Electronic Supplementary Information (ESI)

Selectively recognizing heptad-interfaced G-quadruplexes by a molecular rotor with an ESIPT emission

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Experimental Section

Reagents and Materials

All DNA sequences shown in Table S1 were synthesized by TaKaRa Biotechnology Co., Ltd (Dalian, China) and purified by HPLC. The DNA concentrations were determined by dissolving them in ultrapure water using the absorbance at 260 nm and the extinction coefficients calculated by nearest neighbor analysis. All flavonoids investigated in this work (Table S2) were purchased from MedChemExpress (New Jersey, USA) and we did not perform further purification before use. Phosphate salts for preparing 20 mM PBS buffer were purchased from Aladdin. Ltd. (Shanghai, China). All electrophoresis reagents, including 1 × TBE, ammonium persulfate (APS), Trimethylethylenediamine (TEME), and 30% (w/v) acrylamide/methylene bis-acrylamide solution (29: 1) were purchased from Solarbio Science & Technology Co., Ltd. (Beijing, China). Other reagents including sodium chloride (NaCl), potassium chloride (KCl), lithium chloride (LiCl), potassium bromide (KBr), potassium nitrate (KNO_3) and potassium sulfate (K_2SO_4) were purchased from Sinopharm Chemical Reagent Co. Ltd (Beijing, China) at analytical grade. All of solutions were prepared using ultrapure water from a Millipore Milli-Q water purification system (Billerica, USA) with an electric resistance >18.2 MΩ.

Preparation of nucleic acid structures

An annealing treatment was used to prepare the heptad-interfaced G4 and other nucleic acid structures. Specifically, the single-strand sequences dissolved in 20 mM PBS buffer (containing 100 mM KCl, pH 7.0) were heated to 95 ^{o}C for 5 min in PCR Thermal Cycler (Takara Biomedical Technology Co. Ltd., Beijing, China), and then were slowly cooled to 4 ^{o}C (~1.5 h). The resultant samples were stored in a refrigerator at 4 ^{o}C for later use.

Fluorescence measurements

Fluorescence spectra were acquired using a F2700 spectrofluorometer (Japan) in a quartz cell (with a path length of 1 cm) that was kept at 20 ± 1 ^{o}C using an equipped temperature-controlled circulator (Julabo Labortechnik GmbH, Seelbach, Germany). Flavonoids at the specified concentration were added into the nucleic acid samples for incubation of 15 min with gentle agitation in 20 mM PBS buffer (pH 7.0) containing 100 mM cation ion. The emission spectra were obtained with excitation at 368 nm, while for GGA8-3N, GG6A-6N, GGA8-9N and GGA8-12N, the excitation wavelength was set at 310 nm.

The stoichiometries of Sop binding to the heptad-interfaced G4s (GGA4, GGA8, AGGA8 and GGA8A) was determined by the Job's plot analysis. The total concentration of Sop and G4s was maintained at 2 μM and the Sop-to-G4 concentration ratio was sequentially varied, and the emission spectra were measured under excitation of 368 nm to obtain the fluorescence intensities

at 530 nm at different concentration ratios.

To determine the stability of the heptad-interfaced G4s with addition of the complementary strand (TCC4), TCC4 (2 μM) was mixed with 2 μM GGA4 or 1 μM GGA8 in 20 mM PBS buffer (pH 7.0) containing 100 mM KCl or LiCl, and they were hybridized (heated to 95 ^{o}C for 5 min, and then slowly cooled to 4 ^{o}C). Then, Sop was added to these DNA samples for incubation of 30 min and its final concentration was 5 μM. The emission spectra of Sop were measured with excitation at 368 nm.

UV-Vis absorption spectra measurements

UV-Vis absorption spectra were measured on a UV-2550 spectrophotometer (Shimadzu Corp, Kyoto, Japan) and using a quartz of 1 cm path length. The experiments were conducted at 20 ^{o}C.

DNA melting temperature (T_m) measurements

The melting temperatures (T_m) of GGA8 in the absence and presence of Sop were measured using an UV2550 spectrophotometer (Shimadzu Corp., Kyoto, Japan), which was equipped with a TMSPC-8 T_m analysis system (Shimadzu Corp., Kyoto, Japan) and a micro multi-cell quartz cuvette (with a path length of 1 cm). It can simultaneously control the temperature of eight reservoirs (with each reservoir of about 120 μL). The absorbance of each sample at 295 nm was collected in 20 mM PBS buffer (containing 100 mM KCl, pH 7.0) between 5 to 100 ^{o}C with an increment of 0.5 ^{o}C and a 30 s equilibration time. The resultant data were sigmoidally fitted to obtain the T_m value.

Circular dichroism (CD) spectra measurements

The CD spectra were measured on a MOS-500 CD spectrometer (Bio-Logic Science Instruments, France) using a 2-mm path-length quartz cell. Samples were placed in a temperature-controlled holder (20 ^{o}C) and scanned at a wavelength range of 200-400 nm with a speed of 200 nm/min and 1 nm pitch. The CD spectra of the heptad-interfaced G4s were obtained in 20 mM PBS buffer containing 100 mM KCl, pH 7.0. The DNA-free solution was subtracted from the collected data for blank correction. All given CD spectra were three scans averaged.

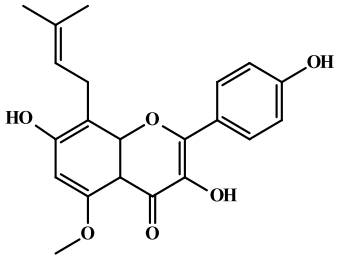
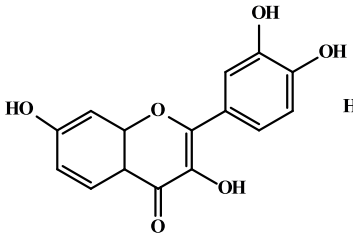
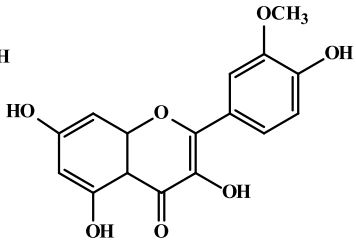
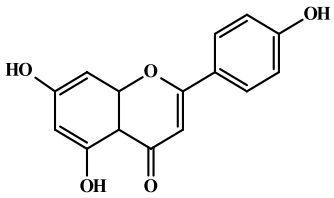
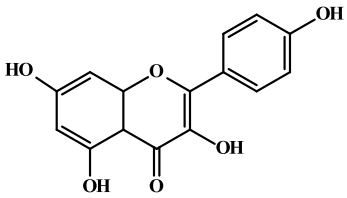
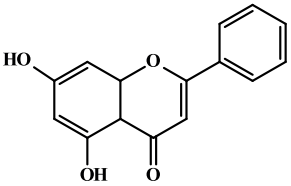
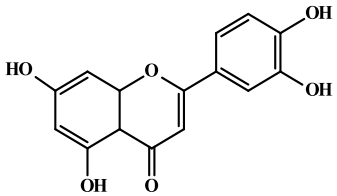
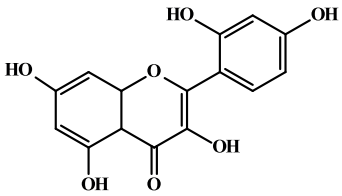
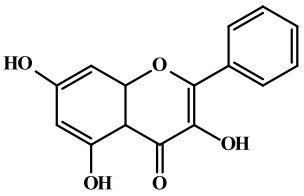
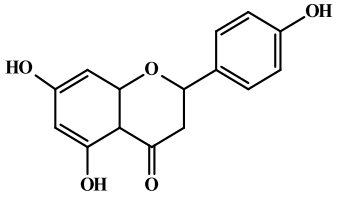
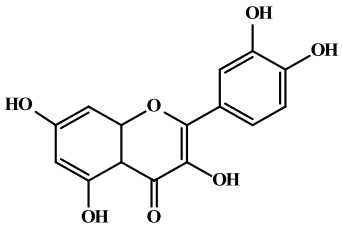
Polyacrylamide gel electrophoresis (PAGE)

Native PAGE (20%) was employed for analysis of the heptad-interfaced G4s in K^+ and Li^+ with the absence and presence of the complementary strand. 10 μL DNA sample (5 μM) was loaded into the gel channel, and electrophoresis was conducted at a constant voltage of 100 V for 5.5 h at 4 ^{o}C in 0.5×TBE buffer (pH 8.0). After electrophoresis, the gel was stained with Stains-All and imaged under ambient light.

Table S1. Sequences of oligonucleotides used in this work

Entry	Sequence (5'-3')	Remark
GGA4	GGAGGAGGAGGA	Heptad-interfaced G4 (N=2-aminopurine)
GGA8	GGAGGAGGAGGAGGAGGAGGAGGA	
GGA8A	GGAGGAGGAGGAAGGAGGAGGAGGA	
AGGA8	AGGAGGAGGAGGAGGAGGAGGAGGA	
GGA8-3N	GGNGGAGGAGGAGGAGGAGGAGGA	
GGA8-6N	GGAGGNGGAGGAGGAGGAGGAGGA	
GGA8-9N	GGAGGAGGNGGAGGAGGAGGAGGA	
GGA8-12N	GGAGGAGGAGGNGGAGGAGGAGGA	
htG4	TAGGGTTAGGGTTAGGGTTAGGG	G4 monomer
2O3M	AGGGAGGGCGCTGGGAGGAGGG	
32R	AGGGCGGTGTGGGAATAGGGAAGAGGGGGAGG	
Z-G4rT	TGGTGGTGGTGGTGTGGTGGTGGTGTT	Intra-stacked G4 dimer
Z-G4	TGGTGGTGGTGGTTGTGGTGGTGGTGTT	
di-htG4	TTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTA GGGTTAGGGTTAGGGTT	Intra-unstacked G4 dimer
22R	AGGGCGGTGTGGGAAGAGGGAA	Inter-stacked G4 dimer
22RT	AGGGCGGTGTGGGAATAGGGAA	
rTel21	GGGUUAGGGUUAGGGUUAGGG	
rTel22	AGGGUUAGGGUUAGGGUUAGGG	
TBA-G3	GGTTGGTGTGG	G-triplex
Hairpin	GAAAAAAAAAAAAAGTTTTCTTTTTTTTTTTTTC	hairpin
10AT-T	AAAAAAAAAATTTTTTTTTTTTTTTTTTTTTTT TTT	triplex
i-motif	CCCTAACCCTAACCCTAACCC	i-motif

Table S2 Chemical structures of flavonoids used in this work

Sophoflavescenol (Sop)	**Fisetin (Fis)**	**Isorhamnetin (Iso)**
		
Apigenin (Api)	**Kaempferol (Kae)**	**Chrysin (Chr)**
		
Luteolin (Lut)	**Morin (Mor)**	**Galangin (Gal)**
		
Naringenin (Nar)	**Quercetin (Que)**	
		

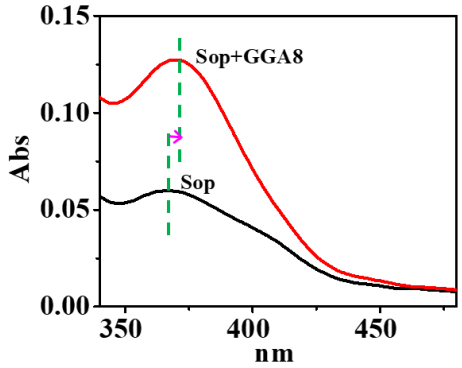


Fig. S1 UV-Vis absorption spectra of Sop (20 μM) in the absence and presence of GGA8 (4 μM) in 20 mM PBS buffer containing 100 mM KCl (pH 7.0).

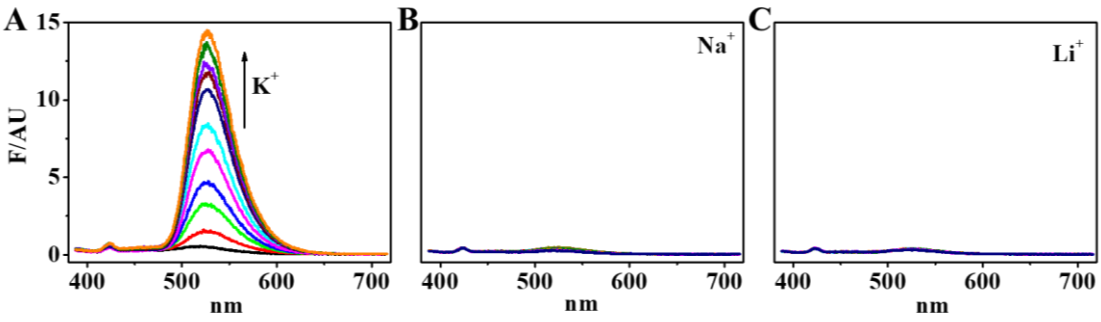


Fig. S2 Emission spectra of Sop (5 μM) in the presence of GGA8 (1 μM) in 20 mM PBS buffer (pH 7.0) containing variant concentrations of (A) KCl, (B) NaCl, and (C) LiCl (0, 1, 2, 3, 5, 7, 10, 20, 30, 50, and 100 mM, respectively).

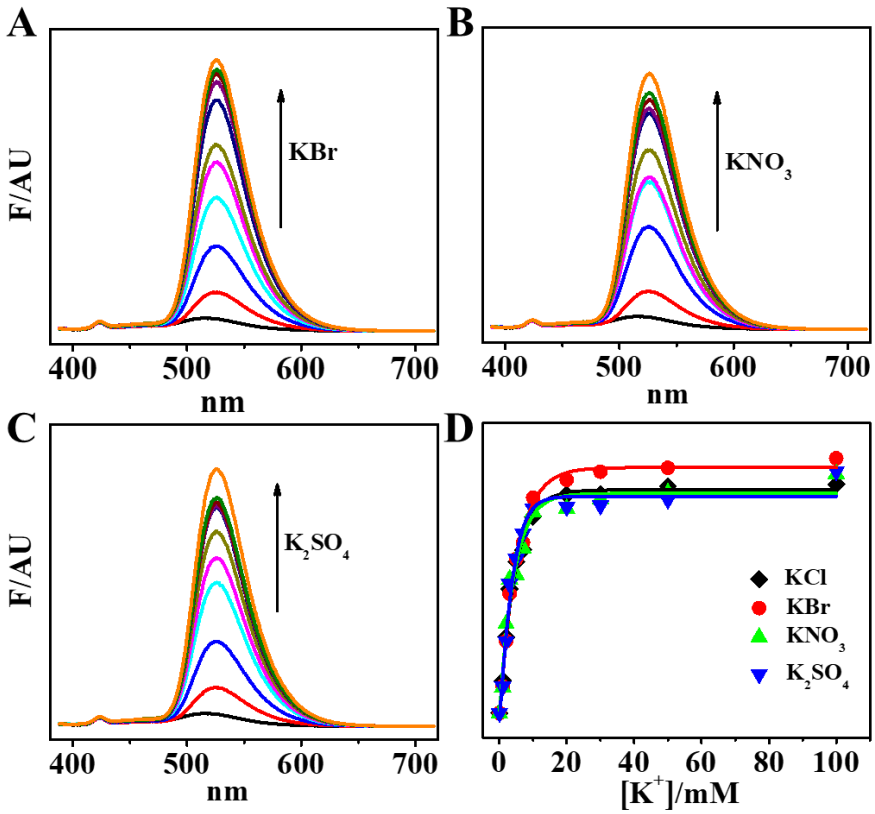


Fig. S3 (A-C) Emission spectra of Sop (5 μM) in the presence of GGA8 (1 μM) in 20 mM PBS buffer (pH 7.0) containing variant concentrations of (A) KBr, (B) KNO_3 and (C) K_2SO_4. (D) Responses of ESIPT emission intensities at 530 nm to the K^+ concentrations.

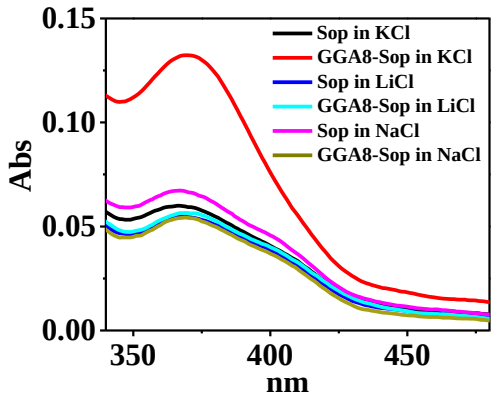


Fig. S4 The UV-Vis spectra of Sop (20 μM) in the absence and presence of GGA8 (4 μM) in 20 mM PBS buffer (pH 7.0) containing 100 mM KCl, LiCl and NaCl, respectively.

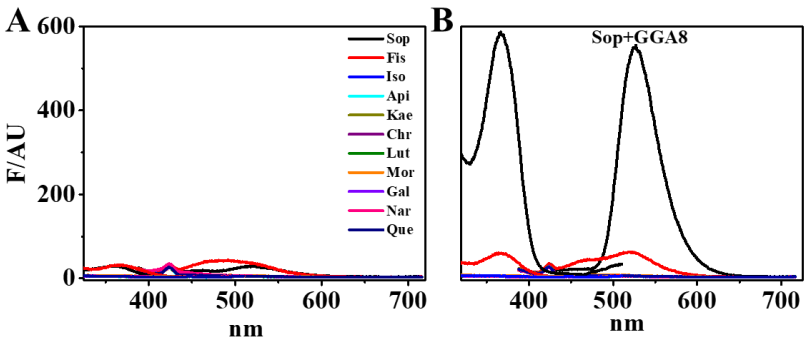


Fig. S5 Excitation and emission spectra of investigated flavonoids (0.5 μM) in the (A) absence and (B) presence of GGA8 (1 μM) in 20 mM PBS buffer containing 100 mM KCl (pH 7.0).

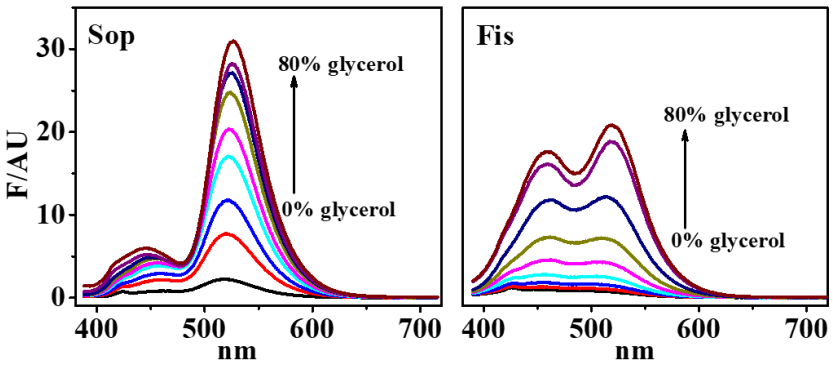


Fig. S6 Emission spectra of (A) Sop and (B) Fis (5 μM) in water/glycerol solvent with increasing the glycerol content (as indicated by arrow from 0 to 80%). 10% ethanol was further added in all cases to increase the ligand solubility in water.

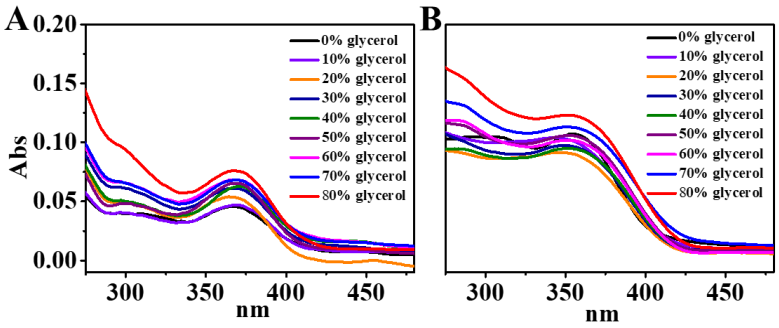


Fig. S7 UV-Vis spectra of (A) Sop and (B) Fis (5 μM) in water/glycerol solvent with increasing the glycerol content (as indicated by arrow from 0 to 80%). 10% ethanol was further added in all cases to increase the ligand solubility in water.

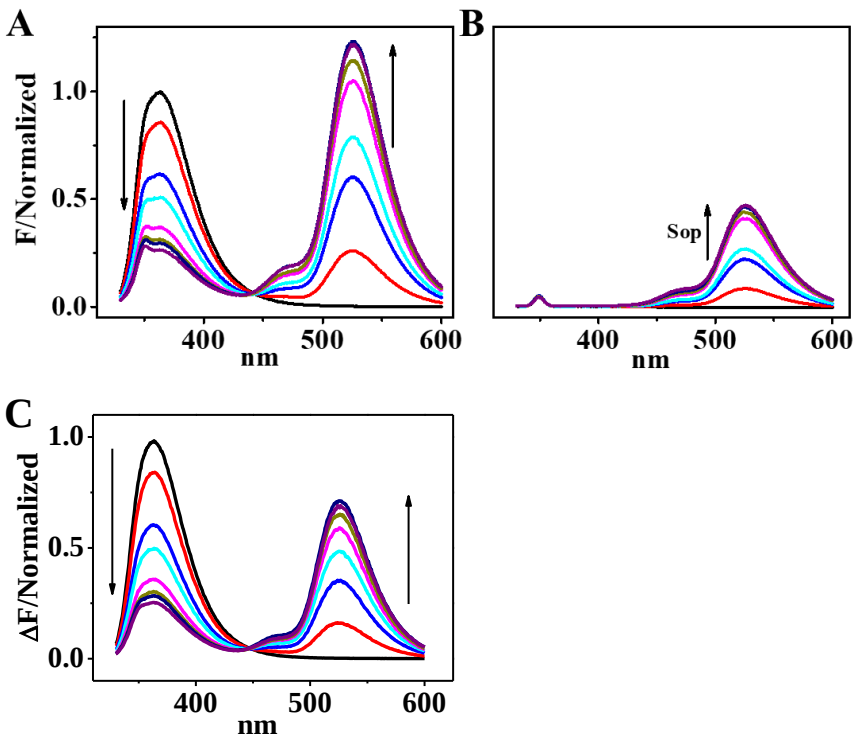


Fig. S8 (A, B) Emission spectra of (A) GGA8-9N and (B) GGA8 (1 μM) under excitation of 310 nm with the Sop concentration at 0, 1, 2, 3, 5, 7, 10, and 13 μM in 20 mM PBS containing 100 mM KCl (pH 7.0). (C) Difference spectra obtained by subtracting the spectra of GGA8 from those of GGA8-9N to get the contribution of FRET (ΔF).

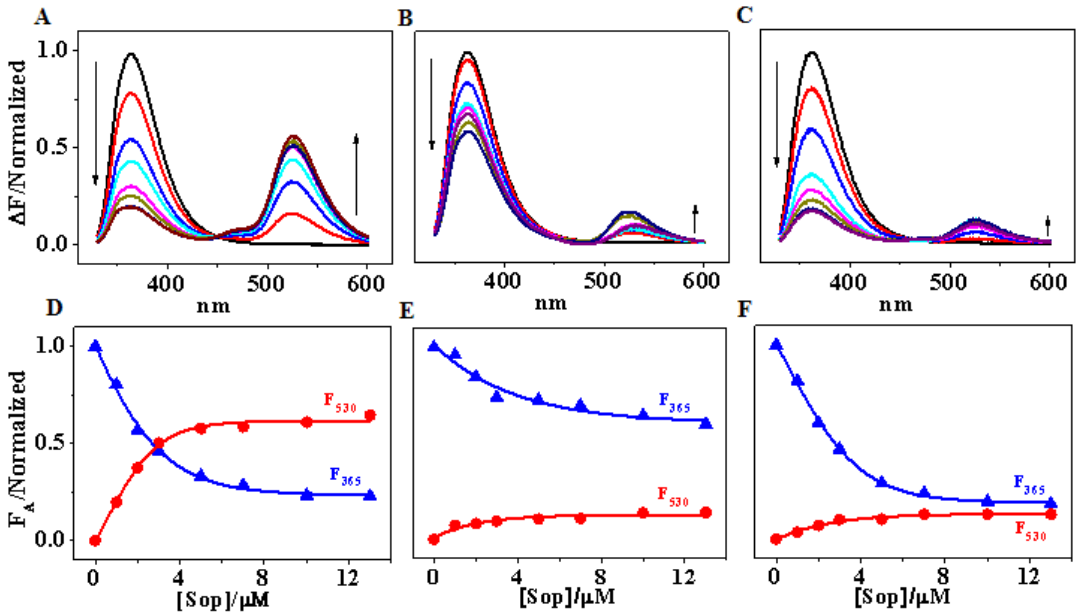


Fig. S9 (A-C) Difference spectra of (A) GGA8-6N, (B) GGA8-3N, and (C) GGA8-12N (1 μM) under excitation of 310 nm with the Sop concentration at 0, 1, 2, 3, 5, 7, 10, and 13 μM in 20 mM PBS containing 100 mM KCl (pH 7.0). The spectra obtained with GGA8 was subtracted from these spectra to get the contribution of FRET (ΔF). (D-F) Band areas of (D) GGA8-6N, (E) GGA8-3N, and GGA8-12N at 365 nm (F_{365}) and 530 nm (F_{530}) with increasing the Sop concentration.

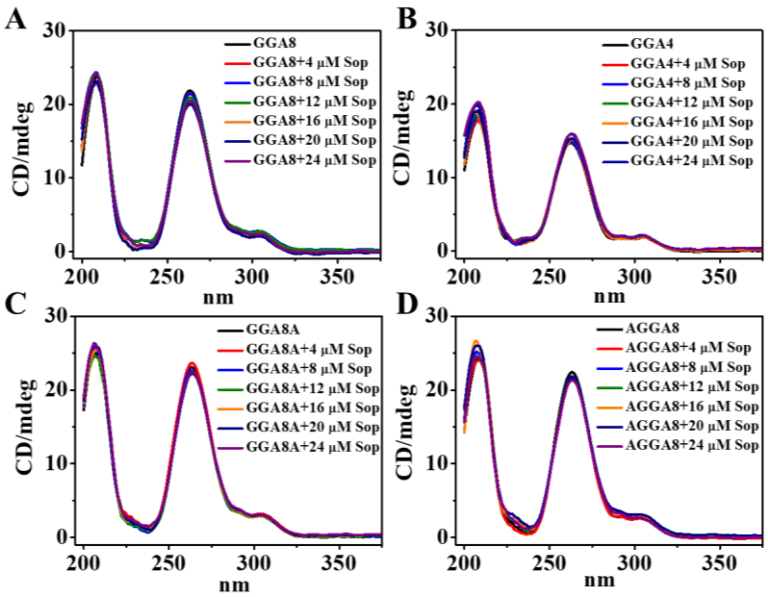


Fig. S10 CD spectra of (A) GGA8 (4 μM), (B) GGA4 (8 μM), (C) GGA8A (4 μM), and (D) AGGA8 (4 μM) with addition of Sop (from 0 to 24 μM) in 20 mM PBS, 100 mM KCl (pH 7.0).

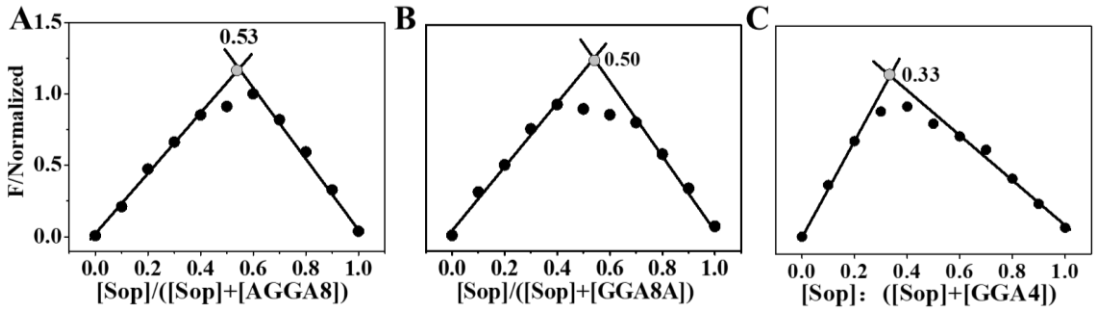


Fig. S11 Job's plots for the stoichiometric analysis of Sop binding to (A) AGGA8, (B) GGA8A, and (C) GGA4 in 20 mM PBS containing 100 mM KCl (pH 7.0). The total concentration of Sop and DNA was kept at 2 μM, and the concentration ratio was systematically changed.

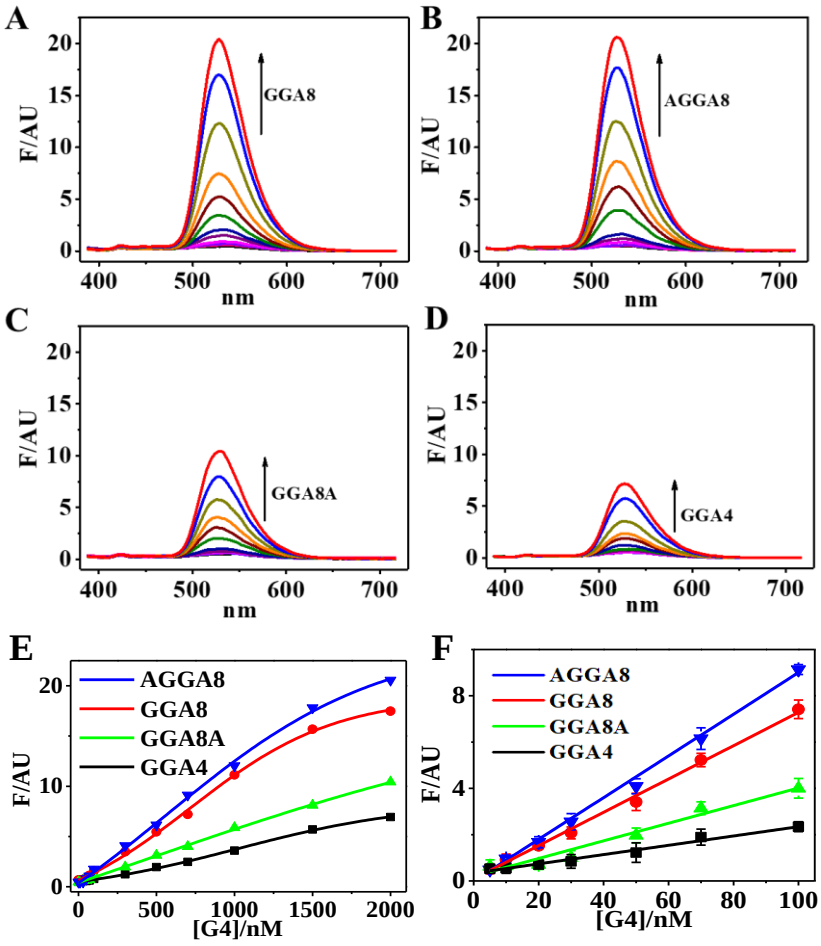


Fig. S12 Emission spectra of Sop (1 μM) with addition of (A) GGA8, (B) AGGA8, (C) GGA8A, and (D) GGA4 in 20 mM PBS buffer (containing 100 mM KCl, pH 7.0). The G4 concentrations were 0, 10, 20, 30, 50, 70, 100, 300, 500, 700, 1000, 1500, and 2000 nM, respectively. (E) Fluorescence titration of Sop (5 μM) at 530 nm upon gradual addition of G4. (F) linear responses to G4 concentration between 0-100 nM.

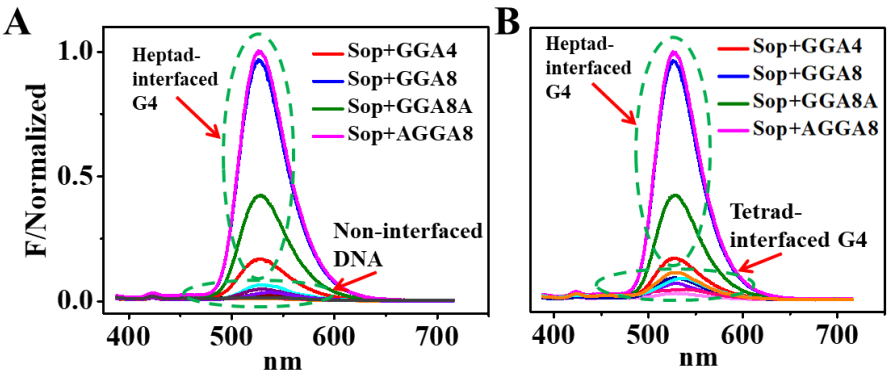


Fig. S13 The emission spectra of Sop (5 μM) in the presence of the heptad-interfaced G4s and comparison with (A) non-interfaced DNA structures and (B) tetrad-interfaced G4s (1 μM) in 20 mM PBS buffer (100 mM KCl, pH 7.0).

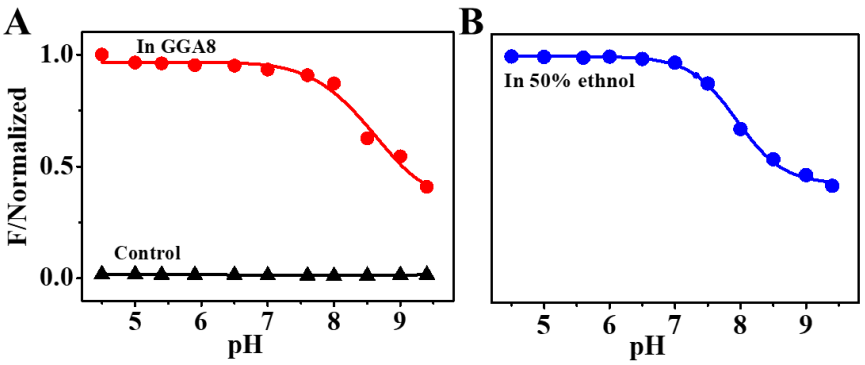


Fig. S14 Dependence of the fluorescence intensity of Sop (5 μM) at 530 nm on pH (20 mM PBS, 100 mM KCl) in the presence of (A) GGA8 (1 μM) and (B) absence of GGA8 but in 50% ethanol, respectively. Control: measured alone in 20 mM PBS buffer (containing 100 mM KCl). Deprotonation of hydroxyl groups at alkalic pH weakened the fluorescence responses due to formation of the negatively charged Sop.

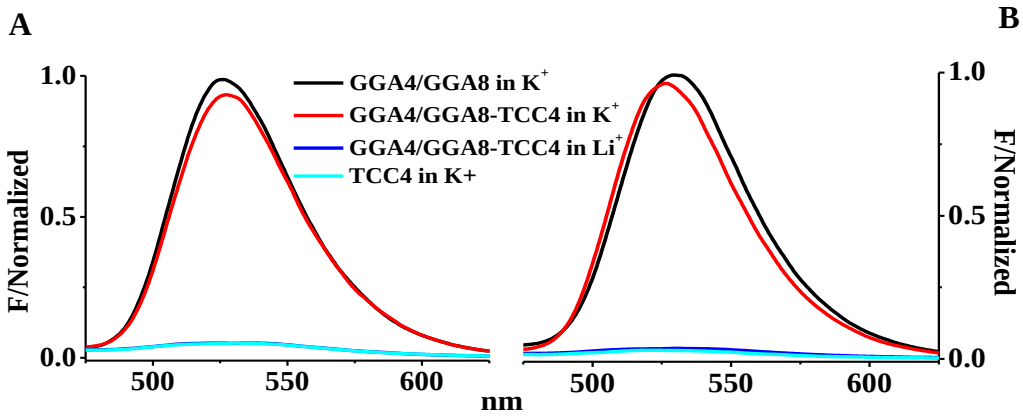


Fig. S15 Fluorescence responses of Sop (5 μM) in the presence of (A) GGA4 (2 μM) and (B) GGA8 (1 μM) with the addition of TCC4 (2 μM) in K^+ or Li^+, respectively.