Electronic Supplementary Material (ESI) for Analyst

Supporting Information

Symmetric Cyanovinyl-PyridiniumTriphenylamine: A Novel Fluorescent Switch-On Probe for Antiparallel G-Quadruplex

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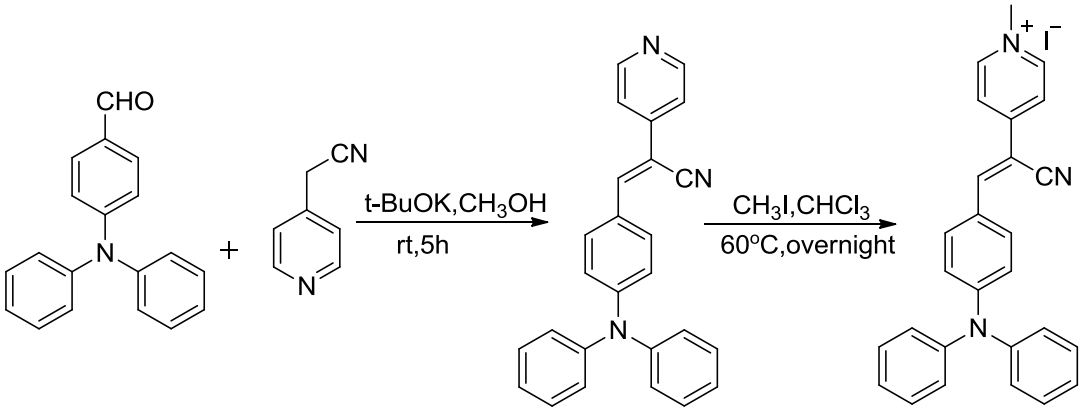
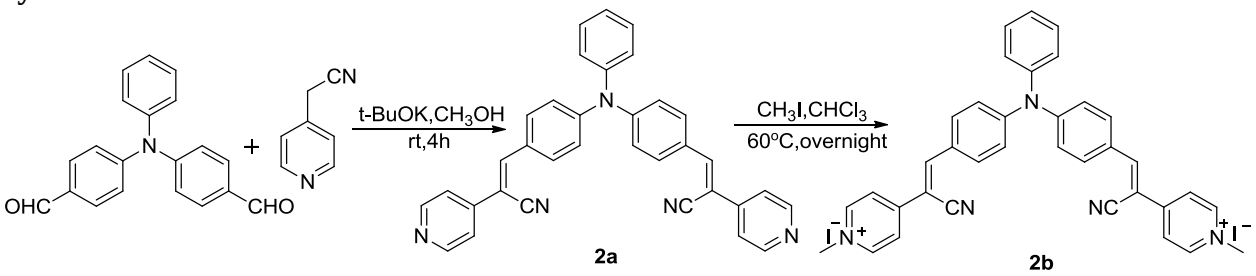
Synthesis of CPT1



4-Formyltriphenylamine (273 mg, 1 mmol) and 4-pyridylacetonitrile (118 mg, 1 mmol) was dissolved in dry CH_3OH (2ml) then potassium tert-butylate (168 mg, 1.5 mmol) was added to the mixture. The reaction mixture was stirred at RT for 5 h. When the reaction was completed, the solvent was removed under pressure affording an orange solid of **1a** (196 mg, 53%), structure of **1a** see in the Supporting Information. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.59 (d, *J*=4.5 Hz, 2H), 7.77 (d, *J*=8.1 Hz, 2H), 7.54 (s, 1H), 7.47 (d, *J*=4.8 Hz, 2H), 7.30-7.26 (m, 4H), 7.13-7.10 (m, 6H), 6.99 (d, *J*=8.1 Hz, 2H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ=151.1, 150.8, 150.2, 146.3, 144.4, 142.6, 131.5, 129.8, 126.2, 125.0, 123.4, 120.2, 120.0, 119.6, 117.9, 104.5 ppm; HRMS (ESI) calcd for $C_{26}H_{20}N_3$ $[M+H]^+$: 374.1657; found: 374.1658.

1a (100 mg, 0.27 mmol) was dissolved in 1 ml chloroform and 1 ml iodomethane was added dropwise. The resulting mixture was heated to 60 °C and refluxed overnight. Then the mixture was filtered under reduced pressure and recrystallized by dissolution in methanol and progressive addition of ether, yielding an orange solid of **CPT1** (78 mg, 56%). ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 8.96 (d, *J*=6.0 Hz, 2H), 8.59 (s, 1H), 8.33 (d, *J*=6.3 Hz, 2H), 8.04 (d, *J*=8.7 Hz, 2H), 7.48-7.43 (m, 4H), 7.30-7.24 (m, 6H), 6.94 (d, *J*=8.4 Hz, 2H), 4.30 (s, 3H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6,): δ=151.9, 150.3, 149.6, 145.1, 133.0, 130.3, 129.9, 126.8, 126.2, 125.7, 123.6, 122.0, 117.83, 116.9, 99.1 ppm; HRMS (ESI) calcd for $C_{27}H_{22}N_3^+$ $[M-I]^+$: 388.1808; found: 388.1807

Synthesis of CPT2



4,4-Diformyltriphenylamine (301 mg, 1 mmol) and 4-pyridylacetonitrile (177 mg, 1.5 mmol) was dissolved in dry CH3OH (2ml) then potassium tert-butylate (258 mg, 2.3mmol) was added to the mixture. Then the mixture was stirred for 4 h at RT and filtered under reduced pressure

to give an orange solid of **2a** (223 mg, 47%), structure of **2a** see in the Supporting Information. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.69 (d, *J*=6.0 Hz, 4H), 7.91 (d, *J*=8.4 Hz, 4H), 7.65 (s, 2H), 7.57 (d, *J*=6.0 Hz, 4H), 7.41 (t, *J*=7.2 Hz, 2H), 7.27-7.18 (m, 7H) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ=150.6, 149.6, 145.5, 144.0, 142.2, 131.5, 130.2, 127.4, 127.0, 126.2, 123.0, 119.8, 117.5, 106.5 ppm; HRMS (ESI) calcd for $C_{34}H_{24}N_5$ $[M+H]^+$: 502.2032; found: 502.2030.

2a (100 mg, 0.20mmol) was dissolved in 1 mL chloroform and 1.5 mL iodomethane was added dropwise. The resulting mixture was heated to 60 °C and refluxed overnight. Then the mixture was filtered under reduced pressure and recrystallized by dissolution in methanol and progressive addition of ether, yielding an amaranth solid of **CPT2** (57 mg, 36%). ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 9.05 (d, *J*=5.7 Hz, 4H), 8.74 (s, 2H), 8.42 (d, *J*= 6.0 Hz, 4H), 8.15 (d, *J*=8.4 Hz, 4H), 7.55 (t, *J*=7.8 Hz, 2H), 7.28-7.40 (m, 7H), 4.34 (s, 6H) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): δ=150.1, 149.8, 149.0, 145.4, 144.3, 132.6, 130.2, 127.1, 126.7, 122.4, 116.4, 101.9 ppm; HRMS (ESI) calcd for $C_{36}H_{29}N_5^{2+}$ $[M-2I]^{2+}$: 531.2412; found: 531.2419.

Materials, reagents and instrumentations

4-Formyltriphenylamine, 2-pyridylacetonitrile and 4,4-Diformyltriphenylamine were bought from Sigma-Aldrich. Other chemicals employed were of analytical grade from SCRC (Shanghai, China). All DNA oligonucleotides were purchased from Invitrogen Technology (Shanghai, China). See Table S1 for complete oligonucleotide sequences.
^{1}H and ^{13}C NMR spectra were recorded on Varian Mercury 300 spectrometers, respectively. HRMS were recorded on a Bruker Daltonics, Inc. APEXIII 7.0 TESLA FTMS, Thermo Fisher LTQ Orbitrap XL and Varian ProMALDI.
Fluorescent spectra were detected by LS55 Perkin Elmer at room temperature with excitation wavelength 521 nm. UV absorption spectra were taken from UV 2550 UV-vis spectrophotometer. CD experiments utilizing a Jasco-810 spectropolarimeter (Jasco, Easton, MD, USA) were measured at room temperature using a quartz cell with a 1 mm path length. In colorimetric Experiments, all the samples were photographed under irradiation of UV light (Vilber Lourmat, Bio-Print, VL).

Fluorescence spectroscopy

Fluorescent spectra were detected by LS55 Perkin Elmer in 1 cm path length quartz cuvette at room temperature with excitation wavelength 521 nm (slit emission and excitation were set at 10 nm).
Fluorescence titrations with different DNA/RNA:
Measurements were performed by adding solutions of pre-formed oligonucleotides (100 μM, containing 50 mM KCl) to a solution of CPT2 (10 μM) in Tris-HCl buffer (10 mM, pH 7.4, containing 50 mM KCl). Samples were left to equilibrate for 0.5 min after each addition to ensure a stable readout.
Fluorescence titrations in different buffers:
Measurements were performed by adding solutions of 22AG (10 μM) in different buffers to a solution of CPT2 (10 μM) in corresponding buffer. Samples were left to equilibrate for 0.5 min after each addition to ensure a stable readout. All titrations were performed in triplicates.

UV spectroscopy

UV absorption spectra were taken from UV 2550 UV-vis spectrophotometer using 10 mm path length quartz cuvettes.

CPT1

Titrations were performed by adding a solution of 22AG (100 µM) in 10 mM Tris (pH 7.4) and 50 mM KCl to a solution of CPT1 (10 µM) in corresponding buffer. Samples were left to equilibrate for 0.5 min after each addition to ensure a stable readout.

CPT2

Titrations were performed by adding a solution of 22AG (100 µM) in 10 mM Tris (pH 7.4) with/without 50 mM KCl to a solution of CPT2 (10 µM) in corresponding buffer. Samples were left to equilibrate for 0.5 min after each addition to ensure a stable readout.

Circular dichroism spectroscopy

CD experiments utilizing a Jasco-810 spectropolarimeter (Jasco, Easton, MD, USA) were measured at room temperature using a quartz cell with a 1 mm path length. All the CD spectra were measured from 220 nm to 320 nm with a scanning speed of 200 nm/min, the 3 nm bandwidth and the 2 s response time. The recorded spectra represent a smoothed average of three scans. Oligonucleotides (10 µM) were dissolved in corresponding buffer containing different concentrations of CPT2. Each spectrum was an average of three measurements.

For melting studies, 10 µM 22AG DNA in corresponding buffer and different concentrations of CPT2 were used. Thermal melting was monitored at 295 nm at a heating rate of 1° C/min.

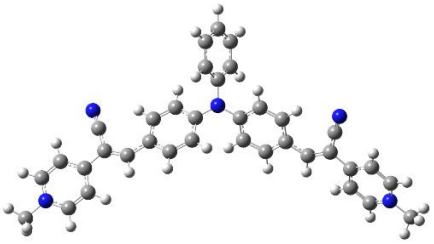
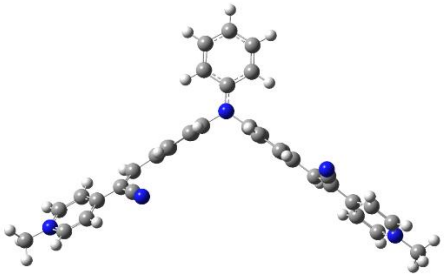
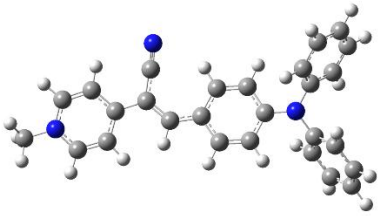
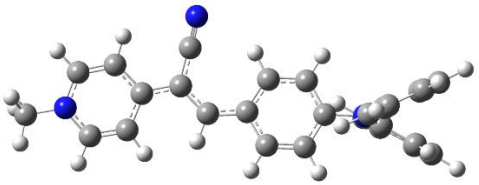
Native Gel Electrophoresis

Native gel electrophoresis was run on 12% polyacrylamide gel containing and 50 mM KCl at 4 °C, 5 V/cm in 1×TBE buffer containing 50 mM KCl. Gels with 5-FAM-labeled oligonucleotides were photographed under irradiation of UV light (Vilber Lourmat, Bio-Print, VL).

DFT optimization on excited state of CPT1 and CPT2

Ground states of CPT1 and CPT2 geometry were optimized at B3LYP/6-31G(d) level. Then the first excited states were optimized using time dependant functional theory model at

TD-B3LYP/6-31G(d) level. Optimized ground state structures were used as initial geometry and no solvation model is used.

	Ground state	Excited state
CPT2		
CPT1		

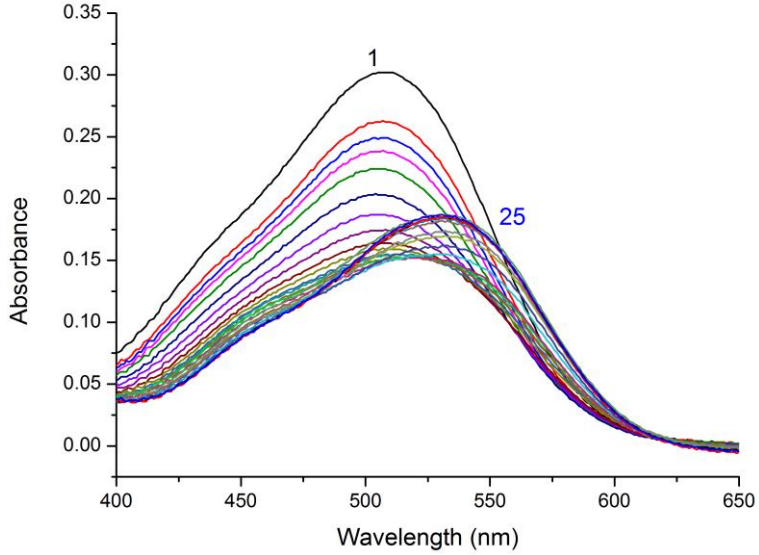


Figure S1. Absorption spectra of CPT2 with 22AG DNA. CPT2 solution (10 μM) containing 50 mM KCl and 10 mM Tris (pH 7.4) with [22AG]/μM: (1) 0, (2) 0.025, (3) 0.05, (4) 0.075, (5) 0.1, (6)0.2, (7) 0.3, (8) 0.4, (9) 0.5, (10) 0.6, (11) 0.7, (12) 0.8, (13) 0.9, (14) 1.0, (15) 1.5, (16) 2.0, (17) 2.5, (18) 3.0, (19) 4.0, (20) 5.0, (21) 6.0, (22) 7.0, (23) 8.0, (24) 9.0, (25) 10.0.

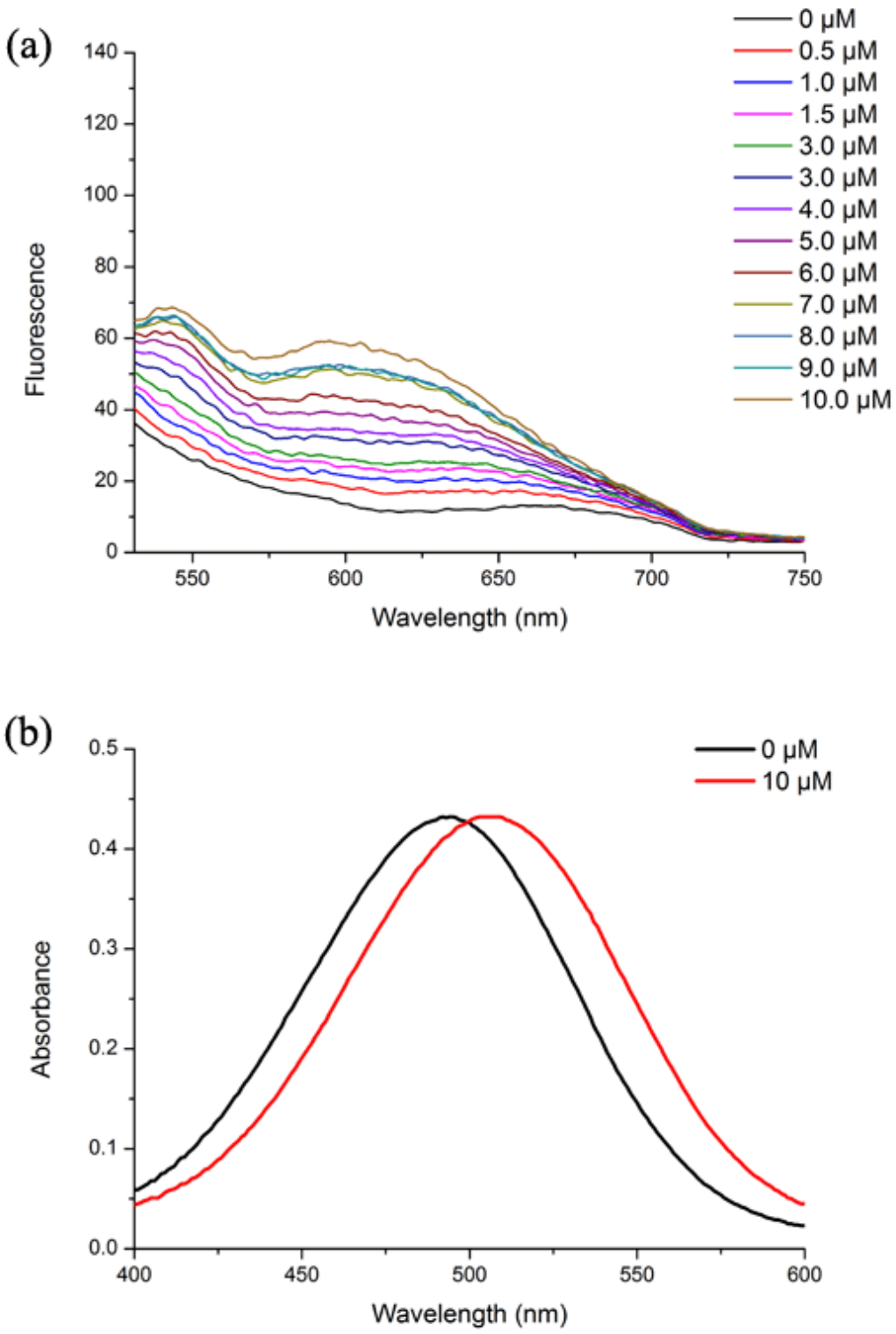


Figure S2. (a) Fluorescence spectra of CPT1 (10 μM) on titration with prefolded 22AG quadruplex DNA in 10 mM Tris (pH 7.4) and 50 mMKCl. [22AG]/μM: (1) 0, (2) 0.5, (3) 1.0, (4) 1.5, (5) 2.0, (6) 3.0, (7) 4.0, (8) 5.0, (9) 6.0, (10) 7.0, (11) 8.0, (12) 9.0, (13) 10.0.λ_{ex} is 493 nm. (b) Absorption spectra of CPT1 (10 μM) with prefolded 22AG quadruplex DNA in 10 mM Tris (pH 7.4) and 50 mMKCl. Concentration of 22AG DNA is 0 μM (black line) and 10 μM (red line).

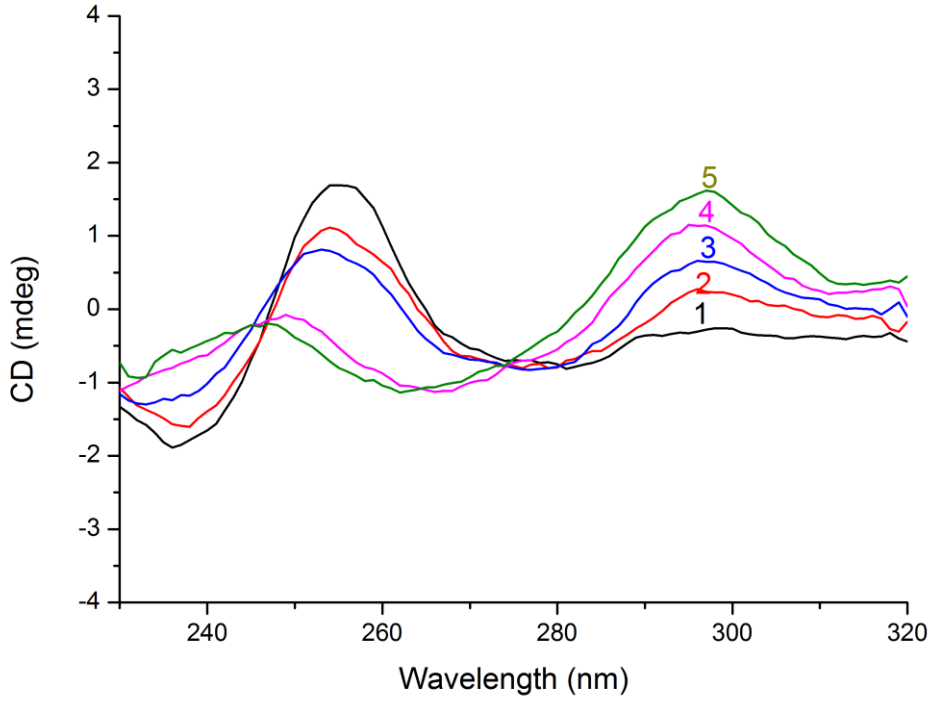
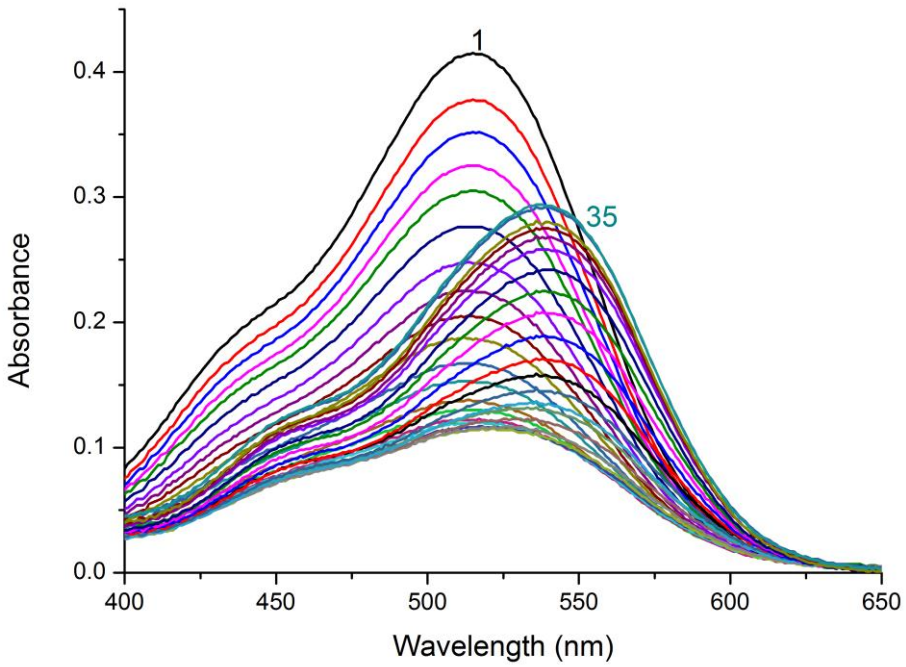


Figure S3. CD spectra for 22AG DNA (10 μM) with the addition of CPT1 buffered with 10 mMTris (pH 7.4); [CPT1]/ μM:(1) 0; (2) 10; (3) 20; (4) 50; (5) 100.



FigureS4. Absorption spectra of CPT2 (10 μM) with [22AG]/μM in 10 mMTris buffer (pH 7.4) without any alkali metal. Concentration of 22AG/μM: 1) 0, 2) 0.025, 3) 0.05, 4) 0.075, 5) 0.100, 6) 0.125, 7) 0.15, 8) 0.175, 9)0.2, 10) 0.225, 11) 0.25, 12) 0.275, 13) 0.3, 14) 0.325, 15) 0.35, 16) 0.375, 17) 0.4, 18) 0.45, 19) 0.5, 20) 0.6, 21) 0.7, 22)0.8, 23) 0.9, 24) 1, 25) 1.5, 26) 2, 27) 2.5, 28) 3, 29) 4, 30) 5, 31) 6, 32) 7, 33) 8, 34) 9, 35) 10.

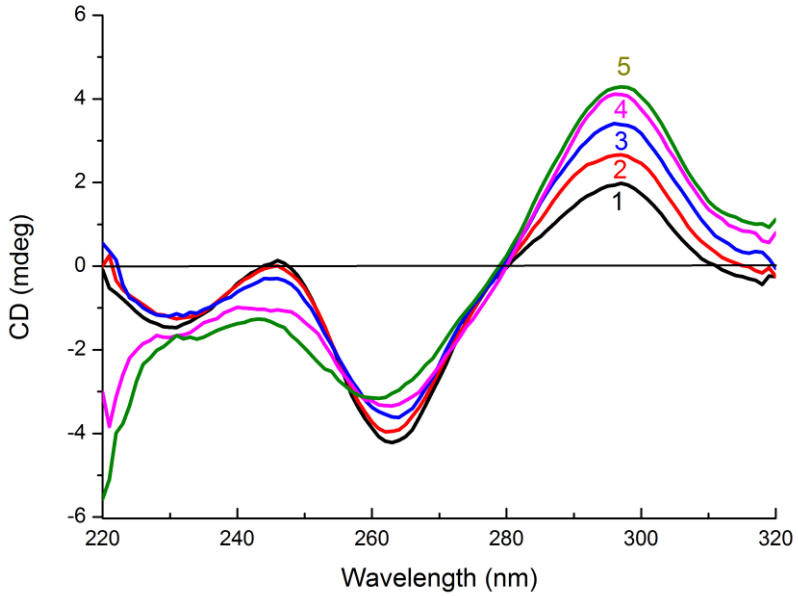


Figure S5. CD spectra for 22AG DNA (10 μM) with the addition of CPT2 in 50 mM NaCl and 10 mM Tris buffer (pH 7.4).[CPT2]/μM:(1) 0; (2) 10; (3) 20; (4) 50; (5) 100.

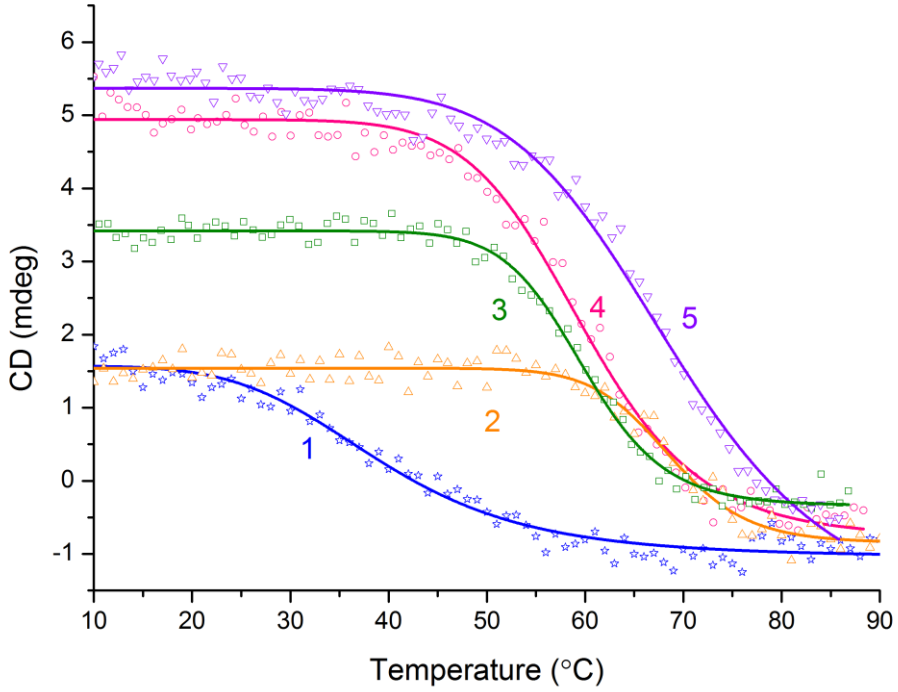


Figure S6. Melting curves for 22AG DNA (10 μM) in different conditions. 1) Solution contains 10 mM Tris (pH 7.4) and 10 μM CPT2. Melting curve provided a T_m of 39.24°C. 2) Solution contains 10 mM Tris (pH 7.4) 10 μM CPT2, and 50 mM KCl. Melting curve provided a T_m of 67.28 °C. 3) Solution contains 10 mM Tris (pH 7.4) and 50 mM KCl. Melting curve provided a T_m of 59.89 °C. 4) Solution contains 10 mM Tris (pH 7.4) and 100 μM CPT2. Melting curve provided a T_m of 59.86 °C. 5) Solution contains 10 mM Tris (pH 7.4), 100 μM CPT2, and 50 mM KCl. Melting curve provided a T_m of 68.96 °C. Melting of antiparallel quadruplex was monitored at 295 nm.

Figure S7. CD spectra for various G-quadruplexes (10 μM) with or without CPT2 in 10 mMTris (pH 7.4) and 50 mM KCl. [CPT2]/μM: 0 (black line); 10 (red line); 20 (blue line); 50 (pink line); 100 (green line).

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 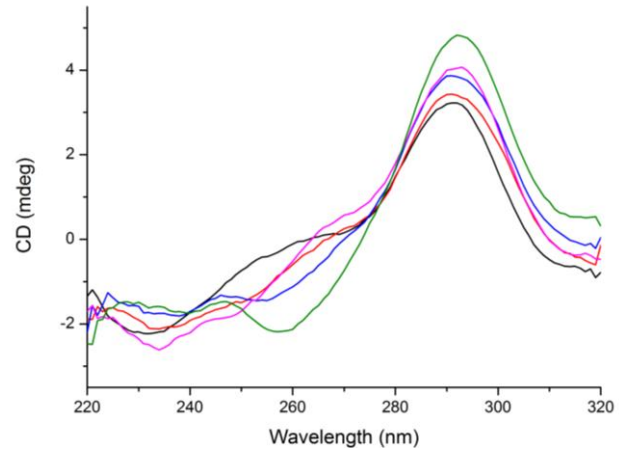

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 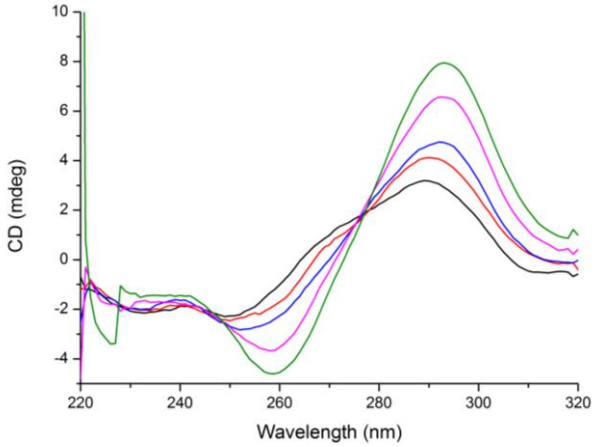

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 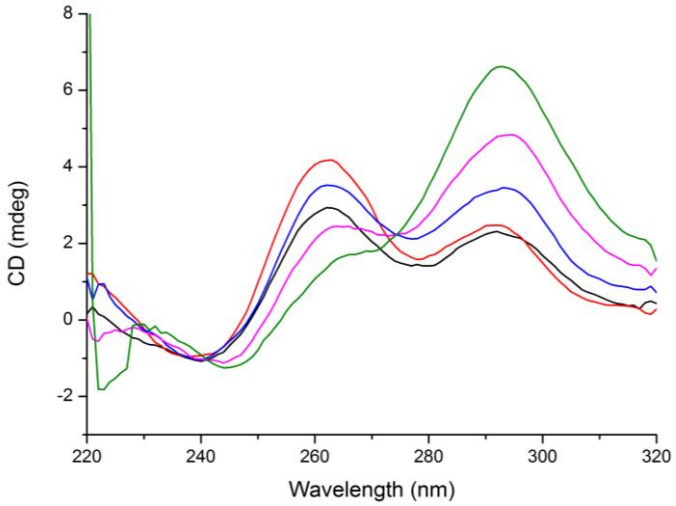

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 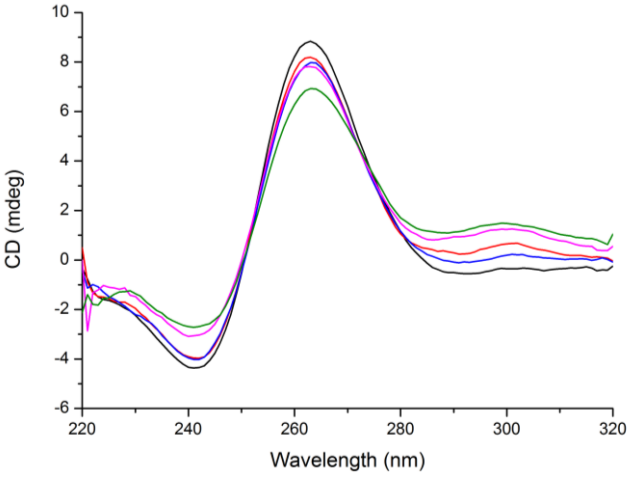

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 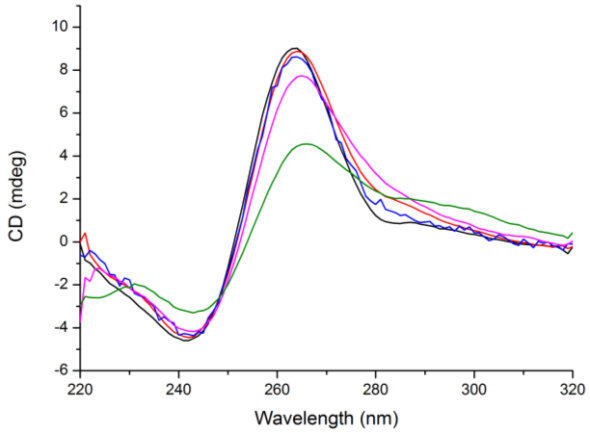

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 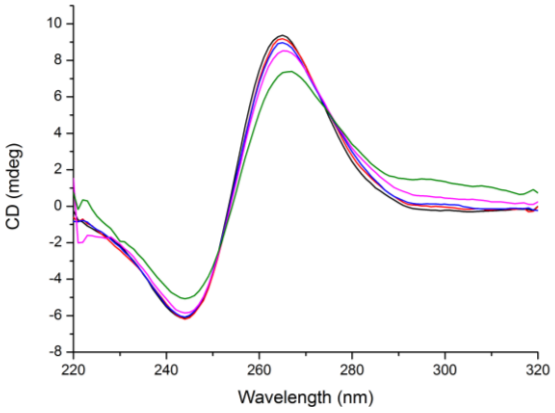

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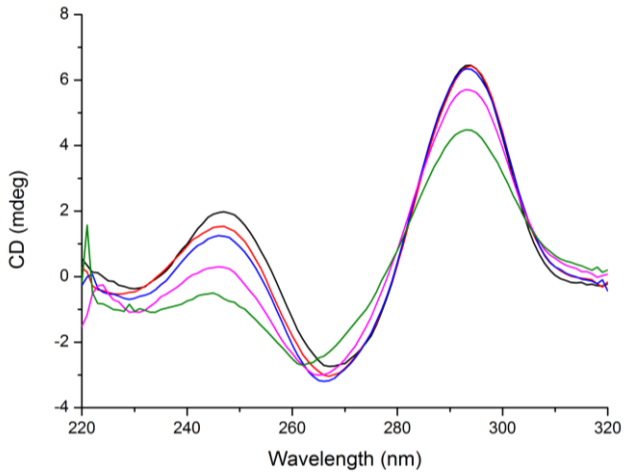

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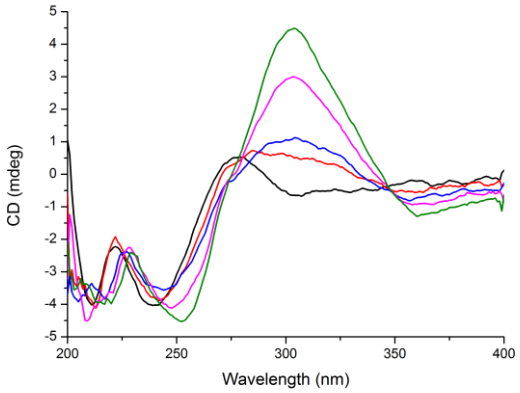

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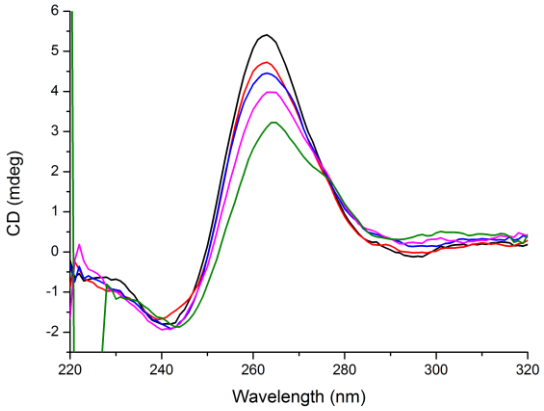

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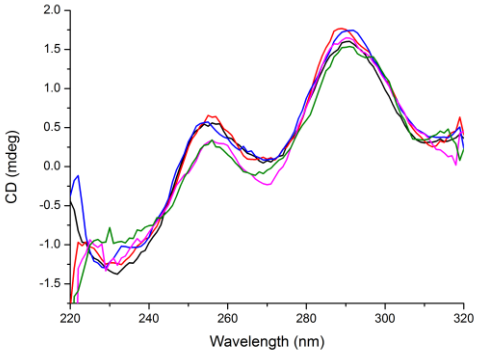

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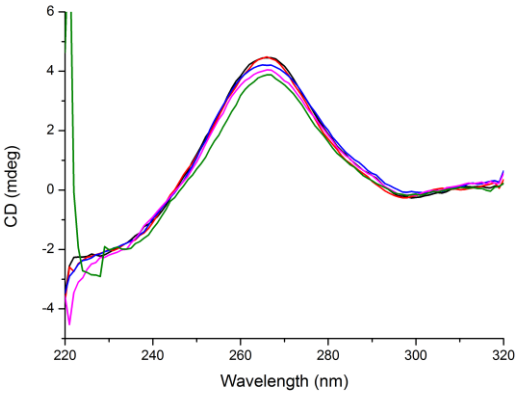

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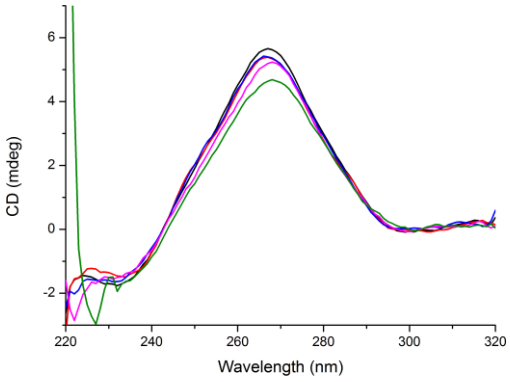

Figure S8
CD spectra for various G-quadruplexes (10 μM)with or without CPT2 in 10 mMTris (pH 7.4). [CPT2]/μM: 0 (black line); 10 (red line); 20 (blue line); 50 (pink line); 100(green line).

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 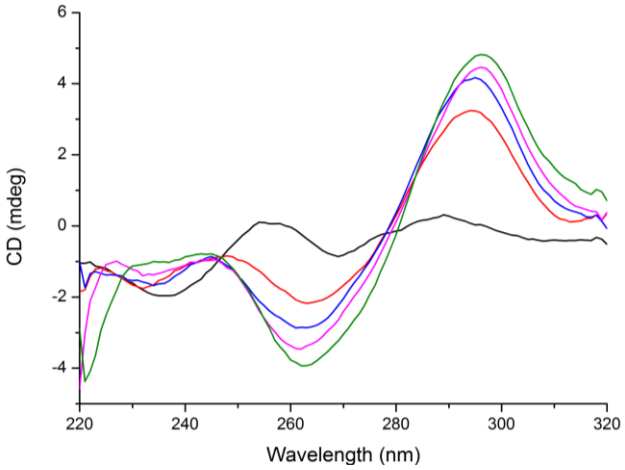

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 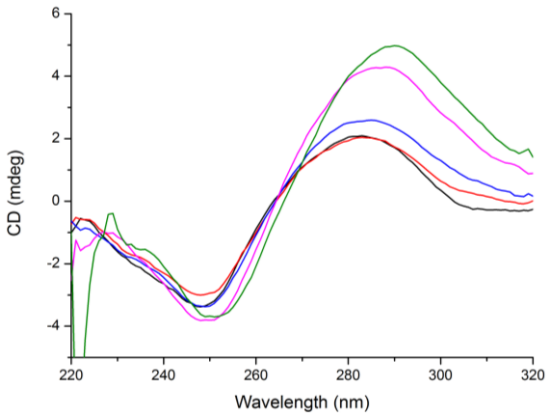

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 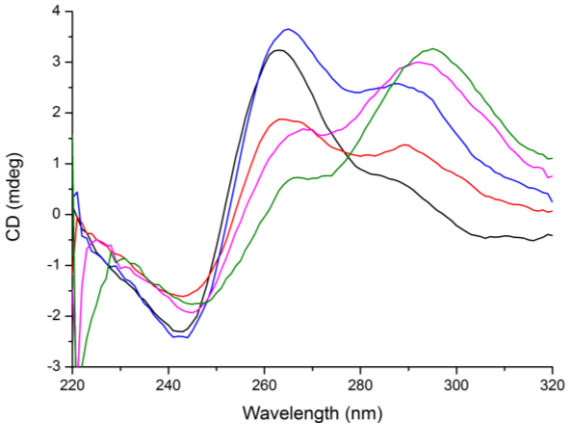

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 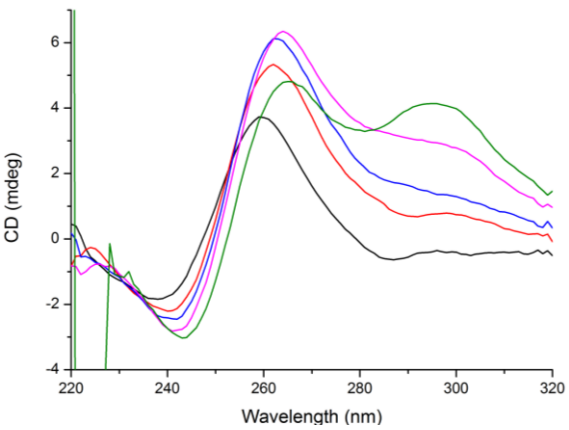

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 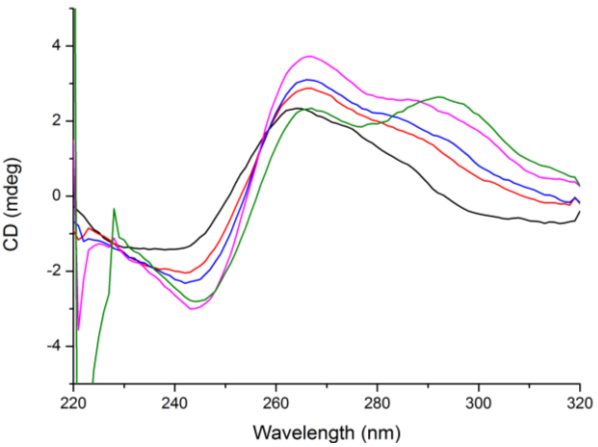

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 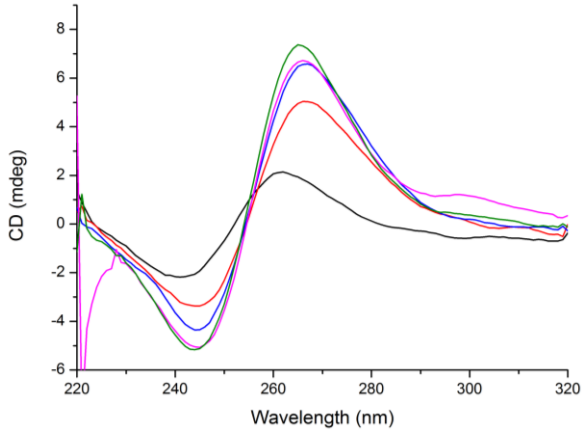

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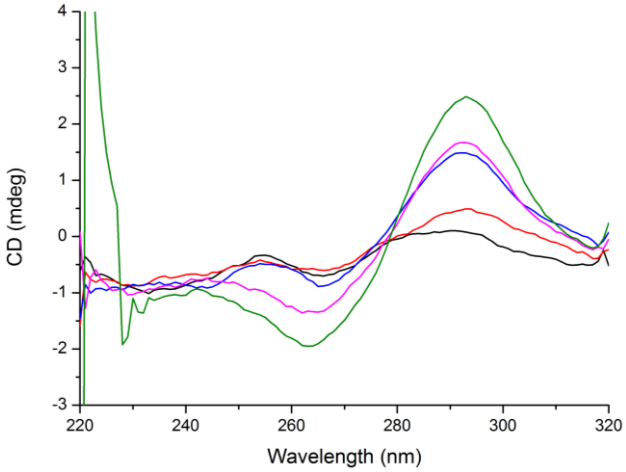
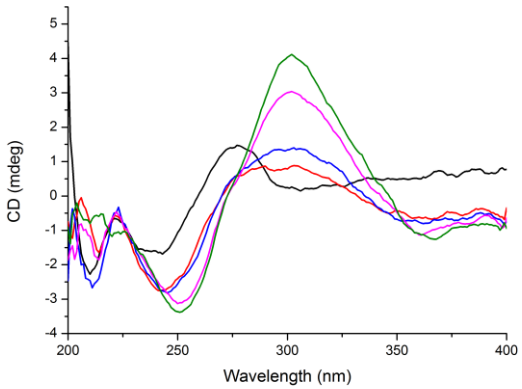
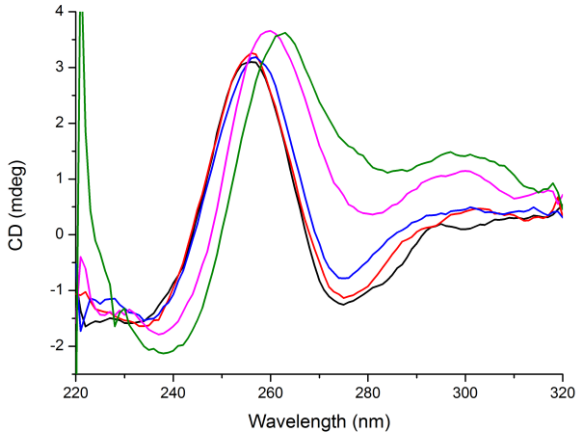


Antiparallel

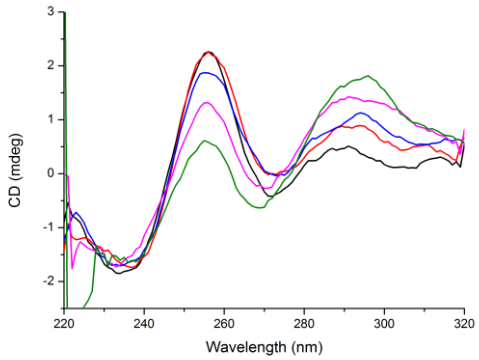
dsDNA



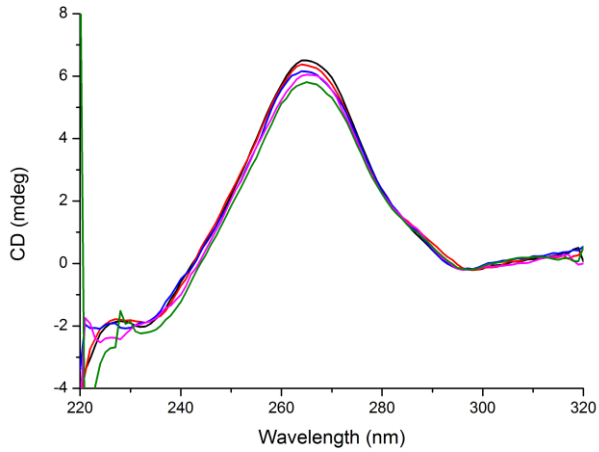
8TG



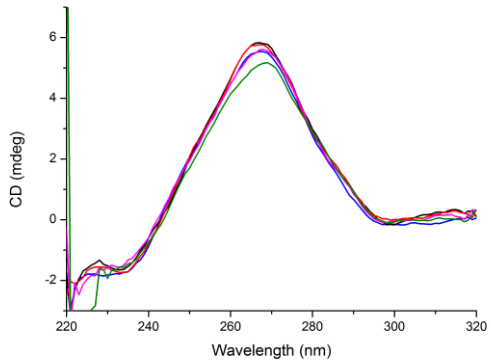
Inter1+3



NRAS



TERRA



The MD simulation

The structure of CPT1 and CPT2 were drawn by Sybyl8.1 package. The molecules were charged with Gasteiger-Marsili method, without changing the formal change before computation. Then the molecules were optimized in energy and geometry using Powell method. The detailed setting was as fellow: Max Iterations: 10000; Max Displacement: 0.01; Min Energy Change: 0.01; Simplex Threshold: 1000.0; LS Accuracy: 0.001; LS Step Size:0.001; Non-bonded Reset: 10; RMS Displacement: 0.001; Gradient: 0.01; Simplex Iterations: 20; Derivative Reset: 100. The minimized structures of CPT1 and CPT2 were applied to the Surflex Dock program to compute the binding mode with G4 DNA structure.

The 3D structures of G4 DNA used in docking study were obtained from PDB database, while the PDB entry of these DNA crystal structure were 1KF1 and 143D. The 1KF1 DNA crystal structure contained three potassium ions and 68 water molecules, and they were all deleted before docking. Docking study was conducted by a Surflex Dock program in Sybyl 8.1 package. The protomol was generated by the Automatic mode. The Threshold and Bloat were changed according to an orthogonal experimental design (Threshold: 0.1 – 0.7, increment 0.1; Bloat: 0 – 10, increment 1). The parameters in docking work were set as follow: Additional Starting Conformation per Molecule: 20; Angstroms to Expand Search Grid: 6; Max Conformation per Fragment: 20; Max Number of Rotatable Bonds per molecule: 100; Density of Search: 3.00; Number of Spins per Alignment: 12. And four flags are considered in calculations: Soft Grid Treatment, Pre-Dock Minimization, Post-Dock Minimization and Molecule Fragmentation.

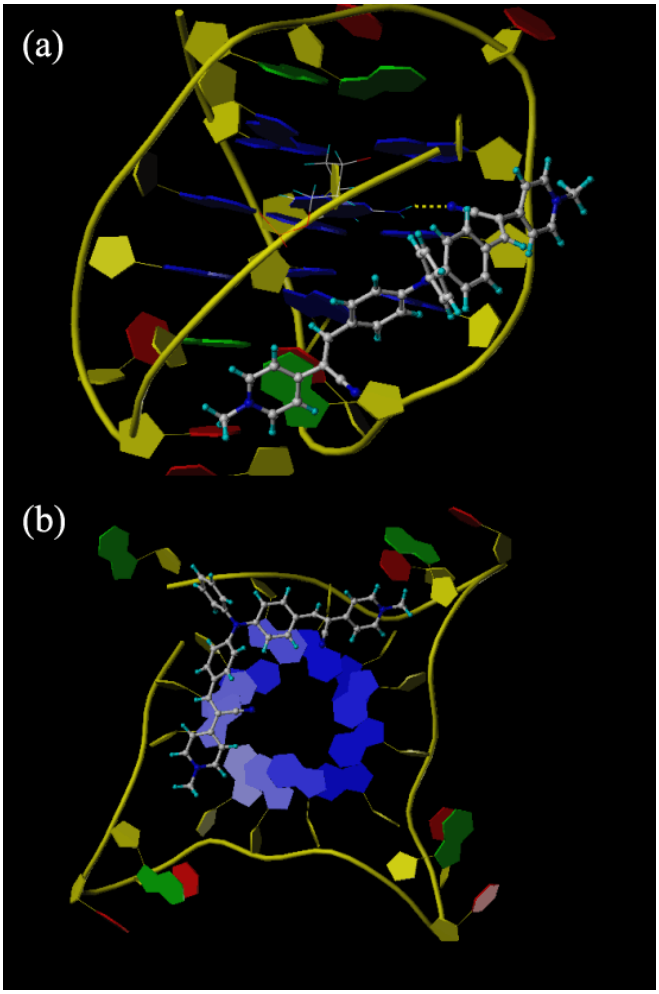


Figure S9. The binding model of CPT2 to anti-parallel [A] and parallel [B] G4 DNA structure. CPT2 is showed in Ball and Stick model, with all the hydrogen atoms are showed. While G4 DNA structure is showed in Ribbon model. A hydrogen binding is observed between N34 in CPT2 and DG21.H22 in 143D DNA structure.

The highest score in docking for CPT2 and 143D system is 4.78 (Threshold 0.5, Bloat 10), while for CPT2 and 1KF1 system is 5.85 (Threshold 0.5, Bloat 8). The highest score in docking for CPT1 and 143D system is 4.14 (Threshold 0.7, Bloat 10), while for CPT1 and 1KF1 system is 4.95 (Threshold 0.3, Bloat 4).

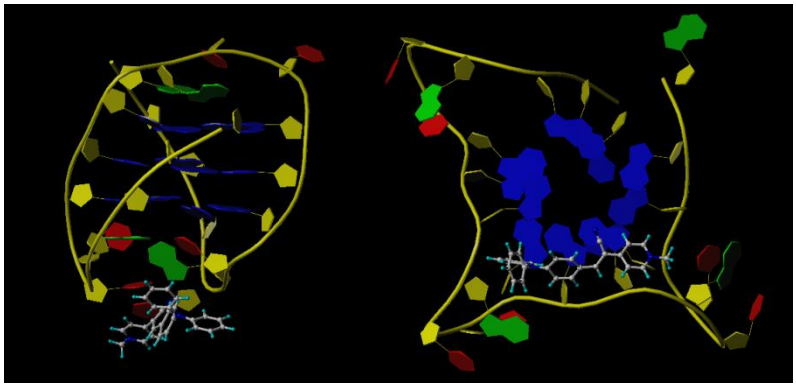


Figure S10. The binding model of CPT1 to anti-parallel [left] and parallel [right] G4 DNA structure. CPT1 is showed in Ball and Stick model, with all the hydrogen atoms are showed. While G4 DNA structure is showed in Ribbon model.

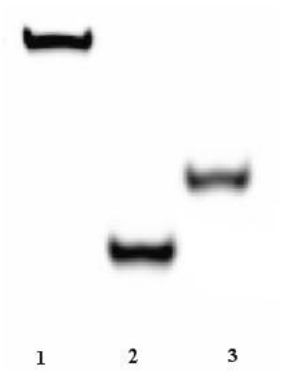


Figure S11 Gel analysis of duplex, G-quadruplex and CPT2-quadruplex complex. Lane 1: 22AG-formed duplexes. Lane 2: 22AG-formed G-quadruplex. Lane 2: 22AG-formed G-quadruplex treated with 10 μM CPT2. Each sample included 1 μM FAM-labeled 22AG DNA. The duplex was formed of 5'-FAM-22AG DNA and its antisense sequence (ssDNA).

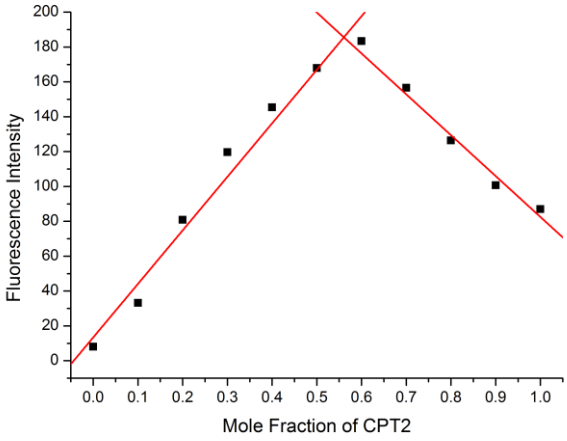


Figure S12 Job Plot of CPT2 with 22AG DNA. Total concentration was maintained at 1μM.
The intersection points obtained in the Job Plot demonstrated binding stoichiometries on the order of one CPT2 per G-quadruplex, indicating that 10 μM was saturated concentration of CPT2 and enough for the fluorescence enhancement.

Oligonucleotides

Table S1 Sequences of DNA used in this study

Name	**Sequence (from 5' to 3')**
22AG	AGGGTTAGGGTTAGGGTTAGGG
G3T3	GGGTTTGGGTTTGGGTTTGGG
ckit1	AGGGAGGGCGCTGGGAGGAGGG
cmyc	TTGAGGGTGGGTAGGGTGGGTAAA
src1	GGGCGGCGGGCTGGGCGGGG
TBA	GGTTGGTGTGGTTGG
ckit*	GGCGAGGAGGGGCGTGGCCGGC
H-RAS	TCGGGTTGCGGGCGCAGGGCACGGGCG
ssDNA	CCCTAACCCTAACCCTAACCCT
dsDNA-1	CCAGTTCGTAGTAACCC
dsDNA-2	GGGTTACTACGAACTGG
Inter1+3-1	TGGGTT
Inter1+3-2	TGGGTTAGGGTTAGGGT
8TG	TGGGGGGT