Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Loop nucleotides impact stability of intrastrand i-motif structures at neutral pH

Taiga Fujii[1], Naoki Sugimoto[1,2]*

[1]Frontier Institute for Biomolecular Engineering Research (FIBER), Konan University, 8–9–1 Minatojima-minamimachi, Chuo-ku, Kobe 650–0047 (Japan)

[2]Graduate School of Frontiers of Innovative Research in Science and Technology (FIRST), Konan University, 8–9–1 Minatojima-minamimachi, Chuo-ku, Kobe 650–0047 (Japan)

Contents

Electronic Supplementary Information

Experimental Sections

Materials. All oligodeoxynucleotides used in this study were high-performance liquid chromatograph grade (Japan Bio Service). Single-strand concentrations of DNA oligonucleotides were determined by measuring the absorbance at 260 nm at 80 °C and using single-strand extinction coefficients calculated from the mononucleotide and dinucleotide data according to the nearest-neighbor approximation model.[1] The absorbance was measured using a Shimadzu 1800 spectrophotometer connected to a thermoprogrammer.

Thermodynamic analysis. Ultraviolet (UV) absorbance was measured on a Shimadzu 1800 spectrophotometer equipped with a temperature controller. Absorbance was measured at 260 nm in in solution containing 10 mM Na_2HPO_4 (pH 6.5), 0.1 mM Na_2EDTA. Samples were heated at a rate of 0.5 °C min^{-1} and curves were the same within experimental error at heating rates between 0.2 °C and 0.5 °C min^{-1} (data not shown). Before the measurements, the DNA samples were heated to 90 °C, cooled to 0 °C at a rate of –1 °C min^{-1}, and incubated at 0 °C for 30 min. All melting curves were fitted to a theoretical equation to obtain thermodynamic parameters for structure formation ($\Delta H°$, $\Delta S°$, and $\Delta G°_{25}$) as described elsewhere.[2] Melting curves were measured for at least three concentrations of DNA strand (from 1.0 μM to 50 μM). Thermodynamic parameters listed in Table 1 and Table 2 are the average values obtained from the curve fitting.

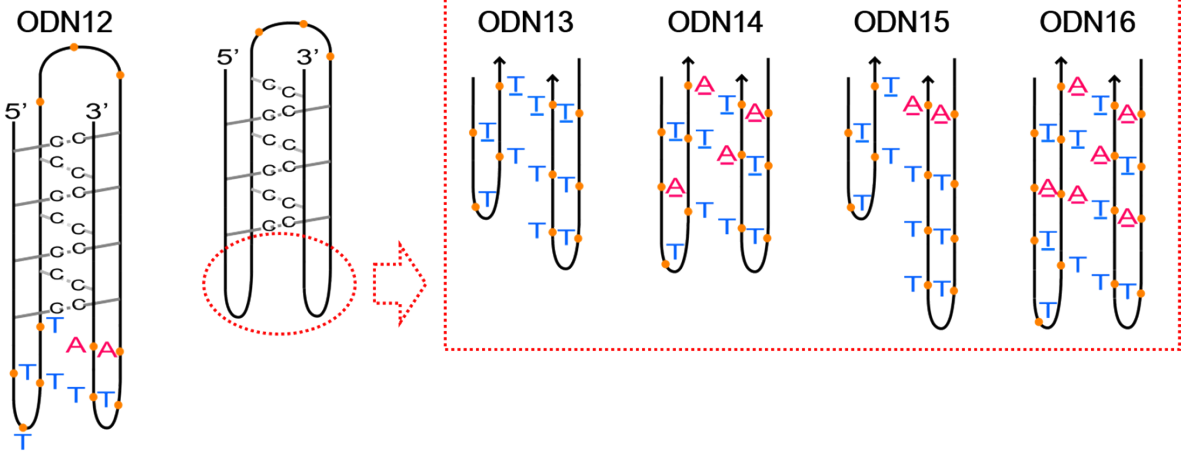
Circular dichroism (CD) measurements. CD measurements were made on JASCO J-820 and J-1500 spectropolarimeters at 20 μM total DNA strand concentration in buffers containing 10 mM Na_2HPO_4 (pH 5.0-8.5), 0.1 mM Na_2EDTA. The spectra at 0-80 °C were obtained by taking at least three scans from 200 to 400 nm in a cuvette with a pathlength of 0.1 cm. The temperature of the cell holder was regulated by a JASCO PTC-424L temperature controller, and the cuvette-holding chamber was flushed with a constant stream of dry N_2 gas to avoid condensation of water on the cuvette exterior. Before the measurement, the sample was heated to 90 °C, cooled at a rate of –1 °C min^{-1}, and incubated at 4 °C for 30 min.

1. E. G. Richards, *In Fasman, G. D. (ed.), Handbook of Biochemistry and molecular Biology: Nucleic Acids*. **1975**, CRC Press, Cleveland, OH, 3rd Ed., vol 1, p597.
2. N. Sugimoto, S. Nakano, M. Katoh, A. Matsumura, H. Nakamuta, T. Ohmichi, M. Yoneyama and M. Sasaki, *Biochemistry*, **1995**, 34, 11211.; S. Nakano, T. Kanzaki and N. Sugimoto, *J. Am. Chem. Soc.*, **2004**, *126*, 1088.

a)

ODN12: 5'-CCCC-TTTT-CCC-$(T)_3$-CCCC-ATTA-CCC-3'
ODN13: 5'-CCC-TTTT-CCC-$(T)_3$-CCC-TTTTTT-CCC-3'
ODN14: 5'-CCC-TATTTA-CCC-$(T)_3$-CCC-ATTTAT-CCC-3'
ODN15: 5'-CCC-TTTT-CCC-$(T)_3$-CCC-ATTTTTTA-CCC-3'
ODN16: 5'-CCC-TATTTATA-CCC-$(T)_3$-CCC-ATATTTAT-CCC-3'

b)



Scheme S1. a) The five additional intrastrand i-motif forming sequences that were used in this study. The NBs in the sequence are underlined. b) Schematics of first and third loop regions of the additional sequences analyzed. The NBs are underlined in the illustrations.

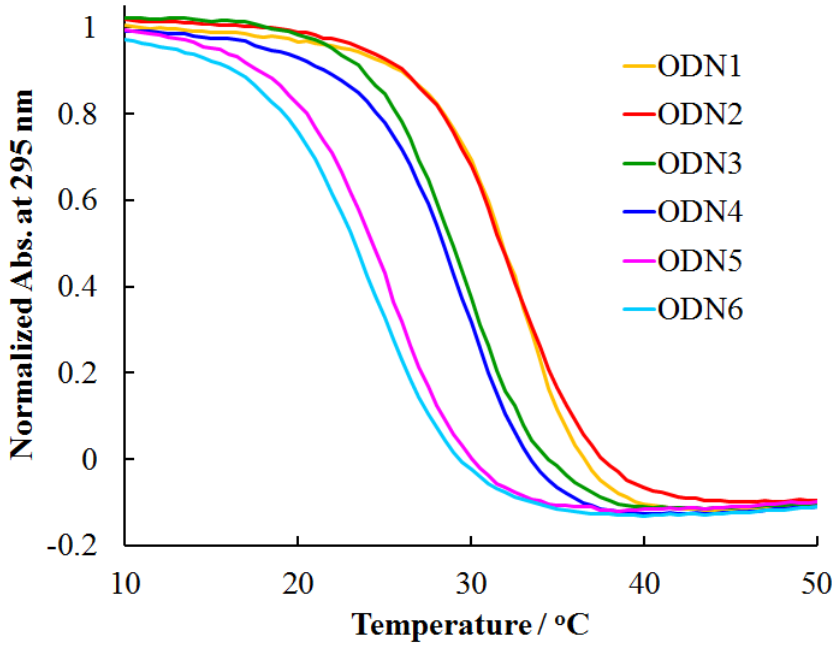


Figure S1. The UV melting curves of i-motif DNA oligonucleotides of different NBs at 5 μM DNA strand at 295 nm. The experiments were carried out in the buffer containing 10 mM Na_2HPO_4 (pH 6.5) and 0.1 mM Na_2EDTA.

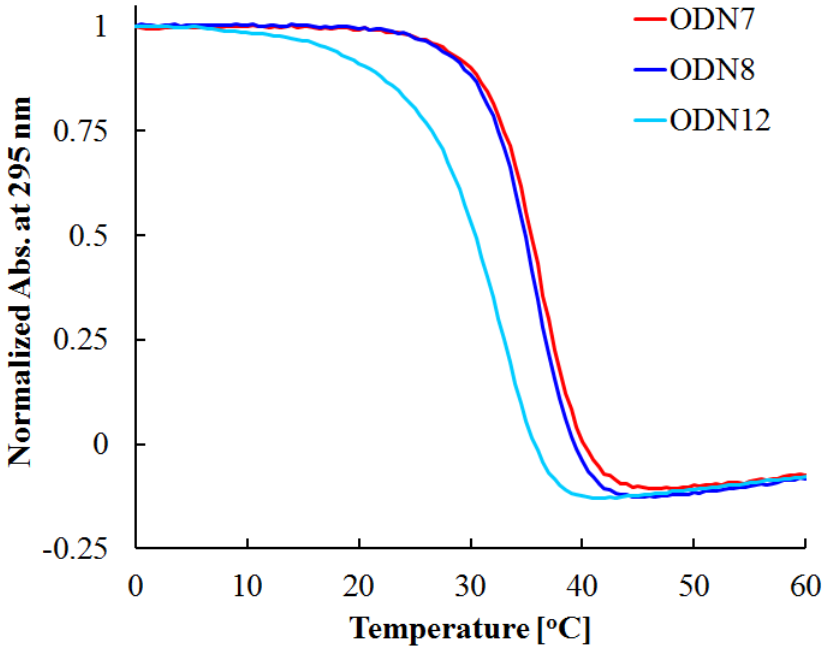


Figure S2. The UV melting curves of 5 μM **ODN7**, **ODN8**, and **ODN12** monitored at 295 nm. The experiments were carried out in the buffer containing 10 mM Na_2HPO_4 (pH 6.5) and 0.1 mM Na_2EDTA.

Table S1. Melting temperatures and thermodynamic parameters for the formation of i-motif quadruplexes with the different numbers of C-C$^+$ base pairs at pH 6.5[a]

Name	C-C$^+$ base pairs	NB [b] [1/3]	$\Delta H°$ [kcal·mol^{-1}]	$T\Delta S°$ [d] [kcal·mol^{-1}]	$\Delta G°_{25}$ [c] [kcal·mol^{-1}]	$-\Delta\Delta G°_{25}$ [c,d] [kcal·mol^{-1}]	T_m [°C]	ΔT_m [d] [°C]
ODN2	6	GG/GG	-73.2±0.8	-71.7±0.9	-1.51±0.28	-	31.3±1.2	-
ODN7	7	GG/GG	-83.9±2.0	-81.0±2.0	-2.98±0.04	+1.47	36.0±0.3	+4.7
ODN8	7	GI/GG	-85.2±2.1	-82.3±2.1	-2.88±0.04	+1.37	35.4±0.3	+4.1
ODN4	6	TT/AA	-72.8±1.1	-72.0±0.9	-0.73±0.30	-	28.0±1.2	-
ODN12	7	TT/AA	-63.7±4.0	-62.3±4.0	-1.35±0.21	+0.62	31.4±0.9	+3.3

[a] Buffer contained 10 mM Na_2HPO_4 (pH 6.5) and 0.1 mM Na_2EDTA. UV melting and annealing curves were measured with three DNA concentrations: 1, 5, and 50 μM. The melting temperatures and thermodynamic parameters are average values obtained fits of curves. [b] NB [1/3] gives NBs in loops 1 and 3, respectively. [c] $T\Delta S^o$ and ΔG^o_{25} were calculated at 298 K (25 °C). [d] $-\Delta\Delta G^o_{25}$ and ΔT_m of **ODN7**, **ODN8**, and **ODN12** were calculated relative to those of **ODN2**, **ODN2**, and **ODN4**, respectively.

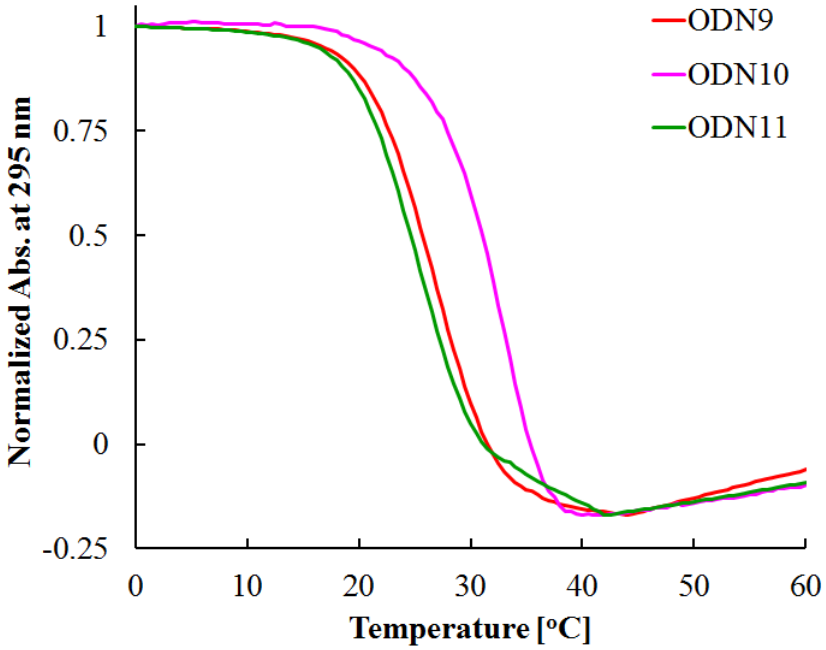


Figure S3. The UV melting curves of 5 μM **ODN9**, **ODN10**, and **ODN11** monitored at 295 nm. The experiments were carried out in the buffer containing 10 mM Na_2HPO_4 (pH 6.5) and 0.1 mM Na_2EDTA.

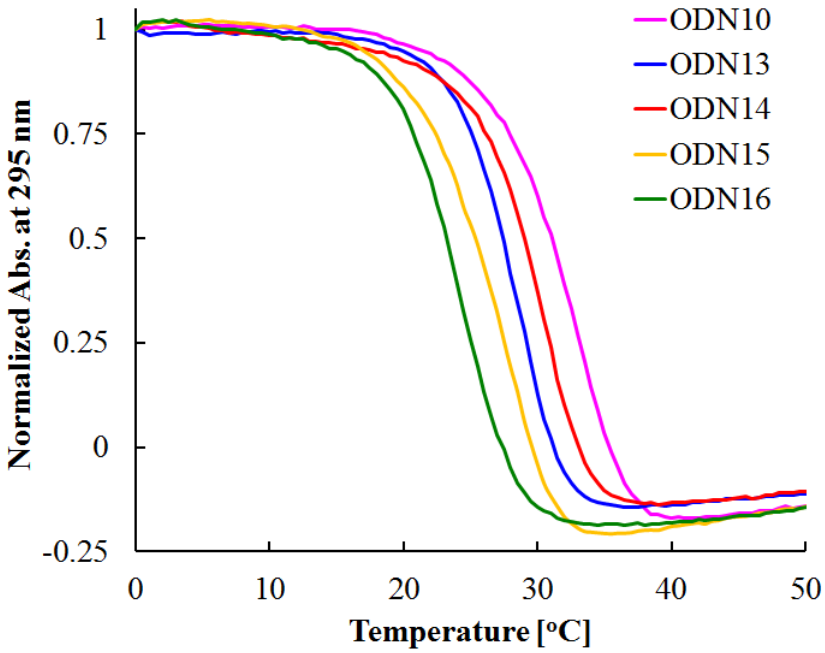


Figure S4. The UV melting curves of i-motif DNA oligonucleotides of different loop lengths at 5 μM DNA strand concentration monitored at 295 nm. The experiments were carried out in the buffer containing 10 mM Na_2HPO_4 (pH 6.5) and 0.1 mM Na_2EDTA.

Table S2. Melting temperatures and thermodynamic parameters for the formation of i-motif quadruplexes with the different loop lengths at pH 6.5[a]

Name	Loop lengths[b]	$\Delta H°$ [kcal·mol^{-1}]	$T\Delta S°$ [c] [kcal·mol^{-1}]	$\Delta G°_{25}$ [c] [kcal·mol^{-1}]	$-\Delta\Delta G°_{25}$ [c,d] [kcal·mol^{-1}]	T_m [°C]	ΔT_m [d] [°C]
ODN13	4/6	-81.4±1.4	-81.0±1.5	-0.42±0.03	-1.14	26.3±0.2	-4.4
ODN1	4/4	-82.1±1.6	-80.6±1.4	-1.56±0.38	-	30.7±1.2	-
ODN10	4/6	-71.2±1.2	-69.7±1.4	-1.44±0.25	+0.71	31.1±1.2	+3.1
ODN4	4/4	-72.8±1.1	-72.0±0.9	-0.73±0.30	-	28.0±1.2	-
ODN14	6/6	-81.9±1.7	-81.3±1.6	-0.64±0.20	-0.09	27.2±0.7	-0.8
ODN15	6/8	-70.8±1.6	-70.6±1.5	-0.13±0.26	-0.60	25.4±1.1	-2.6
ODN16	8/8	-78.0±1.2	-78.6±1.2	0.68±0.18	-1.32	22.4±0.7	-5.6

[a] Buffer contained 10 mM Na_2HPO_4 (pH 6.5) and 0.1 mM Na_2EDTA. UV melting and annealing curves were measured with three DNA concentrations: 1, 5, and 50 μM. The melting temperatures and thermodynamic parameters are average values obtained fits of curves. [b] The number of bases in loops 1 and 3, respectively. [c] $T\Delta S^o$ and ΔG^o_{25} were calculated at 298 K (25 °C). [d] $-\Delta\Delta G^o_{25}$ and ΔT_m of **ODN10**, **ODN14**, **ODN15**, and **ODN16** were calculated relative to those of **ODN4**. $-\Delta\Delta G^o_{25}$ and ΔT_m of **ODN13** were calculated relative to those of **ODN1**.

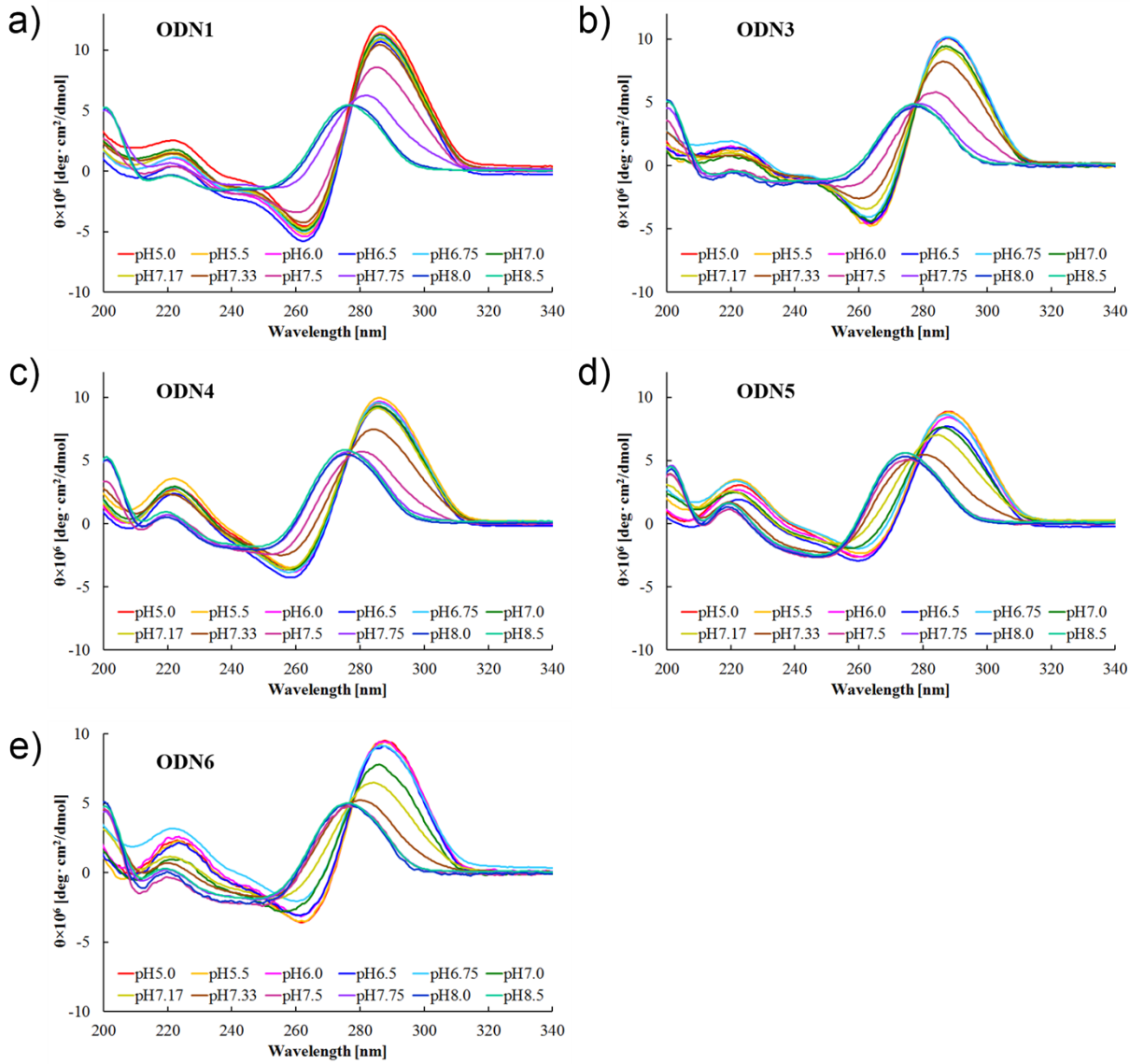


Figure S5. CD spectra of a) **ODN1**, b) **ODN3**, c) **ODN4**, d) **ODN5**, and e) **ODN6** at 4 °C. The experiments were carried out in the buffer containing 10 mM Na_2HPO_4 and 0.1 mM Na_2EDTA at indicated pH at a DNA strand concentration of 20 μM.

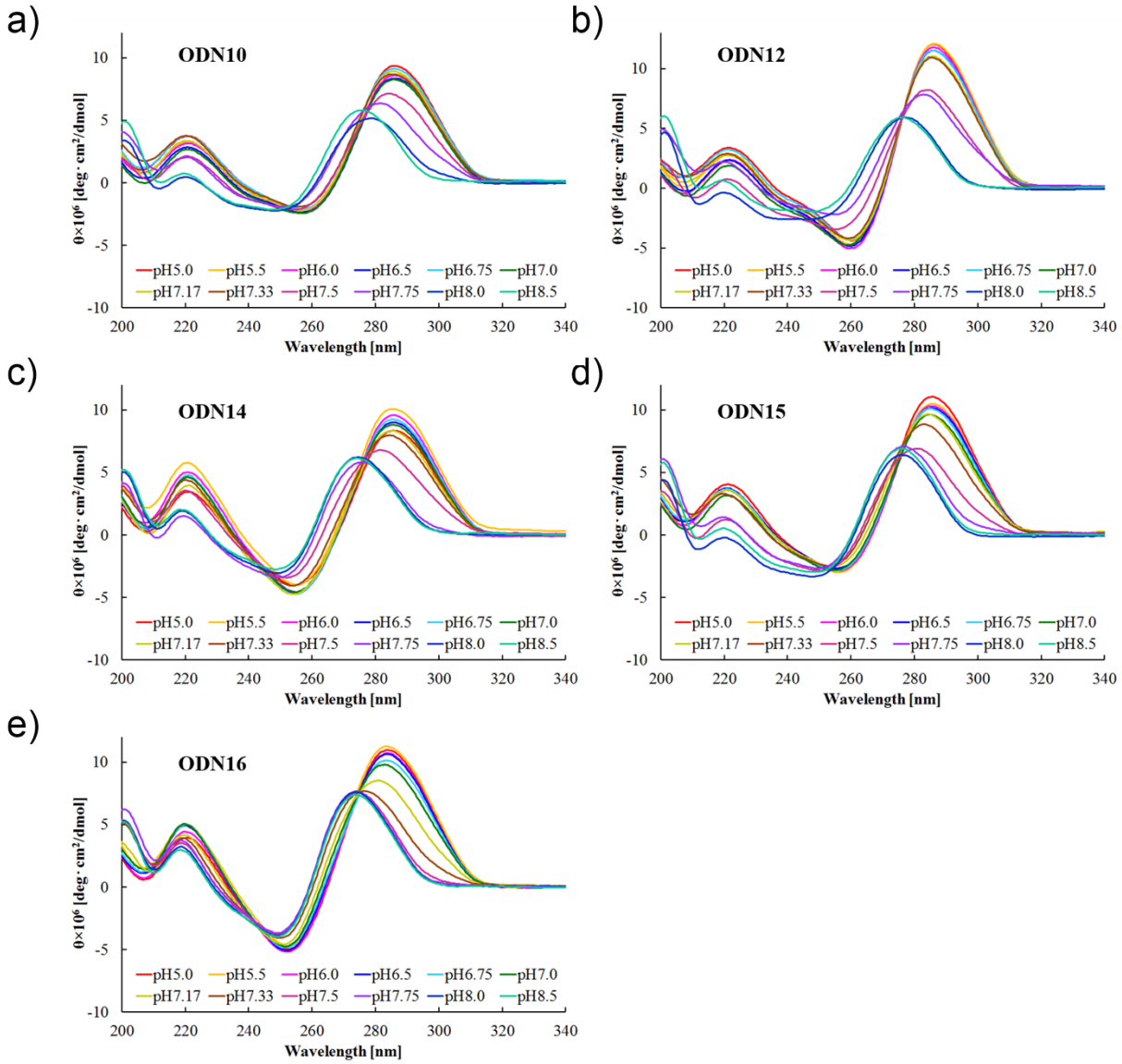


Figure S6. CD spectra of a) **ODN10**, b) **ODN12**, c) **ODN14**, d) **ODN15**, and e) **ODN16** at 4 °C. The experiments were carried out at 20 μM total DNA strand concentration in buffer containing 10 mM Na_2HPO_4 and 0.1 mM Na_2EDTA at indicated pH.

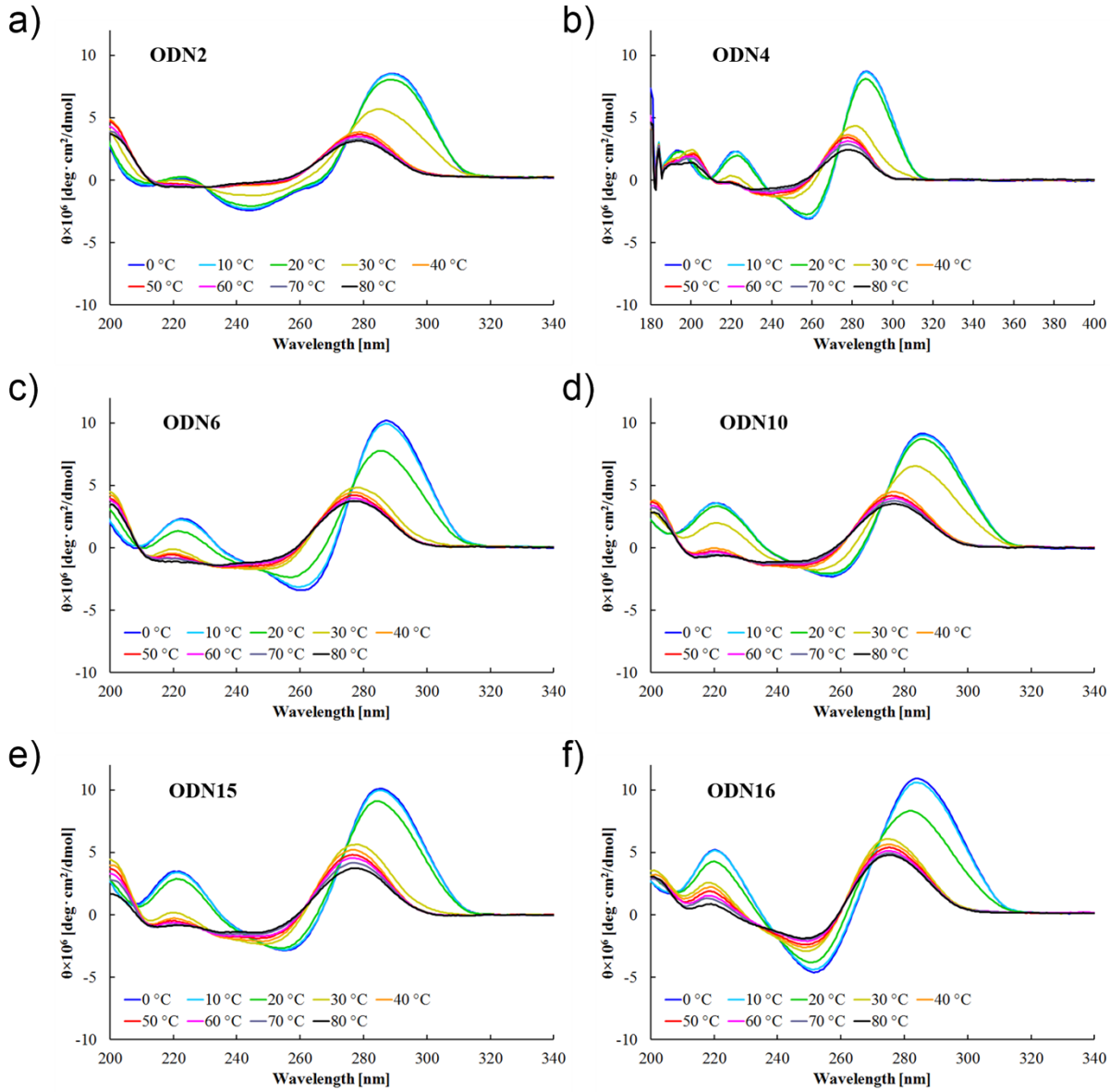


Figure S7. CD spectra of a) **ODN2**, b) **ODN4**, c) **ODN6**, d) **ODN10**, e) **ODN15**, and f) **ODN16** at indicated temperatures. The experiments were carried out in the buffer containing 10 mM Na_2HPO_4 (pH 6.5) and 0.1 mM Na_2EDTA.

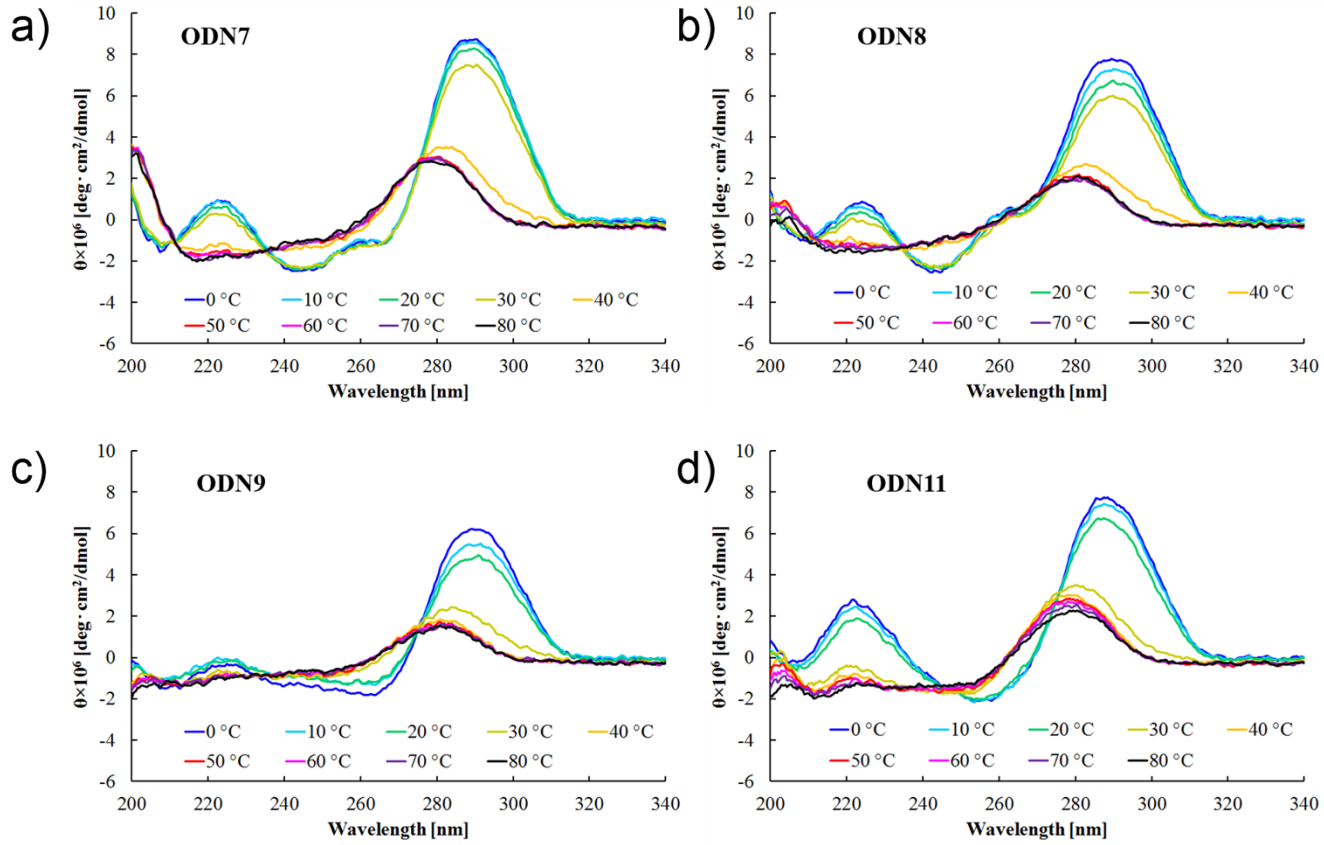


Figure S8. CD spectra of a) **ODN7**, b) **ODN8**, c) **ODN9**, and d) **ODN11** at indicated temperatures. The experiments were carried out in the buffer containing 10 mM Na_2HPO_4 (pH 6.5) and 0.1 mM Na_2EDTA.

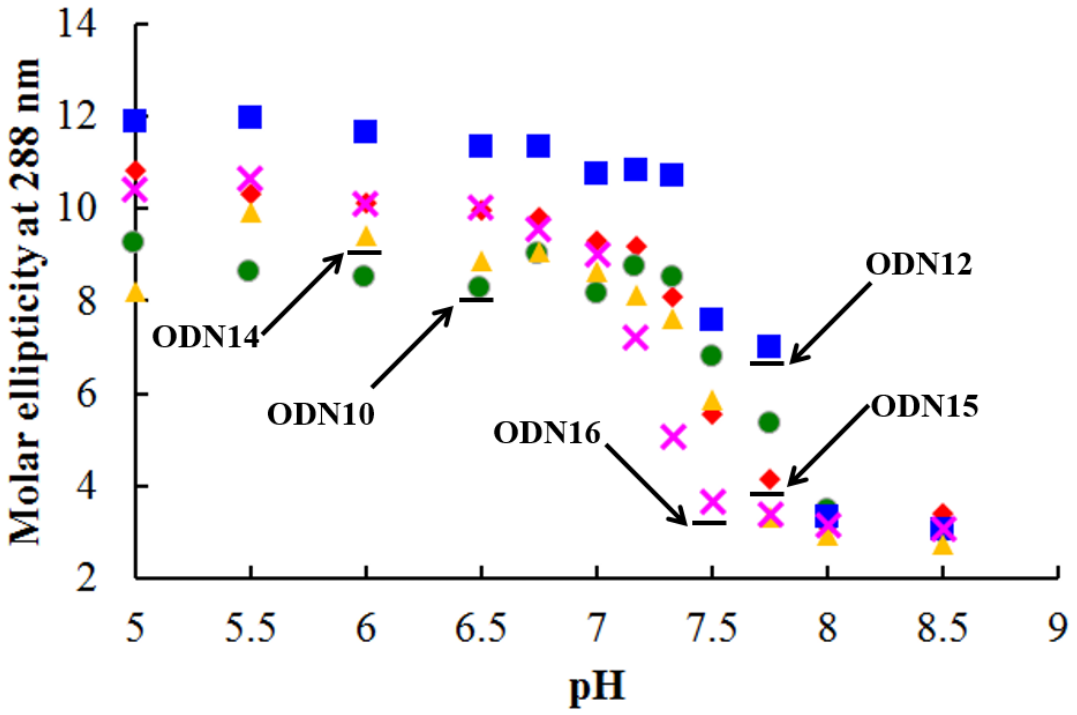


Figure S9. Plots of pH vs. molar ellipticity at 288 nm of **ODN10** (green ●), **ODN12** (blue ■), **ODN14** (yellow ▲), **ODN15** (red ◆), and **ODN16** (pink ×). The experiments were carried out in the buffer containing 10 mM Na_2HPO_4 and 0.1 mM Na_2EDTA at pH values from 5.0 to 8.5.