Electronic Supplementary Material (ESI) for Chemical Communications.


**Supporting Information**

# i-Motifs are more stable than G-quadruplexes in a hydrated ionic liquid

*Hisae Tateishi-Karimata,[a] Miki Nakano,[a] Smritimoy Pramanik[a]*

*Shigenori Tanaka[b] and Naoki Sugimoto[a,c]* ∗

[a] *Frontier Institute for Biomolecular Engineering Research (FIBER), Konan University, 7-1-20, Minatojima-minamimachi, Chuo-ku, Kobe, 650-0047, Japan*

[b] *Graduate School of System Informatics, Department of Computational Science, Kobe University, 1-1, Rokkodai, Nada-ku, Kobe, 657-8501, Japan*

[c] *Graduate School of Frontier of Innovative Research in Science and Technology (FIRST), Konan University, 7-1-20, Minatojima-minamimachi, Chuo-ku, Kobe, 650-0047, Japan*

**Materials.** All oligodeoxynucleotides used in this study were high-performance liquid chromatography grade (Japan Bio Service). Single-strand concentrations of DNA oligonucleotides were determined by measuring the absorbance at 260 nm at 80 °C; single-strand extinction coefficients were calculated from the mononucleotide and dinucleotide data according to the nearest-neighbor approximation model.[1] The absorbance was measured using a Shimadzu 1700 spectrophotometer connected to a thermoprogrammer. The hydrated ionic liquid, choline dihydrogenphosphate (choline dhp), was purchased from Ionic Liquids Technologies Co. Ltd. and used without further purification.

**Thermodynamic analysis.** Ultraviolet (UV) absorbance spectra at 295 nm were measured on a Shimadzu 1700 spectrophotometer equipped with a temperature controller. Melting curves of i-motifs were measured at 295 nm in solution containing 1 mM $Na_2EDTA$ and 0 or 50 mM MES (pH 5.5-6.5) or Tris (pH 7.0) and 100 mM to 4 M NaCl or 4 M choline dhp. Samples were heated at a rate of 0.1 °C $min^{-1}$. The thermodynamic parameters were calculated from the fit of the melting curves obtained from at least four different concentrations of DNA oligonucleotides (2, 5, 10, or 20 μM) to a theoretical equation for an intramolecular association as described previously.[2] The thermodynamic parameters listed in Tables 1 and S2 are the average values obtained from each melting curve and curve fitting analysis. We assumed that ODN1 and ODN2 formed intra-molecular structures because $T_m$ values of ODN1 and ODN2 in 4 M NaCl or in choline dhp solution at different DNA concentrations were almost the same. Before the measurements, the DNA samples were heated to 90 °C, cooled to 0°C at a rate of –0.1 °C $min^{-1}$, and incubated at 0 °C for 30 min. The melting and annealing curves for all DNAs were identical at heating and cooling a rate of 0.1 °C $min^{-1}$. We also measured thermodynamic parameters for ODN2 (i-motif) using melting curves at 265 nm. The thermodynamic parameters at 265 and 295 nm were identical within experimental error (data not shown).

**Fluorescence measurements.** Fluorescence spectra were collected on a JASCO FP-6500 fluorescence spectrophotometer. Concentrations used were 1 μM ODN3 (G-quadruplex), 1 μM ODN4 (i-motif), or 1

μM ODN3 + 1 μM ODN4 or 1 μM ODN5 + 1 μM ODN6 (duplexes) in 4 M choline dhp and 1 mM $Na_2EDTA$ or 100 mM NaCl, 50 mM Tris-HCl (pH 7.0), and 1 mM $Na_2EDTA$.

The spectra from 500 to 600 nm at 25 °C were obtained in a cuvette with a pathlength of 0.1 cm. All measurements were carried out with excitation at 494 nm, the excitation wavelength of 6-FAM (6-carboxylfluorescein). The temperature of the cell holder was regulated by a JASCO EHC-573 temperature controller. Before the measurement, the sample was heated to 80 °C, cooled at a rate of –2 °C $min^{-1}$ to 25 °C, and incubated at 25 °C for 30 min.

**Circular dichroism measurements.** CD measurements were made on a JASCO J-820 spectropolarimeter at 20 μM total DNA strand concentration in buffers containing 1 mM $Na_2EDTA$ and 50 mM MES (pH 5.5-6.5) or Tris (pH 7.0 and 7.5) and 4 M NaCl or 4 M choline dhp. The spectra at 4 °C were obtained by taking at least three scans from 200 to 350 nm in a cuvette with a pathlength of 0.1 cm. The temperature of the cell holder was regulated by a JASCO PTC-348 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 -0.1 °C $min^{-1}$, and incubated at 4 °C for 30 min.

**Water activity measurements.** The water activity was determined by the osmotic stressing method via vapor phase osmometry using a model 5520XR pressure osmometer (Wescor, Logan, UT, USA) at 25°C. [2]

**Molecular dynamics simulations.** Initial structure of G-quadruplexes and i-motif were constructed from the coordinates obtained from the Protein Data Bank (PDB ID 143D [5'-AGGGTTAGGGTTAGGGTTAGGG-3'], referred to as $ODN1_{MD}$, and PDB ID 1EL2 [5'-CCCAATCCCAATCCCAATCCCT-3'] referred to as $ODN2_{MD}$, Table S1). $ODN1_{MD}$ has an A at the 5' end and ODN2 has a T at the 3' end. For $ODN2_{MD}$, we replaced the methylated cytosines with unmethyl-

ated C and replaced uracil with thymine (16U to 16T). DNAs were merged in TIP3P water molecules,[3] and 200 choline ions were added to the solvent. MD simulations were carried out with AMBER12 software package.[4] AMBERff99bsc0 force field was applied for DNA,[5] and the force field of choline ion was generated using antechamber and gaff module included in AMBER Tools.[6] The protocols used for structural optimization and MD simulations were as follows: First, the optimization of water molecules and ions was carried out in 1000 steps with the conformation of DNA fixed. Second, the whole system was energetically minimized in 2500 steps without any constraints. Third, the system was heated to 298 K for 20 ps. Finally, constant-pressure and constant-temperature MD simulations were carried out at 1 atm and 298 K for 20 ns with constraints hydrogen bond lengths using SHAKE algorithm.[7] Throughout these MD simulations, the simulation time steps were set to be 2 fs, and periodic boundary conditions were applied. The non-bonded cutoff length, which was used to limit the direct space sum for particle mesh Ewald, was set at 10 Å. Calculations were performed on the SGI UV 1000 system at the Research Center for Computational Science, Okazaki, and on DELL Precision T5400 system at Kobe University.

**Molecular Mechanics - Generalized Born Surface Area analysis.** The MM-GBSA method is used for energy analysis using continuum solvent models.[8] Using the MM-GBSA method, we estimated the binding free energy between DNA and cations. In this method, the free energy, $G$, for each species is decomposed into a molecular mechanical (MM) energy in gas phase, $E_{gas}$, and a solvation free energy, $G_{solv}$. $E_{gas}$, determined with the sander program from AMBER12, was further decomposed into the internal energies, $E_{INT}$ (bond, angle, and dihedral), electrostatic inter action, $E_{EL}$, and van der Waals interaction, $E_{VDW}$. $G_{solv}$ was also decomposed into polar and non-polar contributions. The polar contribution to the solvation free energy was evaluated using generalized Born (GB) methods, $G_{GB}$; the non-polar contribution to the solvation free energy was determined with solvent-accessible-surface-area-dependent (SA) terms, $G_{SA}$. The contributors to $G$ are as follows:

$$G = E_{gas} + G_{solv} = E_{INT} + E_{EL} + E_{VDW} + G_{GB} + G_{SA} \qquad (1)$$

The binding free energy between DNA and cation in the specified area ($\Delta G_+$) was calculated as follows:

$$\Delta G_+ = G(\text{complex}) - G(\text{DNA structure}) - G(\text{cation}) \qquad (2)$$

The ten closest cations to DNA atoms were selected, and $\Delta G_+$ was calculated for each system. These energy values correspond to the affinity of cations to each DNA structure.

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***Table S1.*** DNA sequences

| Sequence name | sequence (5' to 3') |
|---|---|
| ODN1 | GGGTTAGGGTTAGGGTTAGGG |
| ODN2 | CCCTAACCCTAACCCTAACCC |
| ODN3 | GGGTTAGGGTTAGGGTTAGGG-B [a] |
| ODN4 | F-CCCTAACCCTAACCCTAACCC |
| ODN5 | GCTGTAGAGTGACGTCTAGGG-B |
| ODN6 | F-CCCTAGACGTCACTCTACAGC |
| ODN7 | CCCTAACCC |
| ODN8 | F-CCCTAACCCTAACCCTAACCC-Q |
| $ODN1_{MD}$ | AGGGTTAGGGTTAGGGTTAGGG |
| $ODN2_{MD}$ | CCCTAACCCTAACCCTAACCCT |
| ssODN1 | GGGTTAGGGTTA [b] |
| ssODN2 | CCCTAACCCTAA [b] |

[a] F and B indicated 5' fluorophore (6-carboxylfluorescein) and a 3' quencher (Black Hole Quencher 1), respectively.

[b] Due to technical problems with full-length sequences in molecular dynamic simulations, we used ssODN1 and ssODN2 as the single-stranded ODN1 and ODN2.

***Table S2.*** Thermodynamic parameters for i-motif formation by ODN2 [a]

| | $\Delta H^\circ$ (kcal mol$^{-1}$) | $T\Delta S^\circ$ (kcal mol$^{-1}$) | $\Delta G^\circ_{25}$ (kcal mol$^{-1}$) | $T_m$ [b] (°C) |
|---|---|---|---|---|
| NaCl | | | | |
| pH 5.5 | –42.8±0.6 | –40.0±0.5 | –2.8±0.2 | 45.4 |
| pH 6.0 | –35.0±0.2 | –34.9±2.0 | –0.1±0.6 | 25.5 |
| Choline dhp | | | | |
| pH 5.5 | –61.1±0.6 | –57.2±2.6 | –3.9±0.8 | 45.5 |
| pH 6.0 | –59.3±0.4 | –56.9±1.0 | –2.4±0.2 | 38.1 |

[a] All the experiments were carried out in a buffer containing 1 mM $Na_2EDTA$ and 4 M NaCl or 4 M choline dhp. The pH was controlled using 50 mM MES (pH 5.5 and 6.0). Thermodynamic parameters were evaluated from the average values obtained from curve fitting at different DNA concentrations.

[b] $T_m$ values were measured at 20 μM DNA concentration.

***Table S3.*** The number of cations ($N_+$) and waters ($N_w$) located within 3.5 Å of DNA atoms and the free energy changes due to cation ion binding to DNA structures ($\Delta G_+$)

| DNAs | $N_+$ | $N_w$ | $\Delta G_+$ (kcal mol$^{-1}$)[a] |
|---|---|---|---|
| Choline ions | | | |
| $ODN1_{MD}$ | 30.4 ± 2.5 | 247 ± 10 | −109 ± 16 |
| $ODN2_{MD}$ | 27.5 ± 2.7 | 256 ± 10 | −103 ± 10 |
| ssODN1 | 20.3 ± 2.3 | 189 ± 10 | −85.6 ± 11 |
| ssODN2 | 18.6 ± 2.6 | 211 ± 10 | −73.1 ± 8.1 |
| Sodium ions | | | |
| $ODN1_{MD}$ | 15.2 ± 2.1 | 317 ± 10 | −63.4 ± 9.2 |
| $ODN2_{MD}$ | 15.1 ± 2.5 | 327 ± 10 | −66.2 ± 8.0 |
| ssODN1 | 8.0 ± 2.5 | 240 ± 10 | −18.6 ± 7.1 |
| ssODN2 | 7.9 ± 2.0 | 245 ± 10 | −14.4 ± 7.3 |

[a] Methods for calculation of $\Delta G$ values are described in Supporting Information.

***Table S4.*** Osmolality (mmol/kg) and water activity

| Solution | Osmolality | $a_W$ |
|---|---|---|
| 100 mM NaCl (pH 7.0)[a] | 278±4 | 0.995 |
| 4 M NaCl (pH 6.0)[b] | 7800±12 | 0.868 |
| 4 M NaCl (pH 5.5)[c] | 7836±29 | 0.867 |
| 4 M Choline dhp (without pH control)[d] | 8244±4 | 0.861 |
| 4 M Choline dhp (pH 6.0)[b] | 8256±12 | 0.861 |
| 4 M Choline dhp (pH 5.5) [c] | 8542±29 | 0.856 |

Experiments were performed in a buffer containing [a] 100 mMNaCl, 50 mM Tris (pH 7.0), 1 mM $Na_2EDTA$, [b] 4 M NaCl or 4 M choline dhp, 50 mM MES (pH 6.0), 1 mM $Na_2EDTA$, [c] 4 M NaCl or 4 M choline dhp, 50 mM MES (pH 5.5), 1 mM $Na_2EDTA$, [d] 4 M choline dhp, 1 mM $Na_2EDTA$ and [e] 4 M choline dhp, 50 mM Tris (pH 7.0), 1 mM $Na_2EDTA$. The data were obtained using vapor phase osmometry.

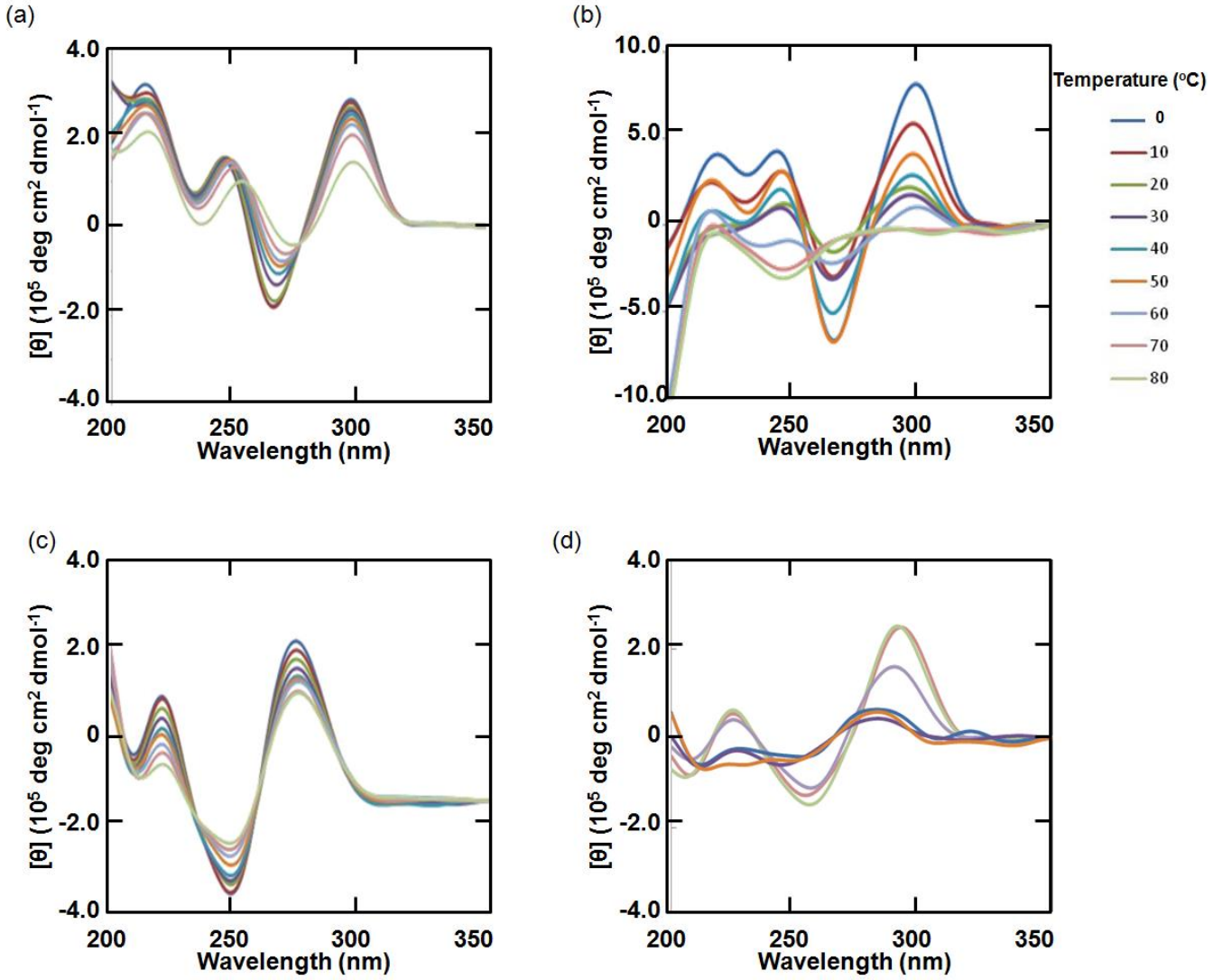


***Fig. S1.*** CD spectra of 20 μM (a, b) ODN1 and (c, d) ODN2 in buffered solution containing (a, c) 100 mM NaCl, 50 mM Tris (pH 7.0), 1mM $Na_2EDTA$, and (b, d) 4 M choline dhp, 1mM $Na_2EDTA$.

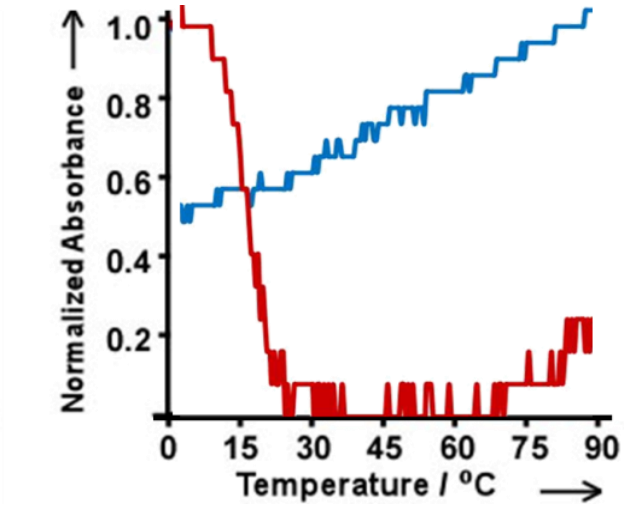


***Fig. S2.*** Normalized UV melting curves at 295 nm for ODN2 in a solution containing 50 mM Tris (pH 7.0), 1 mM $Na_2EDTA$, and 4 M choline dhp(red) and 4M NaCl (blue). DNA strand concentration was 2 μM. The original melting curve of ODN2 in NaCl was used because ODN2 did not show a clear melting transition

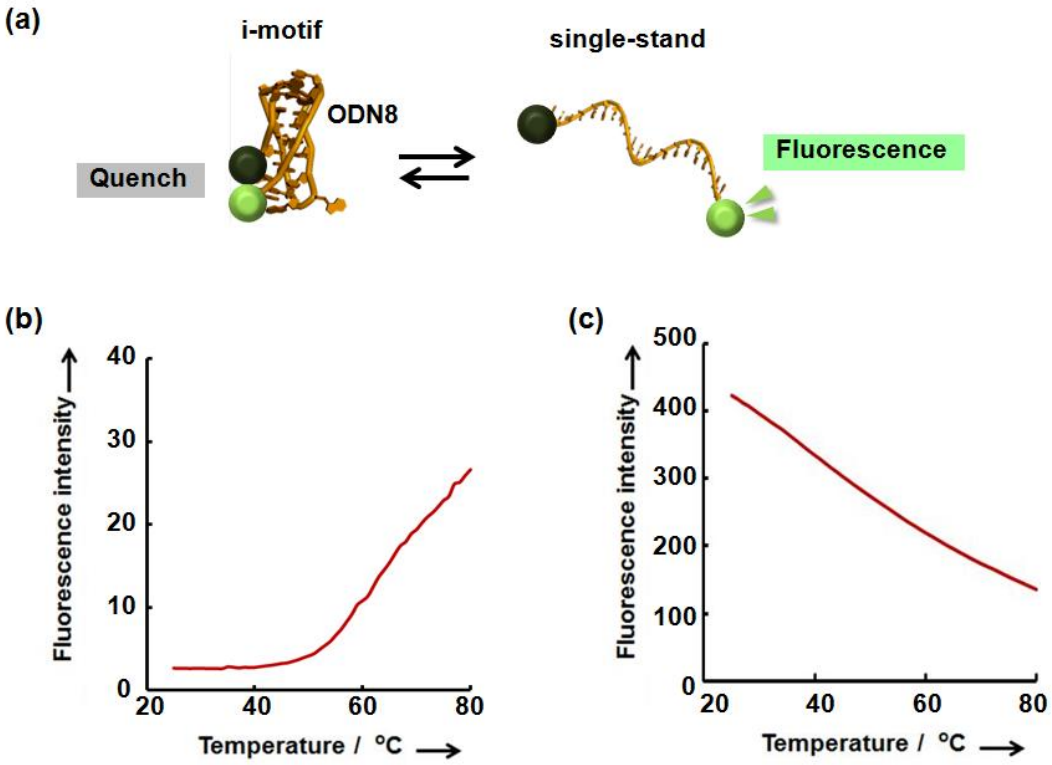


***Fig. S3*** (a) Schematic illustration of structural change for ODN8. When ODN8 forms the i-motif, the fluorescence of ODN8 should be quenched. Fluorescence melting curves for ODN8 in a solution containing (b) 4 M choline dhp and 1 mM $Na_2EDTA$ or (c) 100 mM NaCl, 50 mM Tris (pH 7.0) and 1 mM $Na_2EDTA$ solutions. The $T_m$ value of the ODN8 in choline dhp was 61.0 °C. The ODN8 did not show the clear transition in 4 M NaCl indicating that theODN8 did not form the i-motif.

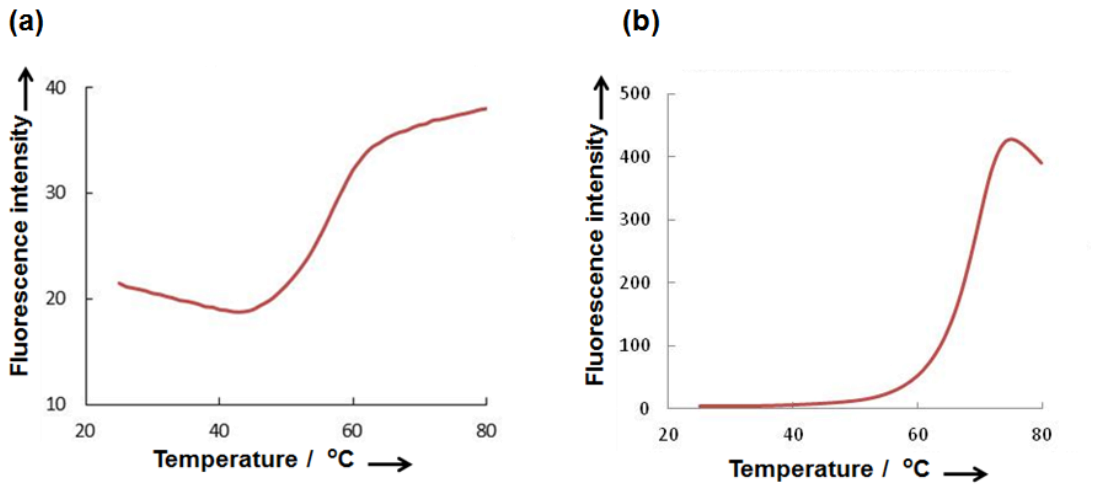


***Fig. S4*** Fluorescence melting curves for duplex (1 μM ODN3 and 1 μM ODN4) in a solution containing (a) 4 M choline dhp and 1 mM $Na_2EDTA$ or (b) 100 mM NaCl, 50 mM Tris (pH 7.0) and 1 mM $Na_2EDTA$ solutions. The $T_m$ values of the duplex in choline dhp were 50.8 and 68.9 °C, respectively.

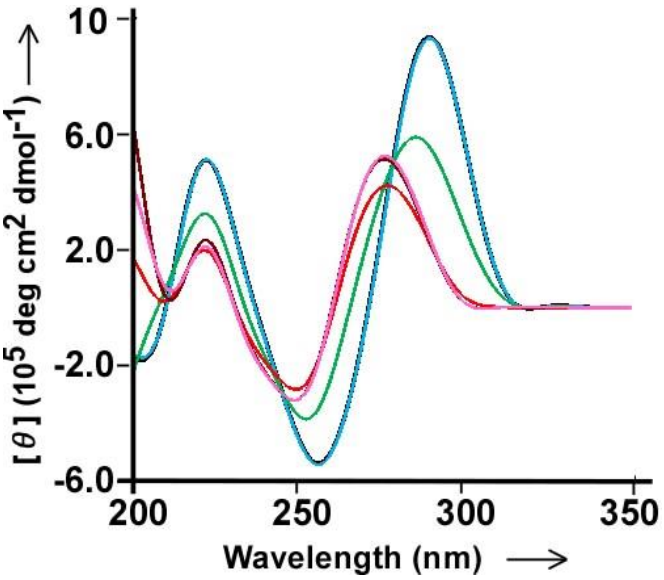


***Fig. S5*** CD spectra of 20 μM ODN2 in buffered solution containing 4 NaCl at the indicated pH: pH 5.5 (black), pH 6.0 (light blue), pH 6.3 (green), pH 6.5 (red), pH 7.0 (brown), and pH 7.5 (pink). The pH was controlled using 50 mM MES (pH 5.0-6.5) or 50 mM Tris (pH 7.0, 7.5).

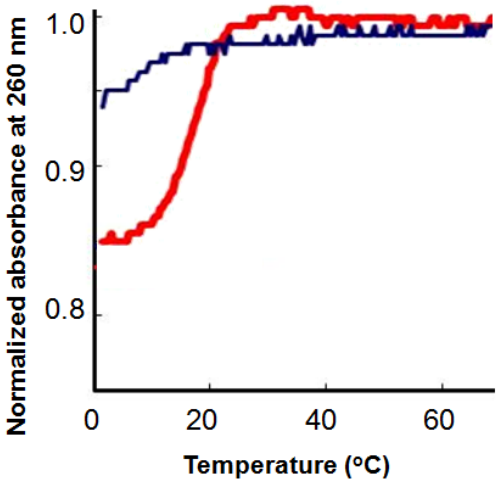


***Fig. S6*** Normalized UV melting curves at 260 nm for 20 μM ODN7 in 4 M NaCl solution (blue) and 4 M choline dhp solution (red). Solutions also contained 50 mM MES (pH 6.0) and 1 mM $Na_2EDTA$. The $T_m$ value of ODN7 in choline dhp was 16.0 °C. The $T_m$ value of ODN7 in NaCl could not be determined.

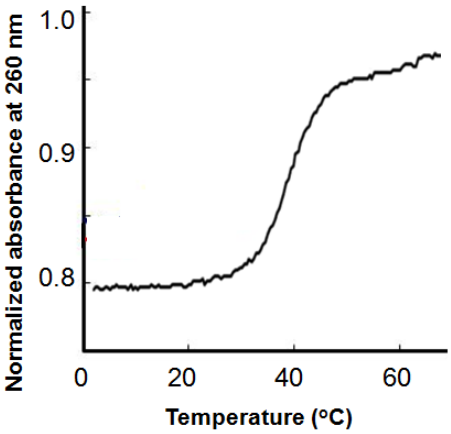


***Fig. S7*** Normalized UV melting curves at 260 nm for 20 μM ODN2 in 4 M choline chloride, 50 mM MES (pH 6.0) and 1 mM $Na_2EDTA$. The $T_m$ value of ODN2 in choline dhp was 37.9 °C.