Supporting Information

Novel Dual-Functional Regulation of a Chair-Like Antiparallel G-quadruplex on Inducing Assembly/Disassembly of Cyanine Dye

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Contents:

S(a) – Experimental Section

Sample preparation. The cyanine dye DMSB was synthesized according to Hamer[1] and Brooker's[2] methods, and the purity was evaluated by mass spectrometry and nuclear magnetic resonance (NMR) [SI, S(a) part]. All oligonucleotides were purchased from Sangon Biotech Co., Ltd. (Shanghai, China) and purified by HPLC (purity 98%). ***CT*** was directly purchased from Sigma Aldrich Inc. (cat# D4522). Analytical grade methanol, KCl, KH_2PO_4 and K_2HPO_4 were purchased from Beijing Chem. Co. (China). Ultrapure water prepared by Milli-Q Gradient ultrapure water system (Millipore) was used throughout the experiments. The stock solution of DMSB is prepared by dissolving it in methanol to 2 mM and then storing in dark at 4 °C. The stock solutions of ***TBA*** was prepared by dissolving them directly into phosphate buffer solution (PBS, 20 mM KH_2PO_4/K_2HPO_4, 100 mM KCl, pH 7.4), filtered by a microfiltration membrane (Φ = 0.22 μm) and then heated to 90 °C for 5 min and then gradually cooled to room temperature at a rate of 1 °C·min^{-1} to form secondary structures. Concentrations of ***TBA*** stock solutions were determined by their absorbance at 260 nm. All DNA samples were stored at 4 °C. The measured samples were prepared by adding different amount of ***TBA*** stock solution into 1mL PBS containing 12 μM DMSB. Then the samples were incubated 2h in darkness at 4 °C before subjected to measurement.

UV-vis and fluorescence spectroscopic measurements. The UV-vis absorption spectra were measured by an Agilent-8453 UV/visible spectrophotometer equipped with a Peltier effect cuvette holder in 10 mm quartz cells at 4 °C. Fluorescence spectra were taken on a Hitachi F-4500 spectrophotometer in a 10 mm quartz cell at room temperature. Xenon arc lamp was used in the excitation light source in fluorescence measurement. The excitation wavelength was 520 nm. Both excitation and emission

[1] F. M. Hamer, *The cyanine dyes and related compounds*, Interscience Publishers, New York, **1964**.

[2] L. G. S. Brooker, F. L. White, *Journal of the American Chemical Society* **1935**, *57*, 547-551.

slits were 5 nm and the voltage was 400 V with a scan speed of 240 nm·min^{-1}.

Circular dichroism (CD) spectra measurements. All the CD spectra were recorded on a JASCO J-815 spectrophotometer in a 10 mm path-length quartz cell at 4 °C. All spectra were collected with scan speed of 200 nm·min^{-1} and a response time of 0.5 s between 700 and 400 nm with 5 scans averaged.

NMR Experiments. The stock solution of DMSB for NMR experiments was prepared by dissolving 22.2 mg DMSB into 1 mL DMSO-d6 (40 mM). The stock solution of ***TBA*** was prepared by dissolving it directly into 0.5 mL NMR buffer solution [20 mM KH_2PO_4/K_2HPO_4, 100 mM KCl, 90% H_2O/10% D_2O (v/v), pH = 7.4] and then dialyzed through a bag with cut-off molecular weight of 500 for 12 hours for desalting purpose. The measured sample for ^{1}H-NMR titration as well as NOESY was prepared by adding different amount of DMSB stock solution to NMR buffer containing 0.5 mM ***TBA***. All NMR spectra were recorded on a Bruker Avance 600 MHz spectrometer which is equipped with a 5mm BBI probe capable of delivering z-field gradients up to 50 G· cm^{-1}. 1D chemical shifts were referenced relative to 3-(trimethylsilyl)-propanoic acid (TSP). The 1D spectra were recorded by the standard Bruker pulse program p3919gp that applies 3-9-19 pulses with gradients for water suppression.[3] All the experiments were acquired 128 scans for each spectrum with a relaxation delay of 2 s at 303 K.

Partial charge calculation and assignment. The three dimensional crystal structure of TBA was obtained from the Brook Haven Protein Data Bank (PDB ID: 1QDF). The partial charges and structure of DMSB was calculated and optimized by Gaussian 03 using DFT method with an HF/6-311++G(d, p) basis set at the B3LYP level with water as the solvent. While the partial charges of the bases on TBA were automatically assigned by the CHARMm forcefield. Then the structures of DMSB

[3] a) M. Piotto, V. Saudek, V. Sklenar, *J. Biomol. NMR* **1992**, *2*, 661-665; b) V. Sklenar, M. Piotto, R. Leppik, V. Saudek, *J Magn Reson Ser A* **1993**, *102*, 241-245.

and TBA were subjected to surface calculation. The wire-mesh style electrostatic surfaces were calculated by using Discovery Studio 3.5 with the interpolated method (Accelrys, San Diego, CA).

S(b) – Identification of cyanine dye DMSB

The structure and purification of cyanine dye DMSB were identified by MS-ESI, NMR and absorption spectroscopy.

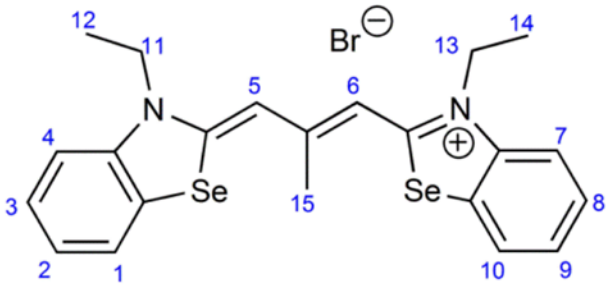

Figure S1. The numbering scheme of molecular structure of DMSB

a) MS-ESI

From the mass spectrum, the measured molecular weight of DMSB is 475.1, which is consistent with the calculated value, 475.5.

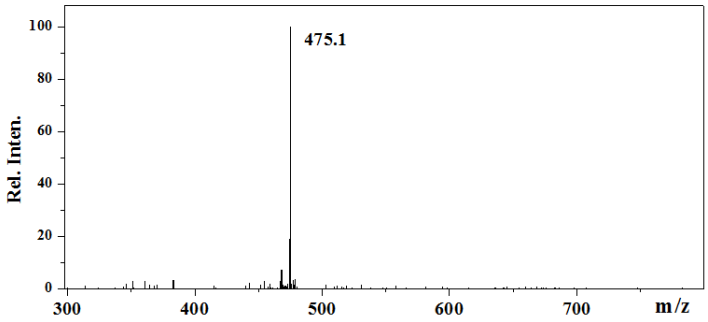

Figure S2. The MS-ESI spectrum of cyanine dye DMSB

b) NMR spectrum

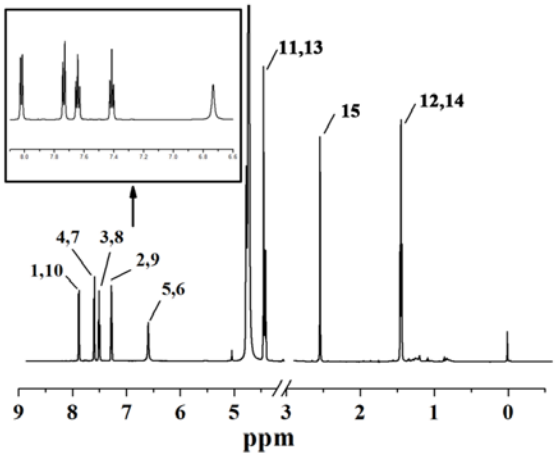

Figure S3. The ^{1}H-NMR spectrum of 1 mM cyanine dye DMSB in CD_3OD

Table S1. The full assignments of the proton resonance signals of DMSB

Proton number	Proton kind	^{1}H peak	Proton number	Proton kind	^{1}H peak
1	Aromatic	7.93-7.91, d	9	Aromatic	7.31, t
2	Aromatic	7.31, t	10	Aromatic	7.93-7.91, d
3	Aromatic	7.54, t	11	Secondary	4.48-4.44, q
4	Aromatic	7.63-7.64, d	12	Primary	1.45, t
5	Tertiary	6.63, s	13	Secondary	4.48-4.44, q
6	Tertiary	6.63, s	14	Primary	1.45, t
7	Aromatic	7.63-7.64, d	15	Primary	2.55, s
8	Aromatic	7.54, t			

c) UV-vis spectrum research and absorption coefficient calculation

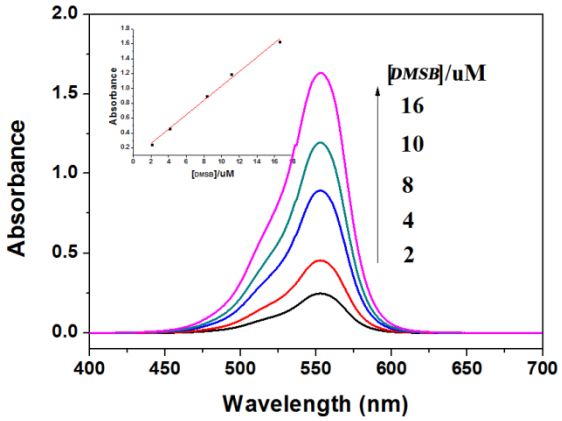

Figure S4. The absorption spectra of DMSB monomer in methanol. Inset gives the curve of the absorbance at 554 nm against the concentration of DMSB. Based on lambert-beer's law, the molar absorption coefficient of DMSB monomer is:

$$\varepsilon^{M}_{1cm,554nm} = 1.083 \times 10^{5} M^{-1} \cdot cm^{-1}.$$

S(c) – UV-vis spectra of DMSB in different conditions

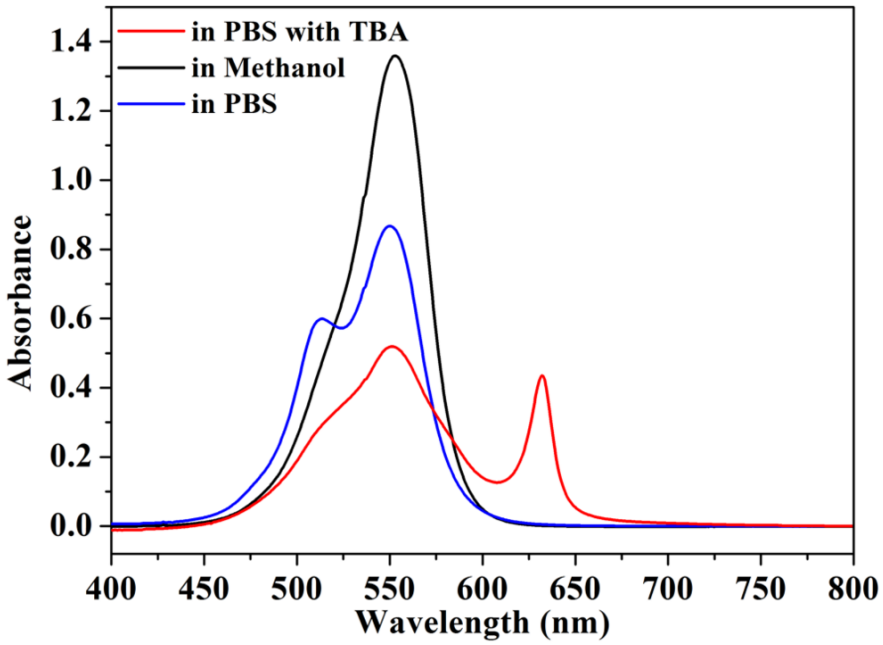

Figure S5. The absorption spectra of 12 μM ***DMSB*** in methanol (black), phosphate buffer solution (PBS, 20 mM K_2HPO_4/KH_2PO_4, 100 mM KCl, pH = 7.4) (blue) and PBS in the presence of 0.6 μM ***TBA*** (red).

S(d) – UV-vis spectra of DMSB in PBS titrated with TBA

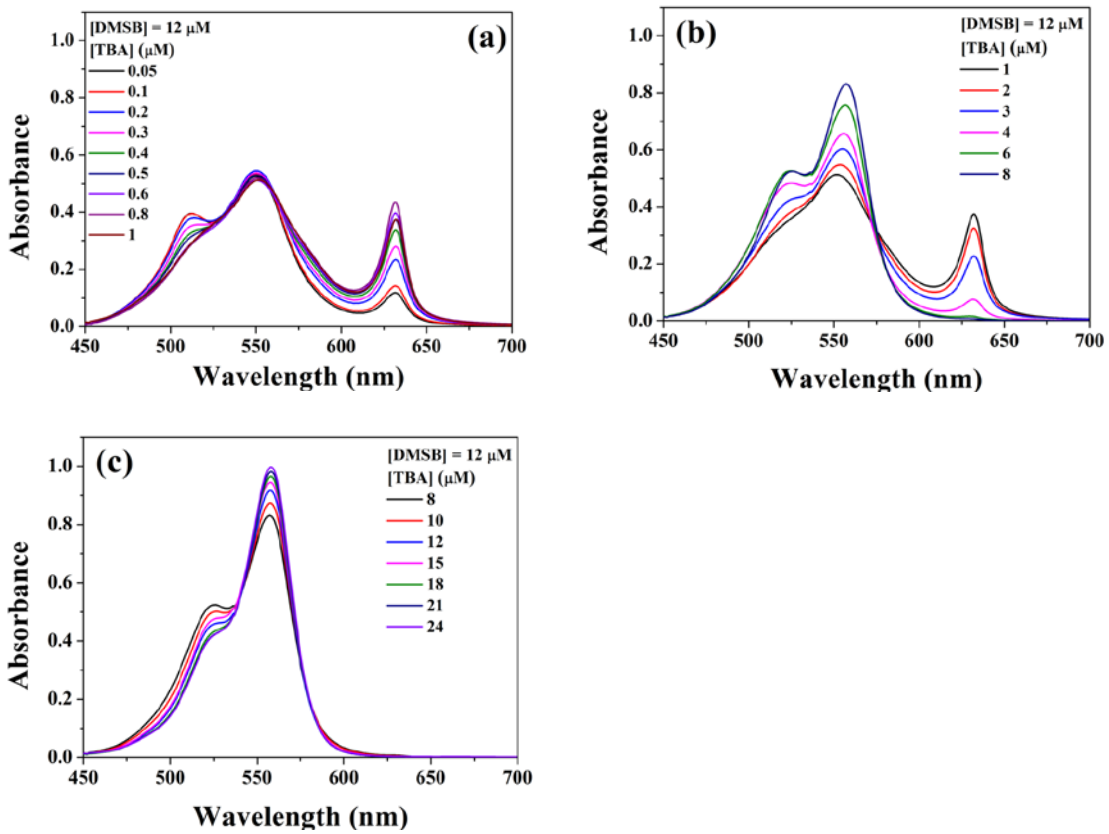

Figure S6. The UV-vis spectra of 12 μM ***DMSB*** in the presence of different concentrations of ***TBA*** in PBS. (a) J-aggregates assembling stage; (b) J-aggregates disassembling stage; (c) dimer disassembling stage.

S(e) – CD spectra of DMSB in PBS titrated with TBA

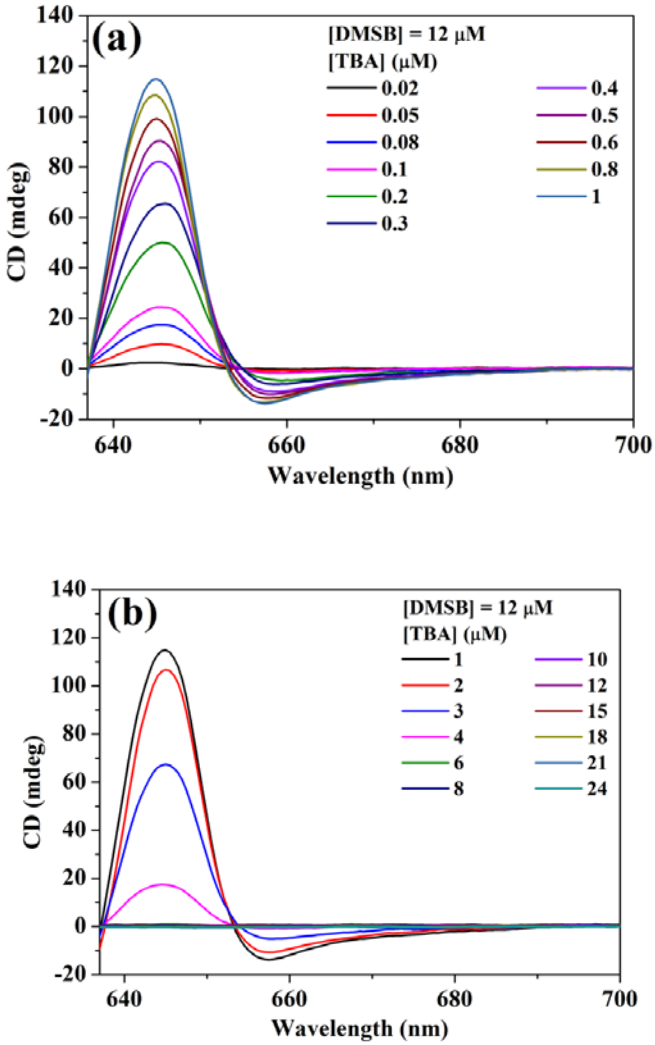

Figure S7. Circular dichroism spectra of (a) J-aggregates assembling stage and (b) J-aggregates disassembling stage of 12 μM ***DMSB*** in the presence of different concentrations of ***TBA*** in PBS.

S(f) – UV and CD spectra of DMSB titrated with BM-19

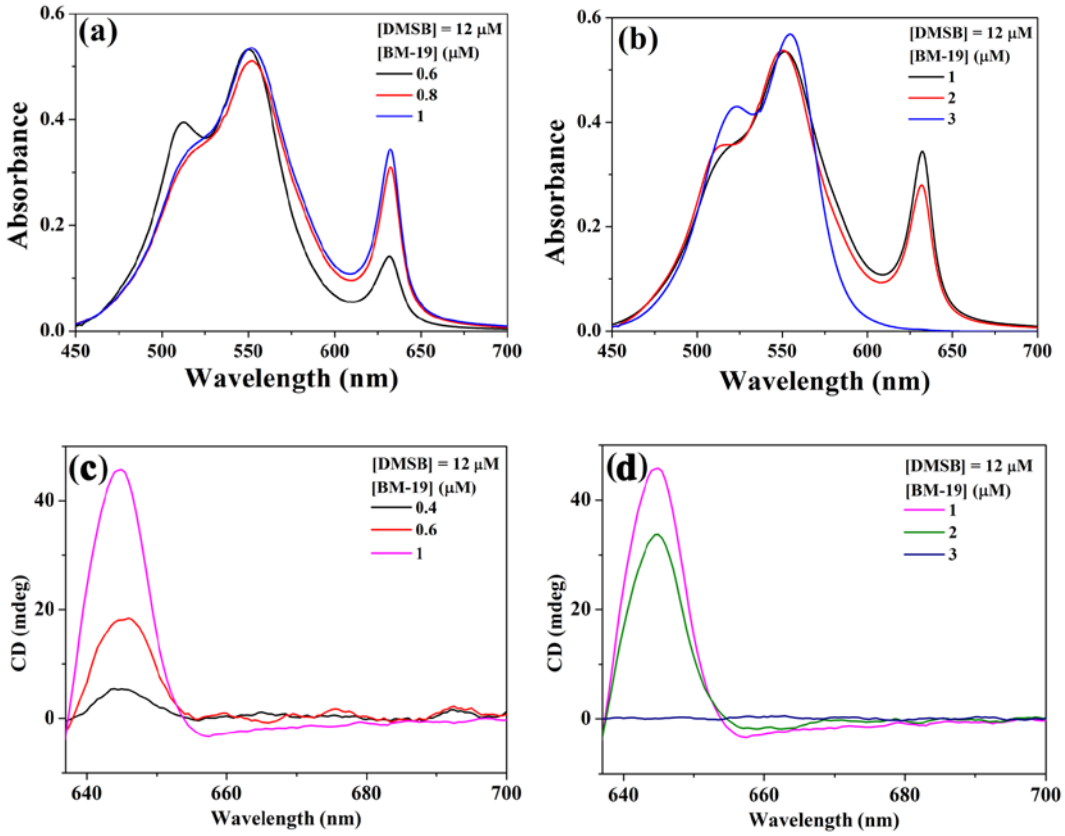

Figure S8. UV-vis spectra of (a) assembling stage and (b) disassembling stage of 12 μM ***DMSB*** in the presence of different concentrations of ***BM-19*** in PBS, and corresponding circular dichroism spectra (c) and (d).

S(g) – Fluorescence spectra of DMSB titrated with TBA

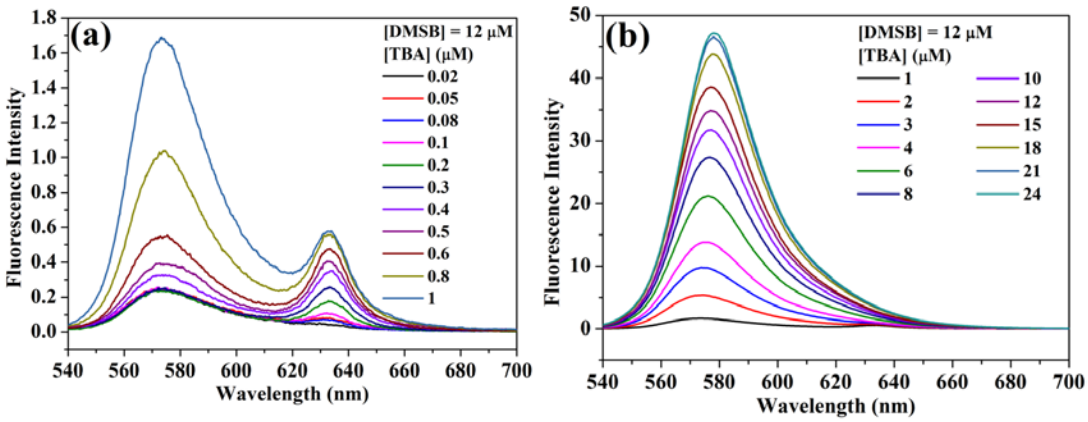

Figure S9. Fluorescence emission spectra of (a) assembling stage and (b) disassembling stage of 12 μM ***DMSB*** in the presence of different concentrations of ***TBA*** in PBS.

S(h) – ^{1}H-NMR spectra of TBA titrated with DMSB

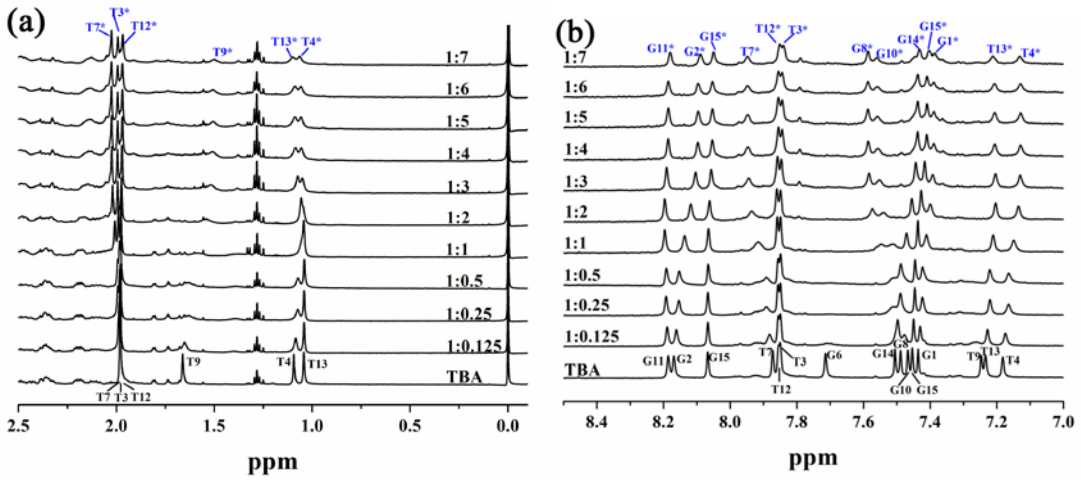

Figure S10. The unambiguous assigned aliphatic region (a) and the aromatic region (b) of ^{1}H-NMR spectra for 0.5 mM ***TBA*** titrated with DMSB in 120mM PBS (K^{+}) at 303 K. The [***TBA***] : [DMSB] mole ratios are shown along the side of each spectrum. The spectrum for ***TBA*** without DMSB added is shown at the bottom of both Figures.

S(i) – TBA sequence mutation experiments

Table S2. Sequences of the six mutated TBA

Name	Sequence
TBA	5′-GGTTGGTGTGGTTGG-3′
T3-mutated TBA	5′-GG**A**TGGTGTGGTTGG-3′
T4-mutated TBA	5′-GGT**A**GGTGTGGTTGG-3′
T7-mutated TBA	5′-GGTTGG**A**GTGGTTGG-3′
T9-mutated TBA	5′-GGTTGGTG**A**GGTTGG-3′
T12-mutated TBA	5′-GGTTGGTGTGG**A**TGG-3′
T13-mutated TBA	5′-GGTTGGTGTGGT**A**GG-3′

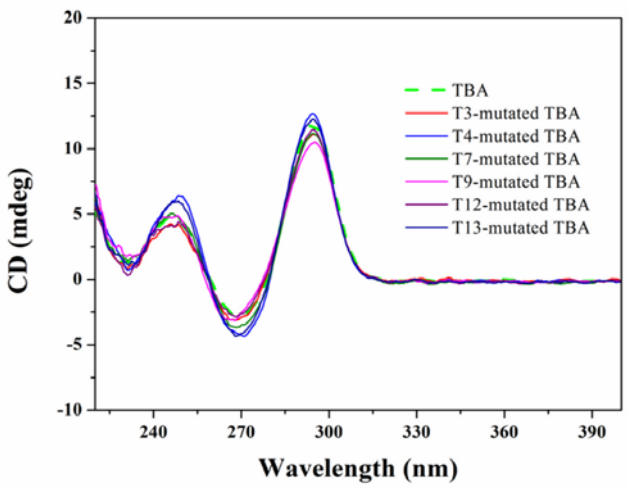

Figure S11. The circular dichroism spectra of the six mutated TBA (3 μM). All the six mutated sequences could form antiparallel G-quadruplexes as TBA (green dash), which indicates that T → A mutations do not change the structure of TBA.