Supplementary information

Hinge stiffness is a barrier to RNA folding

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Supplementary Table 1 Summary of the rate constants derived by time-resolved hydroxyl radical footprinting experiments. Black corresponds to nucleotides from P4-P6, grey corresponds to nucleotides from isolated P5abc. No significant change in solvent accessibility was observed for the double mutant RNA. "p" indicates nucleotides which become *protected*, "e" denotes exposed nucleotides, and "nc" refers to nucleotides which don't show changes in their solvent accessibility profile upon Mg^{2+} mediated folding. "na" stands for "data not available".

Supplementary Figure 1 Exemplary kinetic progress curves describing the Mg^{2+} mediated folding of P4-P6. The continuous lines are the fits to equation <1> as described in Materials and Methods. Each symbol represents an independent experiment. The fractional saturation scale is defined by CE buffer ($\bar{Y} = 0.0$) and CE buffer plus Mg^{2+} ($\bar{Y} = 1.0$). The rates for these progress curves are listed in Supplementary Table 1.

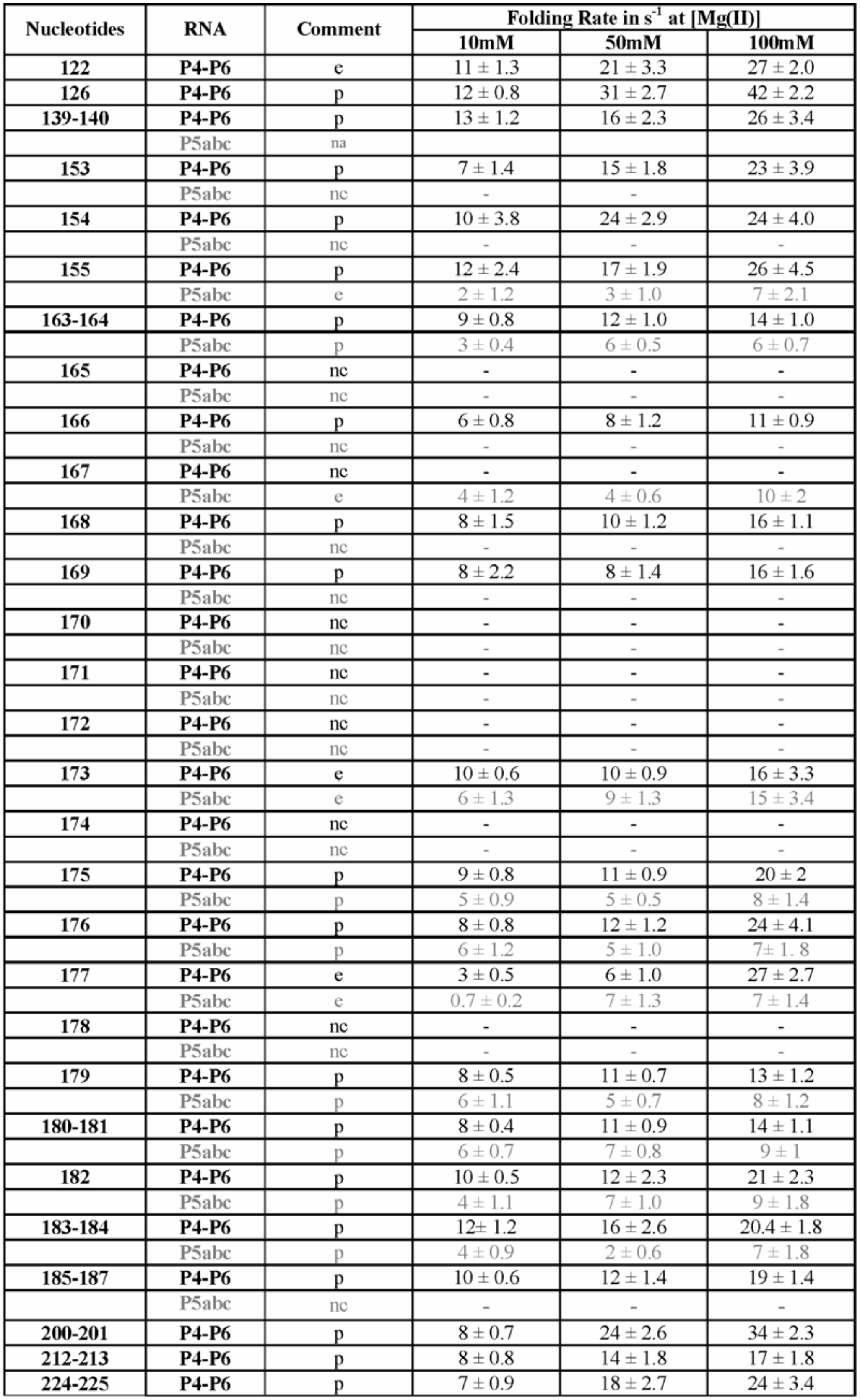
Supplementary Figure 2 Magnesium dependency of wild-type P4-P6 and isolated P5abc domain tertiary structure. Magnesium titrations of RNAs were carried out in 18mM potassium cacodylate (pH 7.0) at 25°C. Midpoints and Hill constants were obtained from fits of fractional saturation versus $MgCl_2$ concentration (mM) to the Hill equation. The protection site at nucleotide 155 showed the largest K_d value for P4-P6 (left) and P5abc (right) RNA of 1.64 ± 0.15 ($n_H = 0.95 \pm 0.1$) and 1.2 ± 0.17 ($n_H = 1.5 \pm 0.18$), respectively. In each panel the squares show the Mg(II) dependent relative extend of protection at nucleotide 155. The solid line correlates with the fit to the Hill equation.

The dotted line corresponds to the lowest Mg(II) concentration used in our experiments (10mM). The equilibrium data demonstrate that at our conditions the P4-P6 and P5abc structures are fully formed.

Supplementary Figure 3 shows all time resolved data acquired on P4-P6 wild type (left) and double mutant (right), displayed as Kratky plots. In this commonly used formalism, compact states exhibit a peak at moderate ‘q’[1]. Wild type P4-P6 begins to compact within ~ 10 milliseconds of the addition of Mg^{2+}. There is no evidence of compaction in the double mutant, at times as long as 160 ms after the initiation of folding.

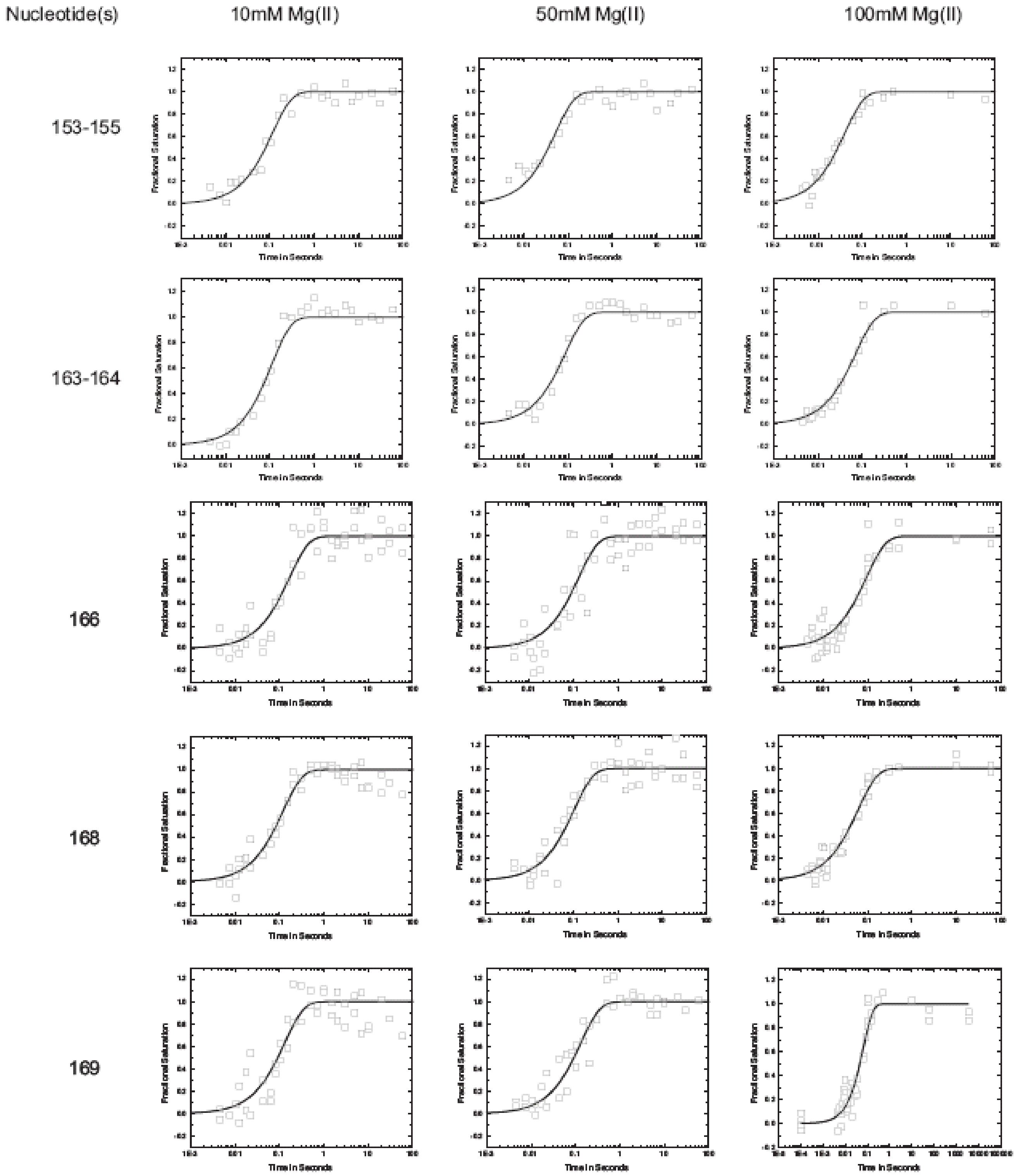
1. Porod, G. (1982). In *Small angle x-ray scattering* (Glatter, O. & Kratky, O., eds.). Academic Press, London.

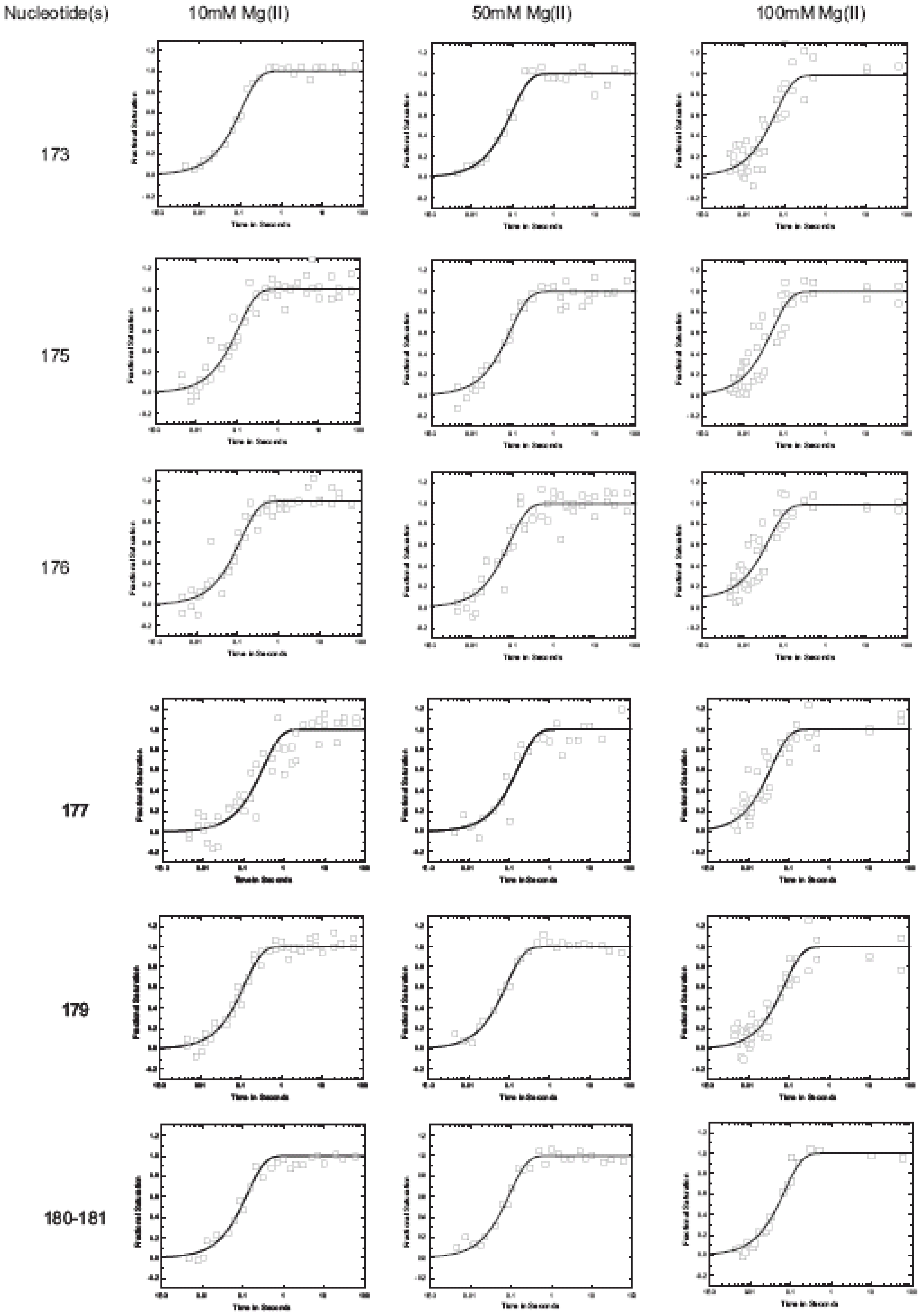
Supplementary Table 1.

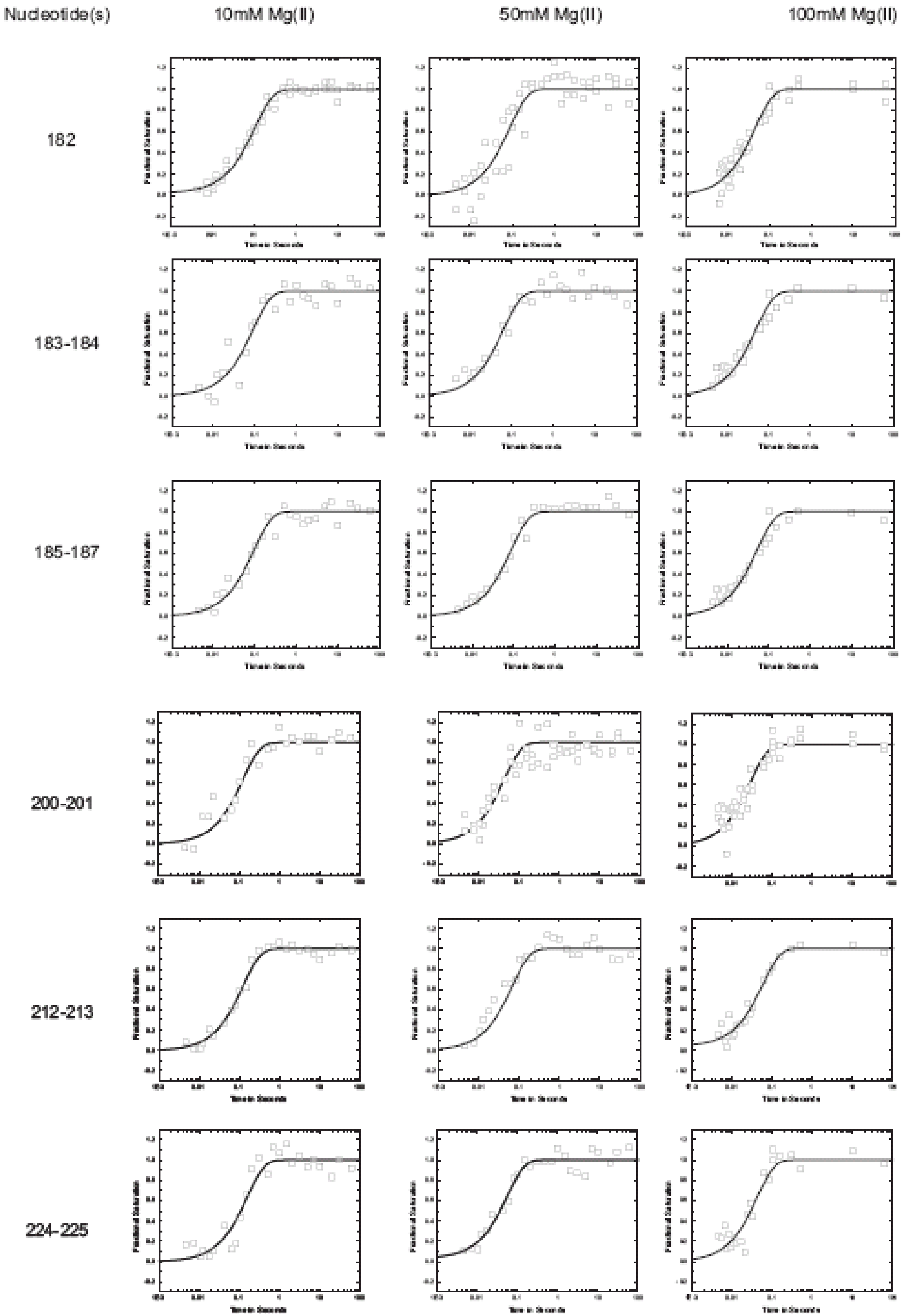


Nucleotides	RNA	Comment	Folding Rate in s^{-1} at [Mg(II)]		
			10mM	50mM	100mM
122	P4-P6	e	11 ± 1.3	21 ± 3.3	27 ± 2.0
126	P4-P6	p	12 ± 0.8	31 ± 2.7	42 ± 2.2
139-140	P4-P6	p	13 ± 1.2	16 ± 2.3	26 ± 3.4
	P5abc	na			
153	P4-P6	p	7 ± 1.4	15 ± 1.8	23 ± 3.9
	P5abc	nc	-	-	
154	P4-P6	p	10 ± 3.8	24 ± 2.9	24 ± 4.0
	P5abc	nc	-	-	-
155	P4-P6	p	12 ± 2.4	17 ± 1.9	26 ± 4.5
	P5abc	e	2 ± 1.2	3 ± 1.0	7 ± 2.1
163-164	P4-P6	p	9 ± 0.8	12 ± 1.0	14 ± 1.0
	P5abc	p	3 ± 0.4	6 ± 0.5	6 ± 0.7
165	P4-P6	nc	-	-	-
	P5abc	nc	-	-	-
166	P4-P6	p	6 ± 0.8	8 ± 1.2	11 ± 0.9
	P5abc	nc	-	-	-
167	P4-P6	nc	-	-	-
	P5abc	e	4 ± 1.2	4 ± 0.6	10 ± 2
168	P4-P6	p	8 ± 1.5	10 ± 1.2	16 ± 1.1
	P5abc	nc	-	-	-
169	P4-P6	p	8 ± 2.2	8 ± 1.4	16 ± 1.6
	P5abc	nc	-	-	-
170	P4-P6	nc	-	-	-
	P5abc	nc	-	-	-
171	P4-P6	nc	-	-	-
	P5abc	nc	-	-	-
172	P4-P6	nc	-	-	-
	P5abc	nc	-	-	-
173	P4-P6	e	10 ± 0.6	10 ± 0.9	16 ± 3.3
	P5abc	e	6 ± 1.3	9 ± 1.3	15 ± 3.4
174	P4-P6	nc	-	-	-
	P5abc	nc	-	-	-
175	P4-P6	p	9 ± 0.8	11 ± 0.9	20 ± 2
	P5abc	p	5 ± 0.9	5 ± 0.5	8 ± 1.4
176	P4-P6	p	8 ± 0.8	12 ± 1.2	24 ± 4.1
	P5abc	p	6 ± 1.2	5 ± 1.0	7± 1. 8
177	P4-P6	e	3 ± 0.5	6 ± 1.0	27 ± 2.7
	P5abc	e	0.7 ± 0.2	7 ± 1.3	7 ± 1.4
178	P4-P6	nc	-	-	-
	P5abc	nc	-	-	-
179	P4-P6	p	8 ± 0.5	11 ± 0.7	13 ± 1.2
	P5abc	p	6 ± 1.1	5 ± 0.7	8 ± 1.2
180-181	P4-P6	p	8 ± 0.4	11 ± 0.9	14 ± 1.1
	P5abc	p	6 ± 0.7	7 ± 0.8	9 ± 1
182	P4-P6	p	10 ± 0.5	12 ± 2.3	21 ± 2.3
	P5abc	p	4 ± 1.1	7 ± 1.0	9 ± 1.8
183-184	P4-P6	p	12± 1.2	16 ± 2.6	20.4 ± 1.8
	P5abc	p	4 ± 0.9	2 ± 0.6	7 ± 1.8
185-187	P4-P6	p	10 ± 0.6	12 ± 1.4	19 ± 1.4
	P5abc	nc	-	-	-
200-201	P4-P6	p	8 ± 0.7	24 ± 2.6	34 ± 2.3
212-213	P4-P6	p	8 ± 0.8	14 ± 1.8	17 ± 1.8
224-225	P4-P6	p	7 ± 0.9	18 ± 2.7	24 ± 3.4

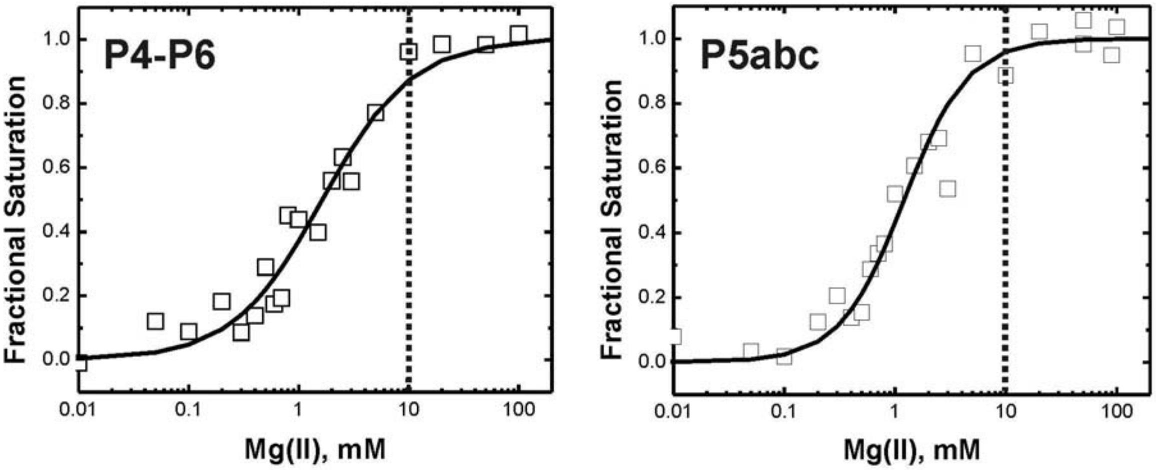
Supplementary Figure 1




Nucleotide(s)
10mM Mg(II)
50mM Mg(II)
100mM Mg(II)
173
175
176
177
179
180-181
Fractional Saturation
Time in Seconds
-0.2
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0.001
0.01
0.1
1
10
100


Nucleotide(s)
10mM Mg(II)
50mM Mg(II)
100mM Mg(II)
182
183-184
185-187
200-201
212-213
224-225
Fractional Saturation
Time in Seconds

Supplementary Figure 2



Supplementary Figure 3