Chelerythrine as a fluorescent light-up ligand for i-motif DNA structure

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Experiments:

Materials. All DNA oligonucleotides were obtained from Invitrogen (Beijing, China), purified by PAGE. The stock solution of the oligonucleotides (sequence information in Table S1) was prepared by directly dissolving the oligonucleotides in 10 mM PBS or PB buffer and annealing in a thermocycler (heating at 90 ℃ for 5 min and cooling down to room temperature slowly). Na_2HPO_4, NaH_2PO_4, K_2HPO_4, KH_2PO_4, $AgNO_3$ were all analytical grade, being purchased from Beijing Chemical Company. Natural isoquinoline alkaloids including chelerythrine (CHE), coptisine (COP), jatrorrhizine (JAT), sanguinarine (SAN) and stephania (STE) were obtained from Sinopharm (China). Ultrapure water, prepared by Milli-Q Gradient ultrapure water system (Millipore), was used in all experiments. The concentrations of DNA stock solutions were determined by measuring their absorbance at 260 nm.

Instruments. Ultraviolet (UV) spectra were measured on an Agilent 8453 UV-visible spectrophotometer at the wavelength range 190-1100 nm using a 1 cm path cell at room temperature (25^{o}C). Ultrapure water was used as reference.

Fluorescence spectra were acquired on a Hitachi F-4600 spectrophotometer in a 10-mm path length quartz cell at room temperature. In fluorescence measurement, xenon arc lamp was used as the excitation light source. The scan speed is 1200 nm/min. In melting assay, the sample was heated over the range of 25-91 °C at a rate of 2 °C/min.

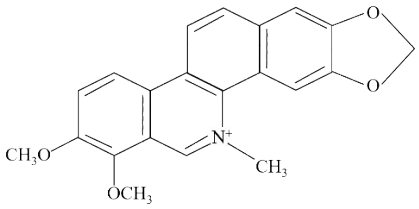
CD spectra were collected from 200 to 350 nm on a Jasco-815 automatic recording spectropolarimeter with a 1 cm path-length quartz cell at 25 ºC. Spectra were collected with scan speed of 500 nm/min. Each spectrum was the average of three scans. A solution containing no oligonucleotide was used as reference, and a buffer blank correction was made for all spectra. For CD melting experiments, all samples were heated over the range of 25-91 °C at a rate of 2 °C/min, and their CD spectra were recorded at 3 °C intervals.

The binding a□nity and stoichiometry. The data from the fluorimetric titrations were analyzed according to the independent-site model[1] by nonlinear fitting to eq 1,[2] in which F_0 is the integral fluorescence intensity of CHE in the absence of i-motif, where F_{max} is the fluorescence intensity upon saturation, $A = (K_a C_{CHE})^{-1}$ and $x = nC_{i\text{-}motif}(C_{CHE}\text{-}1)$, and n is the putative number of binding sites on a given DNA matrix. The parameters, Q and A, were found by Levenberg-Marquardt fitting routine in the Origin 9.0 software, whereas n was varied to obtain a better fit.

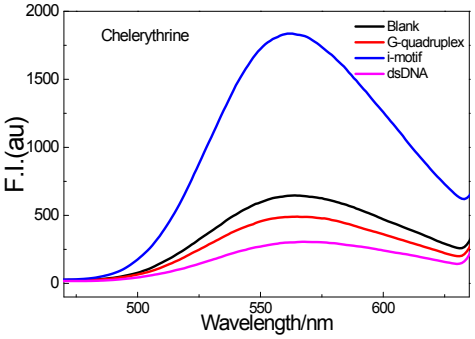
$$\frac{F}{F_0} = 1 + \frac{Q-1}{2}\left[A + 1 + x - \sqrt{(A+1+x)^2 - 4x}\right]$$

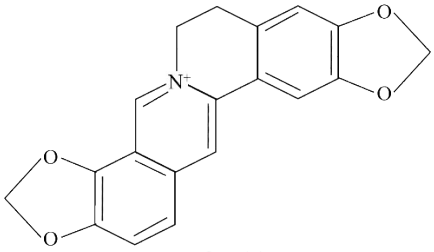
Table S1. Sequences of the oligonucleotides used in this study

Name	Sequence (from5' to 3')
I-motif	
AS1411-C	CCACCACCACCACAACCACCACCACCAAA
H22-C	CCCTAACCCTAACCCTAACCCT
H24-C	TCCCTAACCCTAACCCTAACCCAA
c-myc-C	CCTTCCCCACCCTCCCCACCCTCCCCA
ILPR	TGTCCCCACACCCCTGTCCCCACA
Rb	CCGCCCAAAACCCCCC
G-quadruplex	
Bcl-2	GGGCGCGGGAGGAATTGGGCGGG
AS1411	TTTGGTGGTGGTGGTTGTGGTGGTGGTGG
c-myc	AGGGTGGGGAGGGTGGGG
VEGF	GGGCGGGCCGGGGGCGGG
c-kit	AGGGAGGGCGCTGGGAGGAGGG
ds-DNA	
ds12	CGCGATATCGCG
ds20	CGAATTCGTCTCCGAATTCG
ds22	TTCGCGCGCGTTTTCGCGCGCG
ds26	CAATCGGATCGAATTCGATCCGATTG

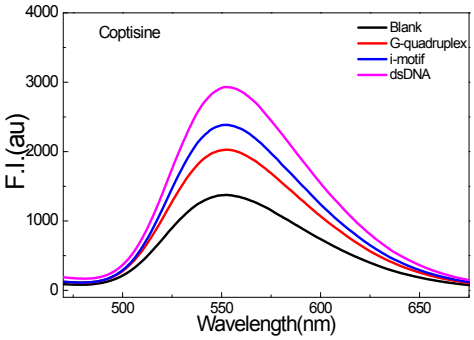


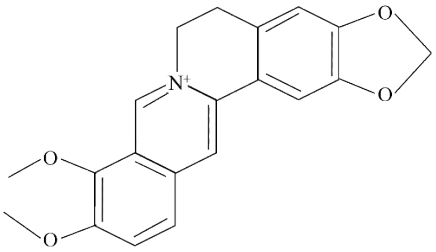
Chelerythrine



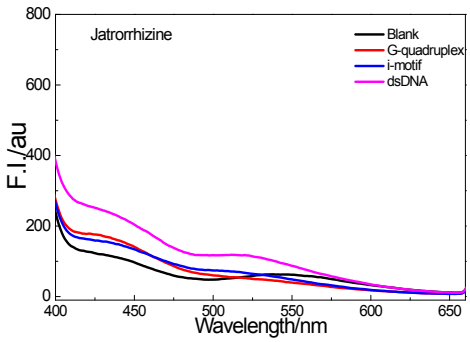


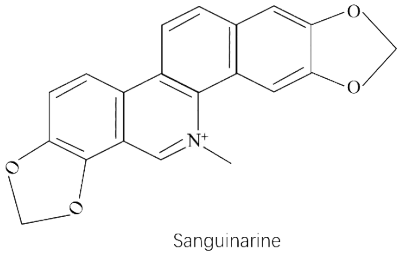
Coptisine



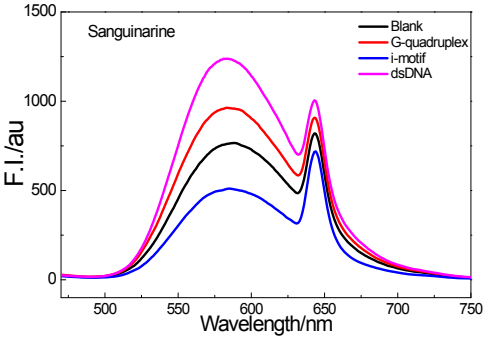


Jatrorrhizine





Sanguinarine



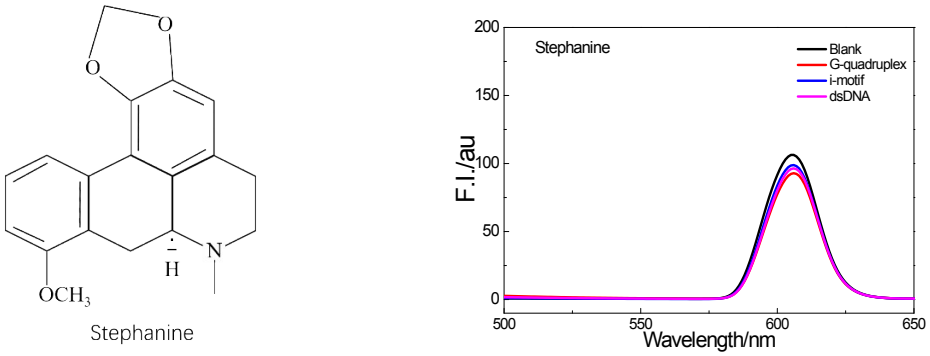


Fig.S1 Structure of isoquinoline alkaloids and fluorescence spectra of isoquinoline alkaloids (2 μM) with G-quadruplex, i-motif and dsDNA (2 μM). I-motif samples were measured in PB buffer solution (10 mM, pH 5.8), other samples were measured in PBS buffer solution (10 mM, pH 7.2). The excititation wavelength of chelerythrine (CHE), coptisine (COP), jatrorrhizine (JAT), sanguinarine (SAN) and stephanine (STE), is 320 nm, 350 nm, , 340 nm 320 nm and 300 nm.

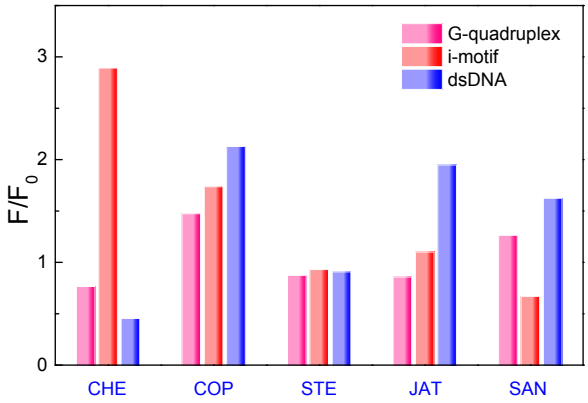


Fig.S2 Dependence of isoquinoline alkaloids (2 μM) fluorescence intensity with G-quadruplex, i-motif and dsDNA (2 μM). I-motif samples were measured in PB buffer solution (10 mM, pH 5.8), other samples were measured in PBS buffer solution (10 mM, pH 7.2).

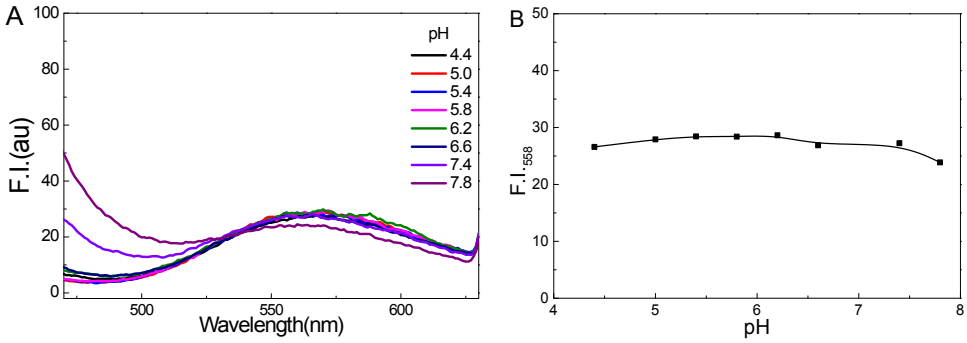
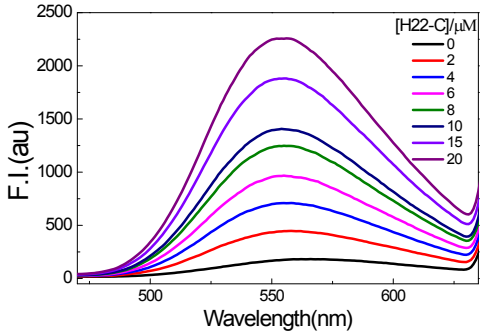
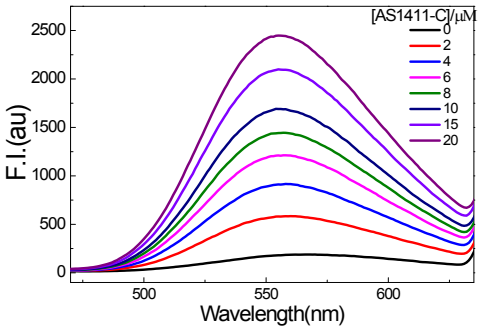
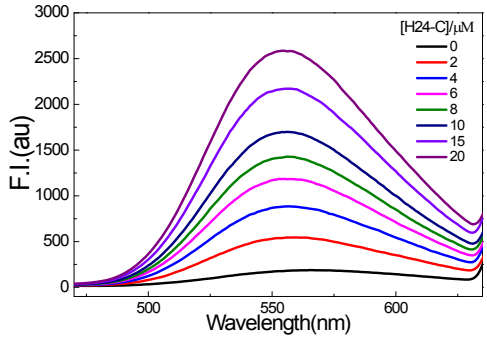


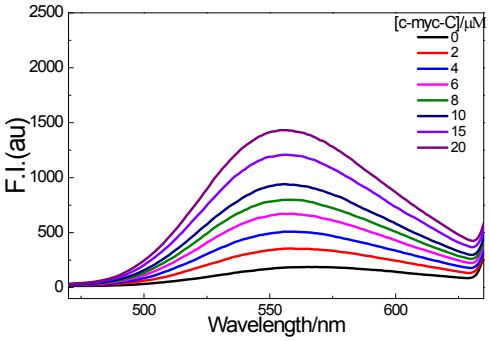
Fig.S3 (A) Fluorescence spectra of CHE (2 μM) in various pH and (B) fluorescence intensity of CHE at 558 nm in various pH. Samples were measured in PB buffer solution. The excititation wavelength of CHE is 320 nm.

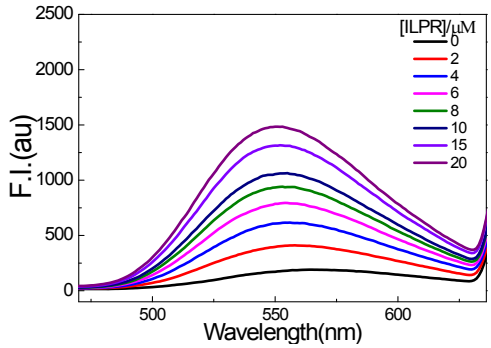
I-motif

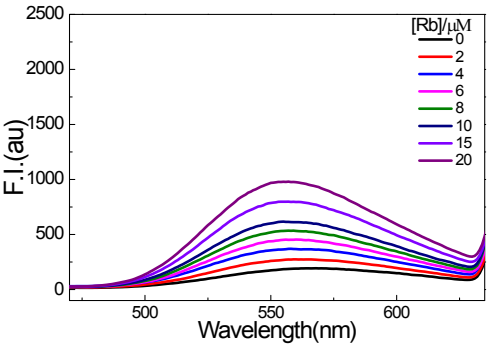




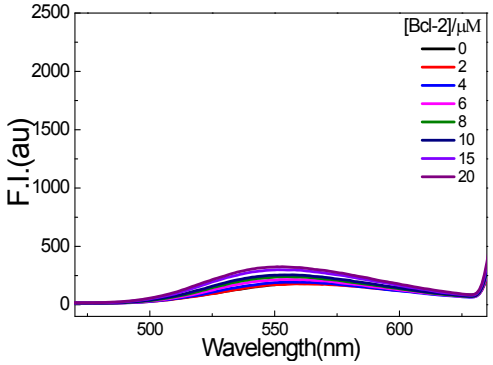


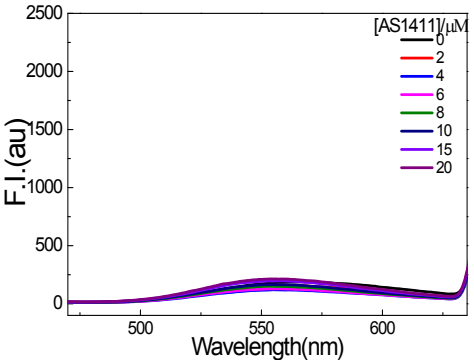


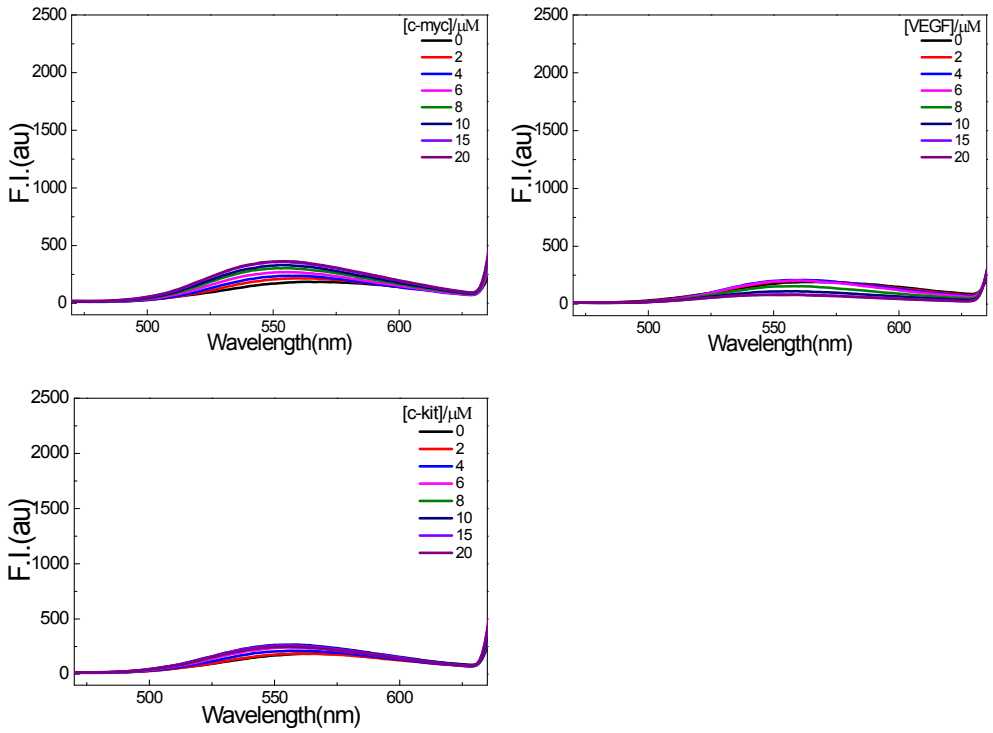




G-quaduplex:







dsDNA

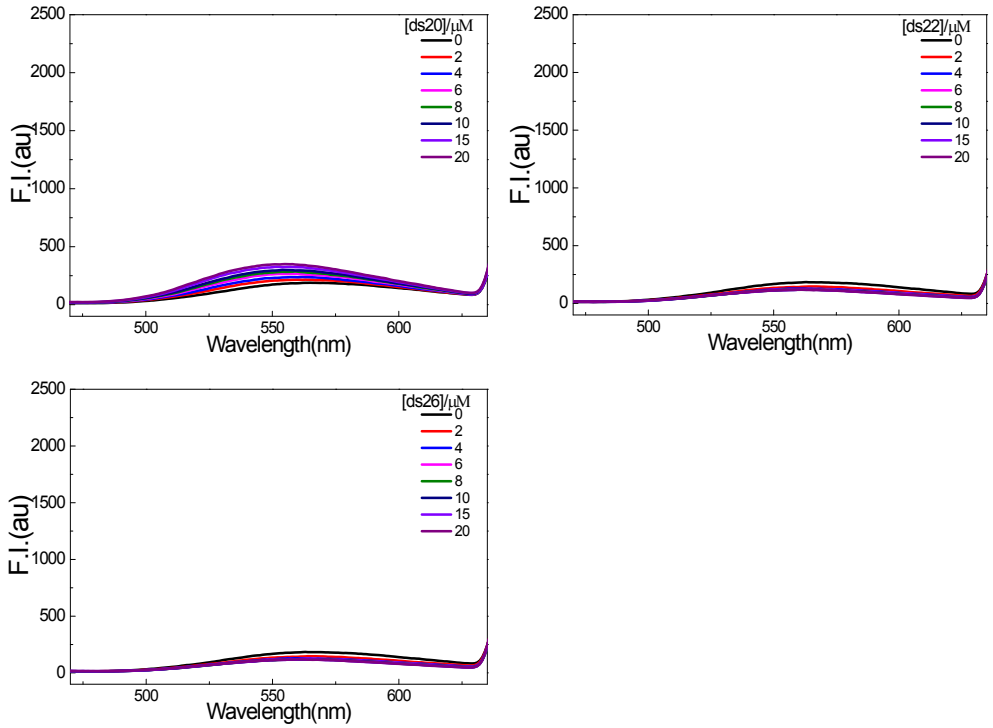


Fig. S4 Fluorescence spectra of CHE (5 μM) with increasing concentration of DNA. I-motif samples were measured in PB buffer solution (10 mM, pH 5.8), other samples were measured in PBS buffer solution (10 mM, pH 7.2).

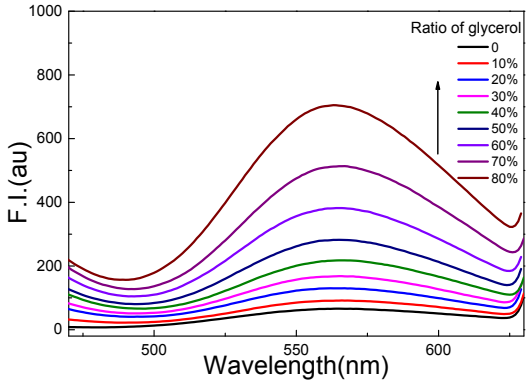


Fig. S5 Fluorescence spectra of CHE in different viscosity. Experimental conditions: [CHE] 2 μM.

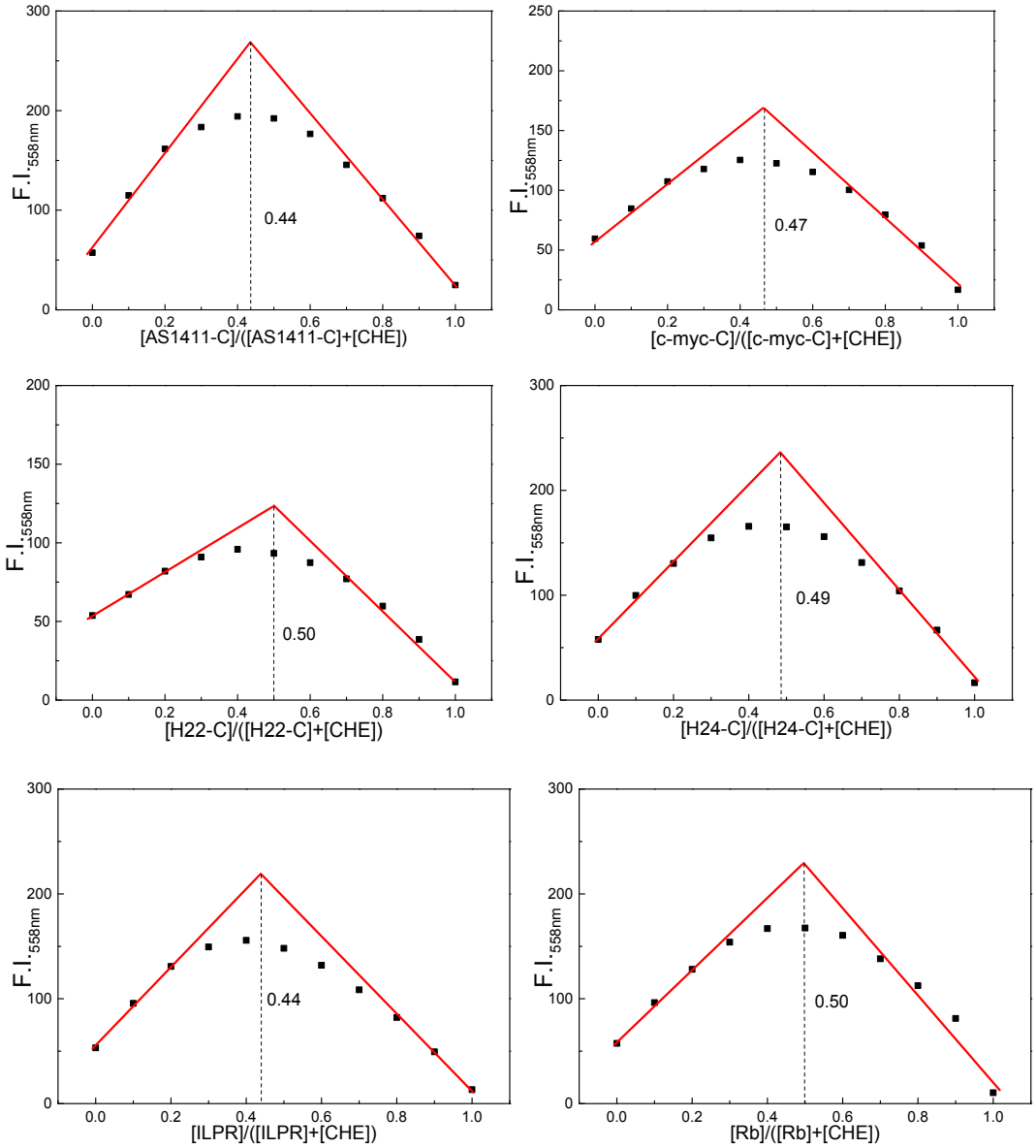


Fig. S6 Jop's Plot analysis of the stoichiometry of i-motif binding with CHE. The total concentration of i-motif and CHE is 2 μM, and samples were measured in PB buffer solution (10 mM, pH 5.8).

Table S2. The binding stoichiometry (n) and apparent binding equilibrium constants (K_a)

Name	n	$K_a(10^4\ M^{-1})$
AS1411-C	1	6.65
H22-C	1	1.92
H24-C	1	3.99
c-myc-C	1	1.94
ILPR	1	6.99
Rb	1	0.83

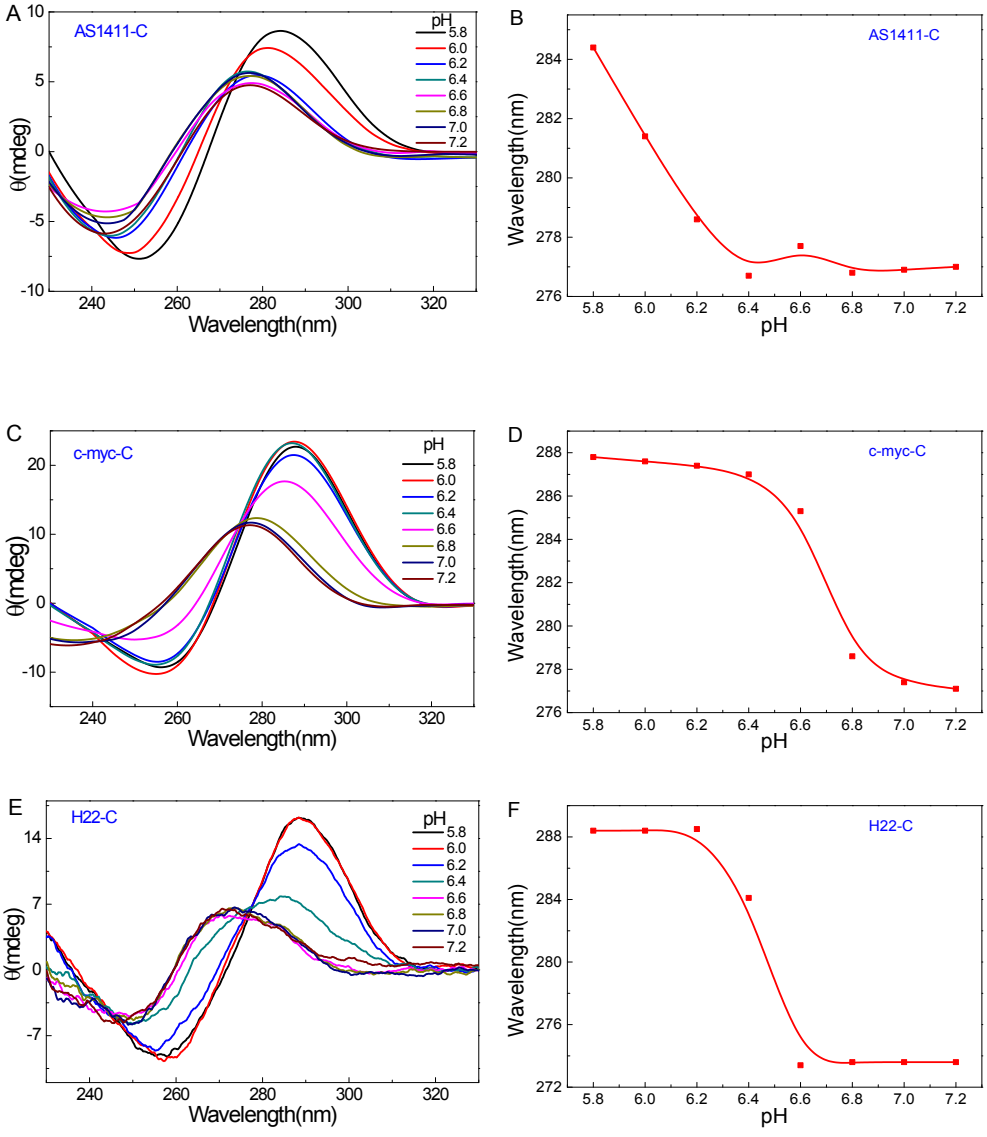


Fig. S7 (A) (C) (E) CD spectra of i-motif (4 μM) sequence with increasing pH. (B) (D) (F) The wavelength of the maximum CD intensity versus pH. Samples were measured in PB buffer solution (10 mM).

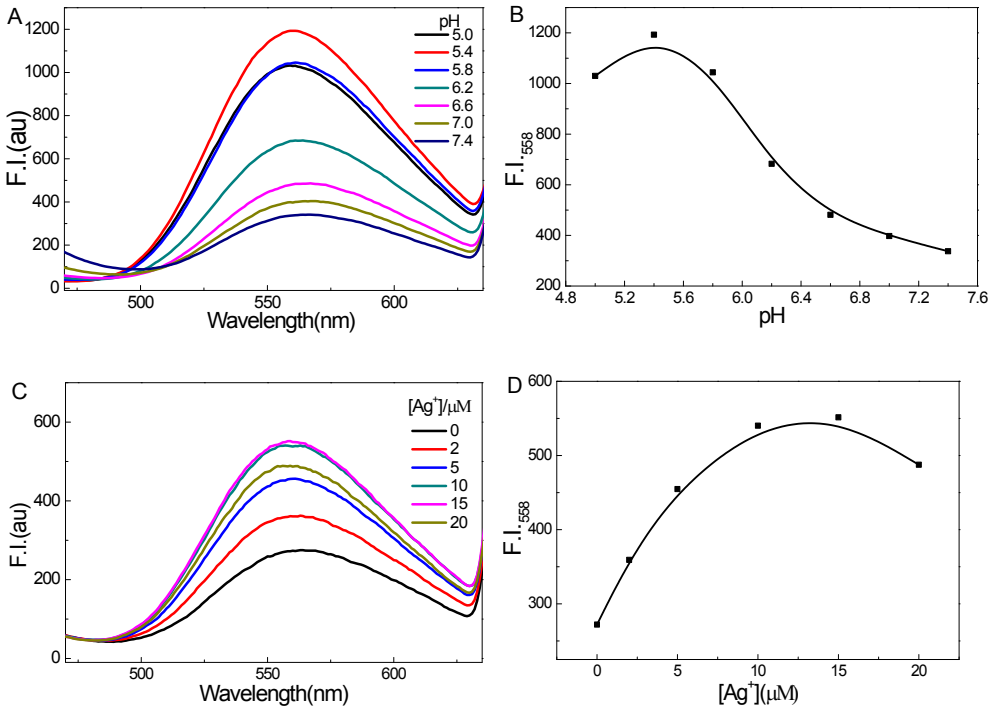


Fig. S8 Fluorescence spectra of CHE-AS1411-C complex in (A) various pH and (C) increasing concentration of Ag^+. Fluorescence intensity of CHE-AS1411-C complex at 558 nm in (B) various pH and (D) increasing concentration of Ag^+. Experimental conditions of (A) and (B): [CHE] 5 μM, [AS1411-C] 5 μM, [PB] 10 mM. Experimental conditions of (C) and (D): [CHE] 5 μM, [AS1411-C] 5 μM, [PB] 10 mM, pH 7.2.

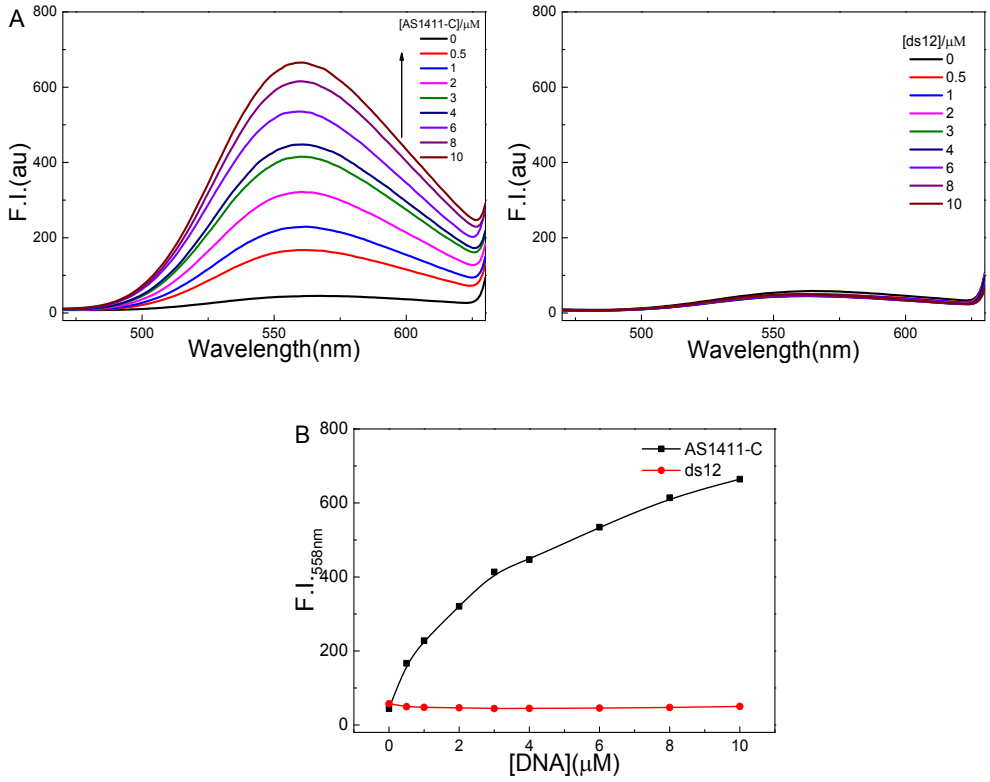


Fig. S9 (A) Fluorescence spectra of CHE (2 μM) with increasing concentrations of AS1411-C and ds12. (B) The plots of the fluorescence intensity of CHE at 558 nm versus the increasing concentration of AS1411-C and ds12. All samples were measured in PB buffer (10 mM, pH 5.8).

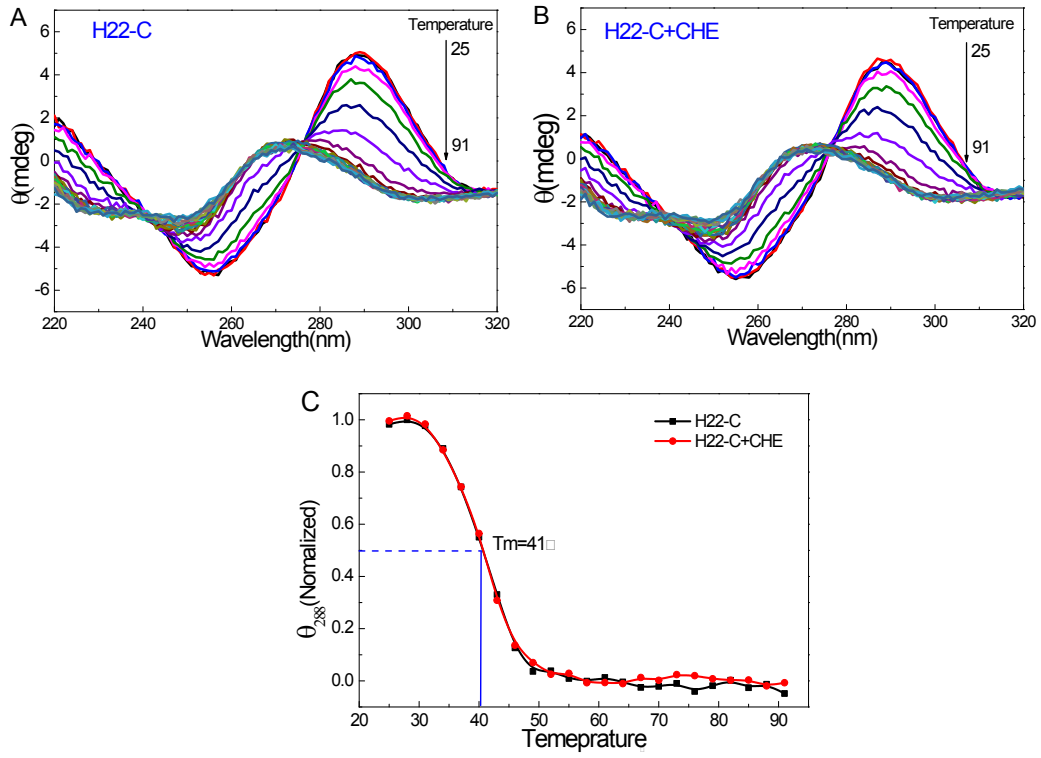


Fig. S10 CD-melting spectra of H22-C in the absence (A) and in the presence (B) of CHE. (C) CD-melting curves at 288 nm of H22-C in the absence and in the presence of CHE. Experimental conditions: [CHE] 4 μM, [H22-C] 2 μM, [PB] 10 mM, pH 5.8.

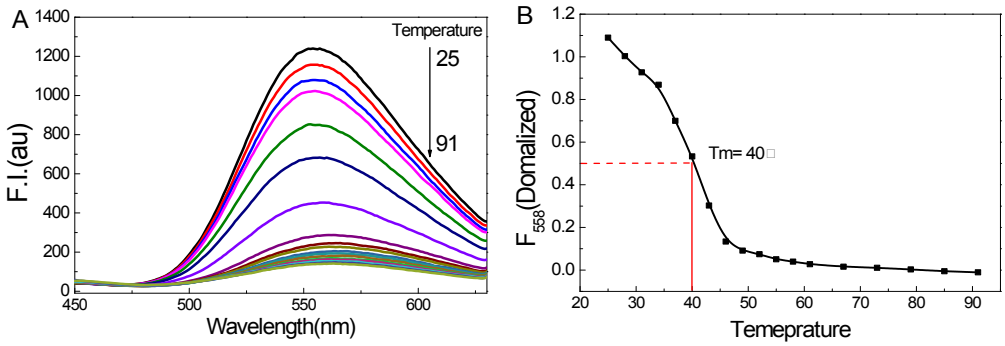


Fig. S11 (A) Melting fluorescence spectra of CHE-H22-C complexs. (B) Fluorescence melting curves at 558 nm of CHE-H22-C complexs. Experimental conditions: [CHE] 5 μM, [H22-C] 5 μM, [PB] 10 mM, pH 5.8.

1. F. Stootman, D. Fisher, A. Rodger and J. Aldrich-Wright, *Analyst*, 2006, **131**, 1145-1151.
2. X. Xie, B. Choi, E. Largy, R. Guillot, A. Granzhan, M. Teulade-Fichou, *Chem.-Eur. J.*, 2013, **19**, 1214-1226.