7.79	(47)
764	B33	GACAuAGUC CUGU_UCAG	-6.48	(47)
765	B34	CAUGAcGCUAC GUACU_CGAUG	-10.58	(47)
766	B35	CAUGUcACUAC GUACA_UGAUG	-8.61	(47)
767	B36	UGAGcGUCA ACUC_CAGU	-8.41	(47)
768	B37	GACUcUGUC CUGA_ACAG	-7.29	(47)
769	B38	UCCUcGAAC AGGA_CUUG	-6.69	(47)
770	B39	GACAcAGUC CUGU_UCAG	-5.93	(47)
771	B40	GACCaaGUC CUGG_uCAG	-9.40	(47)
772	B41	GACGaaGUC CUGC_uCAG	-8.24	(47)
773	B42	GACaaaGUC CUG_uuCAG	-6.87	(47)

774	B43	AGCUggCAG UCGA_cGUC	-9.56	(47)
775	B44	UGAgggUCA ACU_ccAGU	-9.03	(47)
776	B45	AGCAggCAG UCGU_cGUC	-8.59	(47)
777	B46	AGACggCAG UCUG_cGUC	-8.07	(47)
778	B47	GACCuuGUC CUGG_aCAG	-9.28	(47)
779	B48	GACuuuGUC CUG_aaCAG	-7.25	(47)
780	B49	UGAcccUCA ACU_ggAGU	-9.33	(47)
781	B50	UCCUccAAC AGGA_gUUG	-8.41	(47)
782	B51	UCCAccAAC AGGU_gUUG	-8.17	(47)
783	B52	CGGUaGUCU GCCG_CAGA	-8.29	(47)
784	B53	UGAUaCUCA ACUG_GAGU	-5.79	(47)

Supplementary Table 8: Coaxial Stacking Experiment List

Note: The top strand is shown 5’ to 3’. The bottom strand is shown 3’ to 5’. Unpaired nucleotides are in lower case. The top strand forms a hairpin loop whose stem can coaxially stack with the stem formed by base pairing with the bottom strand.

Index	ID#	Duplex	Folding Free Energy Change (kcal/mol)	Reference
785	X1	GGACGCAGUGGCaaaaaGCCACUG CCUGA	-7.79	(48)
786	X2	GGACGCAGUGGCaaaaaGCCACUGA CCUG	-6.28	(48)
787	X3	GGACGAGUGGCgcaaGCCACU CCUGA	-7.41	(48)
788	X4	GGACGAGUGGCgcaaGCCACUA CCUG	-5.87	(48)
789	X5	GGACGAGUGGCgcaaGCCACGA CCUG	-5.69	(48)
790	X6	GGACGAGUGGCgcaaGCCACGA CCUGA	-6.67	(48)
791	X7	GGACACAGUGGCaaaaaGCCACUG CCUGG	-7.79	(48)
792	X8	GGAGCCAGUGGCaaaaaGCCACUG CCUCC	-5.64	(48)
793	X9	GGACCCAGUGGCaaaaaGCCACUG CCUGC	-6.41	(48)
794	X10	GGACGCAGUGGCaaaaaGCCACUGp CCUGAa	-6.34	(48)
795	X11	GGACGCAGUGGCaaaaasGCCACUGaa CCUGA	-6.18	(48)
796	X12	GGACGCAGUGGCaaaaaGCCACUGpa CCUGA	-6.50	(48)
797	X13	GGACGCAGUGGCaaaaaGCCACUGAa CCUGp	-5.64	(48)
798	X14	GGACGCAGUGGCaaaaaGCCACUGAgg CCUG	-5.43	(48)
799	X15	GGACGAGUGGCgcaaGCCACUaa CCUGA	-6.08	(48)
800	X16	GGACGAGUGGCgcaaGCCACGp CCUGAa	-6.22	(48)
801	X17	GGAGCCAGUGGCaaaaaGCCACUGa CCUCCa	-5.12	(48)

802	X18	GGACCCAGUGGCaaaaaGCCACUGa CCUGCa	-5.66	(48)

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