SUPPORTING INFORMATION

A DNA nanosensor for monitoring ligand-induced i-motif formation

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1.0 General information: All chemicals and solvents were of reagent grade and were purchased from Sigma-Aldrich and Thermo Fisher Scientific, Inc. Solutions and buffers were prepared in milliQ water. HPLC purified DNA oligonucleotides and their fluorescent and thiol conjugates were purchased from Sigma Aldrich and Eurofins Scientific. Solid DNA samples were initially dissolved as a stock solution in MilliQ water and further dilutions were carried out in suitable buffer at the respective pH. For annealing, DNA samples were thermally annealed in a heat block at 95°C for 5 minutes, cooled slowly to room temperature and then kept at 4 °C overnight. Graphs were plotted and processed using Origin 8.0.

2.0 Preparation of gold nanoparticles (GNPs): GNPs were prepared by mixing 20 mL 3.5 mg/mL aqueous sodium citrate solution with 200 mL 1 mM aqueous $HAuCl_4$ in a round-bottom flask equipped with nitrogen gas-filled balloon. The solution was heated to 90 °C for 15 min with vigorous stirring. The flask was removed from heating and the stirring was continued for additional 30 min at room temperature. The formed GNPs were isolated from the solution by centrifugation (14000 rpm, 25 min) and washed three times with milliQ water. The prepared GNPs were dispersed in 5 mL milliQ water.

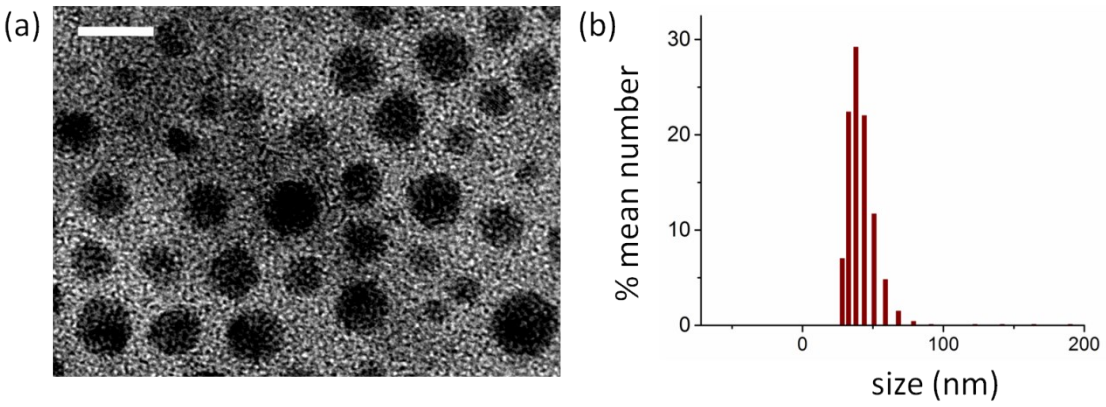

Fig. S1. (a) TEM imaging of the prepared GNPs (scale bar 50 nm). (b) DLS size distribution of GNPs.

The number of C-rich strand on the GNP surface was determined by using a reported protocol.[1] The fluorescence signal of DNA was converted into molar

[1] L. M. Demers, C. A. Mirkin, R. C. Mucic, R. C. Reynolds, R. L. Letsinger, R. Elghanian and G. Viswanadham, *Anal. Chem.* **2000**, *72*, 5535-5541.

concentrations of the DNA functionalized onto GNPs. By dividing the measured oligonucleotide molar concentration by the original GNP concentration, we estimated that there were ~120 strands per particle.

3.0 Immobilization of thiolated FAM-labelled DNA (Td*CF*) on GNPs surface: The thiolated FAM-labelled DNAs were immobilized on GNP surface in milliQ water. The milliQ water was degassed under N_2 stream to avoid oxidative dimerization of thiolated DNA sequences into disulfide dimers. The DNA sequence was immobilized on GNP-surface by incubating 25 µL DNA (concentration 10 µM, not annealed) with 25 µL GNPs in milliQ water. The mixture was then stirred at room temperature in dark for 16 h. The mixed solution was then centrifuged (13000 rpm, 30 min) and washed three times with milliQ water. The DNA immobilized GNPs were dispersed in milliQ water and then used for further experiments.

4.0 Interaction of ligand 1 with i-motif DNA: Fluorescence emission spectra were measured at 25 °C using Fluorolog-3 spectrofluorimeter (HORIBA) using a 0.2 cm path length quartz cuvette. The cuvette was thoroughly washed with methanol : HCl (1:1)

and distilled water to avoid contamination. The emission spectra for each titration were recorded from 300 nm to 550 nm at 0.2 nm/s, 5 nm slit width.

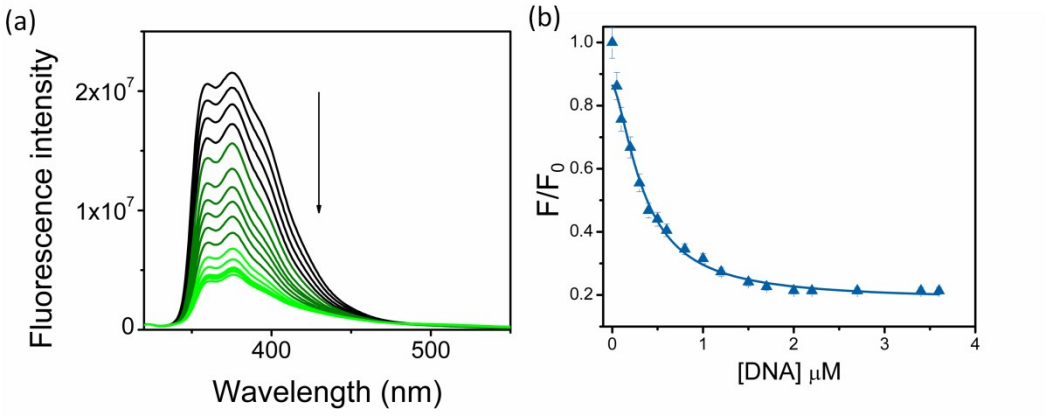

Fig. S2. (a) Fluorescence spectroscopic titrations of ligand **1** (1 μM) with i-motif (iM) DNA. Buffer: 10 mM sodium cacodylate, 10 mM NaCl, pH 5.5 (b) Fluorescence response curves of **1** with incremental addition of iM DNA.

5.0 FRET melting analysis: FRET experiments were carried out with a 200 nM 5'-FAM and 3'-TAMRA-labeled oligonucleotide sequence in milliQ water or in 10 mM sodium cacodylate, 10 mM NaCl buffer, pH 5.5 on a real-time PCR apparatus (LightCycler® 480-II System).

All HPLC purified dual labeled DNA oligonucleotides were purchased from SIGMA-Aldrich. To prepare i-motif DNA, dual-labeled C-rich DNA sequence was annealed at a concentration of 400 nM in 10 mM sodium cacodylate, 10 mM NaCl buffer, pH 5.5 by heating at 95 °C for 5 min followed by gradual cooling to room temperature at a controlled rate of 0.1 °C/min. For unfolded DNA, the sequence was used without any annealing in milliQ water. The dual labeled DNA oligonucleotides (200 nM) were incubated with different concentrations of 1 for 1 hr. Fluorescence measurements were then taken with an excitation wavelength of 483 nm and a detection wavelength of 533 nm at intervals of 1°C over the range of 37-95 °C. Final analysis of melting temperatures was done using LightCycler® 480-II System software and Origin Pro 8 data analysis.

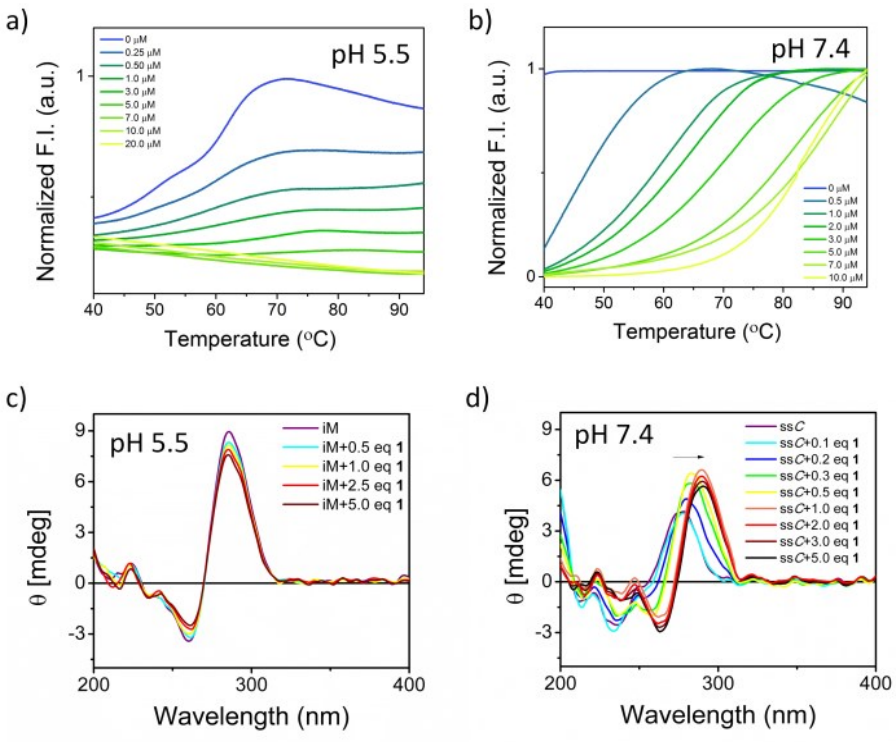

Fig. S3. FRET melting curves for 200 nM a) prefolded **F**d*C***T'** DNA [buffer: 10 mM sodium cacodylate, 10 mM NaCl, pH 5.5; i-motif (iM)] and b) unfolded **F**d*C***T'** DNA [milliQ water; random C-rich DNA (ss*C*)] in the presence of **1**. CD titration spectra of 5 μM C-rich DNA sequence c) at pH 5.5 (iM) and d) at pH 7.4 (*ss*C) with 0-5 equiv. of **1**.

6.0 Circular Dichroism spectroscopy: CD spectra were recorded on a JASCO J-815 spectrophotometer by using a 1 mm path length quartz cuvette. The i-motif DNA sequence was diluted to a 5 μM strand concentration in 10 mM sodium cacodylate buffer (pH 5.5 and milliQ water separately). The i-motif DNA at pH 5.5 was used after proper annealing. All CD spectra were recorded in triplicate, averaged; baseline corrected for buffer signals and smoothed out. Aliquots of **1** (0-10 eq) were added stepwise to pre-annealed i-motif DNA sequence in 10 mM sodium cacadylate buffer, pH 5.5 to investigate the ability of **1** to stabilize or induce i-motif structure. The CD spectrum of un-annealed random coil C-rich oligonucleotide sequence was also obtained in milliQ water to investigate the effect of **1** to change the conformation of the random coil C-rich structure into i-motif DNA.

7.0 Fluorescence spectroscopic titrations:

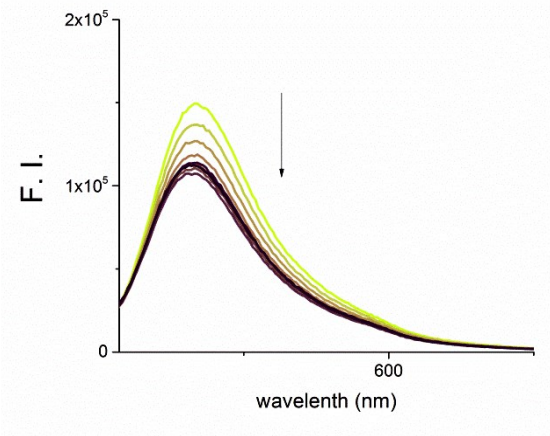

Fig. S4. Fluorimetric titration of 100 nM 5'-thiolated and 3'-FAM-labelled C-rich DNA (**T**d*C***F**) sequence (not grafted on GNPs surface) with 0-1 µM **1** in milliQ water. Excitation at 488 nm.

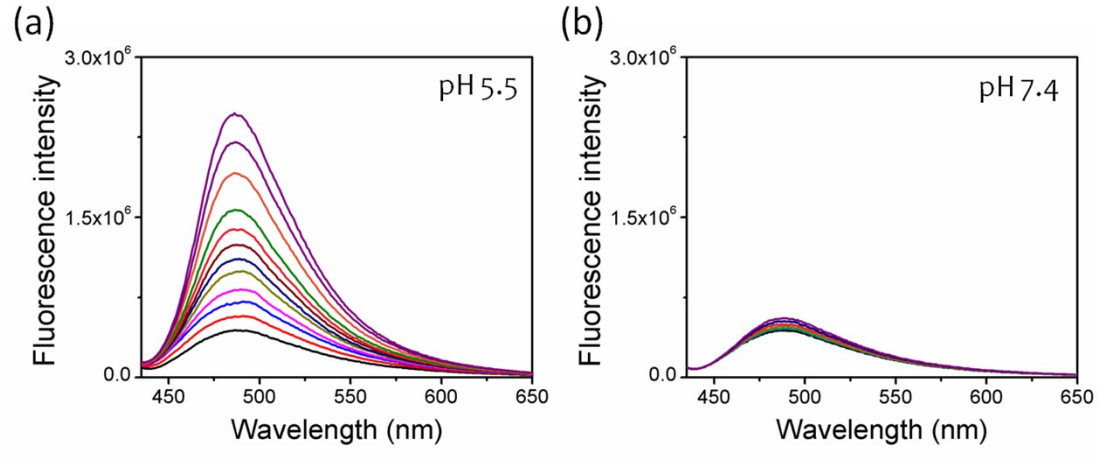

Fig. S5. (a) Fluorimetric titration spectra of ThT (1 µM) with a) i-motif (iM) in 10 mM sodium cacodylate, 10 mM NaCl buffer at pH 5.5 and b) with C-rich oligonucleotide sequence (ss*C*) in milliQ water. The fluorimetric titrations of ThT were performed at 412 nm excitation wavelength and data were collected between 425 nm and 650 nm at 0.2 nm/s; 5 nm slit width.

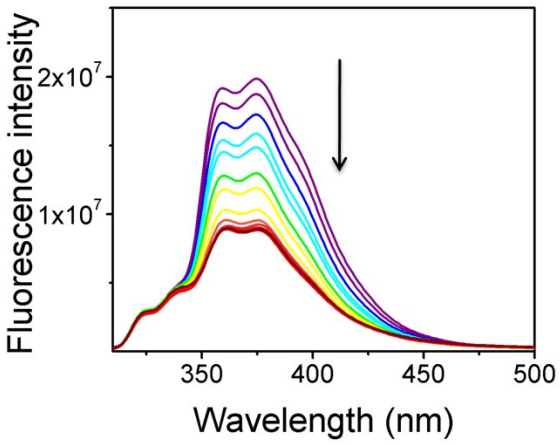

Fig. S6. Fluorescence spectroscopic titration of 1 μM **1** with un-annealed C-rich DNA (0-5 μM) in milliQ water. Excitation at 290 nm.

8.0 Molecular Modeling: Molecular docking studies were performed to explore the binding of 1 and Thioflavin T with i-motif using AutoDock 4.2. The structure of the i-motif tetramer of d(AACCCC) (PDB ID 1YBL)[2] was used as a model i-motif structure to explore the binding of the ligand with i-motif DNA. Charges on each atom of DNA were calculated using Kollman united-atom charge model and then non-polar hydrogen atoms were added. Grid maps were generated using the empirical free-energy scoring function implemented in AutoDock. Grid boxes were chosen such that it covers the entire i-motif. 3-D structures of the ligands, **1** and Thioflavin T, were constructed using the HYPERCHEM 8.0 molecular builder module and optimized using the AM1 semi-empirical method to a convergence limit of 0.001 kcal/(˚A.mol) with the Polak–Ribiere conjugate gradient algorithm. Then, rotatable bonds were assigned for both the ligand and partial atomic charges were calculated using the Gasteiger–Marsili method. Then non-polar hydrogens were merged. 500 Docking runs were carried out for each DNA-ligand system and for each run, a maximum of 2,500,000 GA operations were performed on a single population of 150 individuals. The weights for crossover, mutation and elitism were default parameters of 0.8, 0.02 and 1, respectively. Cluster analysis was performed on the generated docked poses using 2Å RMSD cut-off. Lowest energy structure from the highest population cluster was considered as the possible solution.

[2] N. Esmaili and J. –L. Leroy, *Nucleic Acids Res*., **2005**, *33*, 213-224.

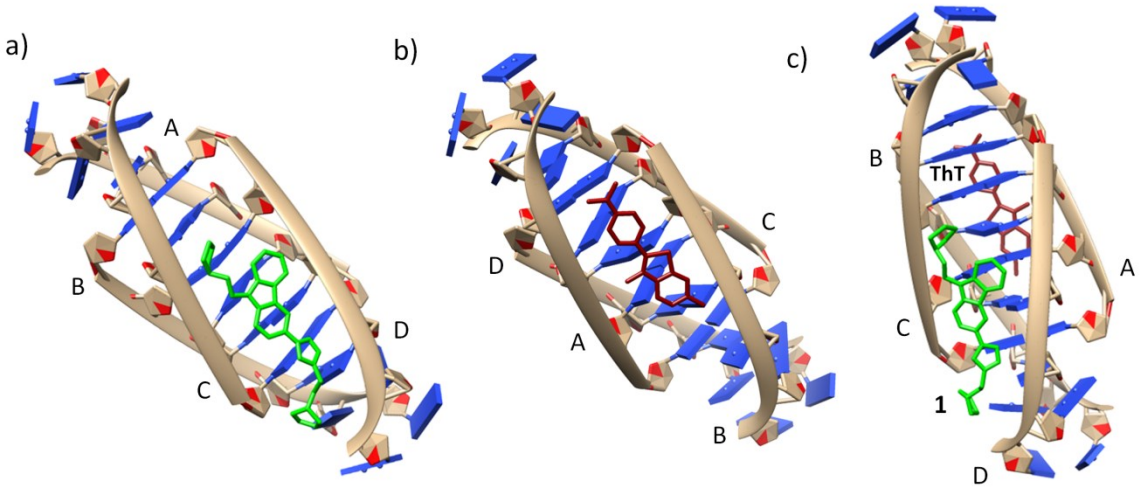

Fig. S7. Docked conformations of a) **1**, b) ThT and b) ThT+**1** with i-motif tetramer structure (PDB ID: 1YBL).

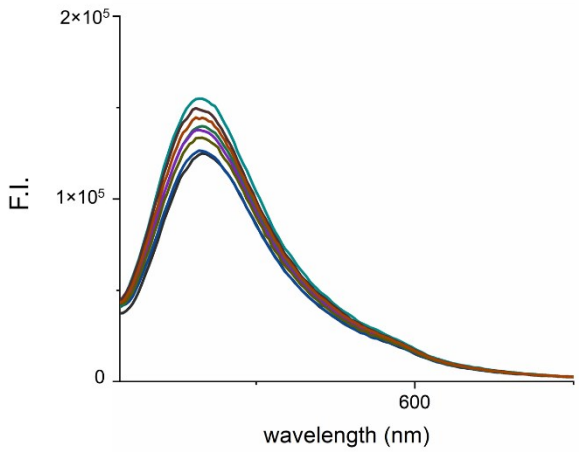

Fig. S8. Fluorimetric titration of 200 nM free FAM-tagged G.C-rich duplex DNA (**T**dC**F**.dG) (not grafted on GNPs surface) with 0-1 µM **1** in 10 mM Tris.HCl buffer, pH 8. Excitation at 488 nm.

9.0 Interaction of Doxorubicin with ligand 1 and different DNA structures:

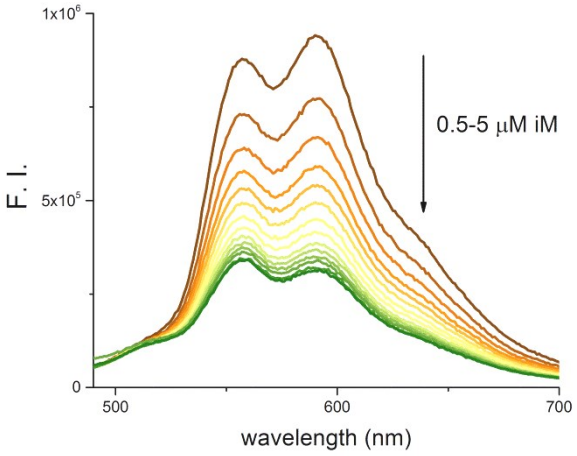

Fig. S9. Fluorimetric titration spectra of Dox (1 μM) with i-motif (0-5 μM) in 10 mM sodium cacodylate, 10 mM NaCl buffer at pH 5.5; excitation wavelength 480 nm.

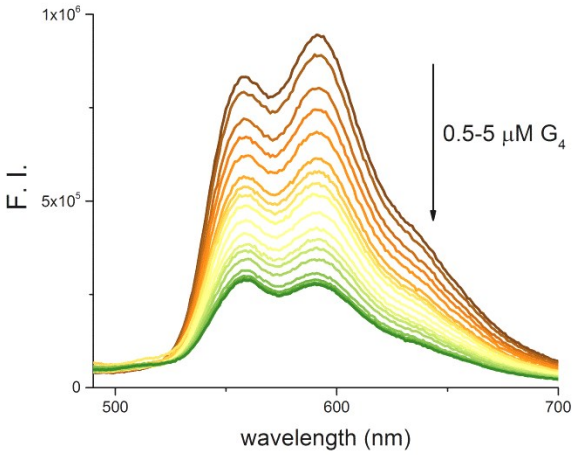

Figure S10. Fluorimetric titration spectra of Dox (1 μM) with G-quadruplex (0-5 μM) in 100 mM Tris.KCl buffer at pH 7.4; excitation wavelength 480 nm.

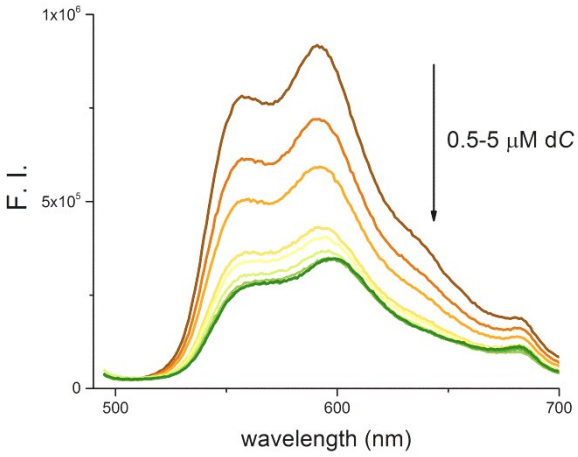

Figure S11. Fluorimetric titration spectra of Dox (1 μM) with C-rich random coil (0-5 μM) in milliQ water; excitation wavelength 480 nm.

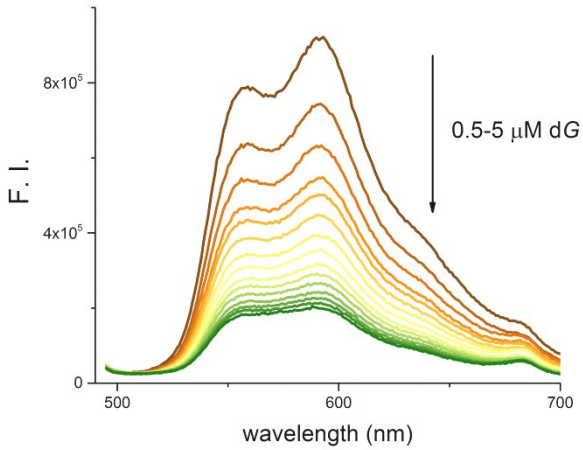

Fig. S12. Fluorimetric titration spectra of Dox (1 μM) with G-rich random coil (0-5 μM) in milliQ water; excitation wavelength 480 nm.

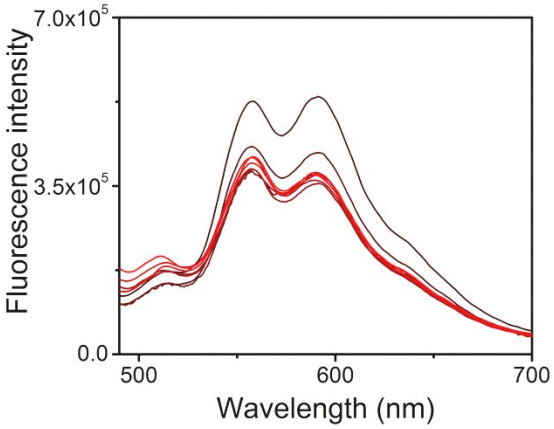

Fig. S13. (a) Fluorimetric titration of Doxorubicin (Dox) (1 μM) with **1** (0-10 μM) in 10 mM Tris.HCl buffer, pH 7.4.

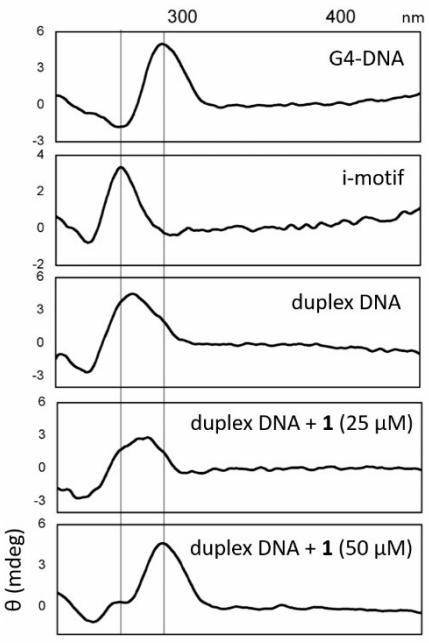

Fig. S14. CD spectra of pre-folded G-quadruplex DNA (graph 1), i-motif DNA (graph 2) and d*C*.d*G* duplex DNA alone (graph 3) and with 25 μM and 50 μM ligand **1** (graph 4-5).

10.0 Chemical structures of carbazole derivatives:

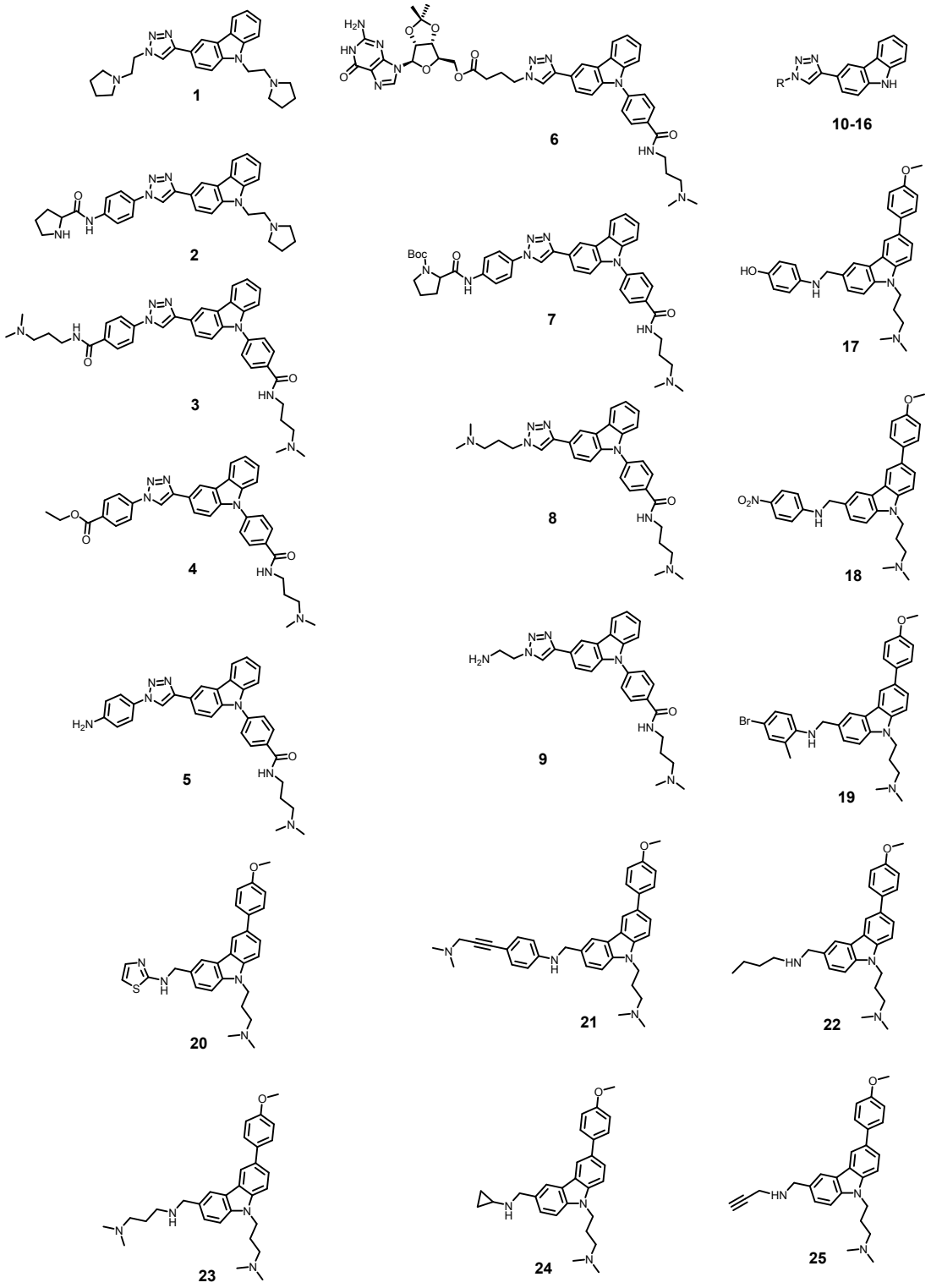

Fig. S15. Structures of the carbazole ligands used in screening assay.

11.0 High-throughput fluorescence screening for i-motif folding ligands:

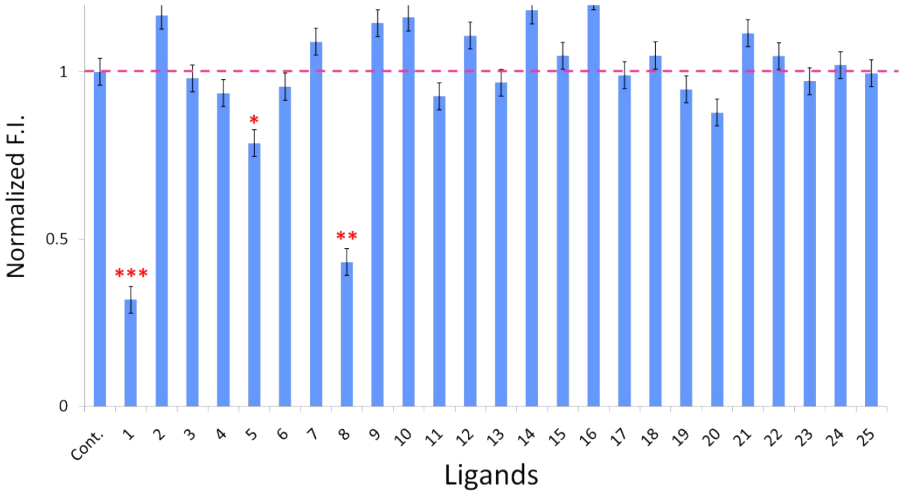

Fig. S16. Normalized fluorescence intensity of **T**d*C***F**-GNP with 1 μM carbazole ligands.