Supporting for

A pH-triggered G-triplex switch with K^+ tolerance

Heng Gao, Xiong Zheng, Tong Yang, Qingqing Zhang, Chenxiao Yan, Xiaoshun Zhou and Yong Shao*

Materials and reagents. Sanguinarine (SG) was purchased from Aladdin Bio-Chem Technology Co., LTD (Shanghai, China). Cucurbit[8]uril (CB8) was obtained from Strem Chemicals, Inc. (Massachusetts, USA). DNAs were synthesized by TaKaRa Biotechnology Co., Ltd and purified by HPLC (Dalian, China). They were first dissolved in pure water for concentration measurement using the 260 nm extinction coefficients calculated by nearest neighbor analysis. All other chemicals were analytical-reagent grade (Sigma Chemical Co., St. Louis, USA) and used without further purification. Milli-Q water (18.2 MΩ; Millipore Co., Billerica, USA) was used throughout experiments.

Fluorescence measurements. Fluorescence spectra were acquired with a F2700 spectrofluorometer (Japan) at 20±1 °C, which was equipped with a temperature-controlled circulator (Julabo Labortechnik GmbH, Seelbach, Germany). Fluorescence was measured in a quartz cell with a path length of 1 cm. To prepare the nucleic acid solution with a thermodynamically stable conformation, the nucleic acid strands were annealed in K^+ at specified pH in a thermocycler (first at 92 °C, then slowly cooled to room temperature) and stored at 4 ^oC overnight. Then, SG at the specified concentration was added into the nucleic acid solution for 30-min incubation. In the case of further addition of CB8, the resulting solution also allowed incubation for another 30 min before fluorescence measurements. Phosphate buffer salt (PBS) containing 100 mM K^+ was used as the appropriate buffer for the G3 switch.

Isothermal titration calorimetry (ITC). ITC experiments were performed at 20 ^oC using an ITC200 microcalorimeter (MicroCal, LLC, Northampton, MA). Both DNA and SG solutions were prepared using an identical PBS buffer containing 0.1 M K^+. DNA (10 μM) in the sample cell was droppingly titrated using 2 μL of 100 μM SG (the first 0.4 μL injection was followed by 19 injections of 2 μL with 20s duration at 150s time intervals). The titration of SG in the syringe into the identical buffer solution in the sample cell without DNA was used as a control to obtain the dilution heat. Origin (version 7.0) software was used for data analysis. The area of each injection peak was automatically integrated. A binding isothermal curve was obtained by plotting the total heat per injection (kcal mol^{-1} of injectant) as a function of the molar ratio of SG to DNA.

Circular dichroism (CD) spectra measurements. The CD spectra were measured on

MOS-500 CD spectrometer (Bio-Logic Science Instruments, France) using a 2-mm path-length quartz cell. Scans were performed at a temperature-controlled holder (20 °C) over a wavelength range of 200-400 nm with a scanning speed of 200 nm/min and 1 nm pitch. The CD spectra were performed in PBS buffer containing 0.1 M K^+ at the specified pH or in 0.1 M KCl using HCl/KOH to adjust pH to the desired value. Solution without DNA was subtracted from the collected data for blank correction. The given CD spectra were three scans averaged and zero-corrected at 400 nm. All scanning was processed at ambient temperature unless otherwise specified.

Thermodynamic melting (T_m) analysis. A solution of TBA-3tG (8 μM) was prepared in PBS containing 0.1 M K^+ at pH 4.92 and 8.6, respectively. The resultant solution in the absence and presence of 8 μM SG and 40 μM CB8 was determined using a UV2550 spectrophotometer (Shimadzu Corp., Kyoto, Japan), equipped with a TMSPC-8 T_m analysis system. The absorbance of the solution at 260 nm as a function of solution temperature between 5 °C and 95 °C was collected in 0.5 °C increments, with a ramp rate of 2 °C/min and a 30-second equilibration time applied after each temperature increment. Melting curves were normalized with regard to the response at 5 ^oC for comparison.

G3 switch regulated by photoacid. 8 μM TBA-3tG was mixed with ETS in an unbuffered 0.1 M KCl solution containing 8 μM SG. The resultant solution was adjusted to pH 8.0 using KOH before illumination using a 310 nm illuminator for 40 min. For 300 μM ETS, the solution pH was changed to 5.9 upon photo-illumination.

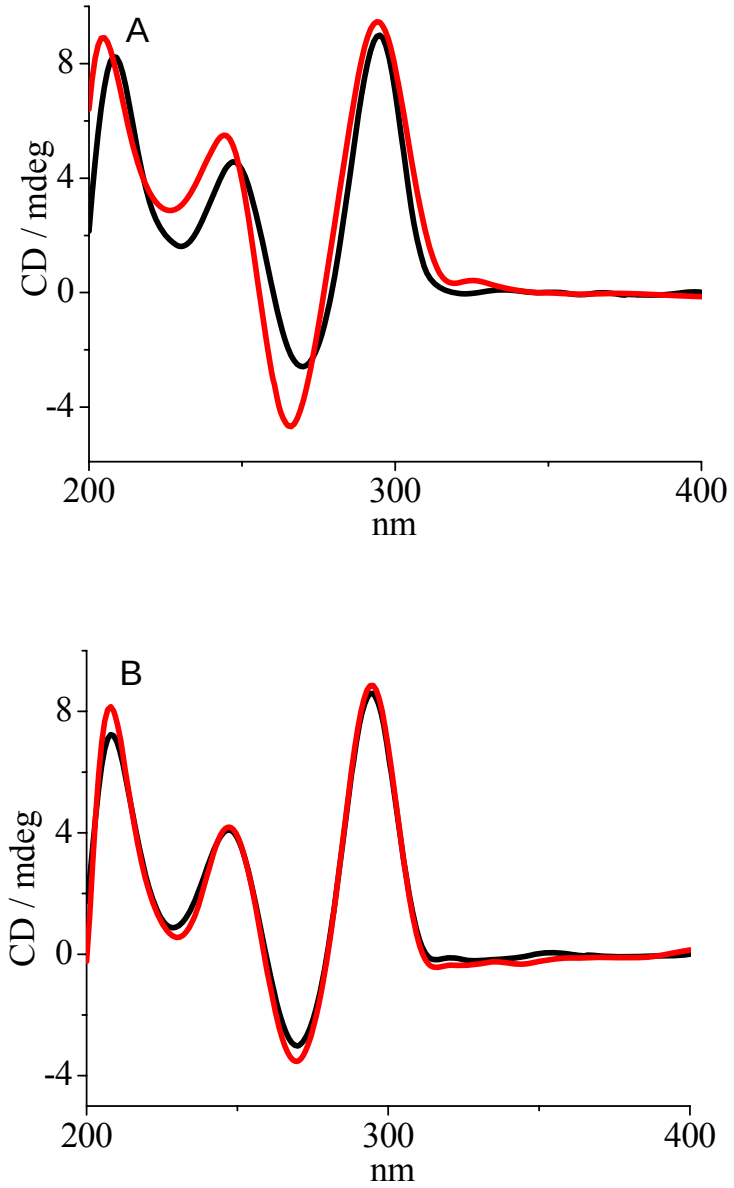


Fig. S1. CD spectra of 8 μM TBA in the absence (black) and presence (red) of 8 μM SG in PBS buffer containing 0.1 M K^+ at (A) pH 4.9 and (B) pH.8.6.

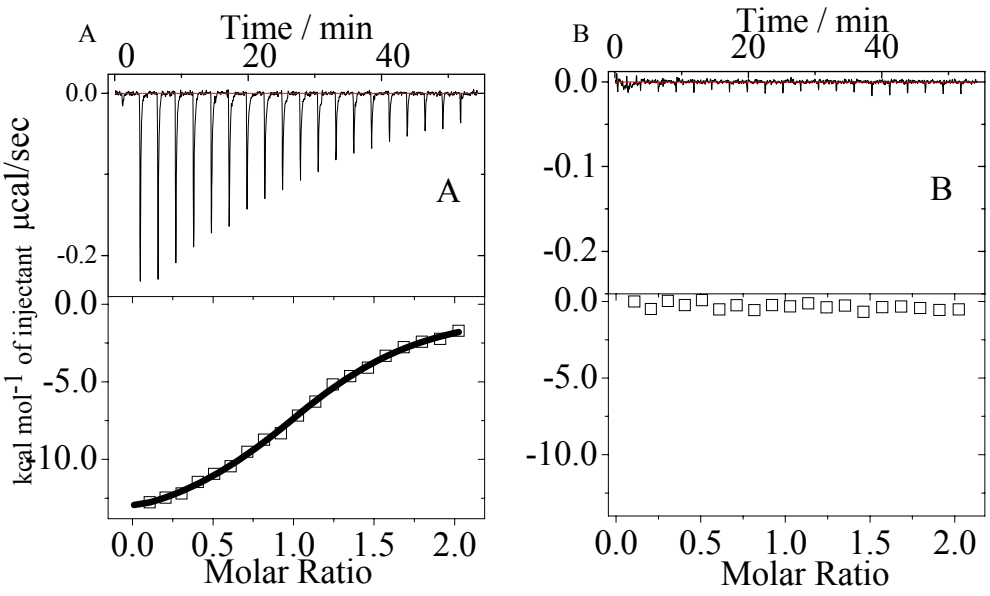


Fig. S2. Binding of SG with TBA evaluated by ITC at (A) pH 4.9 and (B) 8.6. 10 μM TBA was separately titrated by 100 μM SG in PBS buffer containing 0.1M K^+.

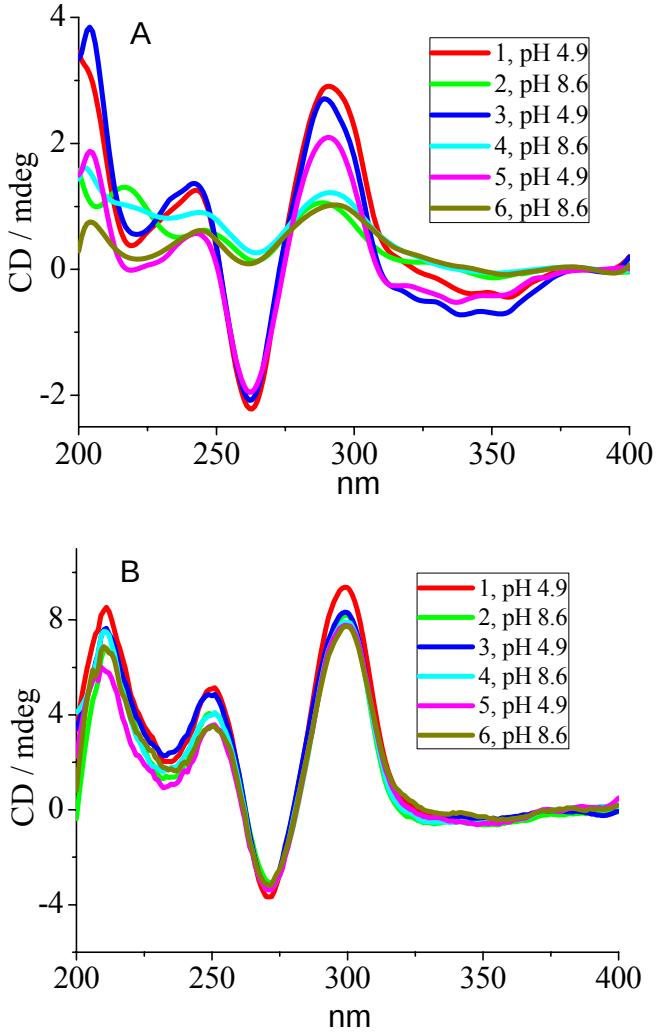


Fig. S3. CD spectra of 8 μM TBA-3tG (A) and TBA (B) in unbuffered 0.1 M KCl containing 8 μM SG by repeatedly changing the solution pH from 4.9 and 8.6. HCl and KOH (4 mM) was used to adjust the solution pH.

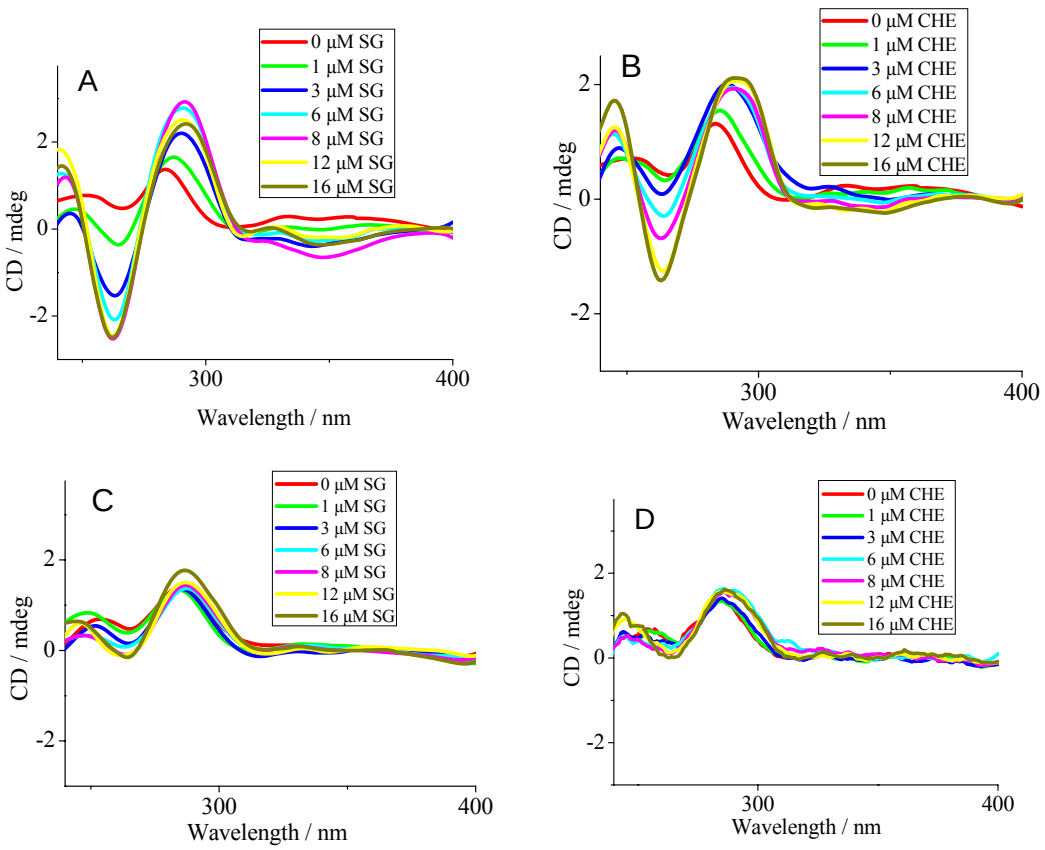
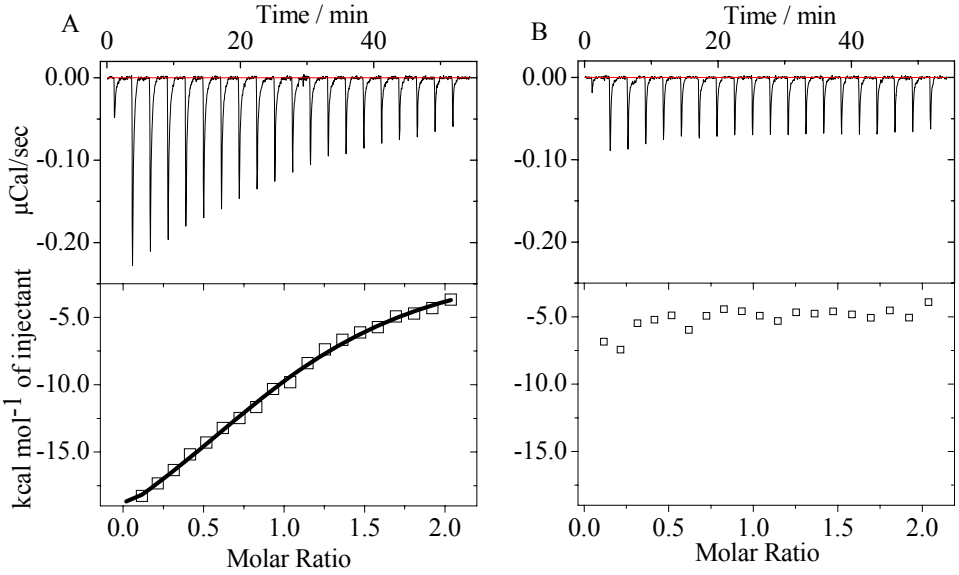


Fig. S4. CD responses of 8 μM TBA-3tG in PBS buffer containing 0.1 M K^+ at (A, B) pH 4.9 and (C, D) pH 8.6 upon increasing the (A, C) SG and (B, D) CHE concentrations, respectively.



	n site	K 10^5/M	△H kcal/mol	△S cal/mol/deg	△G kcal/mol
pH 4.9	1.09 (±0. 01)	2.2 (±0.10)	-26.7 (±1.90)	-66.4	-7.2

Fig. S5. Binding of CHE with TBA-3tG evaluated by ITC at (A) pH 4.9 and (B) pH 8.6. 10 μM TBA-3tG was titrated by 100 μM CHE in PBS buffer containing 0.1M K^+. Inset table: the thermodynamic fitting results.

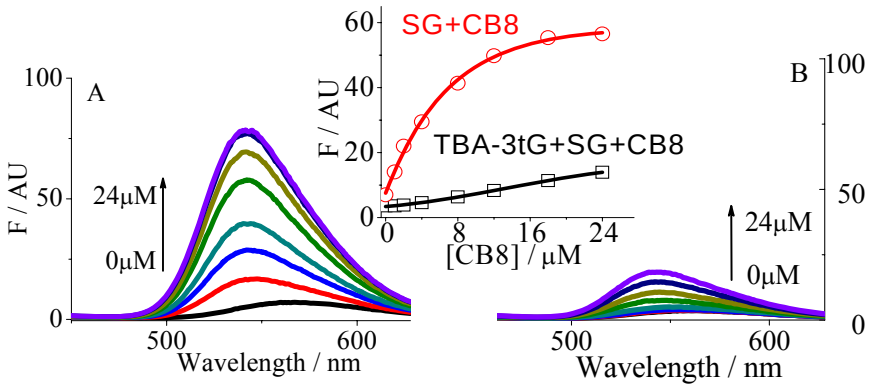


Fig. S6. Effect of CB8 on the fluorescence of SG (0.5 μM) in the (A) absence and (B) presence of 2 μM TBA-3tG at pH 4.9. Inset: the corresponding fluorescence intensity at 566 nm as a function of the CB8 concentration.

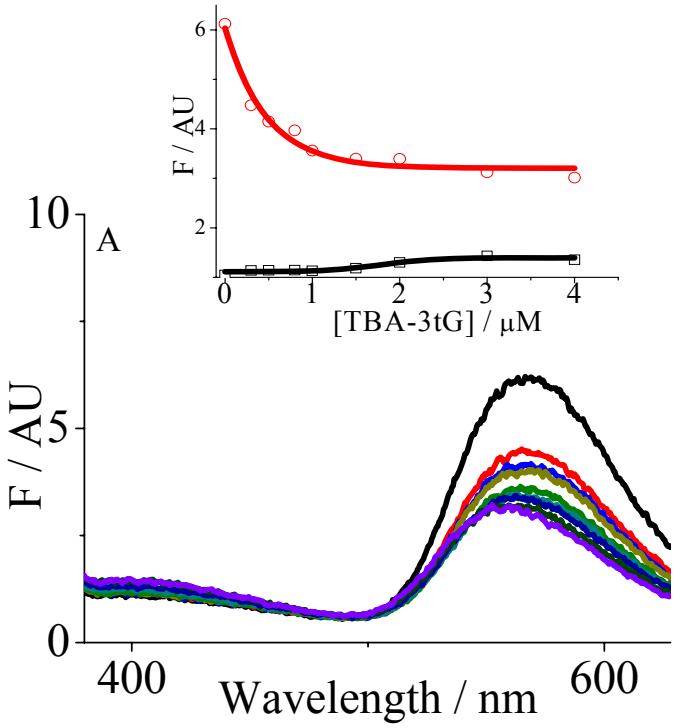


Fig. S7. Fluorescence response of 0.5 μM SG with addition of 0, 0.3, 0.5, 0.8, 1.0, 1.5, 2.0, 3.0, 4.0 μM TBA-3tG at pH 4.9. Inset: the fluorescence intensities at 566 nm (red) and 410 nm (black) as a function of the TBA-3tG concentration, respectively.

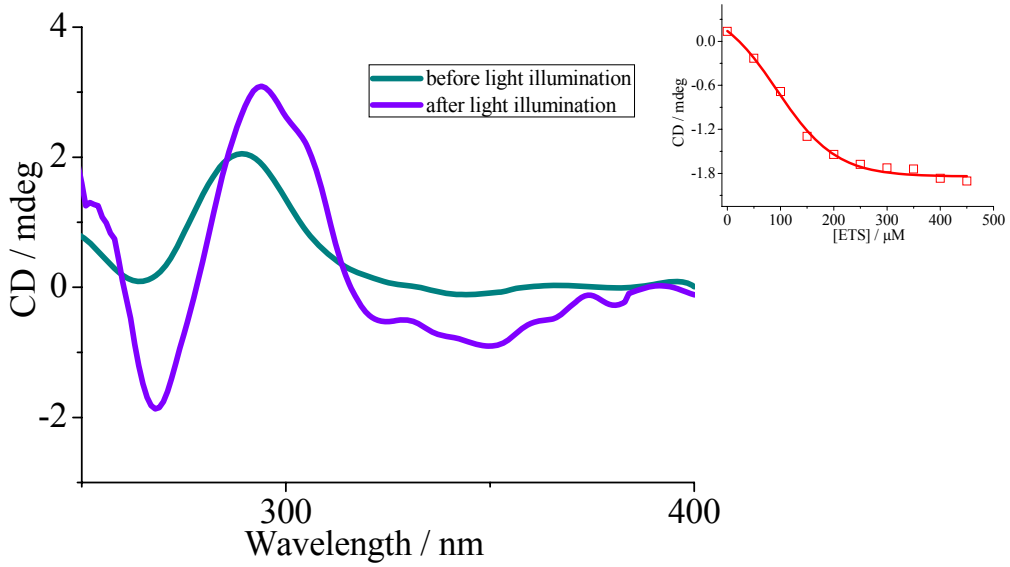


Fig. S8. CD spectra of an unbuffered 0.1 M KCl solution containing 8 μM SG, 8 μM TBA-3tG, and 300 μM ETS before and after photo-illumination at 310 nm. The solution pH was changed from 8.0 to 5.9 upon photo-illumination. Inset: Effect of ETS concentrtation on the CD signal at 267 nm after photo-illumination.