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Predicting Stability of DNA Duplexes in Solutions Containing Magnesium and Monovalent Cations

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Database of average melting profiles, fraction of melted base pairs versus temperature, is available at <http://biophysics.idtdna.com/Paper7/Abstract7.html>.

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DNA sequence (5' to 3')	T_m (°C) at 1M Na ⁺	T_m (°C) at [Mg ²⁺] indicated ^d						
		0.5mM ^c	1.5mM ^c	3.0mM ^c	10mM ^c	20mM ^c	50mM ^d	125mM ^d
CCAGCCAGTCTCTCC	66.7	56.6	59.4	60.5	62.1	62.5	62.9	63.0
GACGACAAAGACCGCG	68.6	59.4	61.7	62.8	64.1	64.6	65.3	64.9
CTCGCGGTGGAAGCG	70.7	61.3	63.9	65.1	66.3	66.9	67.3	66.8
GCGTCGGTCCGGGCT	74.1	65.2	67.5	68.5	69.5	70.3	70.6	70.2
		20-mers						
TAIGTATATTTTGTAAATCAG	59.8 ^e	48.6	51.4	52.6	54.4	55.1	55.8	56.3
TTCAAAGTTAAACAATTTCTATC	61.5	49.9	52.6	53.9	55.6	56.4	57.2	57.8
GAGATTGTTTCCCTTTCAAA	65.3	53.2	56.2	57.5	59.2	60.1	60.9	61.3
ATGCAATGCTACATATTCGC	68.9	57.4	59.9	61.0	62.5	63.1	63.8	63.7
CCACTATACCAATCTATGTAC	64.4	52.9	55.4	56.6	58.0	58.7	59.2	59.5
CCATCAITTGICTACCTCA	68.5	58.1	60.4	61.5	62.9	63.5	64.0	64.1
CGGGACCAACTAAAGGAAAT	68.5	57.6	60.1	61.5	62.9	63.4	64.0	64.4
TAGTGGGATTAGATTCTGC	71.2	60.0	62.5	63.6	64.9	65.6	66.2	66.5
TACTTCCAGTGCTCAGCGTA	73.6	62.2	64.7	65.8	67.3	67.7	68.3	68.4
CAGTGAGACAGCAATGGTCCG	72.5	61.4	63.9	65.0	66.2	66.8	67.2	67.0
CGAGTTATCCCTATCCCTC	70.3	60.0	62.5	63.6	65.0	65.5	65.9	65.9
CGTACTAGCGTTGGTCATGG	71.1	61.1	63.4	64.4	65.8	66.2	66.5	66.5
AAGGCGAGTCAGGCTCAGTG	76.3	66.2	68.5	69.8	70.6	71.2	71.6	71.4
ACCGACGACGCTGATCCGAT	77.3	66.2	68.7	69.5	71.0	71.0	71.6	71.2
AGCAGTCCGCCACACCCCTGA	78.5	67.6	69.9	70.6	72.1	72.5	72.7	72.4
GTGGTGGCCGTGCGGCTCTG	81.0	69.8	72.0	73.2	74.4	74.6	75.0	74.4
GTCCACGCCCCGTGCGGACGG	81.1	70.3	72.5	73.9	74.0	74.5	74.7	74.3

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DNA sequence (5' to 3')	T_m (°C) at 1M Na ⁺ ^b	T_m (°C) at [Mg ²⁺] indicated ^c						
		0.5mM ^c	1.5mM ^c	3.0mM ^c	10mM ^c	20mM ^c	50mM ^d	125mM ^d
CTCAACTGCGGTAAAAFAAATCGCTTAATC	75.5	64.1	66.1	67.2	68.3	68.8	69.6	69.8
TATTGAGAACAAAGTGCCGATTAGCAGAAAA	76.4	65.3	67.4	68.4	69.6	70.1	70.7	70.8
GTCATACGACTGAGTGCAACATTGTTCAAA	76.9	65.5	67.6	68.5	69.8	70.1	70.7	70.8
AACCTGCAACATGGAGTTTTTGTCTCATGC	78.7	67.9	69.9	70.8	71.7	72.3	72.8	72.9
CCGTGCGGTGTACGTTTTTATTTCATCATA	77.6	66.7	68.7	69.6	70.8	71.2	71.6	71.5
GTTCACGTCCGAAAGCTCGAAAAAGGATAC	78.7	68.1	70.2	71.2	72.2	72.7	73.2	73.4
TCGGAGAAATCACTGAGCTGCCTGAGAAAGA	80.9	69.0	71.0	71.9	72.9	73.3	73.8	73.7
CTTCAACGGATCAGGTAGGACTGTGGTGGG	80.1	68.3	70.1	71.0	72.0	72.5	72.9	72.9
ACGCCACAGGATTAGGCTGCCCCACATTG	84.0	73.2	75.0	75.8	76.7	76.9	77.4	77.2
GTTATCCGCAGTCCGATGCCAGCAGGCTC	84.1	72.9	74.8	75.5	76.6	76.9	77.3	77.0
TCAGTAGCGGTGACGCAGAGCTGGCCGATGG	84.6	73.7	75.8	76.0	77.0	77.7	78.0	77.7
CGGCCACGTGTGATCTACAGCCGTTCCGC	84.5	73.9	75.3	76.0	77.1	77.0	77.4	77.0
GCCCCTCCACTGGCCGACGGCAGCAGGCTC	87.7	77.6	79.1	79.8	80.4	80.8	80.9	80.6
CGCCGCTGCCGACTGGAGGAGCGCGGGACG	88.6	78.7	80.1	80.9	81.1	81.6	81.6	81.2

^a $C_i = 2 \pm 0.2$ μ M. Buffers do not contain any KCl. ^bData from reference 33. ^c2mM Tris-HCl buffers. ^d10mM Tris-HCl buffers. ^eThis value was mistyped in reference 33.

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Table S2. Validation set of experimentally measured melting temperatures for 39 DNA duplexes that were not used to determine new T_m magnesium correction equation 16.

Duplex DNA Sequence (5' to 3')	C_t (μM)	N_{bp}	Buffer 1			Buffer 2			
			[Mon ⁺] (M)	[Mg ²⁺] (mM)	Exp. T_m (°C)	[Mon ⁺] (M)	[Mg ²⁺] (mM)	Exp. T_m (°C)	T_m predicted using algorithm from Figure 9
GCCAGTTAA	8.0 ^b	9	0.417	0.0	36.8	0.010	1.0	29.5	28.6
	8.0 ^b	9	0.417	0.0	36.8	0.010	10.0	35.4	38.3
	8.0 ^b	9	0.417	0.0	36.8	0.010	100.0	39.4	39.6
	8.0 ^b	9	0.417	0.0	36.8	0.110	1.0	32.0	32.5
	8.0 ^b	9	0.417	0.0	36.8	0.110	10.0	35.4	38.6
	8.0 ^b	9	0.417	0.0	36.8	0.110	100.0	38.6	39.6
AAAAAAAAA	4.0 ^c	10	1.0	0.0	30.0	0.005	50.0	32.4	30.5
GTAGATCACT	7.3 ^d	10	0.515	0.0	43.0	0.010	50.0	45.7	44.7
GGGGAGGAGA	7.3 ^d	10	0.515	0.0	43.0	0.010	5.0	38.7	41.8
	3.0 ^e	11	0.155	0.0	50.0	0.155	10.0	54.5	54.2
TAGTCAATACT	10.0 ^f	12	1.0	0.0	50.0	0.110	10.0	47.0	47.2
TCCTCTCTCT	0.1 ^g	12	1.025	0.0	53.9	0.025	10.0	47.8	49.6
TTTCCCITCT	0.1 ^g	12	1.025	0.0	54.5	0.025	10.0	48.2	50.2
TTTTTTTTTTTT	2.0 ^h	14	0.815	0.0	48.0	0.113	20.0	46.0	45.8
CTGACGACAAGACT	2.0 ^a	14	1.0	0.0	60.5	0.055	1.5	51.8	52.2
	2.0 ^a	14	1.0	0.0	60.5	0.005	10.0	56.3	56.5

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Duplex DNA Sequence (5' to 3')	C_t (μM)	N_{bp}	Buffer 1			Buffer 2			
			[Mon ⁺] (M)	[Mg ²⁺] (mM)	Exp. T_m (°C)	[Mon ⁺] (M)	[Mg ²⁺] (mM)	Exp. T_m (°C)	T_m predicted using algorithm from Figure 9
GTAACCGGCATGAA	2.0 ^a	14	1.0	0.0	60.8	0.055	20.0	57.0	57.3
	2.0 ^a	14	1.0	0.0	60.8	0.005	20.0	57.0	57.5
CGCTGCTCACCTGA	2.0 ^a	14	1.0	0.0	66.1	0.055	3.0	59.6	60.0
	2.0 ^a	14	1.0	0.0	66.1	0.205	125.0	62.0	62.7
AAAAAAAAATATATAT	1.4 ⁱ	15	0.415	0.0	41.0	0.025	15.0	42.7	40.7
TTTTTTTGGTTTTTTT	8.0 ^j	15	0.110	0.0	37.0	0.010	20.0	49.0	46.6
AAAAGAAAAAGGGGGA	3.0 ^e	16	0.155	0.0	56.5	0.155	10.0	61.0	60.5
CTTCCTTAAAGGGCTT	3.0 ^e	16	0.155	0.0	56.5	0.155	10.0	61.5	60.5
AAGGGCTTCTTCCTTA	3.0 ^e	16	0.155	0.0	58.5	0.155	10.0	63.5	62.6
TCTCAATGGTGITACG	2.0 ^a	16	1.0	0.0	60.4	0.055	1.5	51.8	51.8
	2.0 ^a	16	1.0	0.0	60.4	0.005	0.5	49.0	48.9
TCTCAAACACCACACG	2.0 ^a	16	1.0	0.0	64.4	0.055	3.0	57.7	57.5
	2.0 ^a	16	1.0	0.0	64.4	0.205	10.0	60.0	61.0
TCTCAATGGTGTCACG	2.0 ^a	16	1.0	0.0	63.9	0.055	3.0	56.9	57.0
	2.0 ^a	16	1.0	0.0	67.5	0.055	0.5	57.4	56.7
CCTCTCTCGGCCTT	2.0 ^a	16	1.0	0.0	67.5	0.055	1.5	59.4	59.6
	2.0 ^a	16	1.0	0.0	67.5	0.055	20.0	64.1	63.3
GCCAGTTGGTAAAAACAT	2.0 ^a	17	1.0	0.0	62.5	0.055	1.5	53.7	53.7
	2.0 ^a	17	1.0	0.0	62.5	0.055	3.0	55.6	55.2

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Duplex DNA Sequence (5' to 3')	C_t (μM)	N_{bp}	Buffer 1			Buffer 2			
			[Mon ⁺] (M)	[Mg ²⁺] (mM)	Exp. T_m (°C)	[Mon ⁺] (M)	[Mg ²⁺] (mM)	Exp. T_m (°C)	T_m predicted using algorithm from Figure 9
GGTCAATGGCACTAGCTT	2.0 ^a	18	1.0	0.0	69.5	0.055	20.0	64.7	64.6
	2.0 ^a	18	1.0	0.0	69.5	0.105	1.5	61.2	62.0
ATCTCAGAAATACAGAACTA	2.0 ^a	20	1.0	0.0	64.5	0.055	20.0	59.4	59.3
	2.0 ^a	20	1.0	0.0	66.3	0.055	125.0	62.2	62.0
ATCTCGGAAATACGGAACTA	2.0 ^a	20	1.0	0.0	66.3	0.105	20.0	61.2	61.3
	2.0 ^a	20	1.0	0.0	69.1	0.055	20.0	63.7	63.8
TCGTCCGAGCTCCAGCACCG	2.0 ^a	20	1.0	0.0	79.1	0.055	1.5	70.7	71.0
	2.0 ^a	20	1.0	0.0	79.1	1.005	10.0	77.3	79.1
TAATTTAAAAATTTTAAAAAAA	2.0 ^k	21	0.110	0.0	40.0	0.010	2.0	46.8	43.7
TCGCGAAAGAAAGAAGAACGCT	3.0 ^l	23	0.510	0.0	75.0	0.160	10.0	72.2	71.4
AAAAAAAAAAAAATAAATTTTAAAAATTTT	2.0 ^k	25	0.110	0.0	46.0	0.010	2.0	50.8	49.8
TTTTTTTTTTTAAATAAATTTATAAAA	2.0 ^k	25	0.110	0.0	43.5	0.010	2.0	50.3	47.3
TTTTTTTTTTTTTTTTTTTTTTTTTTTT	2.0 ^h	26	0.815	0.0	62.0	0.113	20.0	58.0	57.0
TAGCGGACACGGCTGACGACCCCTGTG	2.0 ^a	27	1.0	0.0	86.0	0.055	1.5	77.7	77.5
	2.0 ^a	27	1.0	0.0	86.0	0.105	3.0	78.9	79.0
GCAATAGAAAAGAGGAAAATAATAGTTTTAT ATTCGACCTAG	2.0 ^a	40	1.0	0.0	75.4	0.055	0.5	62.2	63.5
	2.0 ^a	40	1.0	0.0	75.4	0.055	1.5	65.1	65.2
GCAATAGAAAAGAGGAAAATAATAGTTTTAT ATTCGACCTAG	2.0 ^a	40	1.0	0.0	75.4	0.055	20.0	69.7	68.0

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Duplex DNA Sequence (5' to 3')	C_t (μM)	N_{bp}	Buffer 1			Buffer 2			
			[Mon ⁺] (M)	[Mg ²⁺] (mM)	Exp. T_m (°C)	[Mon ⁺] (M)	[Mg ²⁺] (mM)	Exp. T_m (°C)	T_m predicted using algorithm from Figure 9
AGCTGACGCCAAAGTCCAAATCTAACCCACA TGCAAGACACG	2.0 ^a	40	1.0	0.0	84.5	0.055	0.5	72.8	73.7
	2.0 ^a	40	1.0	0.0	84.5	0.055	3.0	76.5	75.7
	2.0 ^a	40	1.0	0.0	84.5	0.055	20.0	78.3	76.9
GTCGCATCCCAGAGCCATGTGGTGACC CTGCGCCGCAC	2.0 ^a	40	0.621	0.0	90.1	0.055	1.5	82.0	82.4
	2.0 ^a	40	0.621	0.0	90.1	0.055	3.0	82.9	82.8
	2.0 ^a	40	0.621	0.0	90.1	0.055	20.0	83.9	83.2
GAATATACCAGAAGAATGGTTTGACAG GTTAATTAGAATATTTAGCTGATACAAT TG	2.0 ^a	60	1.0	0.0	79.6	0.055	0.5	66.7	67.8
	2.0 ^a	60	1.0	0.0	79.6	0.055	3.0	71.0	69.9
	2.0 ^a	60	1.0	0.0	79.6	0.055	20.0	73.3	71.5
TTCGGGATTAGCCCTACGCATCGGTTAC AAACGAGGACCTTATGCACCTTGACAGCA TG	2.0 ^a	60	1.0	0.0	88.4	0.055	1.5	79.2	78.7
	2.0 ^a	60	1.0	0.0	88.4	0.055	20.0	81.0	80.0
	2.0 ^a	60	1.0	0.0	89.1	0.055	0.5	77.8	78.8
CGGAGGGACAGCTAGTGCCCTGTGG GGAGTCGCTTATACAAAGCGGAGTGCAAT TTT	2.0 ^a	60	1.0	0.0	89.1	0.055	3.0	81.2	80.0
	2.0 ^a	60	0.220	0.0	87.7	0.055	0.5	82.0	83.3
	2.0 ^a	60	0.220	0.0	87.7	0.055	1.5	84.1	83.8
GGCACACCCATGCGGCTTCCAGGCTTGG ACGGACCTGCCCTTGGAAACCGGTCGGGAC GC	2.0 ^a	60	0.220	0.0	87.7	0.055	3.0	85.2	84.0

^aThis work. ^bNakano, S., Fujimoto, M., Hara, H., and Sugimoto, N. (1999) *Nucleic Acids Res.* 27, 2957-2965. ^cHudson,

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Differential scanning calorimetry of 21 duplexes

Concentrated solutions of DNA duplex oligomers were dialyzed against the magnesium buffer (1.5mM MgCl₂, 10mM Tris-HCl, pH = 8.3 at 25°C) or sodium buffers (33) for 48 hours and diluted to the same single strand concentrations ($C_t = 180\mu\text{M}$). Absorbance of 100× diluted calorimetric samples at 85°C before and after the calorimetric run was used to verify the concentrations. The samples were degassed for 17 minutes at 25 mm Hg vacuum system and injected into a Nano II Differential Scanning Calorimeter (Calorimetry Sciences Corporation, American Fork, Utah). Each DNA duplex was scanned up and down twice at heating rate of 1 °C/min to verify reproducibility of melting profiles. The collected data were analyzed using the CpCalc v2.1 package supplied by the manufacturer. Excess heat capacity curve, ΔC_p^{exc} , versus temperature was obtained by subtraction of the buffer versus buffer scan from the sample versus buffer scan. Reported DSC melting temperatures, T_m (DSC), are temperatures at peak height maxima on excess heat capacity vs. temperature curves. Integrated areas under these curves provided calorimetric transition enthalpies,

$$\Delta H_{\text{DSC}} = \int \Delta C_p^{\text{exc}} dT \quad (\text{S1})$$

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Table S3. Experimentally measured enthalpies using DSC for 21 DNA duplex oligomers ($C_t = 180 \mu\text{M}$) in magnesium and sodium buffers are compared with predictions using the unified nearest-neighbor model (30, 66).

DNA Sequence (5' to 3')	N_{bp}	f_{GC}	ΔH_{DSC} (kcal/mol) in buffer indicated					
			1.5 mM Mg^{2+} ,		69mM Na^+ ,		1M Na^+	
			Experiment	Prediction	Experiment	Prediction	Experiment	Prediction
ATCGTCTGGA	10	0.50	-75.9 ^b	ND ^a	ND ^a	-70.6 ^b	-70.5	
CGATCTGCGA	10	0.60	-75.3 ^b	ND ^a	ND ^a	-78.8 ^b	-76.7	
CTTTCATGTCGGCAT	15	0.47	-123.7	-118.7	-118.7	-107.9	-114.3	
GCAGTGGATGTGAGA	15	0.53	-121.6	-123.9	-123.9	-122.9	-113.6	
GGTCCTTACTTGGTG	15	0.53	-106.3	-115.2	-115.2	-124.7	-112.8	
CGCCTCATGCTCATC	15	0.60	-125.1	-118.3	-118.3	-119.8	-118.1	
ATGCAATGCTACATATTCCG	20	0.40	-180.5	-166.1	-166.1	-168.4	-155.0	
CCATCATTTGTGTACCTCA	20	0.45	-178.5	-169.7	-169.7	-170.0	-151.0	
CGGGACCAACTAAAGGAAAT	20	0.45	-178.4	-152.1	-152.1	-159.8	-151.4	
TAGTGGCGATTAGATTCTGC	20	0.45	-177.6	-164.2	-164.2	-170.1	-153.8	
AGCTGCAGTGGATGTGAGAA	20	0.50	-171.7	-162.2	-162.2	-166.6	-153.2	
CAGTGAGACAGCAATGGTCG	20	0.55	-177.0	-169.9	-169.9	-185.3	-159.0	
AATATCTCTCATGCGCCAAGCTACA	25	0.44	-214.5	-208.6	-208.6	-204.3	-193.4	
CTGGTCTGGATCTGAGAACTTCAGG	25	0.52	-240.1	-215.4	-215.4	-232.9	-193.6	
ACAGCGAATGGACCTACGTGGCCTT	25	0.56	-216.2	-210.2	-210.2	-210.6	-197.3	
GCGAGCCACAGGTTACTTGGCTGAT	25	0.56	-233.3	-229.1	-229.1	-230.8	-200.9	
AAAGGTGTCGGGAGAGTCGTGCTG	25	0.60	-223.9	-216.9	-216.9	-212.6	-203.9	
AACCTGCAACATGGAGTTTTTGTCTCATGC	30	0.43	-283.0	-274.9	-274.9	-302.0	-236.1	
GTTTACGTCCGAAAAGCTCGAAAAAGGATAC	30	0.47	-287.3	-258.3	-258.3	-265.0	-241.8	
AGTCTGGTCTGGA TCTGAGAACTTCAGGCT	30	0.50	-287.2	-280.3	-280.3	-295.4	-231.2	
ACGCCCACAGGATTAGGCTGGCCACATTG	30	0.60	-278.1	-265.5	-265.5	-253.4	-238.8	

^aND = Not determined. ^bThe 10 base pair long oligonucleotides had C_t of 400 μM .

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Table S4. Experimentally measured melting temperatures using DSC for 21 DNA duplex oligomers ($C_t = 180 \mu\text{M}$) in 1.5mM Mg^{2+} and 1M Na^+ buffers (33).

DNA Sequence (5' to 3')	N_{bp}	f_{GC}	DSC melting temperatures ($^{\circ}\text{C}$) in buffer indicated			
			1.5 mM Mg^{2+}			
			1M Na^+ , Experiment	Experiment	Prediction ^b	Error of predictions
ATCGTCTGGA	10	0.50	62.9 ^a	55.1 ^a	56.0 ^a	0.9
CGATCTGCCA	10	0.60	66.9 ^a	59.1 ^a	60.1 ^a	1.0
CTTTCATGTCGCCAT	15	0.47	74.8	66.1	66.4	0.3
GCAGTGGATGTGAGA	15	0.53	74.3	65.5	66.2	0.7
GGTCCTTACTTGGTG	15	0.53	71.5	63.5	63.5	0.0
CGCCTCATGCTCATC	15	0.60	76.8	68.5	68.8	0.3
ATGCAATGCTACATAATTCCG	20	0.40	78.1	68.6	68.9	0.3
CCATCATTTGTGTACCTCA	20	0.45	78.2	69.1	69.1	0.0
CGGGACCAACTAAAAGGAAAT	20	0.45	77.8	69.7	68.8	-0.9
TAGTGGCGATTAGATTCTGC	20	0.45	80.4	71.1	71.3	0.2
AGCTGCAGTGGATGTGAGAA	20	0.50	82.3	72.6	73.3	0.7
CAGTGAGACAGCAATGGTCG	20	0.55	82.1	73.2	73.2	0.0
AATATCTCTCATGCCCAAGCTACA	25	0.44	84.9	75.2	75.3	0.1
CTGGTCTGGATCTGAGAACTTCAGG	25	0.52	84.1	75.1	74.7	-0.4
ACAGCGAATGGACCTACGTGGCCCTT	25	0.56	89.1	80.0	79.6	-0.4
GCGAGCGACAGGTTACTTGGCTGAT	25	0.56	88.0	79.2	78.6	-0.6
AAAGGTGTCCGGAGAGTCGTGCTG	25	0.60	90.8	81.4	81.4	0.0
AACCTGCAACATGGAGTTTTTGTCTCATGC	30	0.43	85.9	76.5	76.0	-0.5
GTTACGTCGAAAAGCTCGAAAAAGGATAC	30	0.47	86.6	77.3	76.8	-0.5
AGTCTGGTCTGGATCTGAGAACTTCAGGCT	30	0.50	87.6	78.2	77.8	-0.4
ACGCCACAGGATTAGGCTGGCCCCACATTG	30	0.60	91.6	82.8	82.0	-0.8

^aThe 10 base pair long oligonucleotides had C_t of 400 μM . ^bMelting temperatures were predicted using the algorithm on Figure 9 and T_m values in 1M Na^+ .

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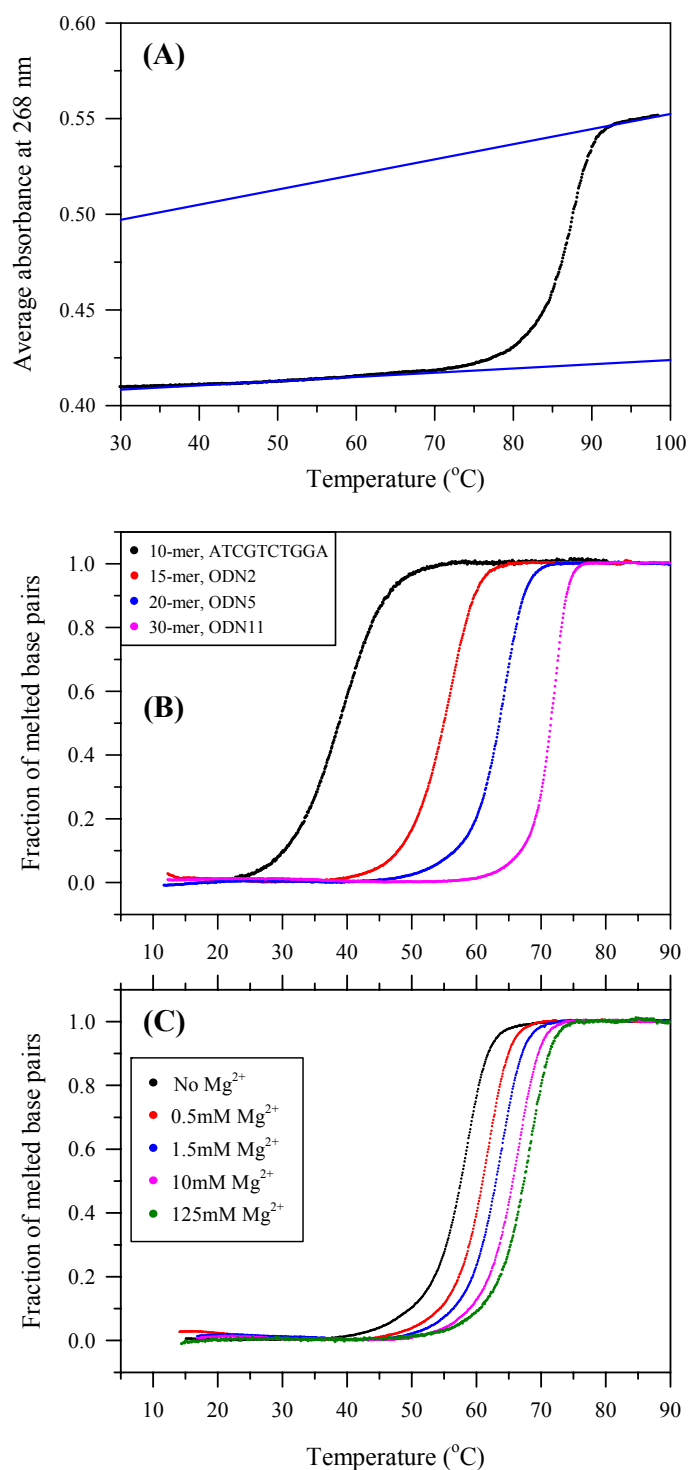


Figure S1. (A) This graph shows lower and upper linear baselines (blue lines) for the ODN12 duplex (black circles) in a buffer of 10mM Tris-HCl and 1M KCl (pH = 8.3 at 25°C). Total single strand concentration was 2 μ M. Although the duplex exhibits a T_m of 86.7°C, it is clearly possible to establish both upper and lower linear sloping baselines from the data. The melting

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transition is narrow for 20-30 base pair duplexes and establishing baselines presents no challenge. The lower and upper baselines were fitted to the melting data in temperature ranges of 30-69.5 and 93.4-98.0 °C, respectively. (B) UV melting profiles of selected duplex DNA oligomers in 1.5mM MgCl₂, 2mM Tris-HCl buffer. Oligomer length is increasing from left to right. Oligonucleotide sequences are listed in Table 4. (C) UV melting profiles of 20 bp long ODN5 duplex in buffers containing 50mM KCl, 10mM Tris-HCl and 0-125mM Mg²⁺.

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DNA structure in sodium and magnesium buffers was investigated by circular dichroism spectroscopy

CD experiments were performed on a Jasco J-810 circular dichroism spectrophotometer equipped with a thermostated 6-cell Peltier holder using a 1 cm pathlength cuvette. Spectra were recorded at 20 °C using 4 seconds response time, 1.0 nm bandwidth, and a scan rate of 20nm/minute. Duplexes were diluted to a total single strand concentration of 4.5 μ M. Five accumulations of spectra were averaged, and the buffer baseline was subtracted from the sample spectra. Analysis was carried out with Spectra Manager software (Jasco, Easton, MD). CD spectra of 12 duplex DNAs were collected in 1M Na⁺ buffer, and in 10mM Tris-HCl buffers with magnesium concentrations from 1.5 to 600mM and with no Na⁺ or K⁺ present.

Representative spectra are displayed in Figure S2 below. Positive peaks at ~275 nm and negative valleys at ~250 nm are indicative of B-DNA conformations. The CD spectrum in magnesium buffers up to 125mM Mg²⁺ concentration is similar to the spectra in 1M Na⁺ buffer. However, the CD spectrum in 600mM Mg²⁺ is different from the spectra in lower magnesium concentrations. The intensity of the band at 275 nm is smaller in 600mM Mg²⁺. This observation suggests that DNA duplexes exhibit subtle changes of secondary structure at very high magnesium concentrations. Interestingly, a similar change of CD spectrum is observed when DNA is dehydrated with 60% ethanol (Gray, D. M., et al. (1992) *Met. Enzym.* 211, 389-406).

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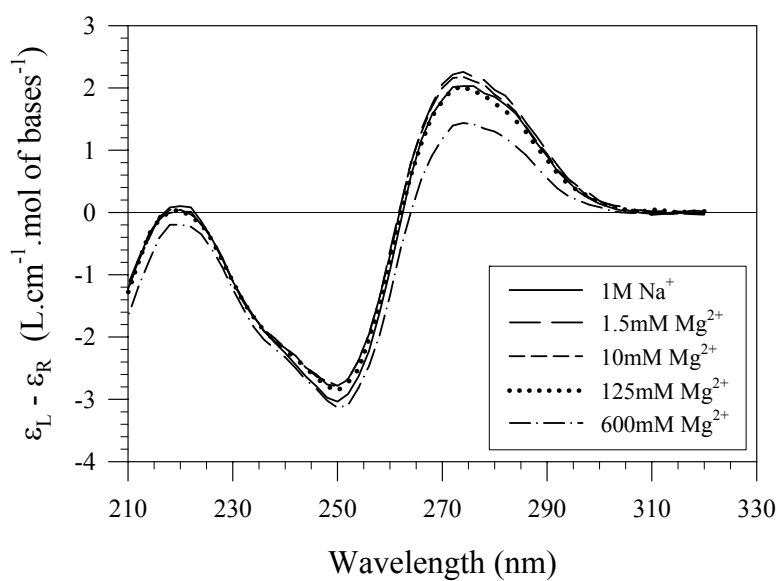


Figure S2. Circular dichroism spectra of the 30 base pair duplex, 5'-TCAGTAGGCGTG-ACGCAGAGCTGGCGATGG-3', in 1M NaCl-phosphate buffer (pH 7) and in buffers containing 10mM Tris-HCl (pH 8.3) and various MgCl₂ concentrations (1.5-600mM).

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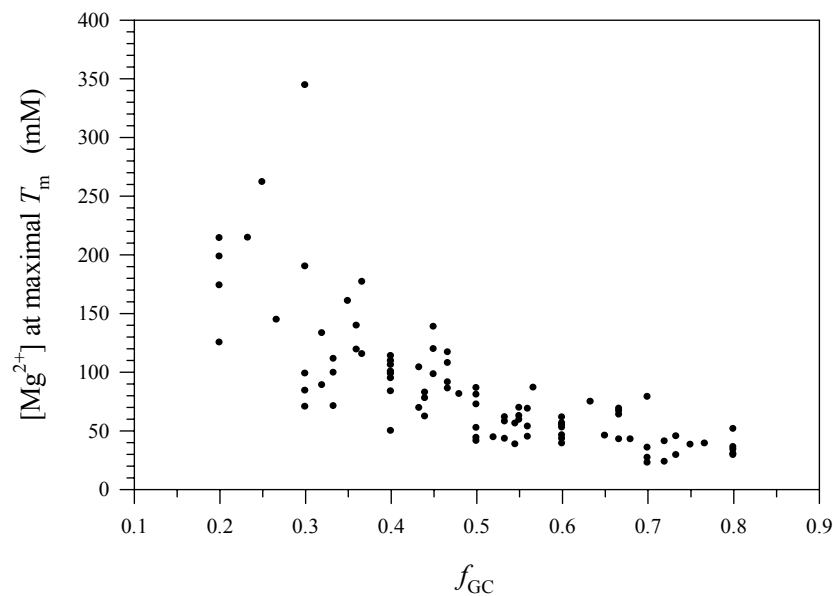


Figure S3. Magnesium concentrations where melting temperatures reach maxima were obtained from quadratic fits of experimental melting temperatures. Buffers did not contain KCl. Tris ions were present at low concentrations and had negligible effects on T_m .

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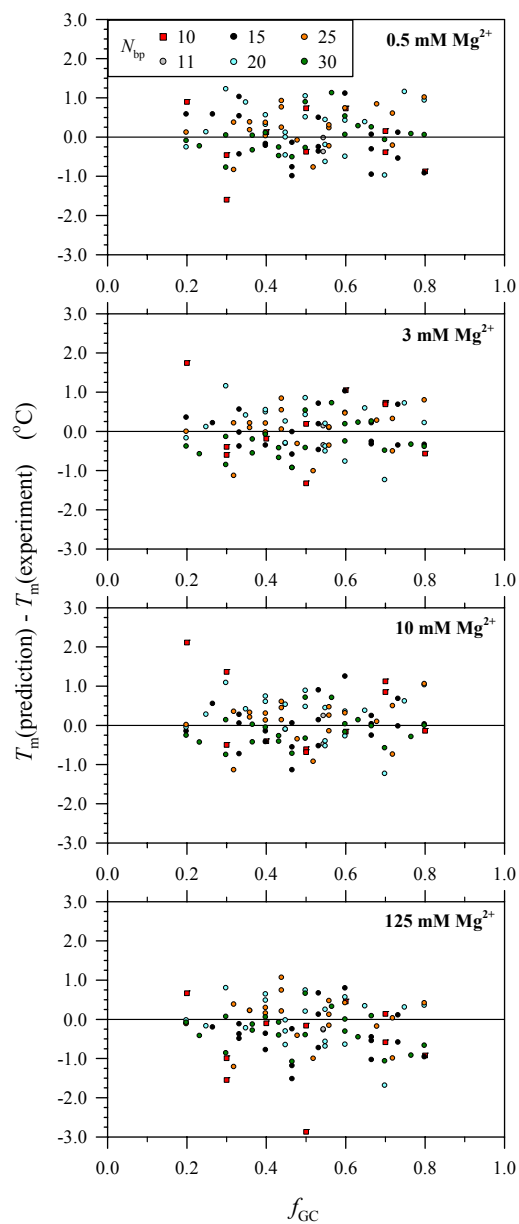


Figure S4. Errors of T_m predictions calculated from the new T_m magnesium correction, equation 16, in 0.5, 3, 10, 125mM Mg^{2+} buffers. Inset shows colored symbols used for oligonucleotides of various lengths.

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Nearest-neighbor model of T_m magnesium correction

Equation 16 can be expanded to include sequence specific nearest-neighbor parameters. Since $f_{GC} + f_{AT}$ is equal to 1, we can rearrange four terms of equation 16,

$$a + b \ln[\text{Mg}^{2+}] + f_{GC} \times (c + d \ln[\text{Mg}^{2+}]) = f_{AT} a + f_{GC} (c + a) + \{f_{AT} b + f_{GC} (b + d)\} \times \ln[\text{Mg}^{2+}] \quad (\text{S2})$$

Equation S2 shows that the parameters a, b, c, d determine dependence of T_m correction on the oligonucleotide sequence. Therefore, their terms can be expressed in the nearest-neighbor fashion,

$$\frac{1}{T_m(\text{Mg}^{2+})} - \frac{1}{T_m(1\text{M Na}^+)} = \sum_{i,j=A,T,C,G,E} A_{ij}^{n-n} f_{ij} + \sum_{i,j=A,T,C,G,E} B_{ij}^{n-n} f_{ij} \ln[\text{Mg}^{2+}] + \frac{1}{2(N_{bp} - 1)} \times [E + F \ln[\text{Mg}^{2+}] + G(\ln[\text{Mg}^{2+}])^2] \quad (\text{S3})$$

where sums are calculated over 10 internal and 2 end nearest-neighbor doublets (44). The A_{ij}^{n-n} and B_{ij}^{n-n} are sequence dependent nearest-neighbor parameters, and E, F, G are additional parameters for end-effects of magnesium ions. The fraction of each nearest neighbor is defined as,

$$f_{ij} = \frac{N_{ij}}{\sum_{i,j=A,T,C,G,E} N_{ij}} = \frac{N_{ij}}{N_{bp} + 1} \quad (\text{S4})$$

The N_{ij} is the number of times the particular nearest-neighbor doublet (e.g., AA/TT, AC/GT, CG/CG, EA/TE) appears in the DNA duplex. The ‘E’ denotes the ends. Using the data set in Table 1 and S1, we constructed a system of 680 linear equations, which can be solved for 27 unknown parameters ($A_{ij}^{n-n}, B_{ij}^{n-n}, E, F,$ and G). The solution was obtained by minimizing the value of χ^2 ,

$$\chi^2 = |(\mathbf{F} \times \mathbf{P}^{n-n} - \mathbf{D}) \times \boldsymbol{\sigma}_D^{-1}|^2 \quad (\text{S5})$$

where \mathbf{D} is the column vector of $1/T_m$ differences shown on left side of equation S3 and $\boldsymbol{\sigma}_D$ is the diagonal matrix whose elements are experimental errors of \mathbf{D} . The \mathbf{F} is the 680 X 27 design matrix (37). Each row of \mathbf{F} comprises the elements for the particular DNA duplex in the specific

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magnesium concentration and each column of \mathbf{F} contains the elements for the particular parameter. The $\mathbf{P}^{n \times n}$ is the column vector of 27 unknown parameters to be solved for. A unique solution for $\mathbf{P}^{n \times n}$ is achieved only if matrix \mathbf{F} is not rank deficient, i.e., it has no singular values. That was verified to be the case in our analysis. The system of linear equations S5 was solved using singular value decomposition (37), which was implemented using Excel Add-in, Matrix.xla package, version 2.3 (Foxes Team, Leonardo Volpi, <http://digilander.libero.it/foxes>).

Resulting parameters are presented in Table S5 below and they were used to scale melting temperatures from 1M Na⁺ buffer to buffers of various magnesium concentrations. When measured melting temperatures in Tables 1 and S1 were compared with values predicted using the nearest-neighbor model (equation S3), values of $\chi_r^2 = 3.7$ and $|\langle T_m \rangle|_{\text{AVE}} = 0.5$ °C were obtained. Because the original T_m correction (equation 16) shows the similar accuracy ($\chi_r^2 = 4.6$ and $|\langle T_m \rangle|_{\text{AVE}} = 0.5$ °C, see Table 3), the incorporation of nearest-neighbor parameters did not significantly improve accuracy of predictions. Although the relationship between magnesium concentration and the DNA melting temperature is sequence dependent, this dependence seems to be sufficiently modeled by a simple relationship with f_{GC} term rather than a more complex equation S3.

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Table S5. The nearest-neighbor T_m correction parameters for magnesium ions were determined using singular value decomposition. Extra significant figures are presented to prevent accumulation of rounding errors in calculations.

Nearest-neighbor sequence (5' to 3')	A_{ij}^{n-n} (K ⁻¹)	B_{ij}^{n-n} (K ⁻¹)
AA/TT	1.22 x 10 ⁻⁵	-1.18 x 10 ⁻⁵
GG/CC	8.52 x 10 ⁻⁵	2.62 x 10 ⁻⁶
AT/AT	5.82 x 10 ⁻⁵	5.50 x 10 ⁻⁷
TA/TA	3.21 x 10 ⁻⁵	-1.85 x 10 ⁻⁵
AC/GT	2.33 x 10 ⁻⁵	-1.13 x 10 ⁻⁶
CA/TG	1.05 x 10 ⁻⁴	-1.86 x 10 ⁻⁶
AG/CT	6.01 x 10 ⁻⁵	1.67 x 10 ⁻⁶
GA/TC	6.41 x 10 ⁻⁵	-6.21 x 10 ⁻⁶
GC/GC	8.69 x 10 ⁻⁵	2.40 x 10 ⁻⁶
CG/CG	1.25 x 10 ⁻⁴	8.59 x 10 ⁻⁶
ET/AE and EA/TE	6.93 x 10 ⁻⁴	3.88 x 10 ⁻⁵
EC/GE and EG/CE	7.18 x 10 ⁻⁴	4.81 x 10 ⁻⁵

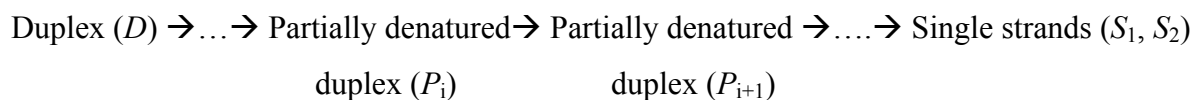
The parameters E , F , G were determined to be $-2.53 \times 10^{-3} \text{ K}^{-1}$, $3.72 \times 10^{-4} \text{ K}^{-1}$, and $8.27 \times 10^{-5} \text{ K}^{-1}$, respectively.

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Analysis of Δn calculation for duplexes that may show deviations from the two-state melting behavior

Unlike the new T_m magnesium correction functions, calculation of released magnesium ions upon duplex denaturation (equation 21) is dependent on the two-state assumption. The two-state approximation means that partially melted duplexes are not present to any significant degree during melting transition. Duplexes that are 10-15 base pairs long are likely to melt in the two-state manner. As length of the duplexes increase, increasing deviations from the two-state behavior have been observed. The exact point where these deviations are considered significant has not been well established in the published literature. However, it is agreed that the transition is not two-state when the melting profile shows more than one transition. In our experiments, all melting profiles exhibited a single, S-shaped transition. No second transition was observed on any melting profile. This is not surprising, since the sequences were designed to minimize the likelihood of this event.

To demonstrate that the calculations of the net released magnesium ions are valid even for duplexes that may show deviations from the two-state approximation, we provide the following analysis. We take into account the possibility of partially bonded strands. Let us assume that there are m partially melted states on the pathway from a duplex to single strands,



The total net released magnesium ions when the duplex melts into the complementary single strands depends solely on the initial and final states. It is the initial and final oligonucleotide structures (or more accurately distributions of structures) that determine association of ions. In other words, association of ions is the state variable. The total of released magnesium ions is therefore the sum of released ions for each melting transition,

$$\Delta n(\text{total}) = \Delta n_{D-P_1} + \dots + \Delta n_{P_i-P_{i+1}} + \dots + \Delta n_{P_m-D} \quad (\text{S6})$$

where $\Delta n_{P_i-P_{i+1}}$ is the number of released ions for duplex transition from the partially melted state P_i to state P_{i+1} , etc. In the scheme above, each reaction between the consecutive states can be

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described using the two-state model. Substitution of each Δn term of equation S6 by equation 21 yields,

$$\Delta n_{\text{Total}} = \frac{d(1/T_t^{D-P_1})}{d \ln [\text{Mg}^{2+}]} \frac{\Delta H_{D-P_1}}{R} + \dots + \frac{d(1/T_t^{P_i-P_{i+1}})}{d \ln [\text{Mg}^{2+}]} \frac{\Delta H_{P_i-P_{i+1}}}{R} + \dots + \frac{d(1/T_t^{P_m-D})}{d \ln [\text{Mg}^{2+}]} \frac{\Delta H_{P_m-D}}{R} \quad (\text{S7})$$

where $T_t^{P_i-P_{i+1}}$ and $\Delta H_{P_i-P_{i+1}}$ is the temperature at midpoint of transition from state P_i to P_{i+1} and the transition enthalpy between states P_i and P_{i+1} , respectively. To calculate derivatives $d(1/T_t)/d \ln[\text{Mg}^{2+}]$, we need to know for each transition the dependence of the transition temperature T_t on magnesium concentration. We turn now to experimental data. Obviously, T_t temperatures must be distributed within the overall melting transition that was experimentally measured. Since the value $d(1/T_t)/d \ln[\text{Mg}^{2+}]$ will depend on oligomer length and GC content according to equation 16, different T_t 's will likely have different dependence on the counterion concentration. This was previously observed by Blake and Delcourt for non-two-state melting transitions of inserts in recombinant plasmids with Na^+ as a counterion (Blake, R. D., and Delcourt, S. G. (1998) *Nucleic Acids Res.* 26, 3323-3332). Their figure 6 shows that differences between T_t values of various melting domains decrease as Na^+ concentrations is increased and the shape of the melting profile changes substantially with increasing counterion concentration.

We now examine the behavior of the short duplexes (10-15 base pairs), which are likely to melt in the two-state fashion, as well as behavior of longer duplexes, which are likely to show deviations from the two-state model. We start by plotting melting profiles for 10, 20 and 30 base pair oligonucleotides as a function of magnesium concentration (Figures S5, S6, S7, S8 and S9 below). All these sequences were used to calculate Δn values in Figure 10B. Figures S5-S9 shown below demonstrate that no significant change in the shape of melting profiles is observed as magnesium concentration is increased from 0.5mM to 125mM even for 30 base pair long duplexes. We can read transition temperatures $T_{10\%}$, $T_{20\%}$, $T_{35\%}$, $T_{50\%}$, $T_{65\%}$, $T_{80\%}$, $T_{90\%}$ at data points where fraction of melted base pairs is 10, 20, 35, 50, 65, 80, 90%, respectively. All of these transition temperatures shows a similar dependence on $\ln [\text{Mg}^{2+}]$ and their derivatives $d(1/T)/d \ln[\text{Mg}^{2+}]$ at 1.5 mM Mg^{2+} are identical within the experimental error ($\pm 12\%$). This result supports two interpretations:

- (1) Melting transitions of duplexes are two-state and the equation 21 for net release of magnesium ions is valid.

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(2) More likely, the partially melted duplexes have similar dependence of melting temperatures on magnesium concentration. This is reasonable when partially melted duplexes differ from the perfectly annealed duplex by two or less melted base pairs. Their $d(1/T_t)/d \ln[\text{Mg}^{2+}]$ values will be similar to $d(1/T_m)/d \ln[\text{Mg}^{2+}]$ values of perfectly annealed duplex. For example, equation 16 predicts that the value of $d(1/T_t)/d \ln[\text{Mg}^{2+}]$ between 20 base pair duplex and partially melted 19 base pair duplex of the same sequence will differ less than 6%. Therefore, the equation S7 can be written as,

$$\Delta n_{\text{Total}} = \frac{d(1/T_m)}{d \ln[\text{Mg}^{2+}]} \frac{1}{R} \times [(\Delta H_{D-P1} + \dots + \Delta H_{P_i-P_{i+1}} + \dots + \Delta H_{P_m-D})] \quad (\text{S8})$$

Since total transition enthalpy is determined by the initial and the final states and it is independent of reaction pathway, $\Delta H_{\text{Total}} = [(\Delta H_{D-P1} + \dots + \Delta H_{P_i-P_{i+1}} + \dots + \Delta H_{P_m-D})]$ and equation S7 will become the equation 21,

$$\Delta n_{\text{Total}} = \frac{d(1/T_m)}{d \ln[\text{Mg}^{2+}]} \frac{\Delta H_{\text{Total}}}{R}$$

This analysis shows that equation 21 is valid for duplexes from our dataset that are up to 30 base pairs long. Equation 21 can be used to calculate net released magnesium ions as long as the shape of the melting profile does not depend significantly on magnesium concentrations.

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10-mer, 50%GC, 5'-ATCGTCTGGA-3'

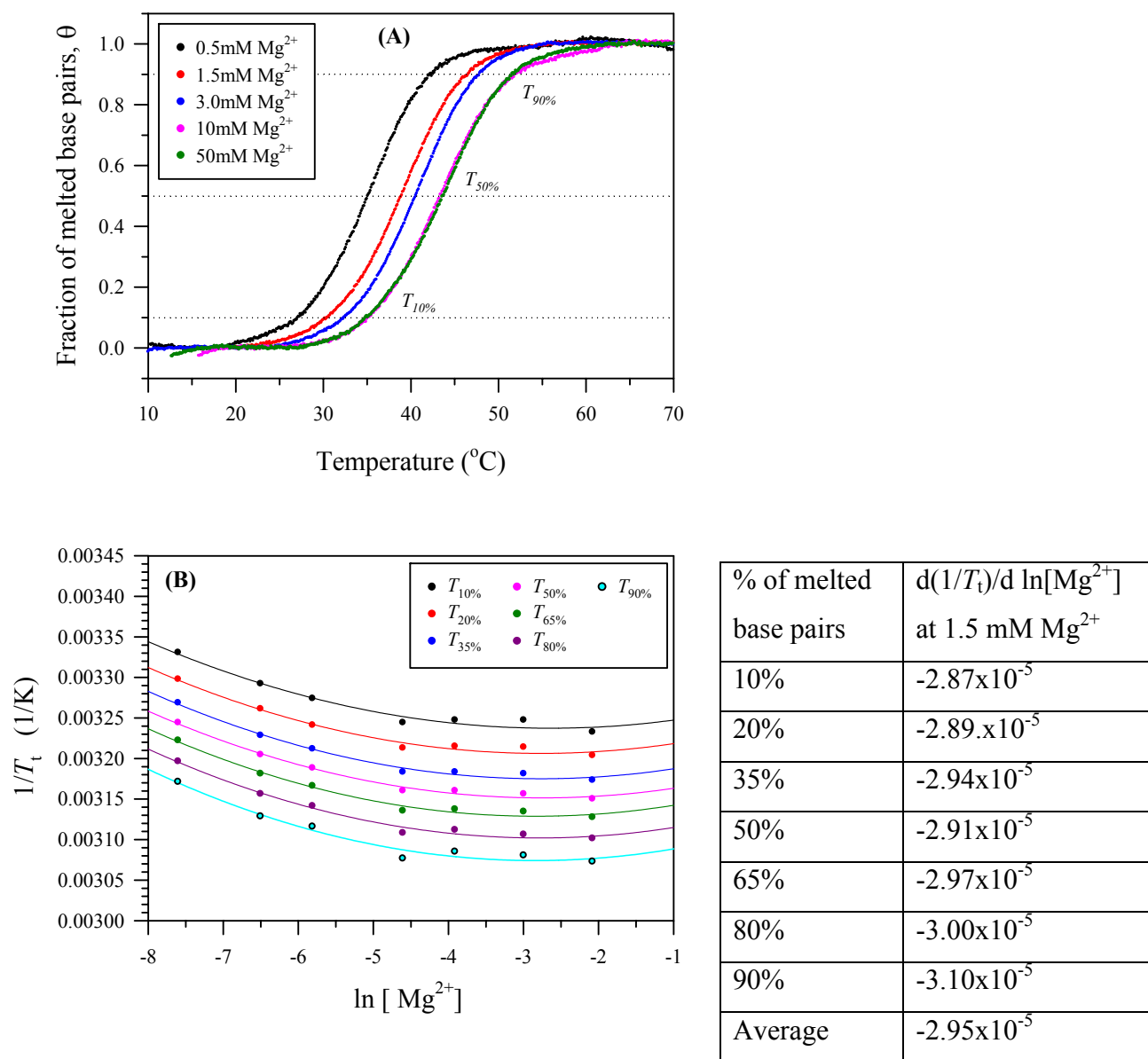


Figure S5. Analysis of the duplex, 5'-ATCGTCTGGA-3'. (A) Melting profiles in buffers of various magnesium concentrations and 2mM Tris-HCl. (B) Transition temperatures read when 10, 20, 35, 50, 65, 80, 90% of base pairs were melted are plotted as a function of magnesium concentrations.

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20-mer, 50%GC, 5'-AGCTGCAGTGGATGTGAGAA-3'

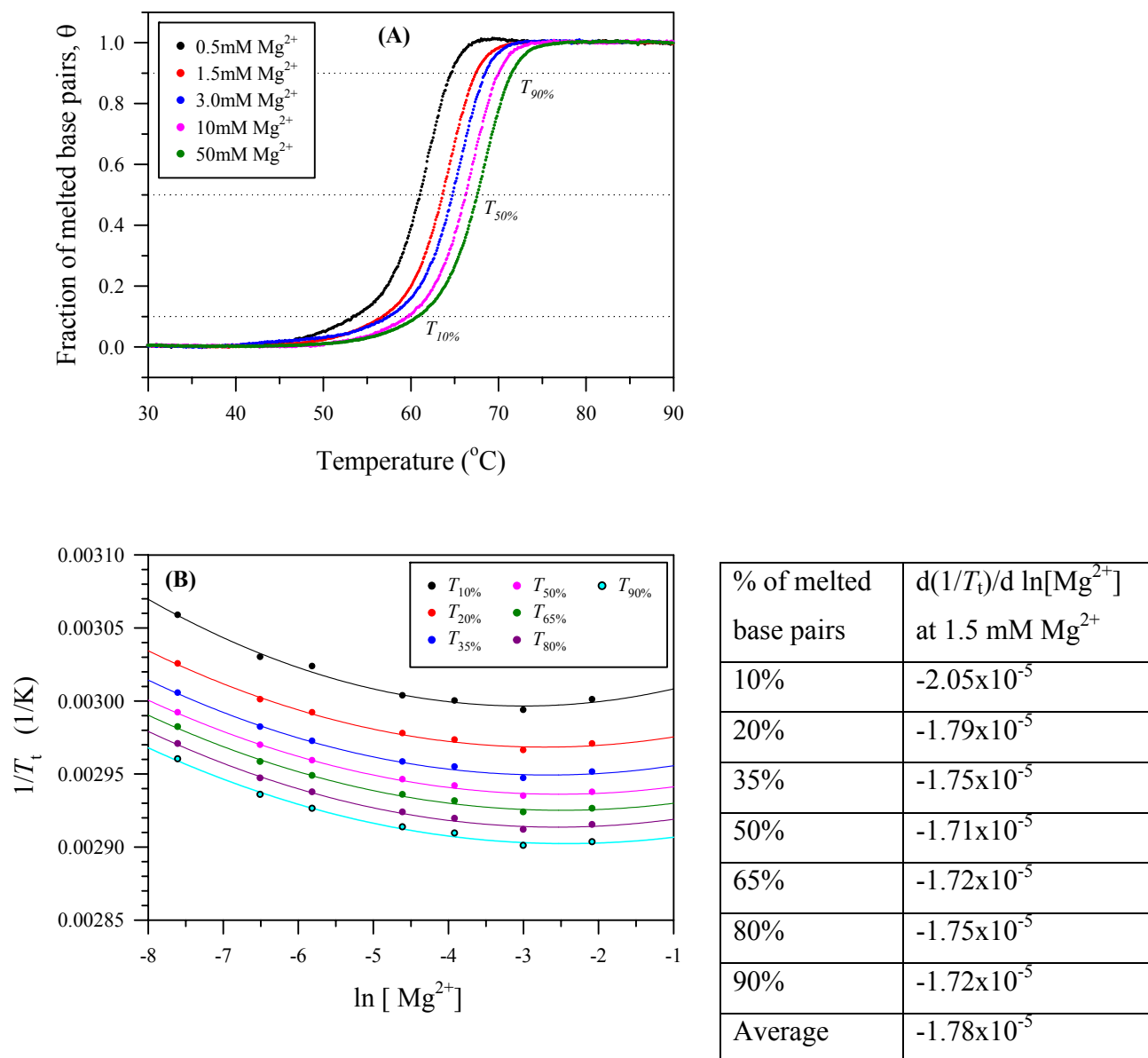


Figure S6. Analysis of ODN5 duplex, 5'-AGCTGCAGTGGATGTGAGAA-3'. (A) Melting profiles in buffers of various magnesium concentrations and 2mM Tris-HCl. (B) Transition temperatures read when 10, 20, 35, 50, 65, 80, 90% of base pairs were melted are plotted as a function of magnesium concentrations.

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30-mer, 43%GC, 5'-AACCTGCAACATGGAGTTTTTGTCTCATGC-3'

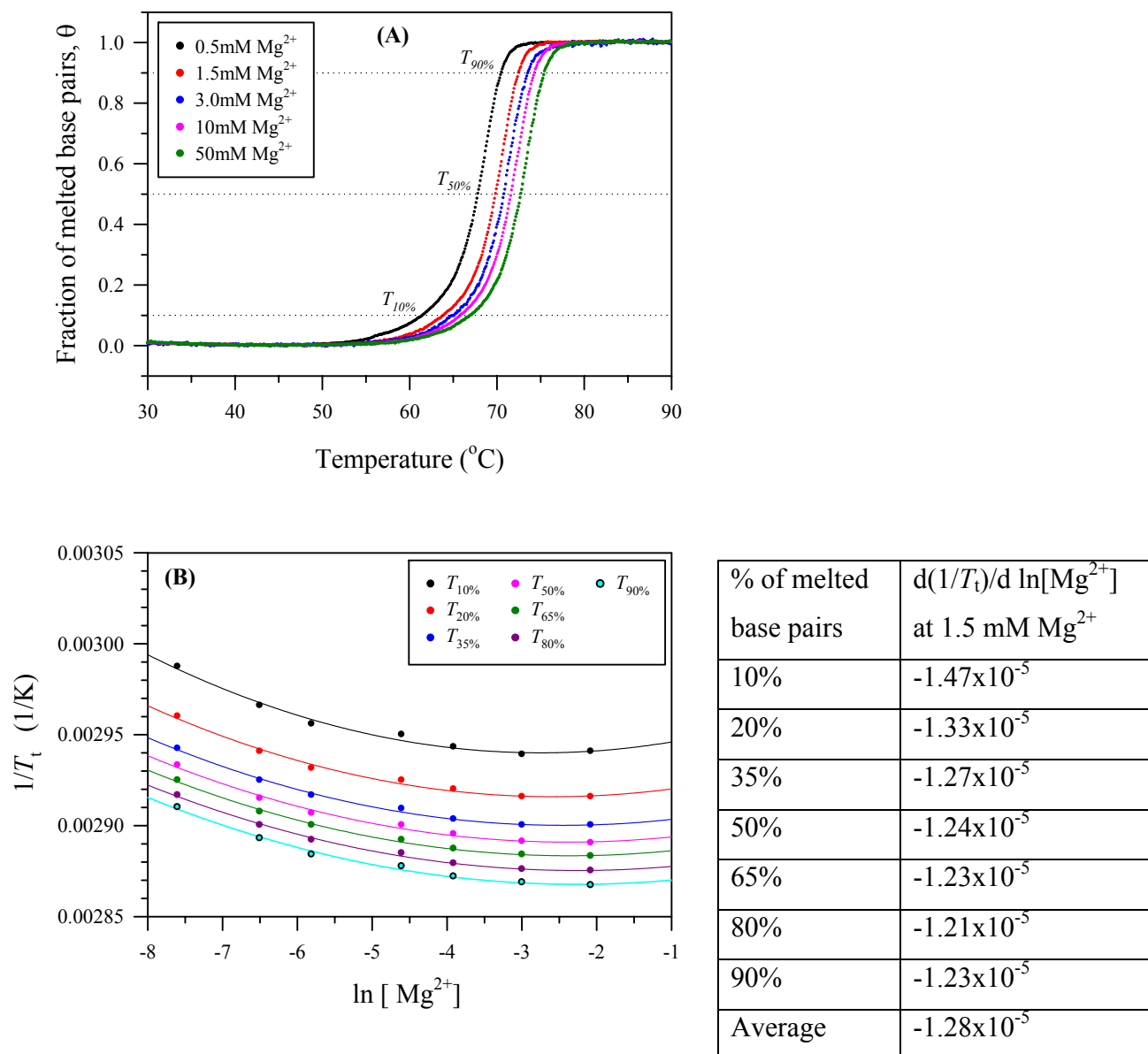


Figure S7. Analysis of the duplex, 5'- AACCTGCAACATGGAGTTTTTGTCTCATGC -3'.

(A) Melting profiles in buffers of various magnesium concentrations and 2mM Tris-HCl.

(B) Transition temperatures read when 10, 20, 35, 50, 65, 80, 90% of base pairs were melted are plotted as a function of magnesium concentrations.

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30-mer, 47%GC, 5'-GTTTCACGTCCGAAAGCTCGAAAAAGGATAC-3'

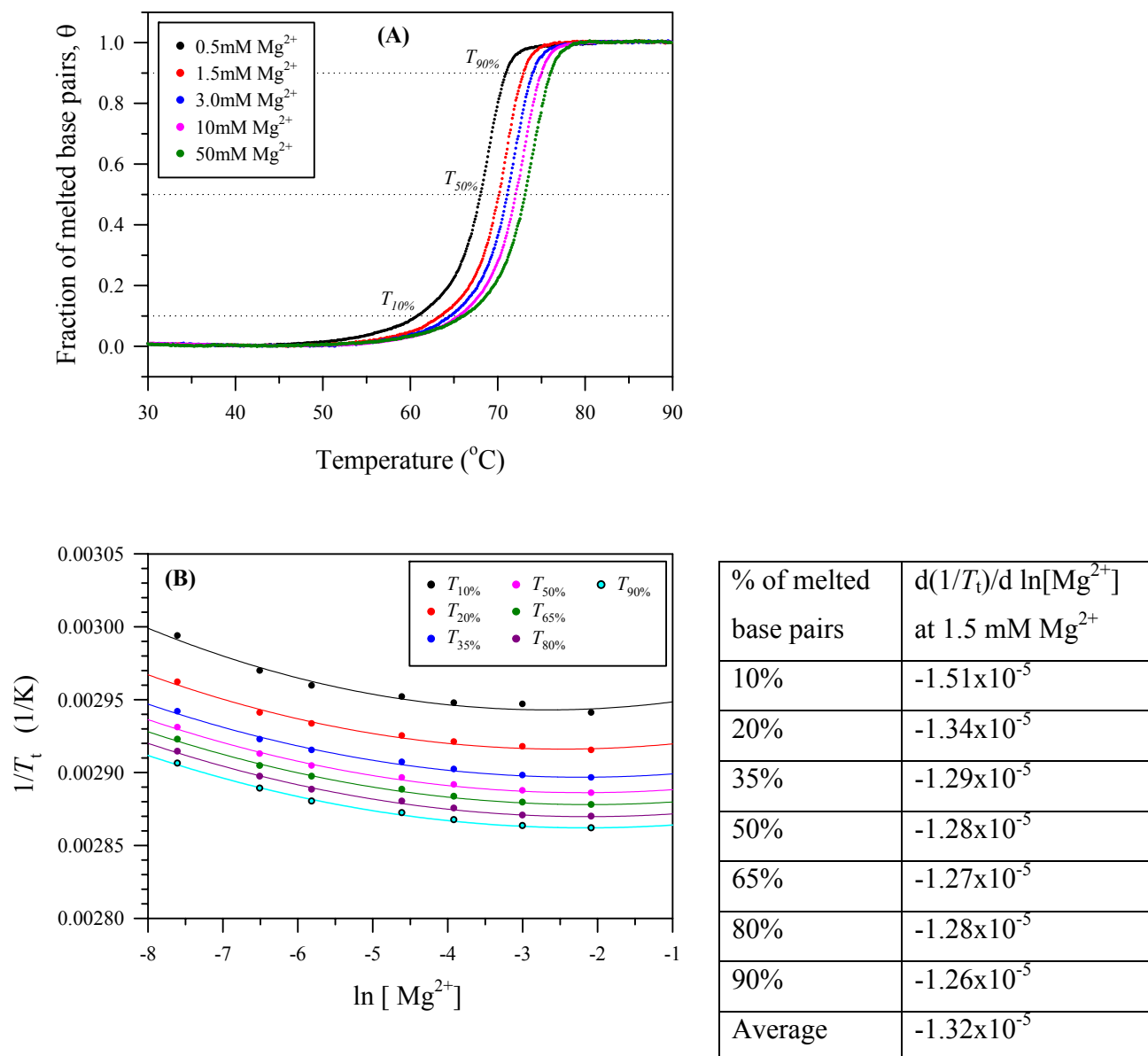


Figure S8. Analysis of duplex, 5'-GTTTCACGTCCGAAAGCTCGAAAAAGGATAC-3'. (A) Melting profiles in buffers of various magnesium concentrations and 2mM Tris-HCl. (B) Transition temperatures read when 10, 20, 35, 50, 65, 80, 90% of base pairs were melted are plotted as a function of magnesium concentrations.

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30-mer, 60%GC, 5'-ACGCCACAGGATTAGGCTGGCCCACATTG-3'

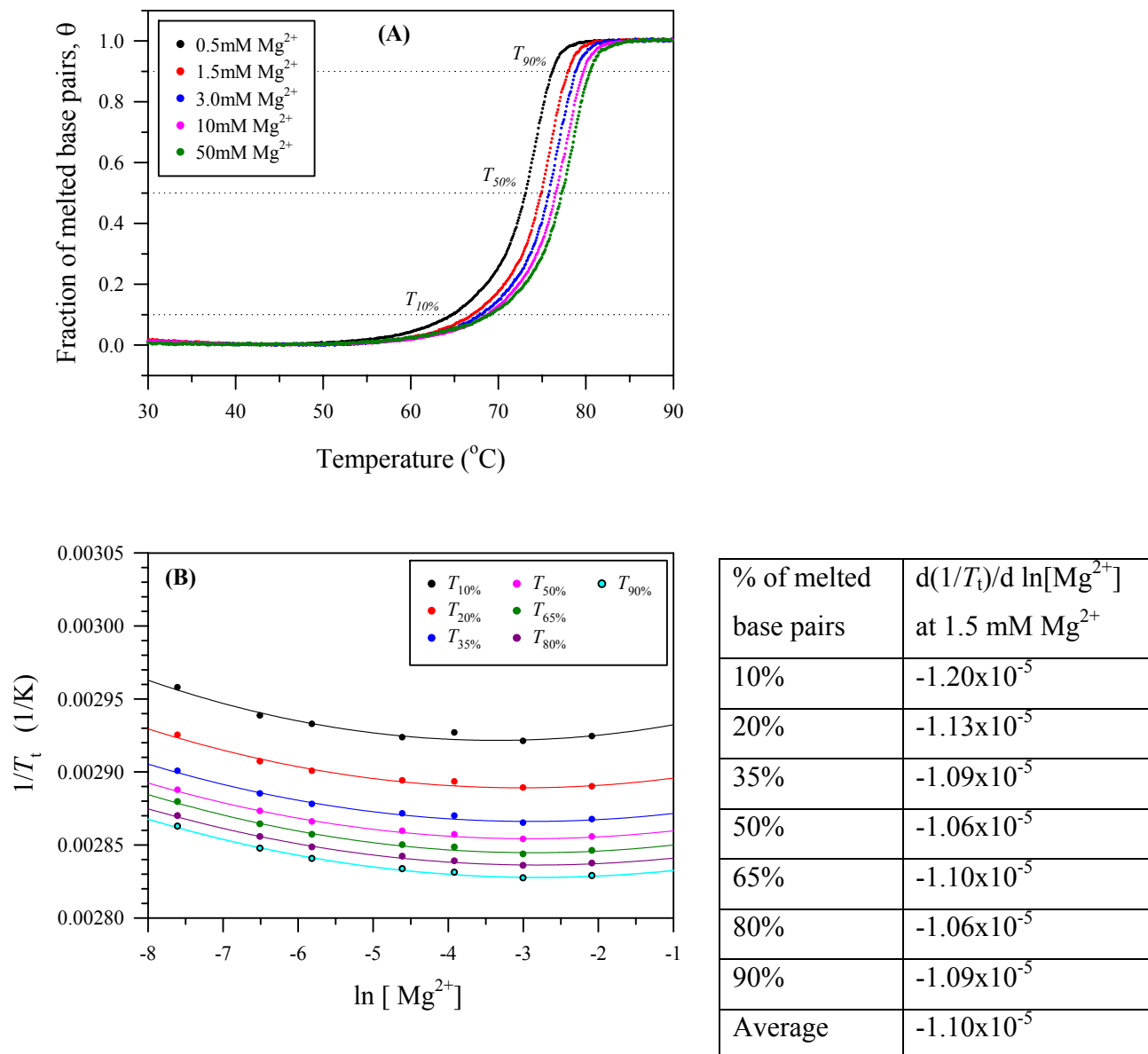


Figure S9. Analysis of duplex, 5'-ACGCCACAGGATTAGGCTGGCCCACATTG-3'. (A) Melting profiles in buffers of various magnesium concentrations and 2mM Tris-HCl. (B) Transition temperatures read when 10, 20, 35, 50, 65, 80, 90% of base pairs were melted are plotted as a function of magnesium concentrations.