Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Multiplex Fluorophore Systems on DNA with New Diverse Fluorescence Properties and Ability to Sense Hybridization Dynamics

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Experimental Methods

Solid-phase oligonucleotide synthesis

The phosphoramidite ^{Py}**A** was introduced as a building block to produce fluorescent oligodeoxynucleotides (ODNs) on a controlled-pore glass (CPG) solid support, using a standard phosphoramidite approach and an automated DNA synthesizer (POLYGEN DNA-Synthesizer). For comparison, unmodified ODNs were also prepared. The synthesized oligonucleotides were cleaved from the solid support after treatment with 30% aqueous NH_4OH (1.0 mL) for 10 h at 55 °C. The crude products from the automated ODN syntheses were lyophilized and diluted with distilled water (1 mL). The ODNs were purified using high-performance liquid chromatography (HPLC; Merck LichoCART C18 column; 10 × 250 mm; 10 μm; pore size: 100 Å). The HPLC mobile phase was isocratic for 10 min (5% MeCN/0.1 M triethylammonium acetate (TEAA) (pH 7.0)) at 2.5 mL/min. The gradient was linearly increased over 10 min from 5 to 50% MeCN/0.1 M TEAA at the same flow rate. The fractions containing the purified ODN were cooled and lyophilized. Subsequently, 80% aqueous AcOH was added to the ODN. After 1 h at ambient temperature, the AcOH was evaporated under reduced pressure. The residue was diluted with water (1 mL); this solution was purified via HPLC using the same conditions as described above. All ODNs were characterized using MALDI-TOF mass spectrometry.

DNA sample preparation for fluorescence and UV spectroscopy experiments

The fluorescence emission spectra for the ODNs were collected with a 368 nm excitation at 20 °C using a quartz cuvette (path length: 1 cm) on a Varian Cary Eclipse spectrometer. The UV spectra were recorded using a Cary 100 Conc UV-Vis spectrophotometer (Varian) and a quartz cell (path length: 1 cm).

Circular dichroism (CD) measurements

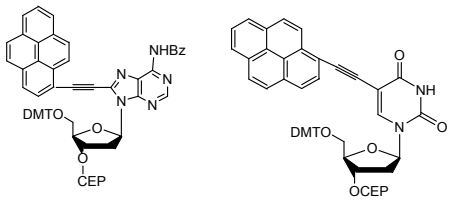
The CD spectra of the ODNs were recorded using a JASCO J-810 spectropolarimeter equipped with a temperature controller. For each sample, five spectral scans were accumulated over wavelengths from 225 to 325 nm at 20 °C. The samples for each ODN were prepared at 0.1 μM in 100 mM Tris-HCl buffer with different pH values.

Table S1 MALDI-TOF mass spectral data.

Sequence	*Calcd. (m/z)*	*Found (m/z)*
MFD1	*5586*	*5587*
MFD2	*6038*	*6038*
MFD3	*6038*	*6043*
MFD4	*6314*	*6314*
MFD5	*5837*	*5837*
MFD6	*6264*	*6263*
MFD7	*5807*	*5808*
MFD8	*6033*	*6035*
MFD9	*6033*	*6034*
MFD10	*6234*	*6234*

Table S2 Oligonucleotide sequences and the structures of **A**^{py} and **U**^{py}

S1	5'd-	**U**^{py}	C**A**^{py}T	TCA	GAG	TGT	CCA
S2	5'd-	**U**^{py}	C**A**^{py}T	**U**^{py}CA	GAG	TGT	CCA
S3	5'd-	**U**^{py}	C**A**^{py}T	**U**^{py}C**A**^{py}	GAG	TGT	CCA
H1	5'd-		TGG	ACA	CTC	TGA	A**U**^{py}G
H2	5'd-		TGG	ACA	CTC	TG**A**^{py}	A**U**^{py}G
H3	5'd-		TGG	ACA	CTC	**U**^{py}G**A**^{py}	A**U**^{py}G



A^{py}

U^{py}

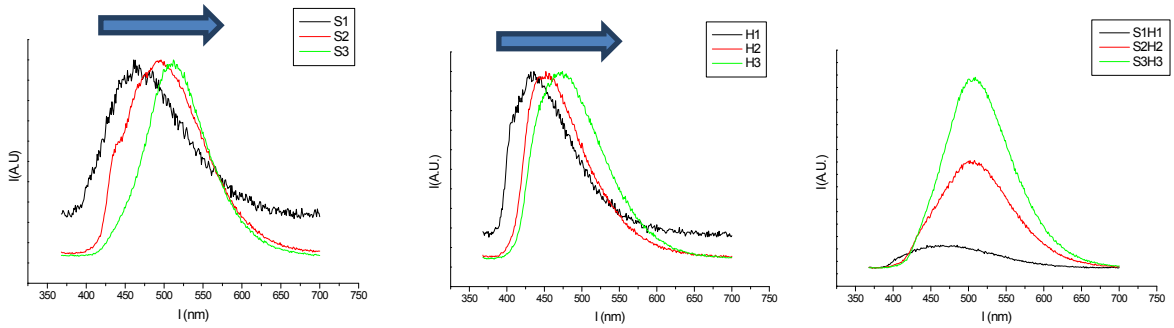


Figure S1 Normalized fluorescence spectra of (A) **ODN Sn**, (B) **ODN Hn**. (C) Fluorescence spectra of **ODN Sn:Hn** duplex, All DNA samples were prepared at 1.0 μM in 100 mM Tris-HCl buffer at 20 °C; fluorescence emission spectra were recorded with a 386 nm excitation.

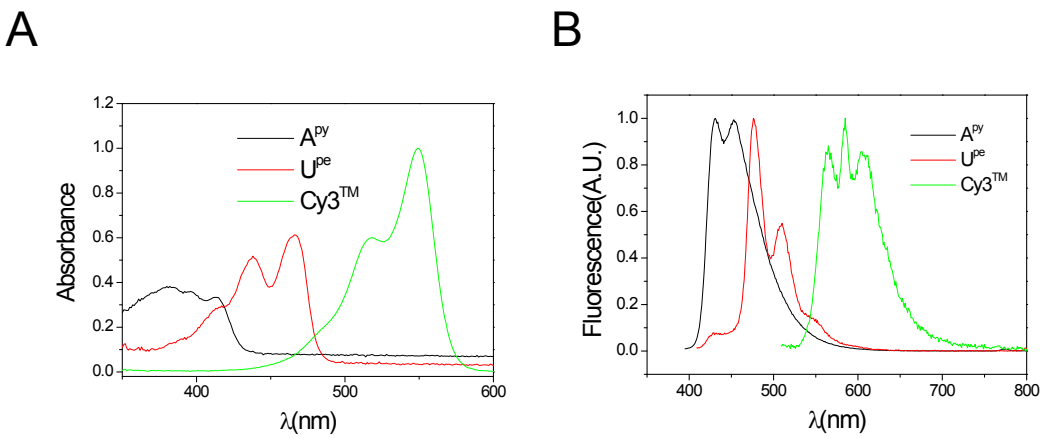


Figure S2 A. Normalized absorption and B. Normalized fluorescence spectra for the A^{py}, U^{pe}, and Cy3™ fluorophores. All fluorophores were prepared in 1 μM solutions at 20 °C; the fluorescence emission spectra were recorded with a 386 nm excitation.

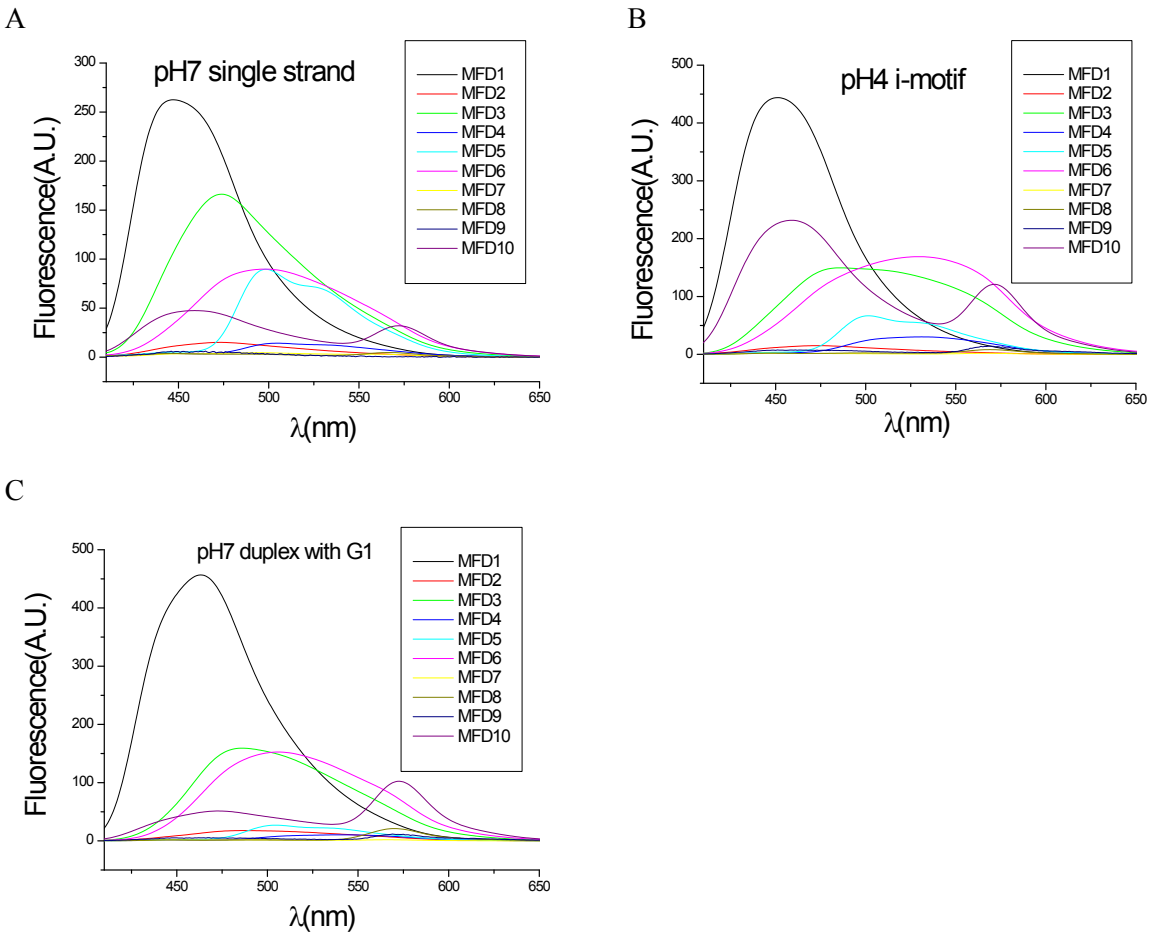


Figure S3 (A–C) Fluorescence spectra for all (A) single stranded ODNs **(MFD1-MFD10)** at pH 7, (B) i-motif structures of the ODNs(**MFD1-MFD10**) at pH 4, (C) duplexes with the G-quadruplex sequences of the ODNs (**MFD1-MFD10**) at pH 4. All DNA samples were prepared at 0.1 μM in 100 mM Tris-HCl buffer at 20 °C.

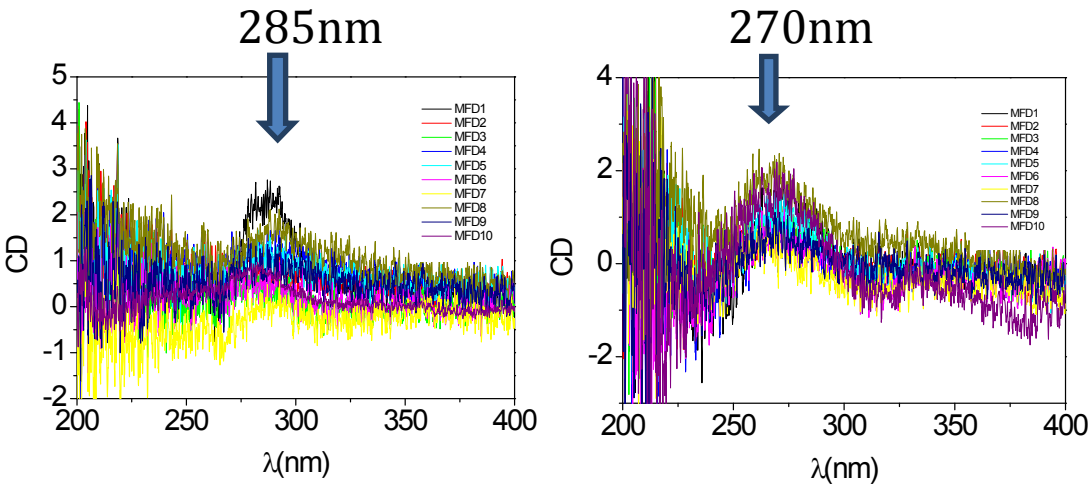
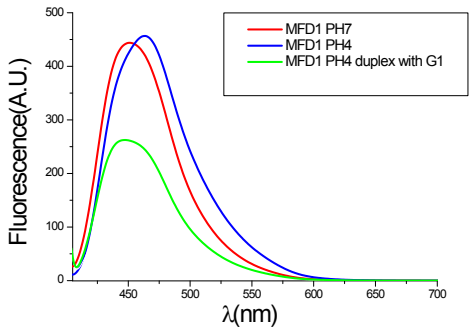


Figure S4 CD spectra of all (A) the i-motif structures of the ODNs (**MFD1-MFD10**) at pH 4 and (B) duplexes with the G-quadruplex sequences of the ODNs (**MFD1-MFD10**) at pH 4. All DNA samples were prepared at 0.1 μM in 100 mM Tris-HCl buffer at 20 °C.

1.



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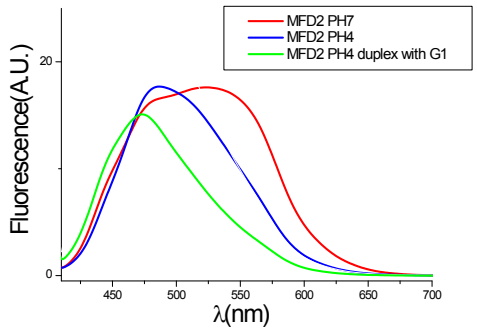

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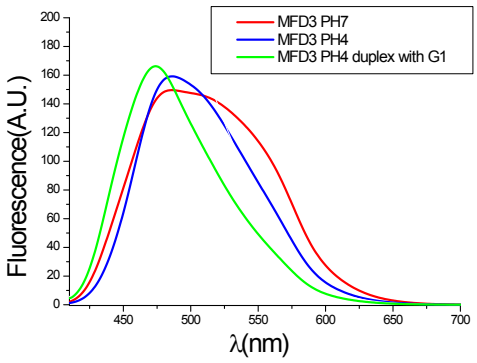

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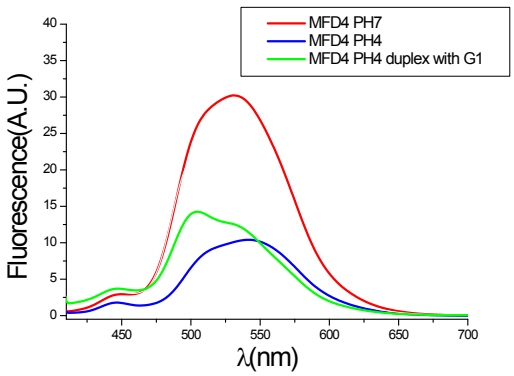

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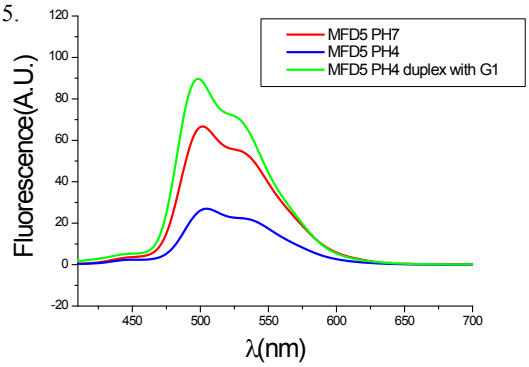

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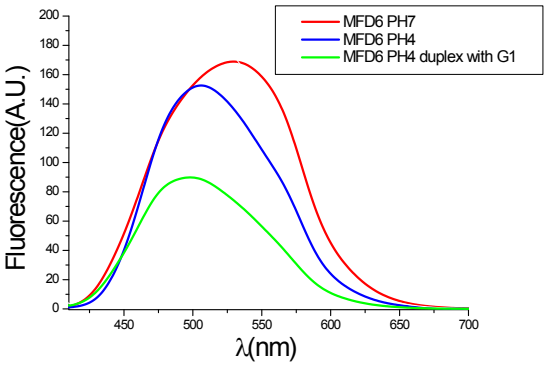

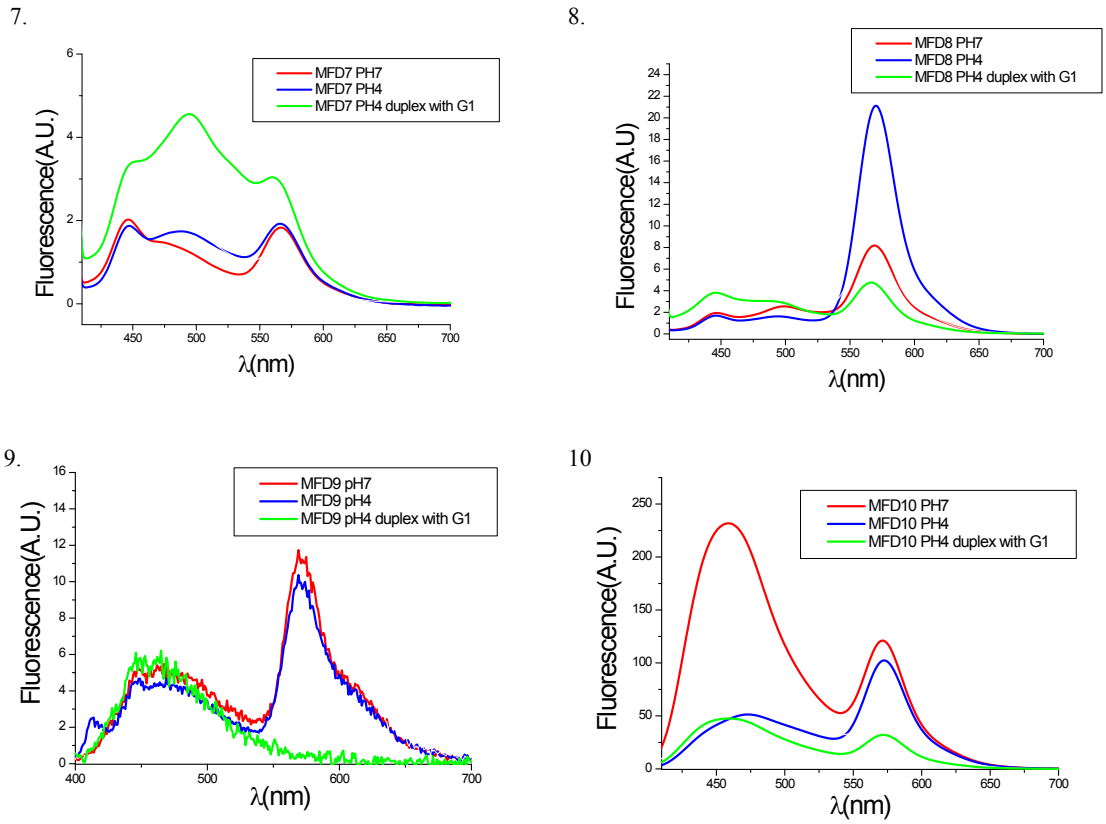

Figure S5 Fluorescence spectra for each MFD sequences (**MFD1-MFD10**). All DNA samples were prepared at 0.1 μM in 100 mM Tris-HCl buffer at 20 °C.

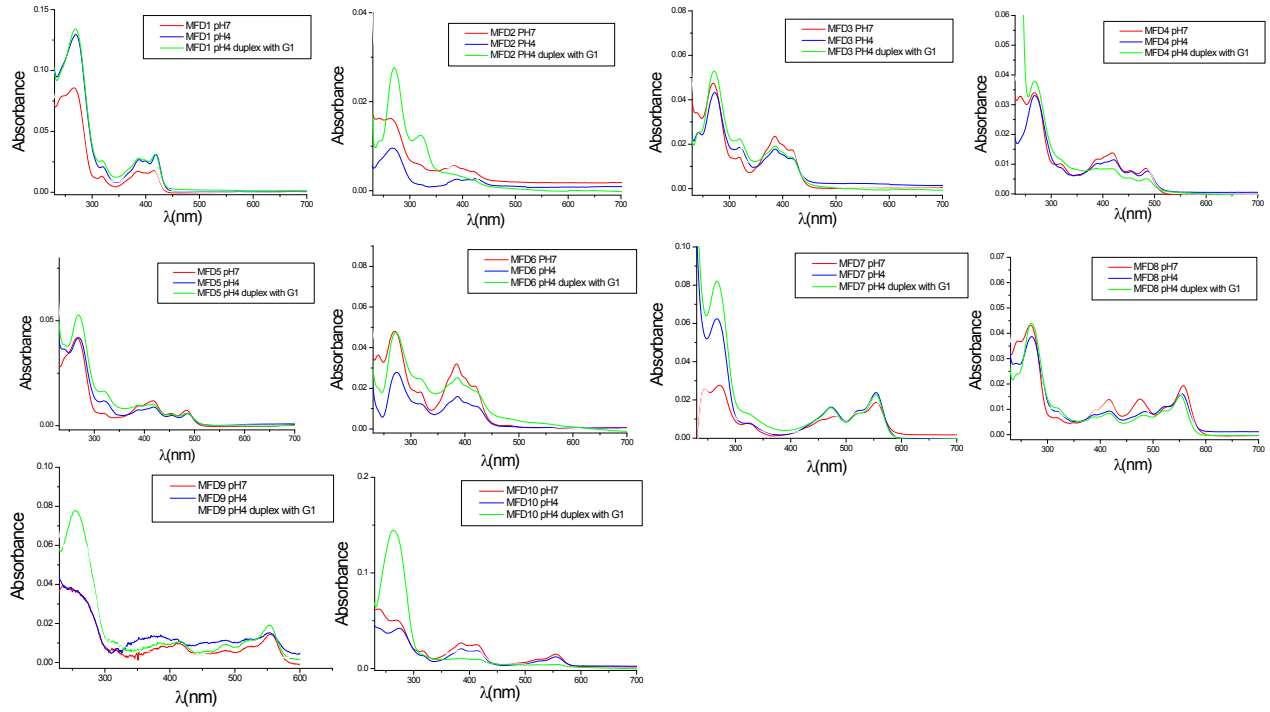

Figure S6. UV absorbance spectra for each MFD sequences (**MFD1-MFD10**). All DNA samples were prepared at 0.1 μM in 100 mM Tris-HCl buffer at 20 °C.

Designed sequence: R1 5'- A^{py}U^{pe}Cy3^{TM}A AAA

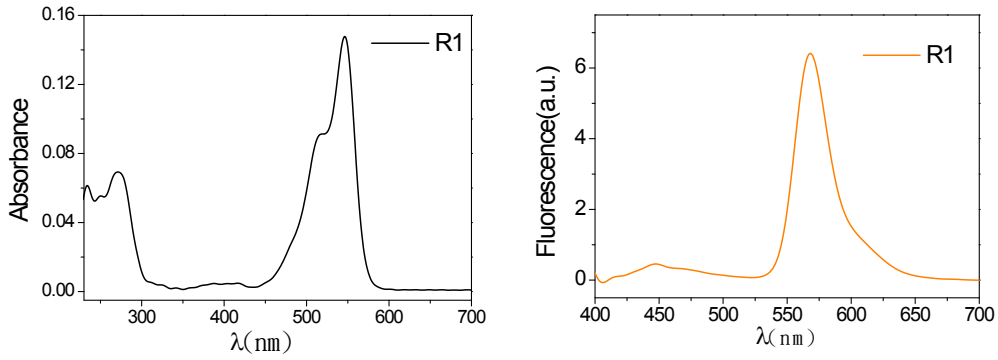

Figure S7) Absorption and fluorescence spectra of R1 sequence. DNA sample was prepared at 0.5 µM in 100 mM Tris-HCl buffer at 20 °C. The fluorescence spectrum was recorded with an excitation at 386 nm.

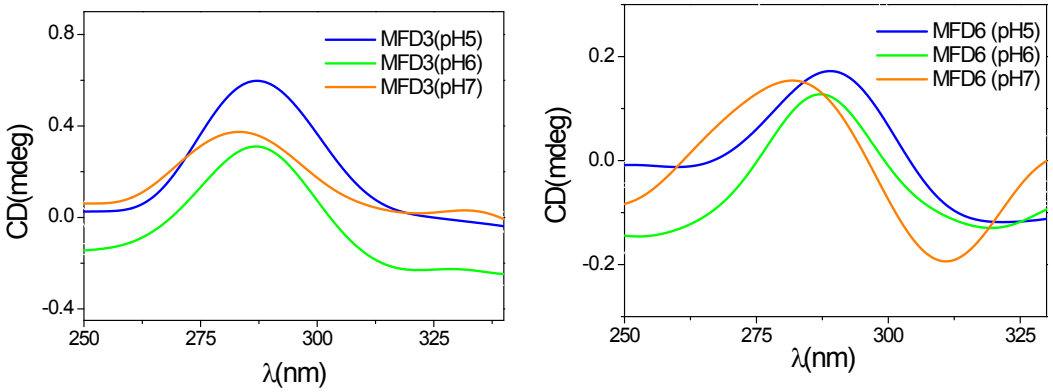

Figure S8) Circular Dichroism data of MFD 3 and MFD 6 depending on a pH. DNA samples were prepared at 0.5 µM in 100 mM Tris-HCl buffer at 20 °C.

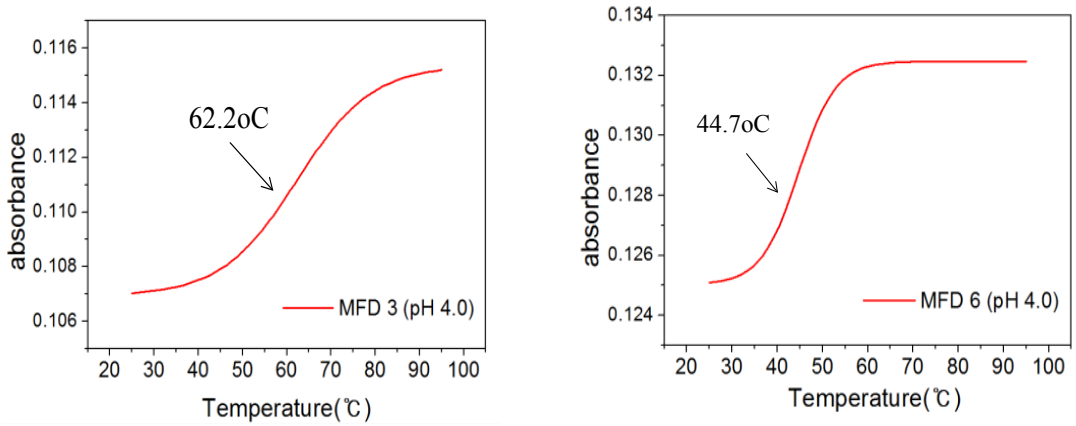

Figure S9) Melting temperature of MFD 3 and MFD 6 at pH 4.0. All ODN samples were prepared at a concentration of 0.5µM in 1 00 mM Tris-HCl buffer and irradiated at 260 nm.

Designed sequences

m1 5'-A^{py}A^{py}U^{T}-GCU GAG AAG TTA GAA CCT ATG CTC AGC-E

m2 5'-A^{py}U^{F}U^{T}-GCU GAG AAG TTA GAA CCT ATG CTC AGC-E

m3 5'-U^{F}U^{T}A^{py}-GCU GAG AAG TTA GAA CCT ATG CTC AGC-E

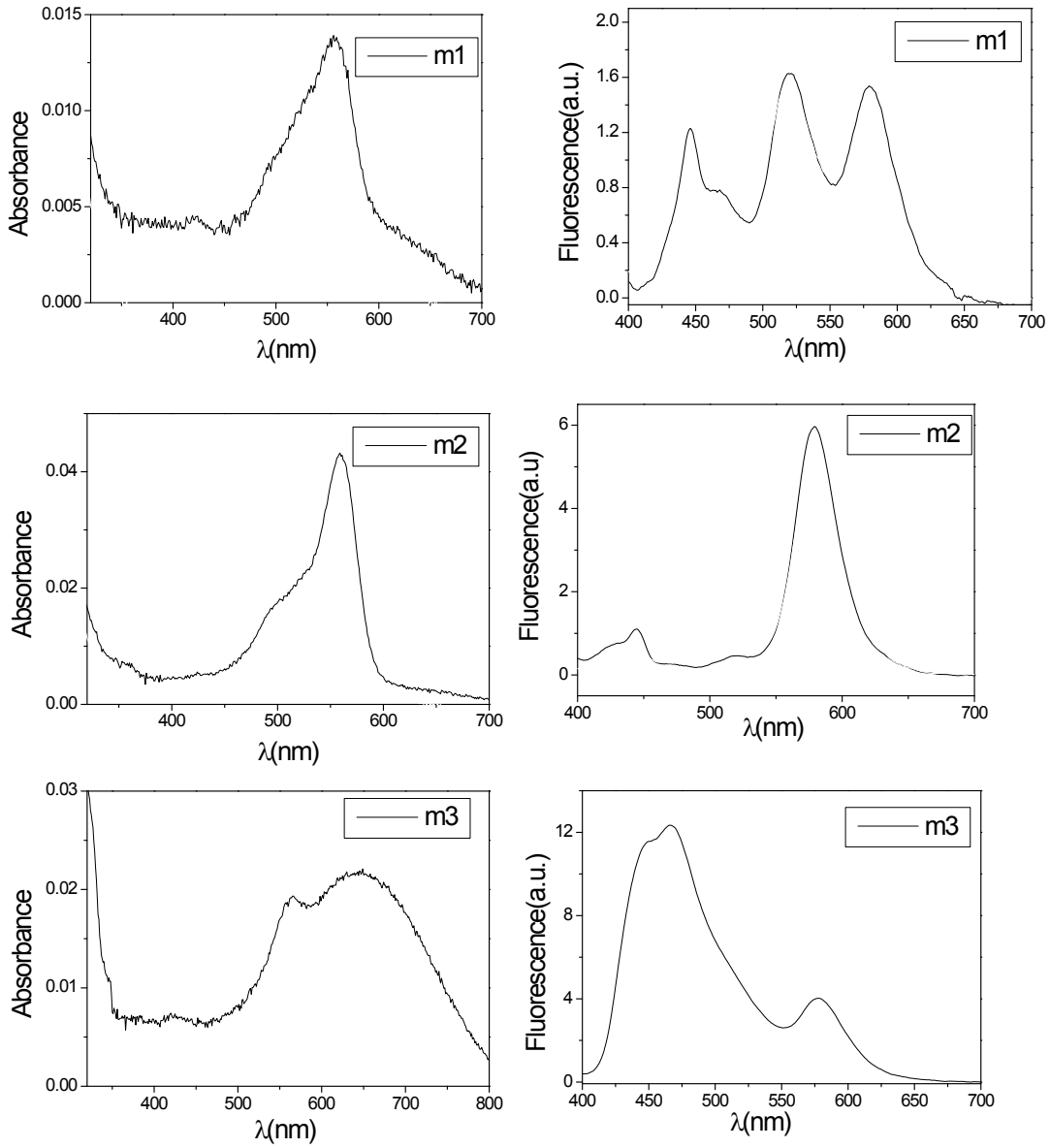

Figure 10) Absorption and fluorescence spectra of m1, m2, and m3 sequence. DNA sample was prepared at 0.5 µM in 100 mM Tris -HCl buffer at 20 °C. The fluorescence spectrum was recorded with an excitation at 386 nm. U^{T}: Tamra attached deoxyuridine, U^{F}: Fluorescein attached deoxy uridine at 5 position.