Supporting Information for World Wide Web Edition

Nondenaturing second-round gels performed with inclusion of various concentrations of monovalent cations (Na^+ or K^+) in the running buffer are shown in Figure 1. Inclusion of monovalents did not change the rank order of mobilities for the various P4-P6 derivatives.

Tables of $\Delta\Delta G^{\circ\prime}$ values with error estimates for all P4-P6 samples are in Table 1. Because $[Mg^{2+}]_{1/2}$ values were determined from the fit values of K and n (manuscript Figure 4), and because $\Delta G^{\circ\prime}$ is related to the logarithm of $[Mg^{2+}]_{1/2}$ by eq (3) of the manuscript, the error on all $\Delta G^{\circ\prime}$ values is in general asymmetrical. However, in all cases for which $\Delta\Delta G^{\circ\prime}$ is < 2.4 kcal/mol (that is, all the data shown in manuscript Figure 5), the plus and minus errors were approximately equal and almost always ≤ 0.2 kcal/mol.

Hill coefficients for the titration data (manuscript Figure 4) were determined in two ways. The first method was to fit the titration data directly (four-parameter fit, as described in Materials and Methods). The second method used the fit values of M_{low} and M_{high} to calculate $\log ((M_{obs} - M_{low})/(M_{high} - M_{obs}))$. A Hill plot was then obtained by plotting this value versus $\log [Mg^{2+}]$; the slope of the plot (central data points only) is the Hill coefficient n. Representative Hill plots determined by this latter method are shown in Figure 2. The values of n calculated by both methods for the various P4-P6 samples of Table 1 are shown in Figure 3; little difference is observed. The Hill coefficients determined by the two methods show a reasonable correlation (Figure 4).

Table 1: Destabilization of P4-P6 RNA Tertiary Folding at 35 °C by 2'-Deoxy and 2'-Methoxy Substitutions[a]

	site(s) substituted	ΔΔG°'[b]	error estimates[b]			site(s) substituted	ΔΔG°'[b]	error estimates[b]	
	Round 1					**Round 2**			
1	C109-2'-OMe	1.06	+0.05	–0.05	8	A183:A184-2'-OMe	1.27	+0.15	–0.11
2	G110-2'-OMe	0.81	+0.08	–0.08	9	C109:A183-2'-OMe	0.68	+0.14	–0.16
3	C109:G110-2'-OMe	1.14	+0.08	–0.07	10	C109:A184-2'-OMe	0.77	+0.12	–0.13
4	C137-2'-OMe	2.42	+0.56	–0.35	11	C109:A183: A184-2'-OMe	0.27	+0.19	–0.22
5	A183-2'-OMe	0.81	+0.06	–0.06	12	G110:A183-2'-OMe	0.02	+0.23	–0.26
6	A184-2'-OMe	0.78	+0.07	–0.08	13	G110:A184-2'-OMe	1.17	+0.14	–0.10
7	A186-2'-OMe	4.13	+1.01	–0.58	14	G110:A183: A184-2'-OMe	0.79	+0.10	–0.10
					15	C109:G110: A183-2'-OMe	0.32	+0.18	–0.21
					16	C109:G110: A184-2'-OMe	0.80	+0.10	–0.11
					17	C109:G110: A183:A184-2'-OMe	–0.11	+0.25	–0.28
					18	C137-2'-OMe	2.74	+0.76	–0.45
					19	A186-2'-OMe	4.14	+1.13	–0.53
	Round 3					**Round 4**			
20	C109-2'-H	0.40	+0.13	–0.13	35	A183-2'-H	0.17	+0.16	–0.16
21	G110-2'-H	0.91	+0.13	–0.12	36	C109:A183-2'-H	0.73	+0.14	–0.14
22	C109:G110-2'-H	1.32	+0.15	–0.16	37	C109:A184-2'-H	1.18	+0.14	–0.13
23	A184-2'-H	1.12	+0.13	–0.14	38	G110:A183-2'-H	1.27	+0.15	–0.14
24	G110:A184-2'-H	1.42	+0.16	–0.18	39	A183:A184-2'-H	1.98	+0.18	–0.17
25	A183:A184-2'-OMe	1.06	+0.13	–0.13	40	C109:G110: A183-2'-H	1.69	+0.19	–0.17

26	G110:A183-2'-OMe	0.12	+0.19	–0.19	41	C109:G110: A184-2'-H	1.84	+0.22	–0.19
27	C109:G110: A183:A184-2'-OMe	–0.20	+0.20	–0.22	42	C109:A183: A184-2'-H	1.75	+0.22	–0.19
28	U107-2'-OMe	0.07	+0.17	–0.18	43	G110:A183: A184-2'-H	2.24	+0.26	–0.22
29	A114-2'-OMe	0.04	+0.19	–0.20	44	C109:G110: A183:A184-2'-H	2.27	+0.25	–0.21
30	G188-2'-OMe	0.29	+0.15	–0.16	45	G110-2'-OMe	0.75	+0.13	–0.13
31	C193-2'-OMe	0.10	+0.17	–0.18	46	C109-2'-H	0.40	+0.12	–0.13
32	C109-2'-OMe	1.03	+0.13	–0.14	47	C109:G110-2'-OMe	1.22	+0.16	–0.15
33	C137-2'-OMe	2.36	+0.34	–0.49	48	G110-2'-H	1.03	+0.14	–0.13
34	A186-2'-OMe	3.99	+0.65	–1.04	49	C109:G110: A183-2'-OMe	0.28	+0.15	–0.16
					50	C109:G110-2'-H	1.55	+0.21	–0.18
					51	G110:A184-2'-H	1.72	+0.28	–0.22

[a] Derivatives are listed in the order shown on the gels of manuscript Figure 3, followed by other samples not shown in those gels. Derivatives are identified by a number for cross-referencing to Supporting Information Figure 3. [b] kcal/mol. We report $\Delta\Delta G^{\circ\prime}$ values to one significant figure more than is arguably appropriate so that rounding errors do not affect comparison of the values.

Supporting Information Figure Legends

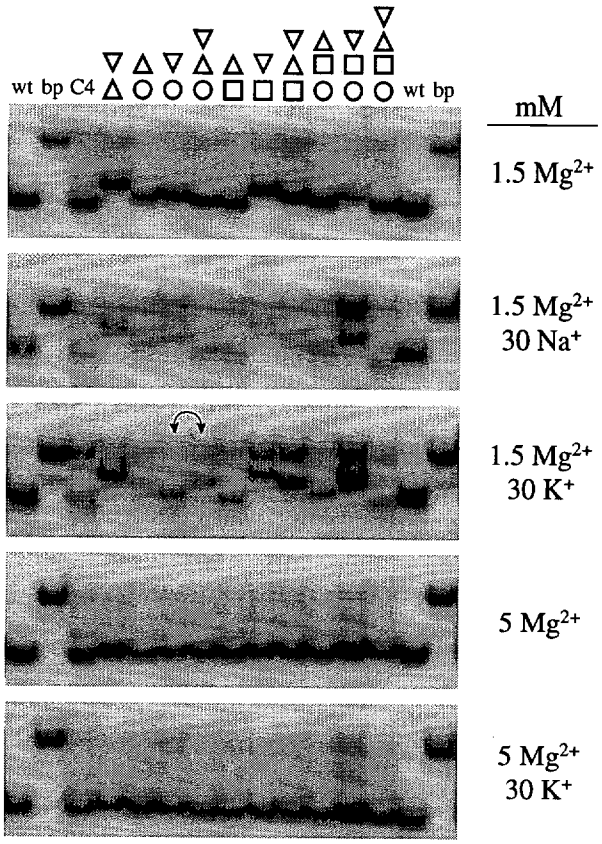
Figure 1: Nondenaturing gels of P4-P6 derivatives with varying concentrations of monovalent ions. Second-round gels with Mg^{2+} plus varying concentrations of Na^+ or K^+ are shown; see manuscript Figure 3 for symbols. The double-headed arrow on the third gel indicates two samples accidentally switched during the gel loading.

Figure 2: Representative Hill plots for the native gel titration data. Six plots from the Round 3 data are shown.

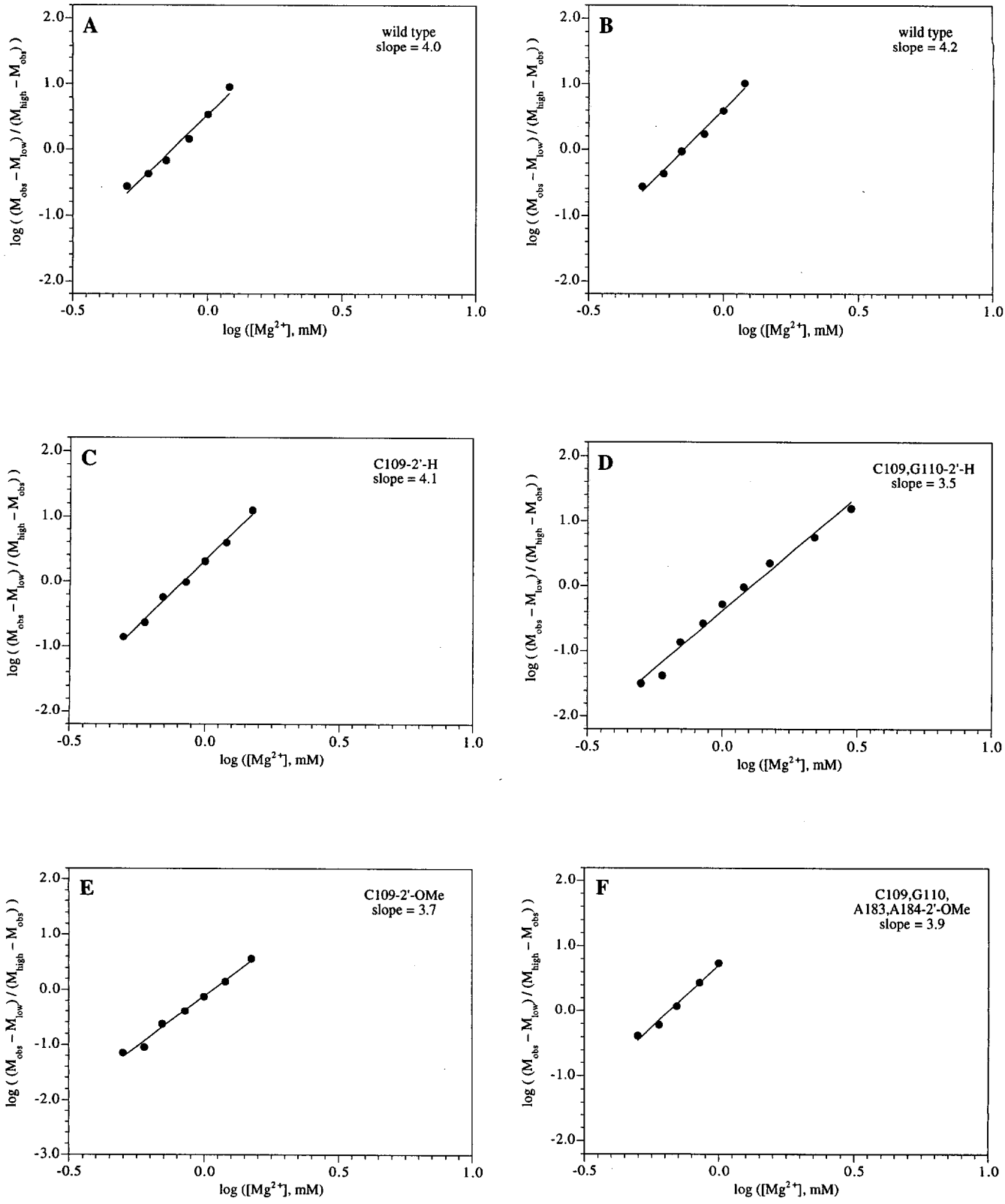
Figure 3: Comparison of Hill coefficients determined from titration data and from Hill plots. (A) Round 1 gels. (B) Round 2 gels. (C) Round 3 gels. (D) Round 4 gels. Error bars are $\pm \sigma$. Samples are labelled with "w" followed by a number for authentic wild-type P4-P6 samples; "c" followed by a number for the control wild-type P4-P6 RNAs C1–C5 prepared by ligation; and with numbers 1–51 for the P4-P6 derivatives as listed in Table 1.

Figure 4: Correlation of Hill coefficients determined from titration data and from Hill plots. Data taken from Figure 2. Different symbols are used for Rounds 1–4 data as shown. The best fit line is $n_{Hill} = -0.213 + 1.054 \cdot n_{titr}$, with correlation coefficient $R = 0.78$.

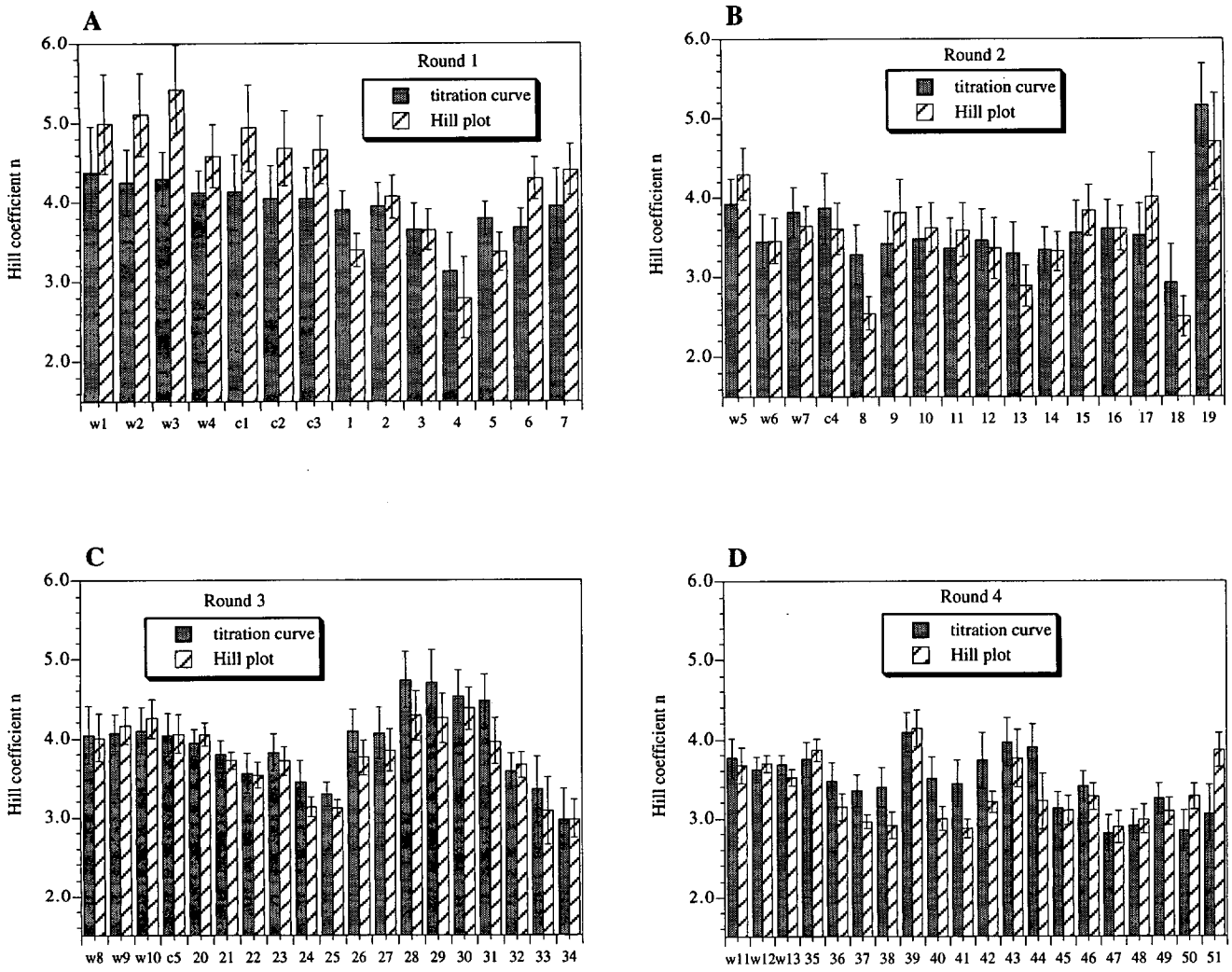
Silverman & Cech
Supp Info
Figure 1



Silverman & Cech
Supp Info
Figure 2



Silverman & Cech
Supp Info
Figure 3



Silverman & Cech
Supp Info
Figure 4