Cascade DNA logic device programmed ratiometric DNA analysis and logic devices based on fluorescent dual-signal probe of G-quadruplex DNAzyme

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Experiment Section

Materials

The oligonucleotides synthesized by Shanghai Sangon Biotechnology Co.(Shanghai, China) were dissolved in distilled water as stock solution and quantified according to the extinction coefficients (ε260 nm, M-1cm-1): A= 15400, G= 11500, C= 7400, T= 8700 by UV-vis absorption spectroscopy, then diluted into desired concentration with distilled water. Scopoletin (Sc) (98%) was obtained from J&K (Beijing, China) and stored in darkness at -20℃ and dissolved in dimethyl sulfoxide (DMSO) to 65 mM stock solution. Hemin was dissolved in dimethyl sulfoxide (DMSO) to the stock solution of 2 mM. Amplex Red (AR) (≥98%) was purchased from Aladdin Industrial Corporation (Shanghai, China) and stored in darkness at -20℃ and dissolved in DMSO to 16 mM stock solution. H_2O_2 (30%) was used to oxidize the fluorescent substrates. Sc, AR, hemin and H_2O_2 were diluted with 1×HEPES buffer (25 mM HEPES, 20 mM KCl, 100 mM NaCl, 0.05% (w/v) Triton X-100, 1% (v/v) DMSO, pH 7.4) to the concentration of 250 μM, 250 μM , 50 μM and 5 mM, respectively. The water used in the experiments was purified through a Millipore system. Other chemicals were reagent grade and were used without further purification.

Sc or AR based DNA-input logic gates construction

For the Sc based DNA logic gates construction, 500 nM hemin, 2.5 μM Sc, 200 μM H_2O_2 were used; for the AR based DNA logic gates construction, 500 nM hemin, 7.5 μM AR, 200 μM H_2O_2 were used. Different DNA strand solutions were heated at 88℃ for 10 min and slowly cooled down to room temperature. Then, 500 nM 1G3, 1G1; 500 nM 2G3, 2G1; 2 μM 1G4, 1C4 were mixed and incubated at room temperature for 30 min. Suitable volume of 1×HEPES buffer (25 mM HEPES, 20 mM KCl, 100 mM NaCl, 0.05%(w/v) Triton X-100, 1% (v/v) DMSO, pH 7.4) was added into the mixture and followed by 500 nM hemin and incubated for another 1h. Then, 2.5 μM Sc or 7.5 μM AR and 200 μM H_2O_2 were added into the mixture. After 8 min's reaction, the fluorescence spectra of Sc or AR were collected.

Cascade logic device programmed target DNA analysis

Sc and AR were simultaneously used in the cascade logic device. For the cascade logic device construction, 1 μM hemin, 2.5 μM Sc, 7.5 μM AR, 200 μM H_2O_2 were used. Different DNA strand solutions were heated at 88℃ for 10 min and slowly cooled down to room temperature. Then, 200 nM S0, 1 μM SA, 1 μM SB were mixed and incubated at room temperature for 30 min. Suitable volume of 1×HEPES buffer (25 mM HEPES, 20 mM KCl, 100 mM NaCl, 0.05%(w/v) Triton X-100, 1% (v/v) DMSO, pH 7.4) was added into the mixture and followed by 1 μM hemin and incubated for another 1h. Then, 2.5 μM Sc and 7.5 μM AR and 200 μM H_2O_2 were added into the mixture. After 8 min's reaction, the fluorescence spectra of Sc and AR were collected separately. For the cascade logic device programmed target DNA analysis, different concentrations of S0 were applied to target DNA detection, and 200 nM S3, S5, S7, S9, S13, S17 were applied to DNA length measurement, respectively.

Fluorescence spectra measurement

The fluorescence emission spectra of different samples were collected on Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies, USA) under room temperature. The emission spectra of Scopoletin were collected from 400 to 600 nm with the excitation wavelength of 380 nm, slit widths for both the excitation and emission were 5 nm. And the emission spectra of Amplex Red were collected from 570 to 650 nm with the excitation wavelength of 560 nm and slit widths for the excitation and emission were 2.5 nm and 5 nm, respectively.

Native polyacrylamide gel electrophoresis (PAGE)

Before strands hybridization reaction, all the DNA solutions were prepared as 40 μM with distilled water and diluted to 20 μM with equal volume of 2×HEPES buffer (50 mM HEPES, 40 mM KCl, 200 mM NaCl, 0.1% (w/v) Triton X-100, 2% (v/v) DMSO, pH 7.4). Then different DNA strand solutions were heated at 88℃ for 10 min and slowly cooled down to room temperature. After that, desired volume of input DNA solutions were mixed and suitable volume of 1×HEPES buffer (25 mM HEPES, 20 mM KCl, 100 mM NaCl, 0.05% (w/v) Triton X-100, 1% (v/v) DMSO, pH 7.4) was added into the mixture to the final volume of 200 μL. For the DNA strands final concentrations, 2 μM 1G3, 1G1, 2G3, 2G1 and 3 μM 1G4, 1C4 were used. After 30 min's incubation, the DNA solutions were analyzed in 15% native polyacrylamide gel after staining with Gel-dye. The electrophoresis was conducted in 1×TBE (pH 8.0) at constant voltage of 130 V for 1 h. The gels were scanned by a UV trans-illuminator.

Circular Dichroism Measurements

Before measurement, different DNA solutions were heated at 88℃ for 10 min and slowly cooled down to room temperature. After that, desired volume of input DNA solutions was mixed and suitable volume of 1×HEPES buffer (25 mM HEPES, 20 mM KCl, 100 mM NaCl, 0.05% (w/v) Triton X-100, 1% (v/v) DMSO, pH 7.4) was added into the mixture to the final volume of 200 μL. All the DNA final concentrations were 2 μM. Then CD spectra were measured on a JASCO J-820 spectropolarimeter (Tokyo, Japan) under room temperature. The spectra were recorded from 220 to 320 nm in 1 mm path length cuvettes and averaged from three scans.

Table S1. Sequences of DNA strands used in this work. G-quadruplex bases have been colored in blue and mismatched bases have been colored in purple.

Strand	Sequence (5' to 3')
1G3	ACTAGAATCTGTCA GGGTAGGGCGGG
1G1	TGGGT TGACAGATTCTAGT
2G3	TCAAGTCTCTGTCA GGGTAGGGCGGG
2G1	TGGGT TGACAGAGACTTGT
1G4	ATTATATACAAACGTCTAACGAAAGGTTCATCG GGGTTTTGGGTTTTGGGTTTTGGG
1C4	CCCAAAACCCAAAACCC CGATGAACCAAACGTTAGACGAAAGTATATAAT
SB	TGGGT GAGATGTATG
SA	GACGTAATAG T GGGTAGGGCGGG
S0	CATACATC TC CTAT TACGTC
S1	CATACATC TC T CTAT TACGTC
S3	CATACATC TC TTT CTAT TACGTC
S5	CATACATC TC TTGTT CTAT TACGTC
S7	CATACATC TC TTGACTT CTAT TACGTC
S9	CATACATC TC TTTGACTTT CTAT TACGTC
S13	CATACATC TC TTTTTGACTTTTT CTAT TACGTC
S17	CATACATC TC AGTTTTGGACCTTTTCT CTAT TACGTC
MS2	CATACATCTG TTAT TACGTC
MS4	CATACATCAG TAAT TACGTC
MS6	CATACATGAG TAGT TACGTC
Random	TATAGTTGACAGAGACTTGT

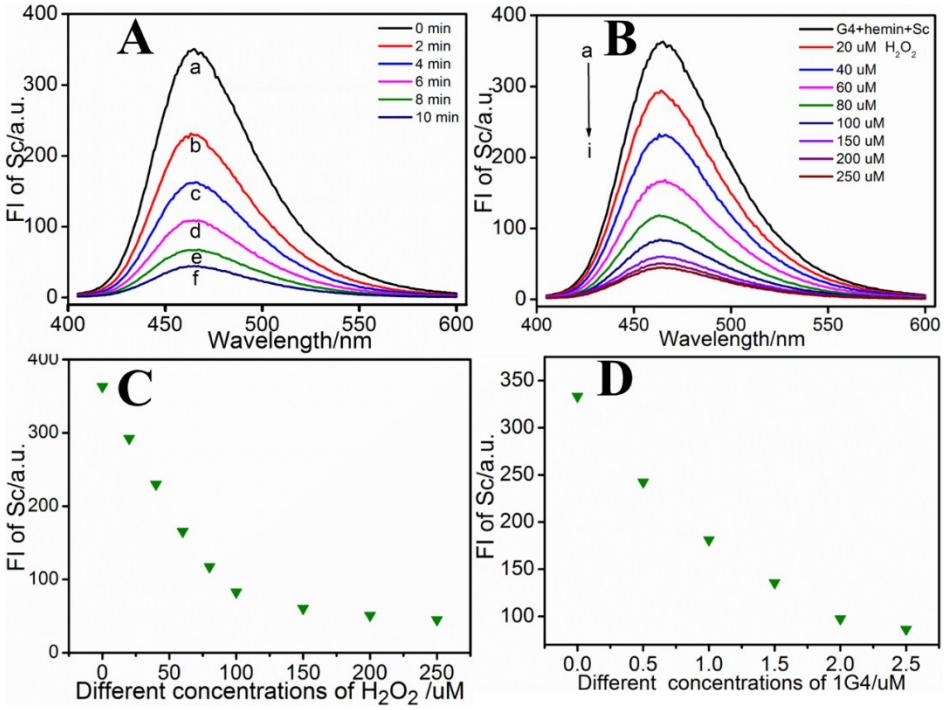

Figure S1. (A) Fluorescence spectra of 2.5 μM Sc in the presence of 500 nM hemin, 500 nM G-quadruplex, 2.5 μM Sc, 200 μM H_2O_2 under different catalysis reaction time (From a to f, 0 min to 10 min); **(B)** Fluorescence spectra of 2.5 μM Sc in the presence of 500 nM hemin, 500 nM G-quadruplex and different concentrations of H_2O_2（From a to i, 0 μM to 250 μM H_2O_2 was used) ; **(C)** Fluorescence responses of 2.5 μM Sc as a function of different concentrations of H_2O_2 in the presence of 500 nM hemin, 500 nM G-quadruplex. **(D)** Fluorescence responses of 2.5 μM Sc as a function of different concentrations of 1G4 in the presence of 500 nM hemin, 200 μM H_2O_2.

As shown in **Figure S1 (A)**, fluorescence intensity of Sc decreased gradually under different catalysis reaction times and reached a very low value at about 8 min, so we select 8 min as the optimized catalysis time. **Figure S1 (B), (C)** showed the fluorescence responses of Sc under different concentrations of H_2O_2 in the presence of 500 nM hemin and 500 nM G-quadruplex, the intensity reached a plain at about 200 μM, then 200 μM H_2O_2 was used in our experiment. **Figure S1 (D)** showed the fluorescence responses of Sc under different concentrations of 1G4 in the presence of 500 nM hemin and 200 nM H_2O_2.

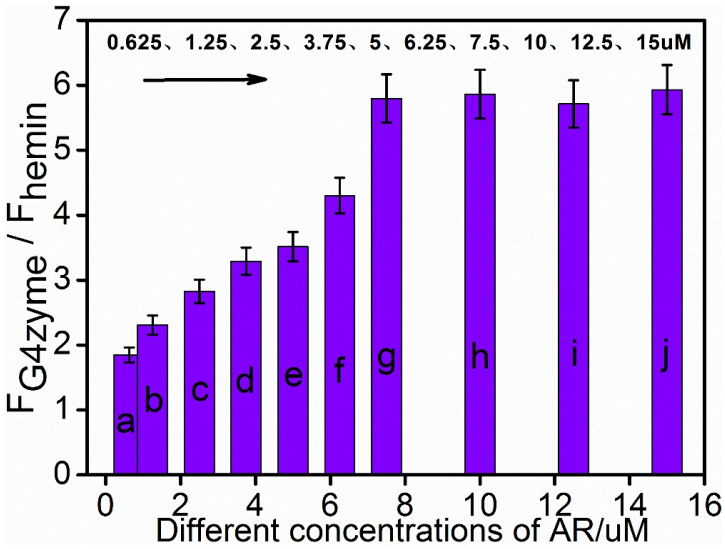

Figure S2. Column bars of S/N ratio values in the presence of different concentrations of AR under the same catalysis conditions with Sc (500 nM hemin, 500 nM G-quadruplex, 200 μM H_2O_2). (From a to j, 0.625 μM to 15 μM AR was used)

Herein, S/N= F_{G4zyme}/F_{hemin}. F_{G4zyme} represent the fluorescence intensity of different concentrations of AR at 585 nm in the presence of 500 nM G-quadruplex, 500 nM hemin, 200 μM H_2O_2. F_{hemin} represent the fluorescence intensity of different concentrations of AR at 585 nm in the presence of 500 nM hemin, 200 μM H_2O_2.

As can be seen in **Figure S2,** the S/N ratio increased gradually with the improved concentration of AR and reached a stable value at about 7.5 μM. To save experiment cost, we select 7.5 μM AR in our experiment.

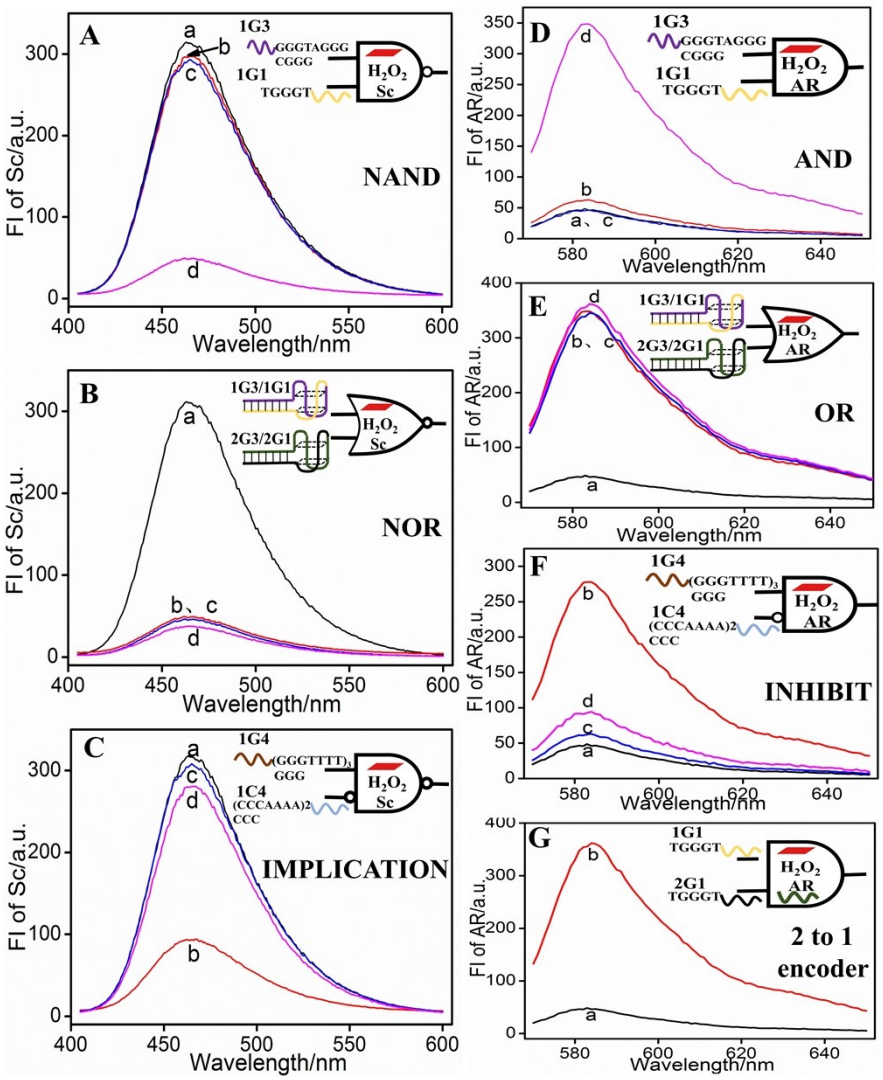

Figure S3. (A) Fluorescence spectra of the NAND logic gate under different inputs, 2.5 μM Sc in the absence of any input **(A, a)**, in the presence of 500 nM 1G3 **(A, b)**, 500 nM 1G1 **(A, c)**, 500 nM 1G3 and 500 nM 1G1 **(A, d)**. **(B)** Fluorescence spectra of the NOR logic gate under different inputs, 2.5 μM Sc in the absence of any input **(B, a)**, in the presence of 500 nM 1G3/1G1 **(B, b)**, 500 nM 2G3/2G1 **(B, c)**, 500 nM 1G3/1G1 and 500 nM 2G3/2G1 **(B, d)**. **(C)** Fluorescence spectra of the IMPLICATION logic gate under different inputs, 2.5 μM Sc in the absence of any input **(C, a)**, in the presence of 2 μM 1G4 **(C, b)**, 2 μM 1C4 **(C, c)**, 2 μM 1G4 and 2 μM 1C4 **(C, d)**. **(D) (E) (F)** Fluorescence spectra of AR corresponding AND, OR, INHIBIT logic gate under different inputs. **(G)** Fluorescence spectra of the 2 to 1 encoder, 7.5 μM AR in the presence of 500 nM 1G1 **(G, a)**, 500 nM 2G1 **(G, b)**. (The inset presented the logic circuits of different logic gates)

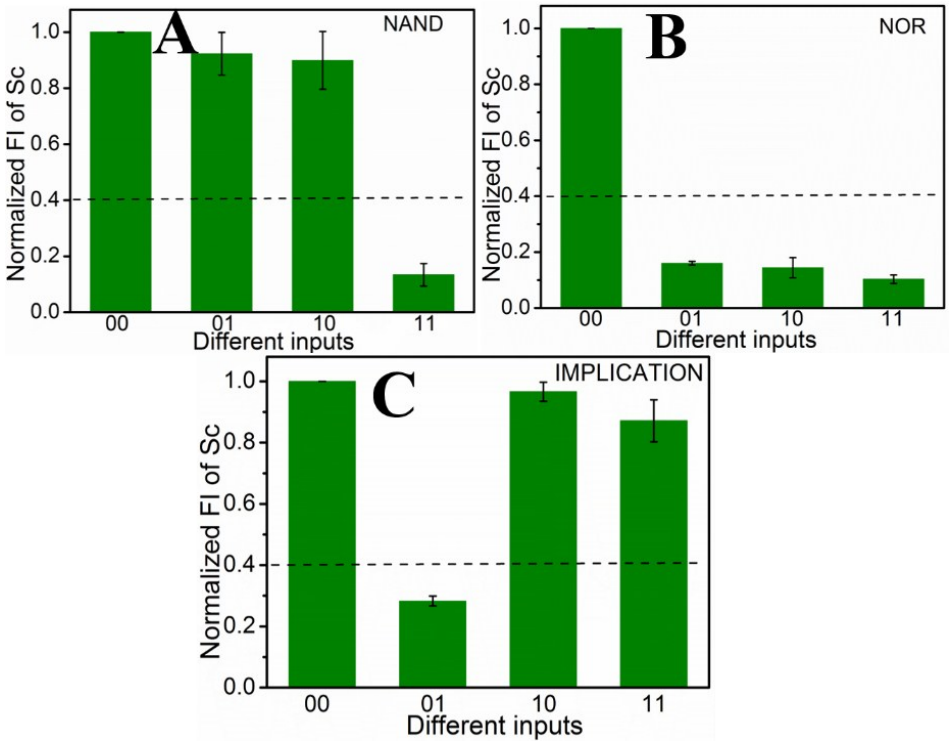

Figure S4. (A) Column bars of the NAND logic gate based on Sc, 2.5 μM Sc in the absence of any input **(00)**, in the presence of 500 nM 1G1 **(01)**, 500 nM 1G3 **(10)**, 500 nM 1G3 and 500 nM 1G1 **(11)**. **(B)** Column bars of the NOR logic gate based on Sc, 2.5 μM Sc in the absence of any input **(00)**, in the presence of 500 nM 1G3/1G1 **(01)**, 500 nM 2G3/2G1 **(10)**, 500 nM 1G3/1G1 and 500 nM 2G3/2G1 **(11)**. **(C)** Column bars of the IMPLICATION logic gate based on Sc, 2.5 μM Sc in the absence of any input **(00)**, in the presence of 2 μM 1G4 **(01)**, 2 μM 1C4 **(10)**, 2 μM 1G4 and 2 μM 1C4 **(11)**. The highest values in **Figure S3 (A, a), (B, a), (C, a)** were set as 1.0. Error bars were calculated through three independent experiments.

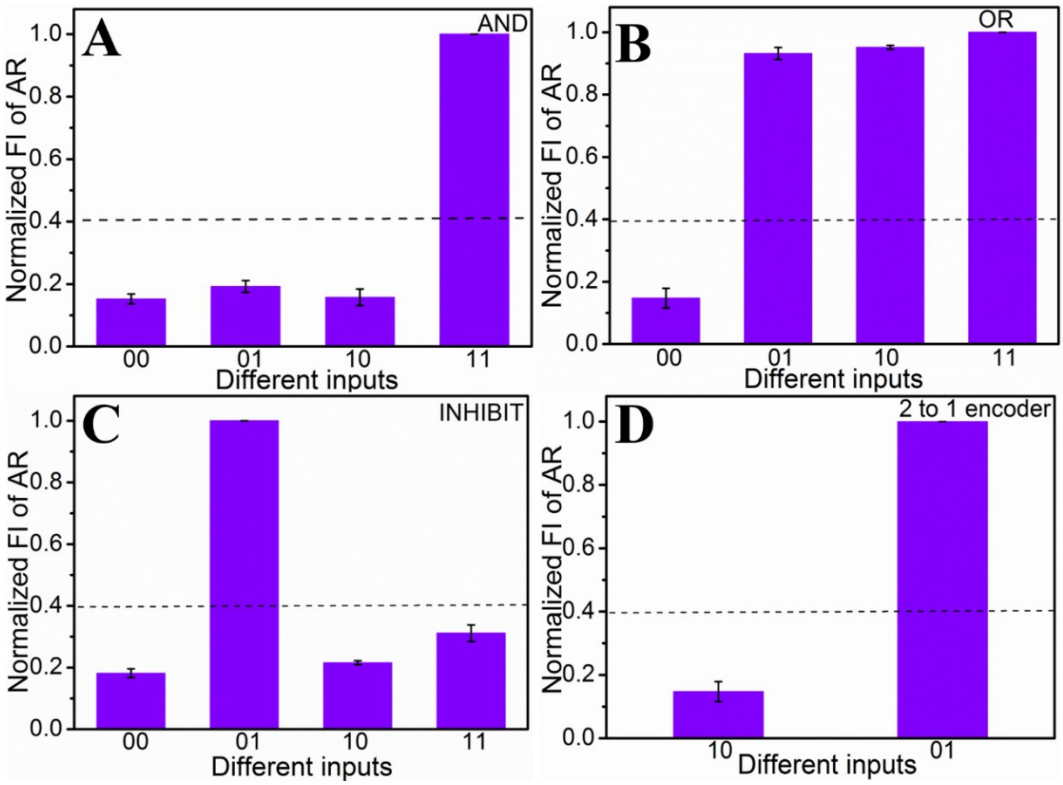

Figure S5. (A) Column bars of the AND logic gate based on AR, 7.5 μM AR in the absence of any input (00), in the presence of 500 nM 1G1 (01), 500 nM 1G3 (10), 500 nM 1G3 and 500 nM 1G1 (11). **(B)** Column bars of the OR logic gate based on Sc, 7.5 μM AR in the absence of any input (00), in the presence of 500 nM 1G3/1G1 (01), 500 nM 2G3/2G1 (10), 500 nM 1G3/1G1 and 500 nM 2G3/2G1 (11). **(C)** Column bars of the INHIBIT logic gate based on AR, 7.5 μM AR in the absence of any input (00), in the presence of 2 μM 1G4 (01), 2 μM 1C4 (10), 2 μM 1G4 and 2 μM 1C4 (11). **(D)** Column bars of the 2 to 1 encoder based on AR, 7.5 μM AR in the presence of 500 nM 1G1 (10), 500 nM 2G1 (01). The highest values in **Figure S3 (D, d), (E, d), (F, b), (G, b)** were set as 1.0. Error bars were calculated through three independent experiments.

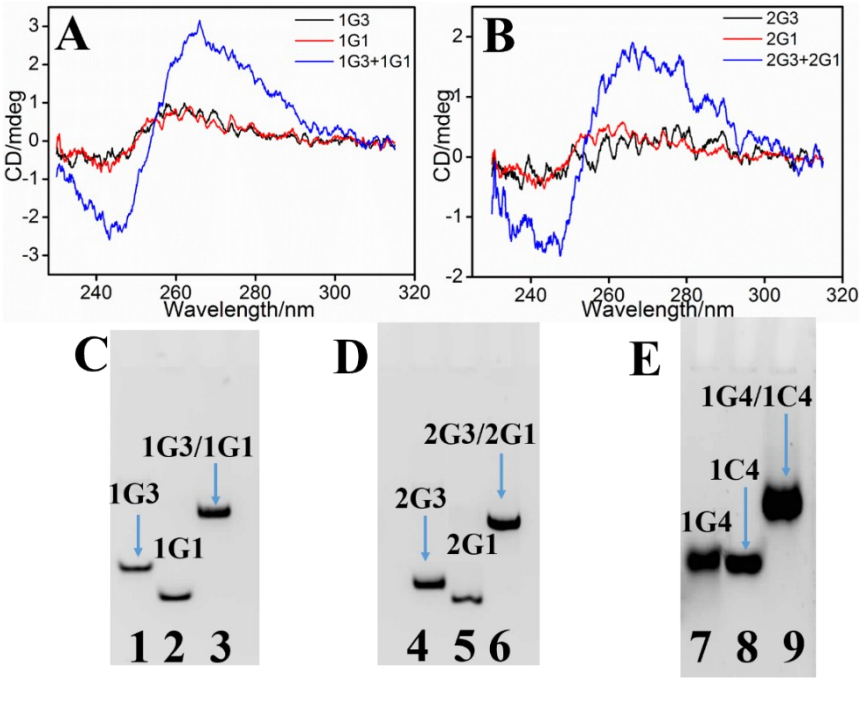

Figure S6. (A) The CD spectra of different DNA strands: 1G3(black), 1G1(red), 1G3 with 1G1(blue); **(B)**The CD spectra of different DNA strands: 2G3(black), 2G1(red), 2G3 with 2G1(blue) **(C)**15% PAGE analysis of the interaction between different DNA strands, 1G3(Lane 1), 1G1(Lane 2), 1G3 with 1G1(Lane 3); **(D)**15% PAGE analysis of the interaction between different DNA strands, 2G3(Lane 4), 2G1(Lane 5), 2G3 with 2G1(Lane 6); **(E)** 15% PAGE analysis of the interaction between different DNA strands, 1G4(Lane 7), 1C4(Lane 8), 1G4 with 1C4(Lane 9)

As shown in **Figure S6 (A), (B)**, the black and red lines in the CD spectrum have no obvious peaks in the presence of 1G3, 1G1, 2G3, 2G1, respectively. While after the mix of 1G3 and 1G1; 2G3 and 2G1, a positive peak at 265 nm and a negative peak at 244 nm appeared, which features the characteristic peaks of parallel G-quadruplex configuration. Thus, the formation of G-quadruplex was validated.

Figure S6(C), (D), (E) showed the polyacrylamide gel analysis of the interaction between different DNA strands. Lane 1 to Lane 9 shows the DNA bands of 1G3, 1G1, 1G3 with 1G1; 2G3, 2G1, 2G3 with 2G1; 1G4, 1C4, 1G4 with 1C4, respectively. New bands appeared in Lane 3, Lane 6 and Lane 9, indicated the hybridization between 1G3 and 1G1, 2G3 and 2G1, 1G4 and 1C4, respectively.

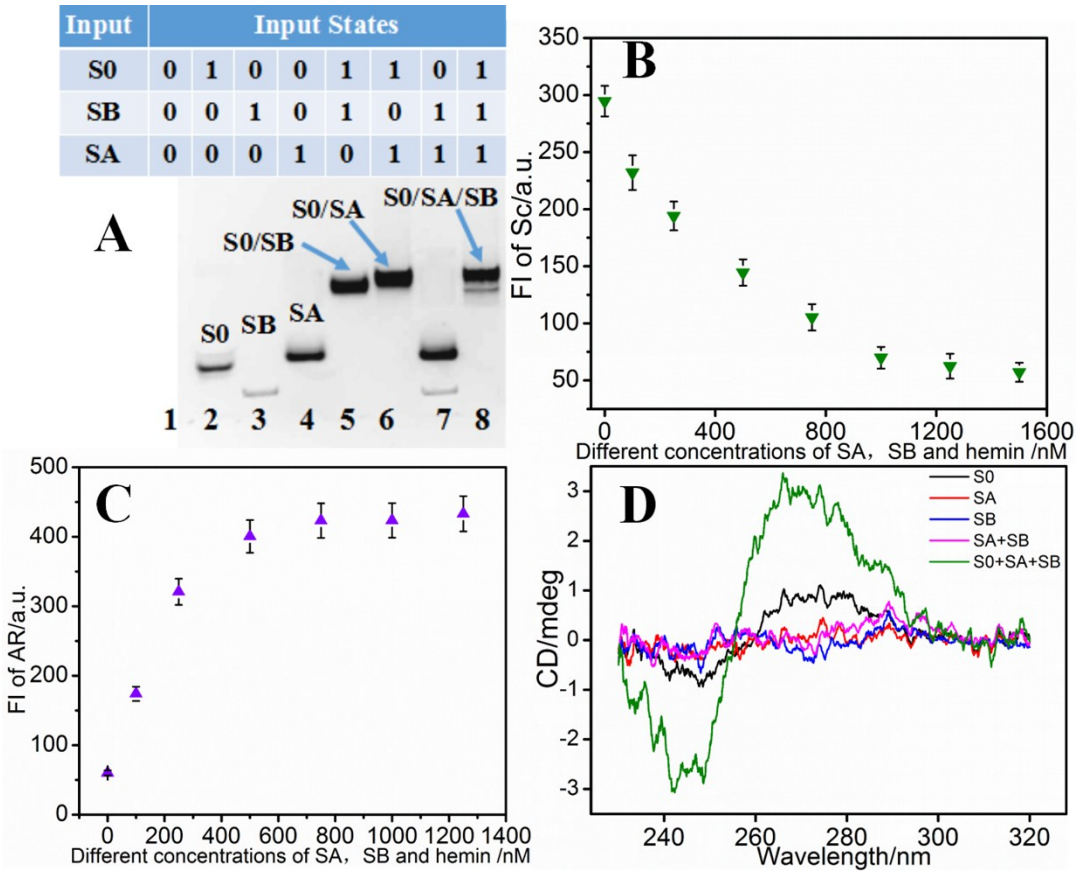

Figure S7. (A) Different output states shown in the 15% PAGE photos in the presence of various input states: in the absence of any input strand (Lane 1), in the presence of S0 (Lane 2), SA (Lane 4), SB (Lane 3), S0 and SA (Lane 6), S0 and SB (Lane 5), SA and SB (Lane 7), S0, SA and SB (Lane 8). **(B)** Fluorescence responses of 2.5 μM Sc as a function of different concentrations of SA, SB and hemin in the presence of 200 nM S0, 7.5 μM AR, 200 μM H_2O_2 with error bars; **(C)** Fluorescence responses of 7.5 μM AR as a function of different concentrations of SA, SB and hemin in the presence of 200 nM S0, 2.5 μM Sc, 200 μM H_2O_2 with error bars; **(D)** The CD spectra of different DNA strands: S0 (black), SA(red), SB (blue), SA with SB(pink), S0 with SA with SB(green). (The error bars were obtained through three parallel experiments)

Figure S7 (B) showed the fluorescence responses of 2.5 μM Sc under different concentrations of SA, SB and hemin in the presence of 200 nM S0, 7.5 μM AR, 200 μM H_2O_2, the intensity decreased gradually with the addition of SA, SB and hemin, and reached a plain at about 1μM. **Figure S7 (C)** showed the fluorescence responses of 7.5 μM AR under different concentrations of SA, SB and hemin in the presence of 200 nM S0, 2.5 μM Sc, 200 μM H_2O_2, the intensity increased gradually with the addition of SA, SB and hemin, and reached a plateau at about 800 nM. To use the two substrates simultaneously, we select 1 μM SA, 1 μM SB and 1 μM hemin in our experiment.

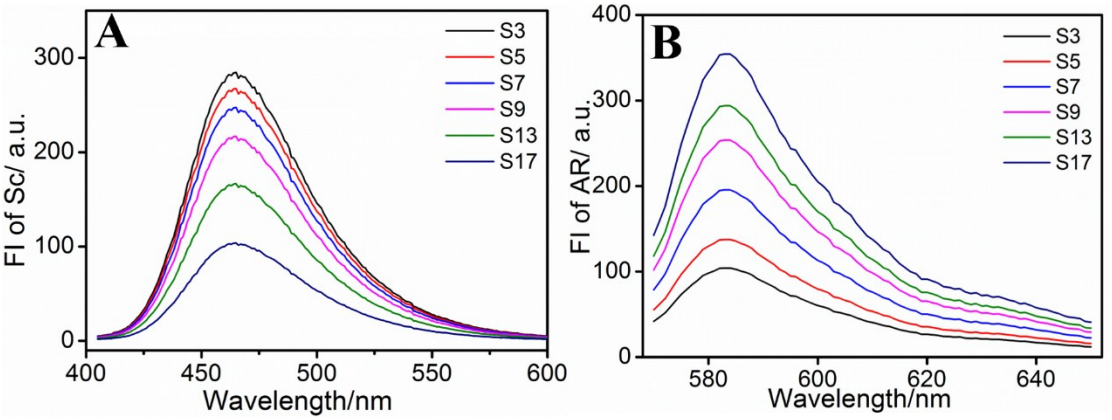

Figure S8. (A) Fluorescence spectra of 2.5 μM Sc in the presence of 1 μM SA, 1 μM SB, 1 μM hemin, 200 μM H_2O_2, 7.5 μM AR and 200 nM different length of target DNA strands (S3, S5, S7, S9, S13, S17). **(B)** Fluorescence spectra of 7.5 μM AR in the presence of 1 μM SA, 1 μM SB, 1 μM hemin, 200 μM H_2O_2, 2.5 μM Sc and 200 nM different length of target DNA strands (S3, S5, S7, S9, S13, S17).

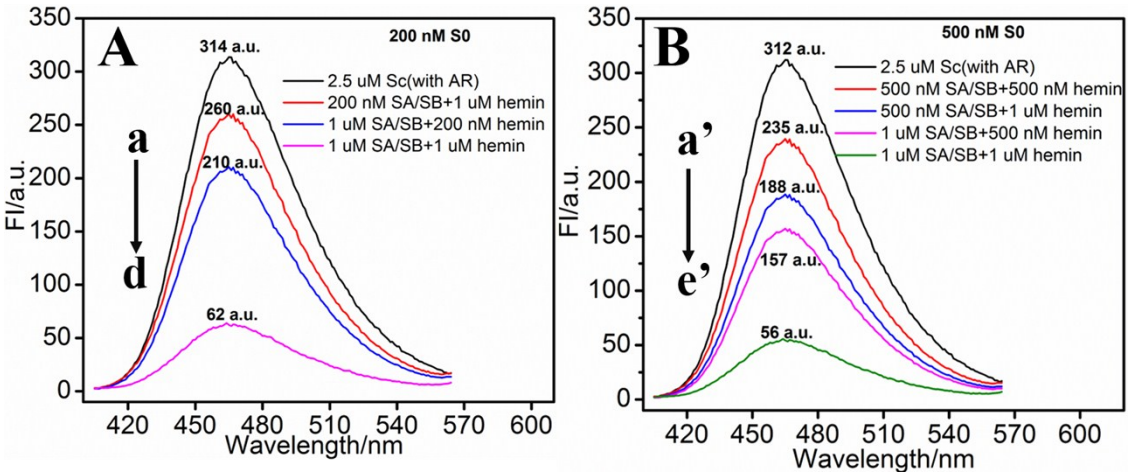

Figure S9. (A) Fluorescence spectra of 2.5 μM Sc and 200 nM S0, 7.5 μM AR **(a)** in the presence of 200 nM SA, 200 nM SB and 1 μM hemin **(b)**, 1 μM SA, 1μM SB and 200 nM hemin **(c)**, 1 μM SA, 1μM SB and 1 μM hemin **(d)**. **(B)** Fluorescence spectra of 2.5 μM Sc and 500 nM S0, 7.5 μM AR **(a')** in the presence of 500 nM SA, 500 nM SB and 500 nM hemin **(b')**, 500 nM SA, 500 nM SB and 1 μM hemin **(c')**, 1 μM SA, 1μM SB and 500 nM hemin **(d'),** 1 μM SA, 1μM SB and 1 μM hemin **(e')**. (200 μM H_2O_2 and 8 mins were controlled as constant in all the experiments.)

We performed verification experiments under different combinations for different concentrations of S0, SA/SB, hemin to explore the reason why Sc's fluorescent response in the presence of "200 nM S0, 1 μM SA/SB, 1 μM hemin, 200 μM H_2O_2 and 7.5μM AR" is different with that in the presence of "500 nM S0, 500 nM SA/SB, 500 nM hemin, 200 μM H_2O_2 and 7.5 μM AR", which were shown in Figure S9. As can be seen in Figure S9 (A), in the "200 nM S0 system" (which means 200 nM S0, 200 μM H_2O_2 and 7.5μM AR in the presence of different concentrations of SA/SB

and hemin), neither the fluorescence signal of Figure S9 (A, b) (in the presence of 200 nM SA/SB and 1 μM hemin) nor that of Figure S9 (A, c) (in the presence of 1 μM SA/SB and 200 nM hemin) showed obvious decrease, which indicated that higher concentrations of SA/SB and hemin are all essential to obtain an obvious response of Sc in the presence of 200 nM S0 and 7.5 μM AR. Figure S9 (B) presented the fluorescence spectra of "500 nM S0 system" (which means 500 nM S0, 200 μM H_2O_2 and 7.5μM AR in the presence of different concentrations of SA/SB and hemin), the same phenomenon with "200 nM S0 system" could also be obtained (Figure S9B, a' to d'). After comparison of Figure S9 (A, d) and (B, e'), both of Sc's responses in "200 nM S0 system" (62 a.u.) and "500 nM S0 system" (56 a.u.) presented obvious decreases in the presence of 1 μM SA, 1μM SB and 1 μM hemin, which indicated that 200 nM S0 is enough to catalyze Sc and AR in the presence of 1 μM SA/SB and 1 μM hemin and H_2O_2.

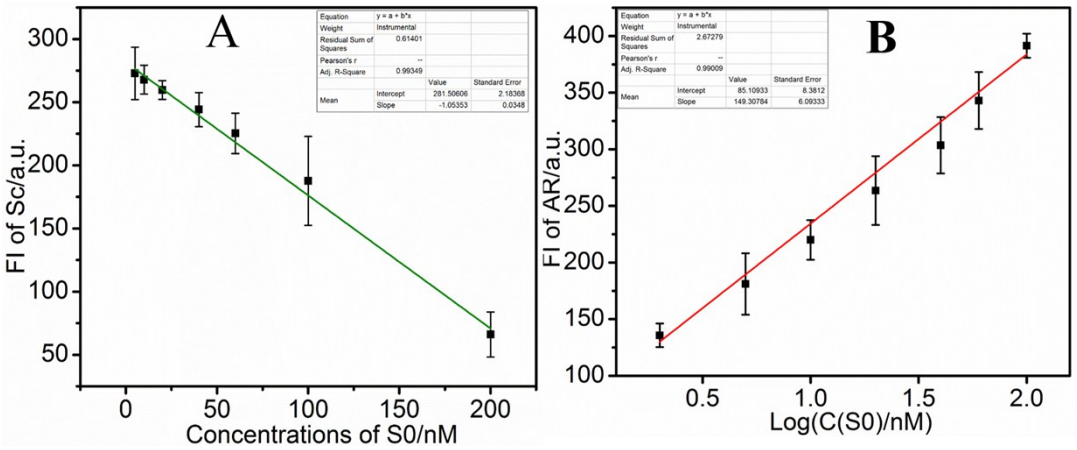

Figure S10. (A) Linear relationship (R^2=0.993) between fluorescence intensities of Sc and different concentrations of S0. (From left to right, 5 nM, 10 nM, 20 nM, 40 nM, 60 nM, 100 nM, 200 nM S0 were used). **(B)** Linear relationship (R^2=0.990) between fluorescence intensities of AR and different concentrations of S0. (From left to right, 2nM, 5 nM, 10 nM, 20 nM, 40 nM, 60 nM, 100 nM S0 were used).

As can be seen in **Figure S10**, single Sc or AR based target DNA detection was performed to compare with our constructed dual-signal ratiometric detection method. For Sc, linear range was obtained from 5 nM to 200 nM with detection limit of 5 nM; for AR, linear range was from 2 nM to 100 nM with detection limit of 2 nM. After comparison, both of the linear range and limit of detection of our constructed ratiometric platform were superior to the signal-single based methods.

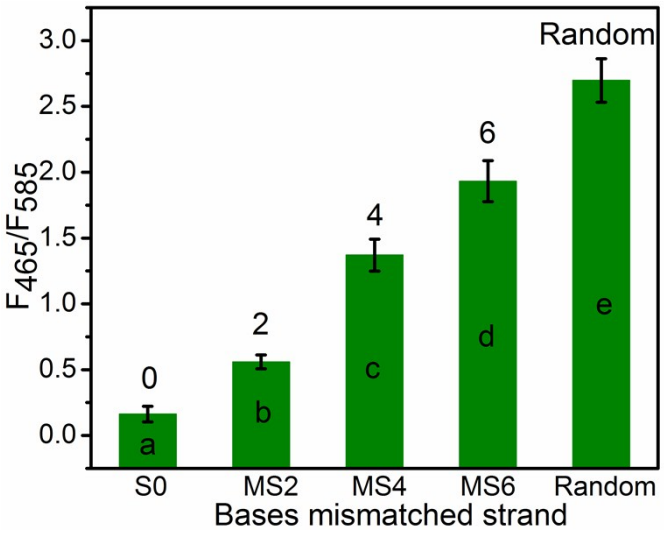

Figure S11. Ratiomertric column bars of different Sx strand with different amounts of mismatched bases. (From left to right, 200 nM S0, MS2, MS4, MS6 and Random strand were used).

Figure S11 presented ratiometric fluorescence values of different bases mismatched strand of S0. As the increase of mismatched bases, the ratio showed distinct values, which verified the good selectivity of our constructed ratiometric platform.