Probing the competition between duplex and G-quadruplex/i-motif structures using a conformation-sensitive fluorescent nucleoside probe

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Electronic Supplementary Information

Contents	Page
1. Materials	S2
2. Instrumentation	S2
Fig. S1 HPLC chromatogram of modified ONs	S2
3. MALDI-TOF mass of modified ONs	S2
Table S1 ε_{260} and mass data of modified DNA ONs	S3
Fig. S2 Individual curve fits obtained for ON **2** by steady-state fluorescence (**A**–**C**) and lifetime (**D**–**F**) analysis	S3
Table S2 *tpH* values obtained for ONs **2** and **3** by steady-state fluorescence and lifetime analysis	S3
Fig. S3 Representative excited-state decay profile for DNA ON **2** at near *tpH* (pH 7.0) and below *tpH* (pH 5.0)	S4
Table S3 Lifetime values of iM forming DNA ONs **2** and **3** at different pH	S4
Fig. S4 CD spectra and UV-thermal melting profile of C-rich DNA ONs at different pH	S4
Table S4 T_m values of modified ONs **2, 3** and unmodified ONs **4, 5** at acid pH	S5
Fig. S5 Individual curve fits obtained for ON **3** by steady-state fluorescence (**A**–**C**) and lifetime (**D**–**F**) analysis	S5
Fig. S6 Fluorescence spectra of nucleoside **1** and control modified DNA ON **6** at different pH	S5
Fig. S7 Fluorescence spectra (1 μM) of benzofuran-modified C-rich DNA ON **2** and H-Telo DNA ON **3** and corresponding hybrids with complementary ONs at basic and acid pH	S6
Fig. S7 Schematic diagram showing the possible conformation of **1** in iM structure of H-Telo DNA ON **3**	S6
Table S5 T_m values of control unmodified and modified G-rich DNA ONs, and C-rich-G-rich DNA hybrids at different pH	S6
Fig. S9 Fluorescence spectra (1 μM) of non-iM-forming benzofuran-modified control DNA ON at different pH	S7
4. References	S7

1. Materials: Benzofuran-modified nucleoside analog **1** and corresponding phosphoramidite substrate for solid-phase oligonucleotide (ON) synthesis were synthesized as per our earlier reports.[1] *N*-benzoyl-protected dA, dT, *N*,*N*-dimethylformamidine-protected dG and *N*-acetyl-protected dC phosphoramidite substrates for DNA synthesis were purchased from ChemGenes. Solid supports for the DNA synthesis were obtained from ChemGenes. All other reagents for solid-phase ON synthesis were obtained from ChemGenes and Sigma-Aldrich. Synthetic DNA ONs were purchased from Integrated DNA Technologies, Inc. and purified by polyacrylamide gel electrophoresis (PAGE) under denaturing condition, and desalted on Sep-Pak Classic C18 cartridges (Waters Corporation). Chemicals (BioUltra grade) for preparing buffer solutions were purchased from Sigma-Aldrich. Autoclaved water was used for the preparation of all buffer solutions and fluorescence analysis. Solutions of emissive ONs at different pH were prepared in phosphate (5.8–8.2) or acetate buffer (5.0–5.6), which is commonly used to study iM formation at different pH.[2]

2. Instrumentation: Mass measurements were recorded on Applied Biosystems 4800 Plus MALDI TOF/TOF analyzer. Modified DNA ONs were synthesized on Applied Biosystems RNA/DNA synthesizer (ABI-394). Absorption spectra were recorded on a PerkinElmer, Lambda 45 UV-Vis spectrophotometer. RP-HPLC analysis was performed using Agilent Technologies 1260 Infinity. UV-thermal melting studies of ONs were performed on a Cary 300Bio UV-Vis spectrophotometer and CD analysis was performed on JASCO J-815 CD spectrometer. Steady-state and time-resolved fluorescence experiments were carried out in a micro fluorescence cuvette (Hellma, path length 1.0 cm) on Fluoromax-4 and a TCSPC instrument (Horiba Jobin Yvon, Fluorolog-3), respectively.

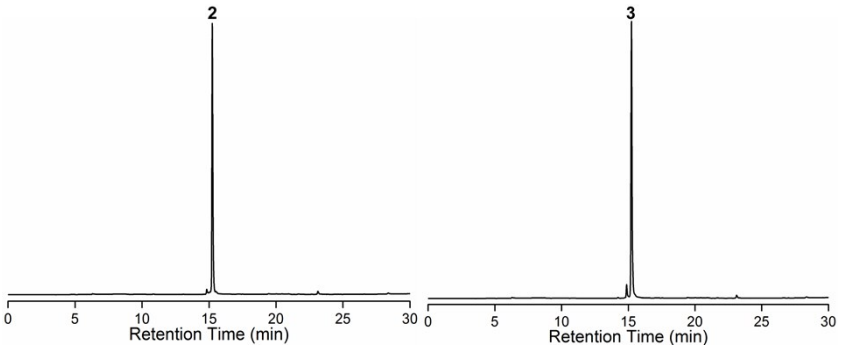

Fig. S1 RP-HPLC chromatograms of PAGE purified fluorescent ONs **2** and **3** at 260 nm. Mobile phase A = 100 mM triethylammonium acetate buffer (pH 7.6), mobile phase B = acetonitrile. Flow rate = 1 mL/min. Gradient = 0–10% B in 10 min and 10–100% B in 20 min. HPLC analysis was performed on Agilent Technologies 1260 Infinity using Phenomenex Luna C18 column (250 x 4.6 mm, 5 micron).

3. MALDI-TOF mass of DNA ONs: Molecular weight of benzofuran-modified DNA ONs was determined using Applied Biosystems 4800 Plus MALDI TOF/TOF analyzer. 2 μL of the modified ON (200 μM) was combined with 1 μL of ammonium citrate buffer (100 mM, pH 9), 1.5 μL of a DNA standard (200 μM) and 4 μL of saturated 3-hydroxypicolinic acid solution. The samples were desalted using an ion-exchange resin (*Dowex 50W-X8*, 100-200 mesh, ammonium form), spotted on the MALDI plate, and air dried. The resulting spectrum was calibrated relative to an internal DNA ON standard.

Table S1 ε_{260} and mass data of modified DNA ONs.

Modified ON	ε_{260} ($M^{-1}cm^{-1}$)[a]	Calculated mass $[M]^+$	Observed mass $[M]^+$
2	1.79 x 10^5	7040.6	7041.5
3	2.25 x 10^5	7232.8	7233.0
6[3]	2.33 x 10^5	7068.6	7067.9

[a]Molar absorption coefficient (ε) of modified ONs was determined by using OligoAnalyzer 3.1. ε of nucleoside **1** (ε_{260} = 12613 $M^{-1}cm^{-1}$) was used in place of thymidine.[1,3]

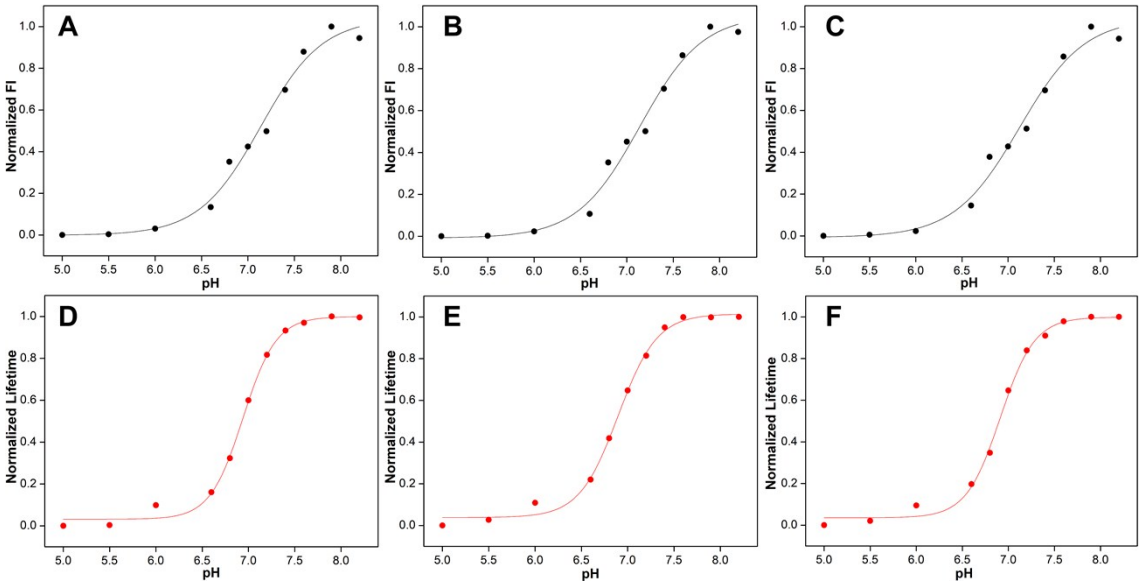

Fig. S2 *tpH* value was determined by fitting the curve obtained by plotting normalized fluorescence intensity at emission maximum (black) or lifetime (red) against pH. Individual curve fits obtained for ON **2** by steady-state fluorescence (**A**–**C**) and lifetime (**D**–**F**) analysis. See Table S2 for *tpH* values for the individual curve fits.

Table S2 *tpH* values obtained for ONs **2** and **3** by steady-state fluorescence and lifetime analysis.[a]

ON	technique[a]	*Average tpH*
2	steady-state fluorescence *tpH* = 7.14; 7.14; 7.12	7.13 ± 0.01
	lifetime *tpH* = 6.94; 6.90; 6.91	6.92 ± 0.02
3	steady-state fluorescence *tpH* = 5.79; 5.79; 5.78	5.79 ± 0.01
	lifetime *tpH* = 5.78; 5.80; 5.81	5.80 ± 0.01

[a]All experiments were performed in triplicate. For individual curve fits see Fig. S2 and S5.

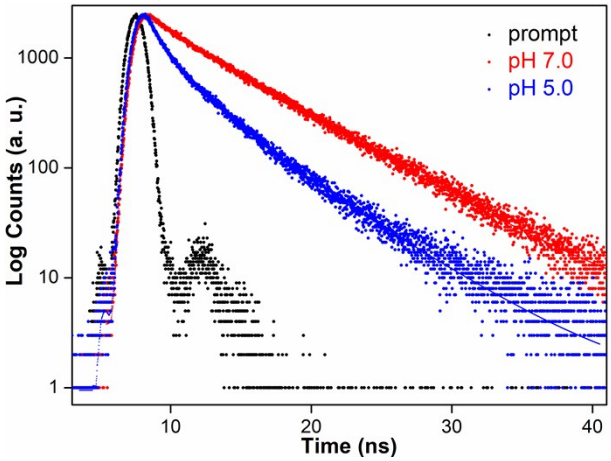

Fig. S3 Representative excited-state decay profile for C-rich DNA ON **2** (1 µM) at near *tpH* (pH 7.0) and below *tpH* (pH 5.0). Samples were excited using 339 nm LED source. Laser profile is shown in black (prompt). Curve fits are shown in solid lines.

Table S3 Lifetime values of iM forming DNA ONs **2** and **3** at different pH.

ON **2**	τ_{ave} (ns)[a]	ON **3**	τ_{ave} (ns)[a]
pH 5.0 [b]	1.49	pH 5.0	3.55
pH 5.5 [b]	1.56	pH 5.2	3.55
pH 6.0 [b]	1.90	pH 5.5	3.63
pH 6.6 [b]	2.28	pH 5.6	3.68
pH 6.8 [b]	2.99	pH 5.8	4.26
pH 7.0	4.09	pH 6.0	4.41
pH 7.2	4.88	pH 6.6	4.65
pH 7.4	5.32	pH 7.0	4.56
pH 7.6	5.54	pH 7.4	4.70
pH 7.9	5.61	pH 7.9	4.68
pH 8.2	5.60	pH 8.2	4.68

[a]Standard deviations for τ_{ave} (lifetime) are ≤ 0.18 ns. [b]The excited-state decay kinetics was found to be triexponential. All other samples exhibited biexponential excited-state decay kinetics.

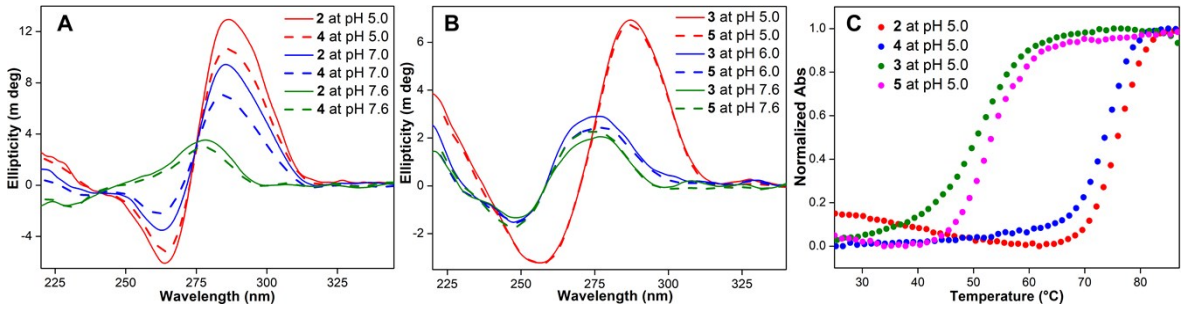

Fig. S4 CD spectra of C-rich DNA ONs at below, near and above respective *tpH*. (**A**) CD spectrum (5 µM) of modified DNA ON **2** and control unmodified DNA ON **4** at pH 5.0, 7.0 and 7.6. (**B**) CD spectrum (5 µM) of modified H-Telo DNA ON **3** and control unmodified H-Telo DNA ON **5** at pH 5.0, 6.0 and 7.6. (**C**) UV-thermal melting profile (1 µM at 260 nm) of iM form of modified ONs (**2** and **3**) and unmodified ONs (**4** and **5**) at pH 5.0.

Table S4 T_m values of modified ONs **2, 3** and unmodified ONs **4, 5** in 30 mM acetate buffer (pH 5.0, 100 mM NaCl).

Fluorescently-modified DNA ON	T_m (°C)	Control unmodified DNA ON	T_m (°C)
2	75 ± 0.7	**4**	74 ± 1.2
3	52 ± 0.8	**5**	53 ± 1.1

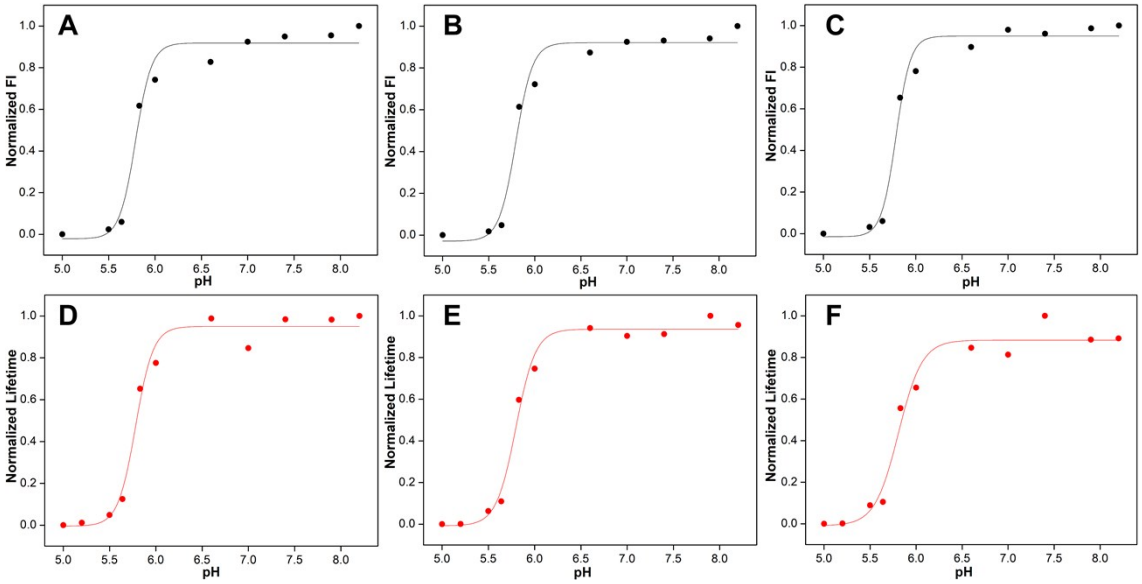

Fig. S5 *tpH* value was determined by fitting the curve obtained by plotting normalized fluorescence intensity at emission maximum (black) or lifetime (red) against pH. Individual curve fits obtained for ON **3** by steady-state fluorescence (**A**–**C**) and lifetime (**D**–**F**) analysis. See Table S2 for *tpH* values for the individual curve fits.

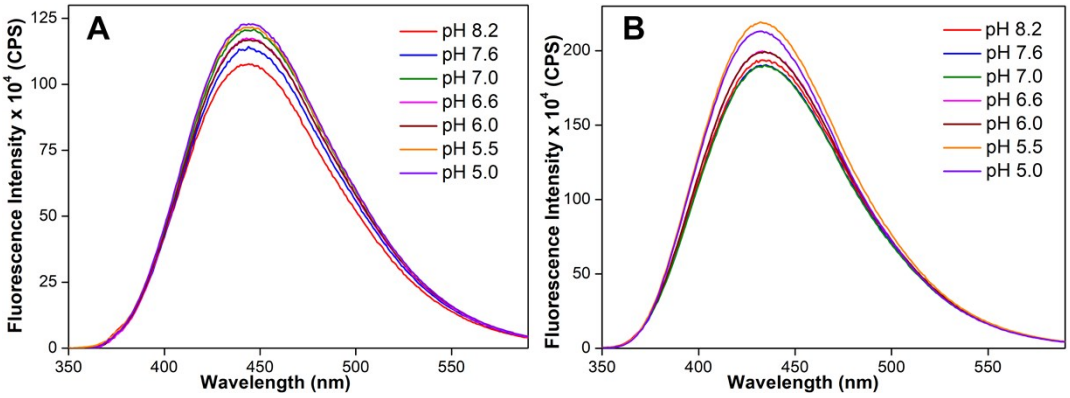

Fig. S6 (**A**) Fluorescence spectra (1 μM) of nucleoside **1** at different pH. Excitation and emission slit widths were kept at 3 nm and 6 nm, respectively. (**B**) Fluorescence spectra (1 μM) of a control benzofuran-modified DNA ON **6**, which does not fold into iM structure at different pH. Excitation and emission slit widths were kept at 3 nm and 4 nm, respectively. All samples were prepared in 30 mM phosphate buffer (pH 6.0–8.2) or 30 mM acetate buffer (pH 5 and 5.5) containing 100 mM NaCl. All samples were excited at 330 nm.

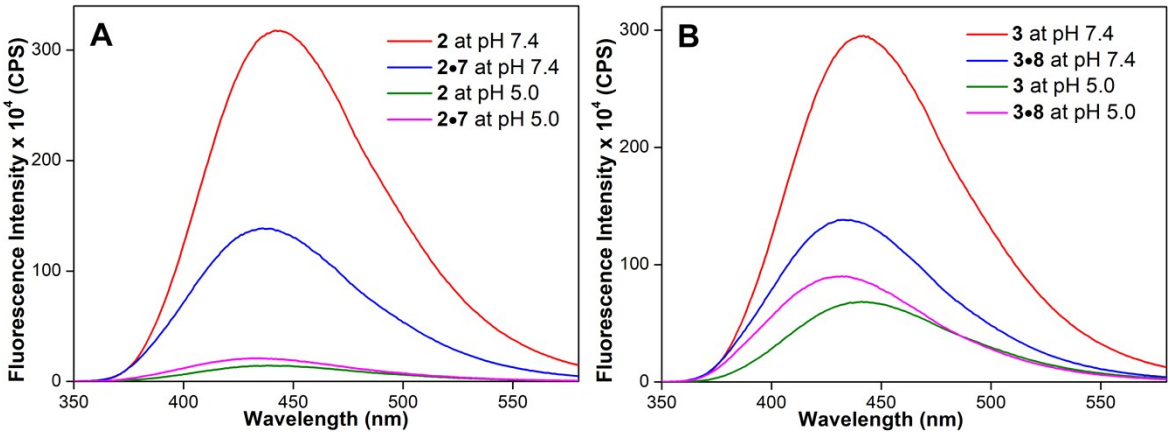

Fig. S7 Fluorescence spectra (1 μM) of benzofuran-modified C-rich DNA ON **2** and H-Telo DNA ON **3** and corresponding hybrids with complementary ONs at basic and acid pH. All samples were excited at 330 nm. Excitation and emission slit widths were kept at 3 nm and 4 nm, respectively.

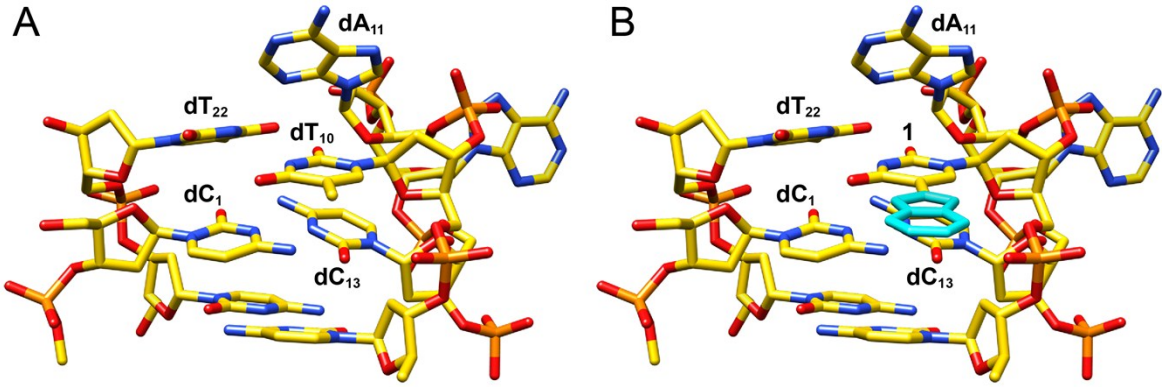

Fig. S8 (**A**) The conformation of dT_{10} residue in the iM structure of H-Telo DNA repeat is shown (PDB: 1EL2). For clarity, hydrogen atoms have been omitted and iM core associated with the second loop is only shown. The dT_{10} residue is stacked between the iM core and dA_{11} residue.[4] (**B**) A schematic diagram showing the possible conformation of the emissive nucleoside **1** (in place of dT_{10} residue). Benzofuran ring is shown in cyan color. In this conformation, the nucleoside analog also should experience similar stacking interaction with adjacent bases as that of the dT_{10} residue in the native iM structure. This stacking interaction between the emissive base and adjacent bases in the iM structure could be the possible reason for fluorescence quenching.

Table S5 T_m values of control unmodified (**7**, **8**), modified (**6**) G-rich DNA ONs and C-rich-G-rich DNA hybrids (**2•7, 3•8** and **6•5**) at different pH.

G-rich DNA ON	T_m (°C) pH 7.4	T_m (°C) pH 5.0	C-rich-G-rich DNA hybrid	T_m (°C) pH 7.4	T_m (°C) pH 5.0
7	73 ± 0.2	73 ± 0.5	**2•7**	77 ± 0.8	76 ± 0.8
8	63 ± 0.6	62 ± 1.1	**3•8**	68 ± 0.3	62 ± 1.4
6	59 ± 1.2	58 ± 1.0	**6•5**	67 ± 0.9	62 ± 0.6

Thermal melting of ONs **7**, **8** and **6** gave a typical reverse sigmoidal profile at 295 nm for a GQ structure, which was not affected by changes in pH.

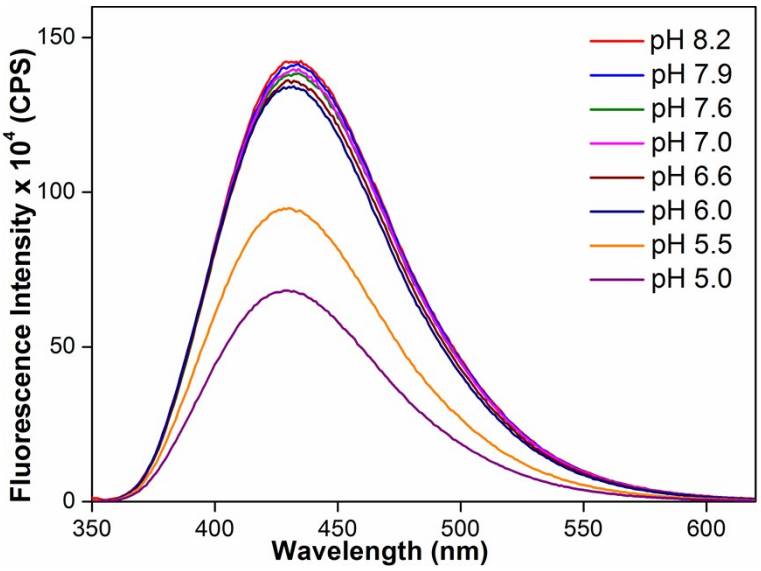

Fig. S9 Fluorescence spectra (1 μM) of non-iM-forming benzofuran-modified control DNA ON (5′ GCGATCAC**1**CACTAGCG 3′), where benzofuran-modified nucleoside **1** is flanked in-between C-residues. All samples were prepared in 30 mM phosphate buffer (pH 6.0–8.2) or 30 mM acetate buffer (pH 5 and 5.5) containing 100 mM NaCl. All samples were excited at 330 nm. Excitation and emission slit widths were kept at 2 nm and 3 nm, respectively. At pH 5.5 and 5.0 a decrease in fluorescence intensity was observed, which is not as dramatic as in the case of i-motif forming ON sequences **2** and **3**. Compare with Fig. 3A and 4A.

4. References

1. (*a*) A. A. Tanpure and S. G. Srivatsan, *ChemBioChem*, 2012, **13**, 2392–2399; (*b*) A. A. Tanpure and S. G. Srivatsan, *Nucleic Acids Res.*, 2015, **43**, e149.
2. (*a*) S. Modi, A. H. Wani and Y. Krishnan, *Nucleic Acids Res.*, 2006, **34**, 4354–4363; (*b*) A. L. Lieblein, J. Buck, K. Schlepckow, B. Furtig and H. Schwalbe, *Angew. Chem. Int. Ed.*, 2012, **51**, 250–253.
3. S. Manna, C. H. Panse, V. A. Sontakke, S. Sangamesh and S. G. Srivatsan, *ChemBioChem*, 2017, **18**, 1604–1615.
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