Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information for

Single Thiazole Orange Forms Exciplex in DNA i-motif

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Experimental

Materials and Methods. All the chemicals were purchased from Aldrich, Alfa Aesar or Merck. Standard reagents and CPG columns (1 μmol) for DNA syntheses were purchased from Glen Research. All of the reagents and chemicals were used as purchased.

Samples used for CD, fluorescence, lifetime decay and static UV measurements were 1μM of TO-DNA in 20 mM sodium cacodylate, 180 mM NaCl, pH 5.0 or 7.3 buffer or 1 μM duplex DNA in 20 mM tris-Cl, pH 7.3 buffer.

CD spectra were recorded on a Jasco J-810 CD Spectropolarimeter equipped with a temperature controller with the scan speed of 200 nm/min. Each measurement was an automatic average of 10 repeated scans at 20 ^{o}C.

Static state fluorescence spectra were recorded on Cary Eclipse fluorescence spectrophotometer equipped with a temperature controller. Fluorescence emission spectra were obtained at 20 ^{o}C with excitation wavelength at 490 nm and excitation spectra were recorded with emission wavelength at 620nm. Quantum yields (Φ_F) of thiazole orange (TO) labeled DNA were determined by fluorescent peak area from 500 nm to 700 nm and calculated with reference to free TO upon intercalating to ctDNA at 25 ^{o}C (Φ_F = 0.11).[1] Dissociation constants of i-motif (*p*Ka) were obtained by fitting CD intensity at 286 nm or fluorescent intensity at 625 nm against pH to sigmoidal curves. The standard deviations of the data were calculated over at least three sets of experimental repeats.

Cycling of exciplex formation and dissociation was monitored by the fluorescence intensity at 580 nm as well as the CD intensity at 286 nm. Stock solution of **TO-cHT22** was dissolved in a buffer solution (450 mL) containing 20mM sodium cacodylate, 180mM NaCl at pH5.0 to yield a final concentration of 1μM in a 10 mm $\times$ 4 mm quartz fluorescence cuvette. The pH value of the buffer was cycled between 7.3 and 5.0 by alternatively adding 7.2 μmoles NaOH and HCl at 20 ^{o}C. After each addition of acid or base, **TO-cHT22** sample was incubated at 20 ^{o}C for 5 min before the measurement to allow the completion of exciplex formation and dissociation. The fluorescence intensities at 580 nm and CD intensities at 286 nm were corrected by DNA concentration due to the dilution of adding acids and bases.

The time-resolved fluorescence lifetime was measured using a time-correlated single photon counting (TCSPC) spectrofluorimeter (FluoroCube, Horiba Jobin Yvon) equipped with a pulsed diode laser (NanoLED-470L, Horiba Jobin Yvon). The samples were excited at 466 nm and the emissions were monitored at 580 nm for TO exciplex at pH 5.0 and at 530 nm for TO monomer at pH 7.3 and in the duplex DNA. All measurements were performed at ambient conditions. The fluorescence decay profiles were analyzed using the Horiba Jobin Yvon Datastation software with consideration of the reduced chi-square value and the randomness of the weighted residuals. All parameters were achieved by fitting the fluorescence decay curves to multiple exponential decay function with χ^2 values around 1. The mean fluorescence lifetime was calculated according to the equation as, $\tau = \Sigma\tau_i A_i$.

Preparation of DNA.

Unmodified DNA oligonucleotides, **HT22** and **cHT22** (sequences shown in Table S1) with HPLC grade of purity were purchased from Sangon (Shanghai, China) and used as received without further purification. The strand concentration in 5 mM tris-Cl, pH 7.3 buffer was determined by the UV absorbance at 260 nm on a Cary100 UV-Visible Spectrophotometer at room temperature. TO modified DNA sequences were synthesized with standard solid phase phosphoramidite chemistry on a MerMade-4 DNA synthesizer. TO phosphoramidite was synthesized according to the reported protocol.[2,3] The incorporation of normal bases followed standard protocol, while the incorporation of TO building block was performed with 4 couplings of 18 minutes incubation each time. After synthesis, the trityl-off oligonucleotide was cleaved from the resin and deprotected by treatment with 28% NH_4OH at room temperature for 24 hours. The modified oligonucleotides were purified by

HPLC equipped a Varian Dynamax 250 × 10 mm C18 reverse phase column using the following conditions: **A**= NH_4OAc buffer (50 mM), pH = 7; **B**= acetonitrile; gradient 0-35% **B** over 45 min with a flow rate of 3.5 ml/min. The eluent with both 260 nm and 500 nm absorption was collected (Figure S1). The oligonucleotide was lyophilized and quantified by the absorbance in 5 mM tris-Cl, pH7.3 buffer at 260nm at room temperature. The modified DNA was characterized by ESI-MS spectrometry.

The duplex of **TO-cHT22** were prepared by heating the 1:1 equivalent of **TO-cHT22** with the complementary strand, **HT22**, at 90 °C for 5 minutes in 20 mM tris-Cl pH 7.3 buffer followed by slowly cooling down to ambient temperature over 2 hours. The samples of **TO-cHT22** under various pHs were prepared by directly diluting the stock solution of **TO-cHT22** in 20 mM sodium cacodylate, 180 mM NaCl at various pHs.

Table S1. The oligonucleotide sequences used in this study and the corresponding molecular weights.

DNA	Sequence	Mw Found (calculated)[b]
HT22	5'-AGGGTTAGGGTTAGGGTTAGGG-3'	6966.8 (6966.5)
cHT22[a]	5'-CCCTA$_5$A$_6$CCCTAACCCT$_{16}$A$_{17}$A$_{18}$CCCT-3'	6503.9 (6504.3)
TO-cHT22	5'-CCCTAACCCTAACCC-**TO**-AACCCT-3'	6698.3 (6698.6)
5T	5'-CCCT-**T**-ACCCTAACCC-**TO**-AACCCT-3'	6689.6 (6689.6)
6T	5'-CCCTA-**T**-CCCTAACCC-**TO**-AACCCT-3'	6689.3 (6689.6)
17T	5'-CCCTAACCCTAACCC-**TO**-**T**-ACCCT-3'	6689.7 (6689.6)
18T	5'-CCCTAACCCTAACCC-**TO**-A-**T**-CCCT-3'	6688.9 (6689.6)

[a]: The subscript letters of **cHT22** annotate nucleobase positions in the sequence.

[b]: The calculated molecular weight is shown in the parentheses.

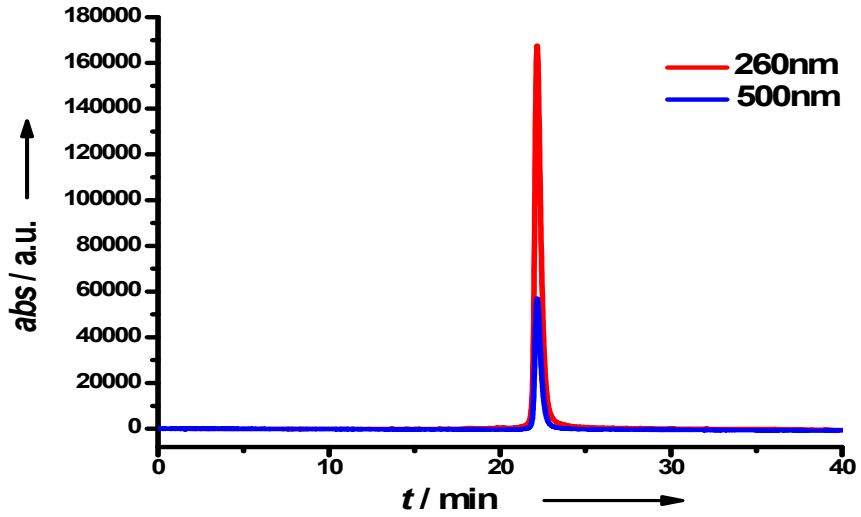

Figure S1. HPLC trace of **TO-cHT22**. Red curve is the UV absorption trace of **TO-cHT22** in 260 nm channel; blue curve is that of **TO-cHT22** in 500 nm channel.

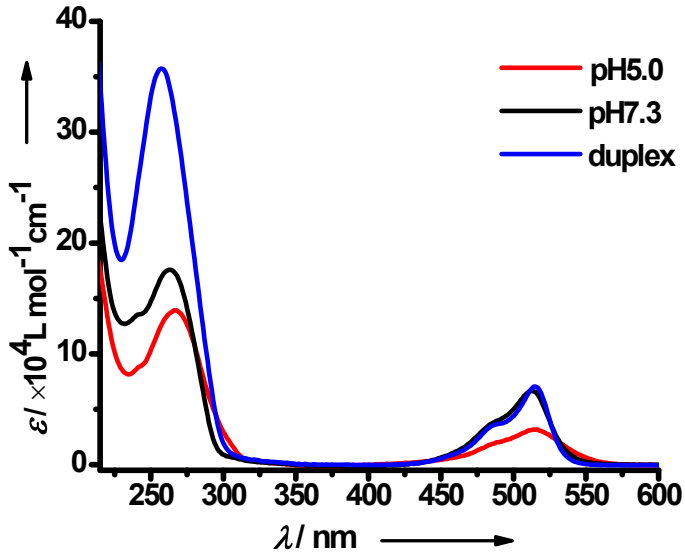

Figure S2. UV-vis spectra of **TO-cHT22** at pH 5.0 (red, i-motif structure), at pH 7.3 (black, random coil) and duplex (blue, **TO-cHT22** + **HT22**) at pH 7.3.

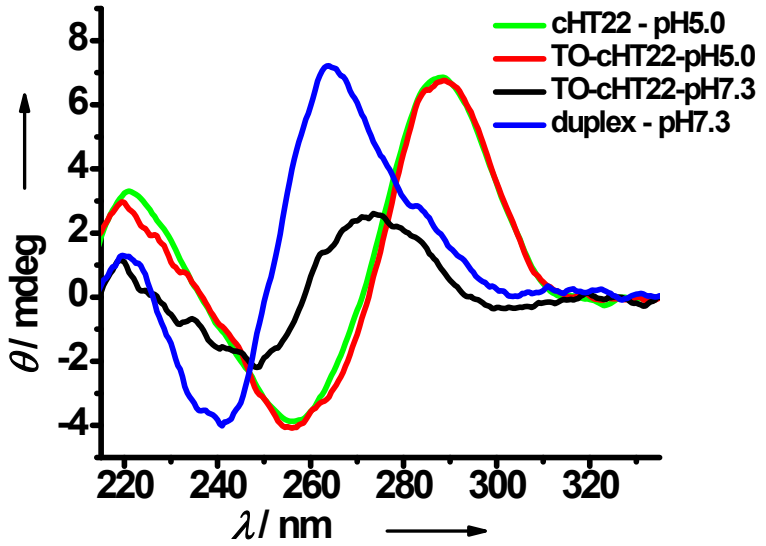

Figure S3. CD spectra of **cHT22** (green) and **TO-cHT22** (red) at pH 5.0 (i-motif structure), **TO-cHT22** at pH 7.3 (black, random coil) and duplex (blue, **TO-cHT22** + **HT22**) at pH 7.3.

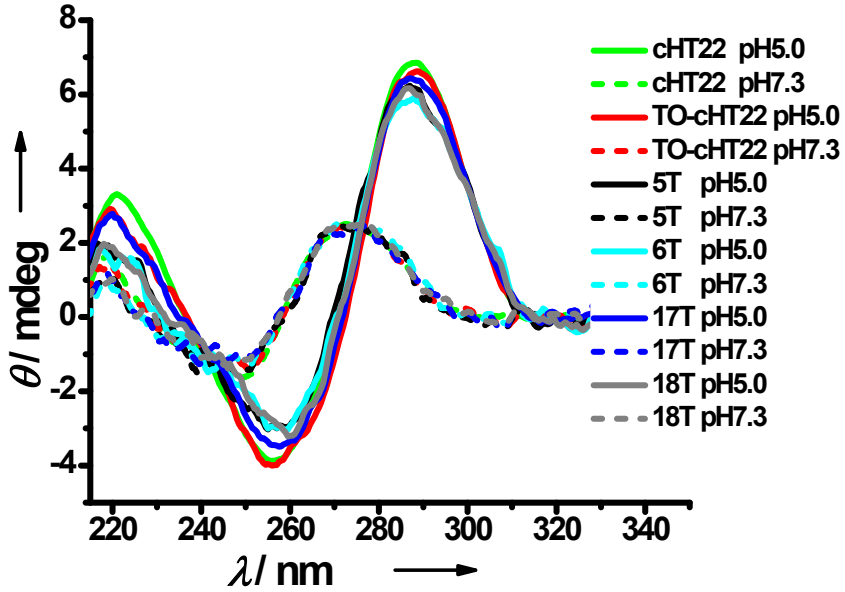

Figure S4. CD spectra of **cHT22** and T-substituted **TO-cHT22** analogues in i-motif structure at pH 5.0 (short dash) and random coil at pH7.3 (solid line).

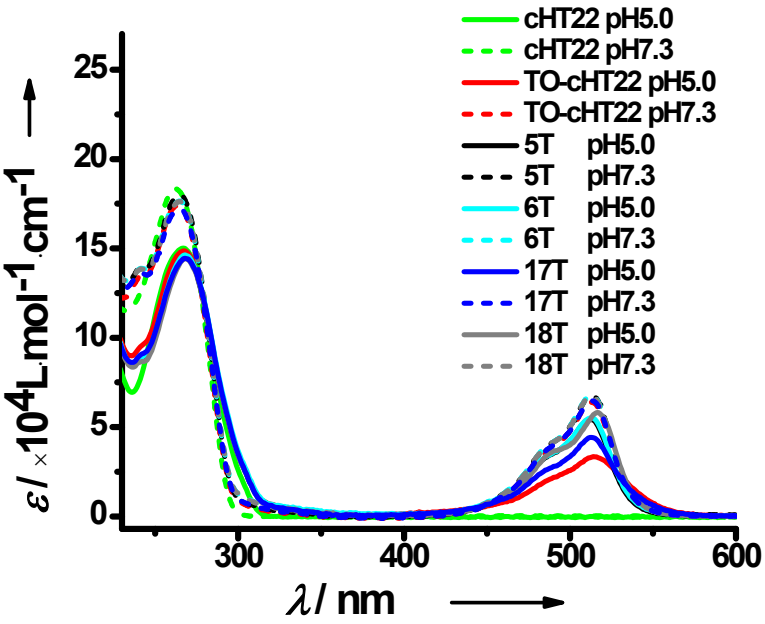

Figure S5. UV-vis spectra of **cHT22** and T-substituted **TO-cHT22** analogues in i-motif structure at pH 5.0 (short dash) and random coil at pH7.3 (solid line).

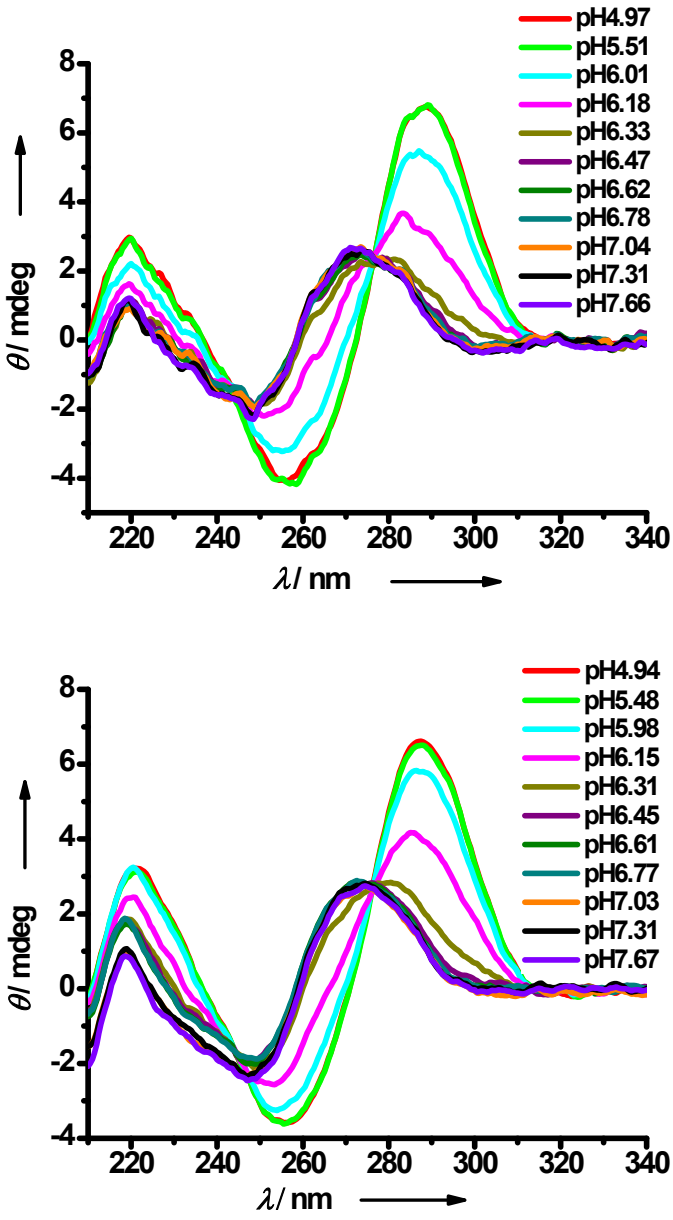

Figure S6. CD spectra of **TO-cHT22** (*upper)* and **cHT22** (*bottom*) upon pH titration.

References

(1) J. Nygren, N. Svanvik, M. Kubista, *Biopolymers* **1998**, *46*, 39-51.

(2) Y. Hara, T. Fujii, H. Kashida, K. Sekiguchi, X. G. Liang, K. Niwa, T. Takase, Y. Yoshida, H. Asanuma, *Angew. Chem., Int. Ed.* **2010**, *49*, 5502-5506.

(3) H. Asanuma, X. Liang, H. Nishioka, D. Matsunaga, M. Liu, M. Komiyama, *Nat. Protoc.* **2007**, *2*, 203-212.