Electronic Supplementary Material (ESI) for Materials Horizons.

Supplementary Information for

An intelligent universal system yields double results with half the effort for engineering a DNA "Contrary Logic Pairs" library and various DNA combinatorial logic circuits

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This ***Supporting Information*** includes:

Experimental section

Chemicals. The DNAs were synthesized by Shanghai Sangon Biotechnology Co. (Shanghai, China) and the sequences were shown in **Table S1**. All the DNAs were dissolved in distilled water and quantified by UV-vis absorption spectroscopy via the extinction coefficients (ε260 nm, M^{-1} cm^{-1}): A= 15400, G= 11500, C= 7400, T = 8700. 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 as stock solution. 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 as stock solution. Hemin was dissolved in dimethyl sulfoxide (DMSO) to the stock solution of 2 mM. H_2O_2 (30%) was used to oxidize the fluorescent substrates. N-methylmesoporphyrin IX (NMM) was purchased from J&K (Beijing, China). AR, SC, 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 as stock solutions, respectively. The water used in the experiments was purified through a Millipore system.

Fluorescence measurement: The fluorescence emission spectra of different samples were collected on Cary Eclipse Fluorescence Spectrophotometer (Agilent Technologies, USA) at room temperature. The emission spectra of AR 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. The emission spectra of SC were collected from 400 to 600 nm with the excitation wavelength of 380 nm, slit widths for the excitation and emission were all 5 nm. For the emission spectra of NMM, they were collected from 550 to 750 nm after excited at 399 nm. The slit widths for the excitation and emission were all 10 nm.

Native polyacrylamide gel electrophoresis (PAGE): The DNA stock solution was diluted to desired concentrations by 2×TE buffer (100 mM Tris, 10 mM EDTA and 200 mM $MgCl_2$). After that, they were heated at 90 ℃ for 10 mins and slowly cooled down to room temperature. Then, different combinations of DNA (2 μM) were mixed and suitable volume of 1×TE buffer (50 mM Tris, 5 mM EDTA and 100 mM $MgCl_2$, pH 8.0) was added into the mixture to the final volume of 50 μL. After incubated at room temperature for more than 30 mins, the DNA samples were analyzed in 15% native polyacrylamide gel. The electrophoresis was conducted in 1×TBE buffer (18 mM Tris, 18 mM Boric Acid and 1 mM EDTA, pH 8.0) at constant voltage of 130 V for 1 h. The gels were scanned by a UV transilluminator after staining with Gel-Red.

CD spectra and Tm value Measurements: CD spectra were measured on a JASCO J-820 spectropolarimeter (Tokyo, Japan) under room temperature. Spectra were recorded from 220 to 320 nm in 1 mm pathlength cuvettes and averaged from three scans. For the collection of thermal denaturation curves of duplex (*Tm* values), the absorbance of different duplexes was monitored at 260 nm, the interval temperature is 1 ℃, the ramp rate is 1 ℃ min^{-1} and the temperature range is 20 to 90 ℃.

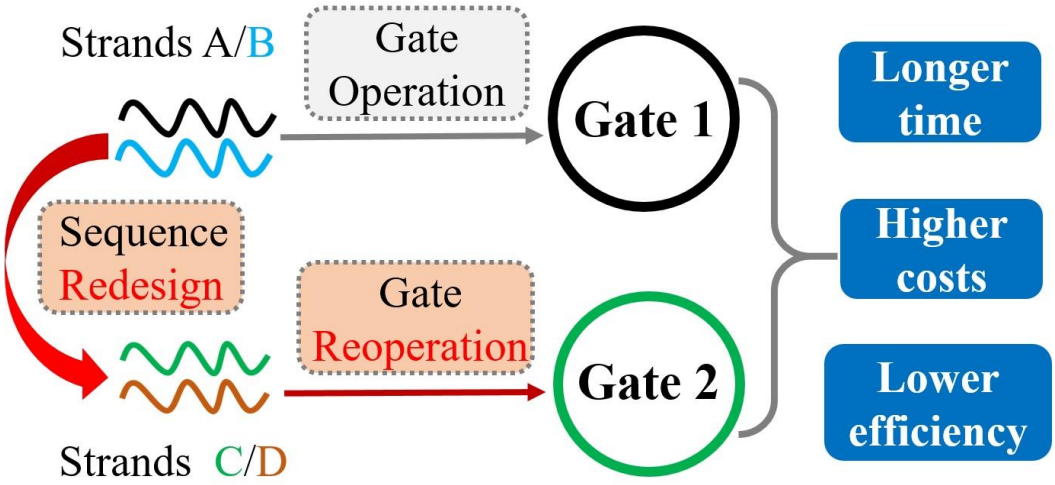
Operation of various "CLPs": The mixture of 7.5 μM AR, 2.5 μM SC, 1 μM hemin, 200 μM H_2O_2 were used as the universal system of all the contrary logic pairs. Different DNA strand solutions were heated at 90 ℃ for 10 min and slowly cooled down to room temperature.

For the elementary logic pairs, 500 nM G3, G1; 500 nM T30695, PW17; 1 μM C4 were alternatively mixed. For the "XOR∧XNOR" logic pair: In Label 1, 500 nM XP (10 μM) was used; in Label 2 or Label 3, 500 nM XP was mixed with 500 nM XB, 1 μM CA (or 500 nM XA, 1 μM CB); in Label 4, 500 nM XB and 1 μM CA were premixed with 500 nM XA, 1 μM CB and

reacted at room temperature for 20 mins, then 500 nM XP was added. For the "MAJ∧MIN" logic pair, 400 nM MA, 300 nM MB and 350 nM MC were alternatively added to the platform strands (the mixture of 300 nM P1+P2+P3) to achieve the function.

After different DNA combinations and incubating at room temperature for about 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, AR, SC and H_2O_2 were added into the mixture. After 10 mins' reaction, the fluorescence spectra of AR and SC were collected.

Schemes, Tables and Figures.



Scheme S1. The operating principle of previously reported systems for constructing DNA logic gates with different functions, in which the gates' redesign/reoperation were obligatory.

Table S1. Sequences of DNA strands used in this work. Poly-G parts were colored in red. The colors of DNAs used in XOR∧XNOR, MAJ∧MIN logic pairs were all consistent with that in **Scheme 3** and **Scheme 4**.

Gates Operation	Strand	Sequence (5' to 3')
Elementary Logic pairs	T30695	GGGT GGGT GGGT GGGT
	G3	ACTAGAATCTGTCA GGGTAGGGCGGG
	G1	TGGGT TGACAGATTCTAGT
	C4	ACCC ACCC ACCC ACCC
XOR∧XNOR	XP	ATACAGTAATAG GGGTGGG ATAAGAAAGTACAA
	XA	TGGGTGGG CTATTACTGTAT
	CA	ATACAGTAATAG CCCA
	XB	TTGTACTTTCTTAT GGGTGGGT
	CB	ACCC ATAAGAAAGTACAA
MAJ∧MIN	P1	ACGAACATCG GGGTGGG CTACAACGAC
	P2	AACGATAGTC GGGTGGG AGTGACGCAC
	P3	AATCCGCGAA GGGTGGG TACTGAATGT
	MA	GGG CGATGTTCGT ACATTCAGTA GGG
	MB	GGG GACTATCGTT GTCGTTGTAG GGG
	MC	GGG TTCGCGGATT GTGCGTCACT GGG

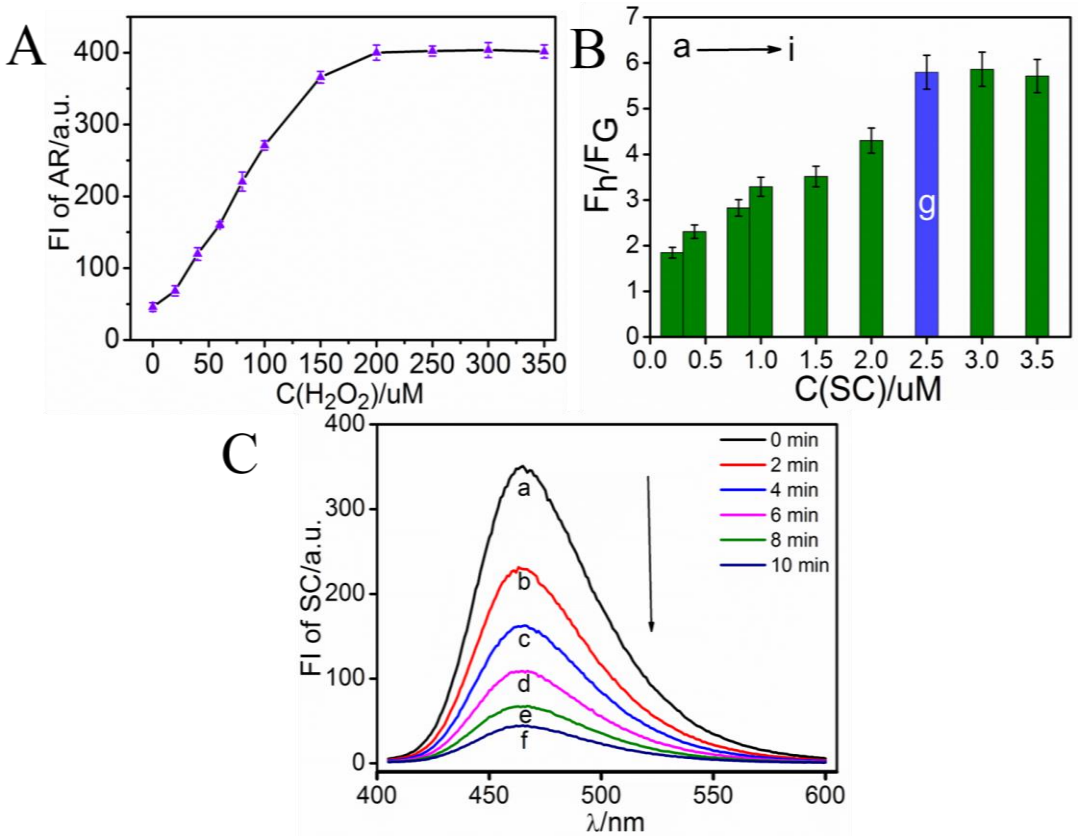


Figure S1. (A) Fluorescence changes of 7.5 μM AR in the presence of 1 μM hemin, 500 nM G4 with different concentrations of H_2O_2; **(B)** Column bars of *S/N* ratio values in the presence of different concentrations of SC under the same catalytic conditions with that of AR (1 μM hemin, 500 nM G4, 200 μM H_2O_2) (from **a** to **i**, 0.2 μM to 3.5 μM SC were used; herein, $S/N = F_h / F_G$, F_h represented the fluorescence intensity of different concentrations of SC at 465 nm in the presence of 1 μM hemin, 200 μM H_2O_2; F_G represented that in the presence of 500 nM G4, 1 μM hemin, 200 μM H_2O_2; **(C)** Fluorescence spectra of 2.5 μM SC in the presence of 1 μM hemin, 500 nM G4, 200 μM H_2O_2 under different catalytic reaction time (from **a** to **f**, 0 to 10 min).

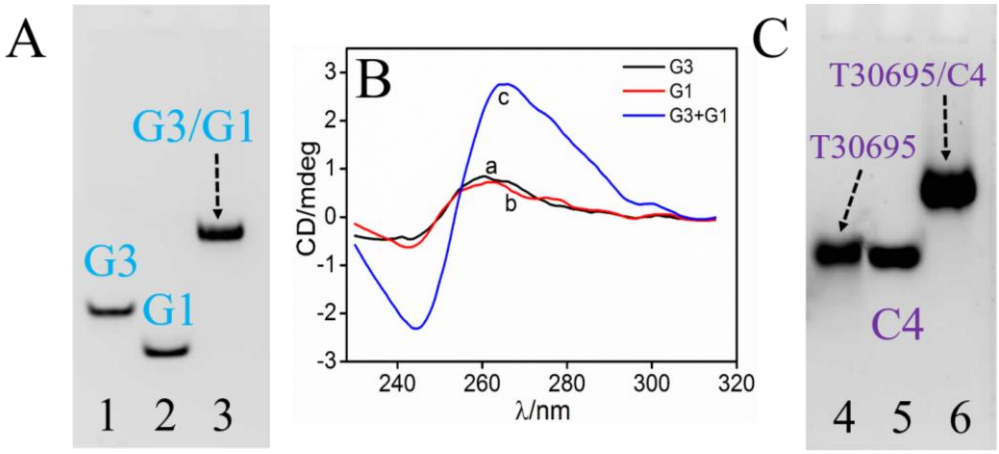


Figure S2. (A)15% PAGE analysis of the interaction between different DNA strands, G3 (Lane 1), G1(Lane 2), G3 and G1 (Lane 3); **(B)** The circular dichorism (CD) spectra of different DNA strands: G3 **(a)**, G1 **(b)**, G3 and G1 **(c)**; **(C)** 15% PAGE analysis of the interaction between different DNA strands, G4 (T30695, Lane 4), C4 (Lane 5), G4 and C4 (Lane 6).

A

Inputs	Out (Pos) YES	Out (Neg) NOT
T30695		
0	0	1
1	1	0

B

Inputs		Out (Pos) AND	Out (Neg) NAND
G3	G1		
0	0	0	1
0	1	0	1
1	0	0	1
1	1	1	0

C

Inputs		Out (Pos) OR	Out (Neg) NOR
T30695	PW17		
0	0	0	1
0	1	1	0
1	0	1	0
1	1	1	0

D

Inputs		Out (Pos) INH	Out (Neg) IMP
T30695	C4		
0	0	0	1
0	1	0	1
1	0	1	0
1	1	0	1

Figure S3. Truth tables of four elementary logic pairs constructed in this work. **(A)** YES∧NOT; **(B)** AND∧NAND; **(C)** OR∧NOR; **(D)** INH∧IMP. (The colors of different gates in the truth table were in accord well with the equivalent logic circuits in **Figure 1**, respectively)

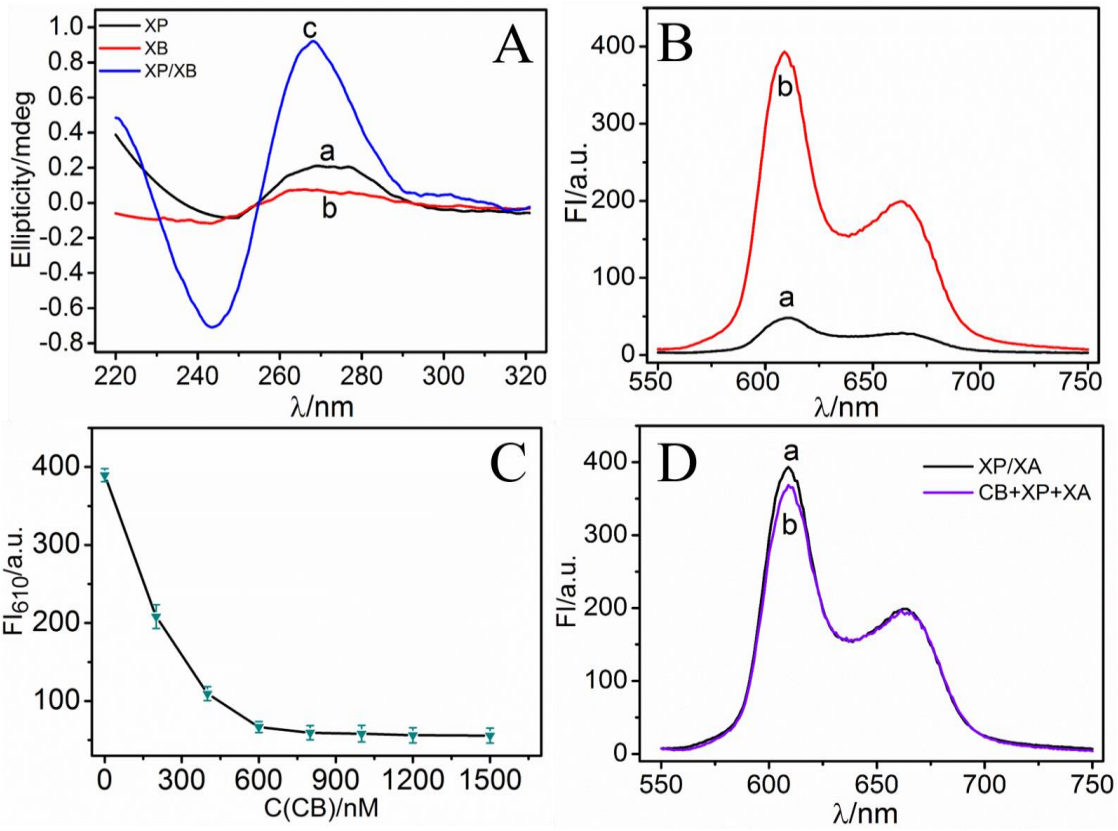


Figure S4. (A) CD spectra of XP **(a)**, XB **(b)**, XP/XB **(c)**; **(B)** Fluorescent spectra of NMM in the presence of 500 nM XP **(a)**, 500 XP and 500 nM XB **(b)**; **(C)** FI_{610} changes of NMM (500 nM XB was premixed with increasing concentrations of CB before the addition of 500 nM XP); **(D)** Fluorescent spectra of NMM in the presence of 500 nM XP and 500 nM XA **(a)**, 500 nM XP, 500 nM XA and 1 μM CB **(b)**.

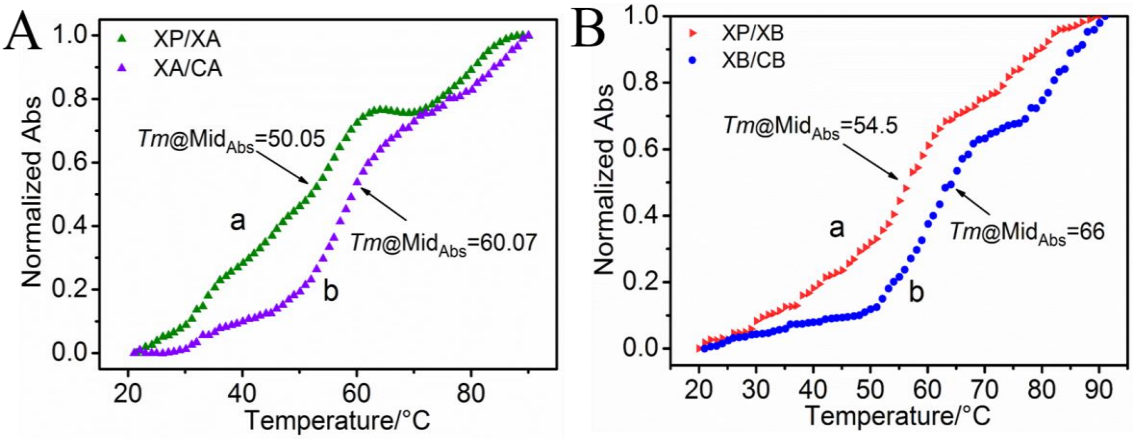


Figure S5. (A) Normalized thermal denaturation curves of duplex XP/XA **(a)** and XA/CA **(b); (B)** Normalized thermal denaturation curves of duplex XP/XB **(a)** and XB/CB **(b)** (The *Tm* values were obtained according to the **Middle-Abs** (**Mid_{Abs}**) of each curve).

The *Tm* values were roughly estimated according to the **Mid_{Abs}**, in which **Mid_{Abs}**= (Abs@90 ℃+Abs@20 ℃)/2. The temperature that was in accordance with the **Mid_{Abs}** was identified as the *Tm* value.

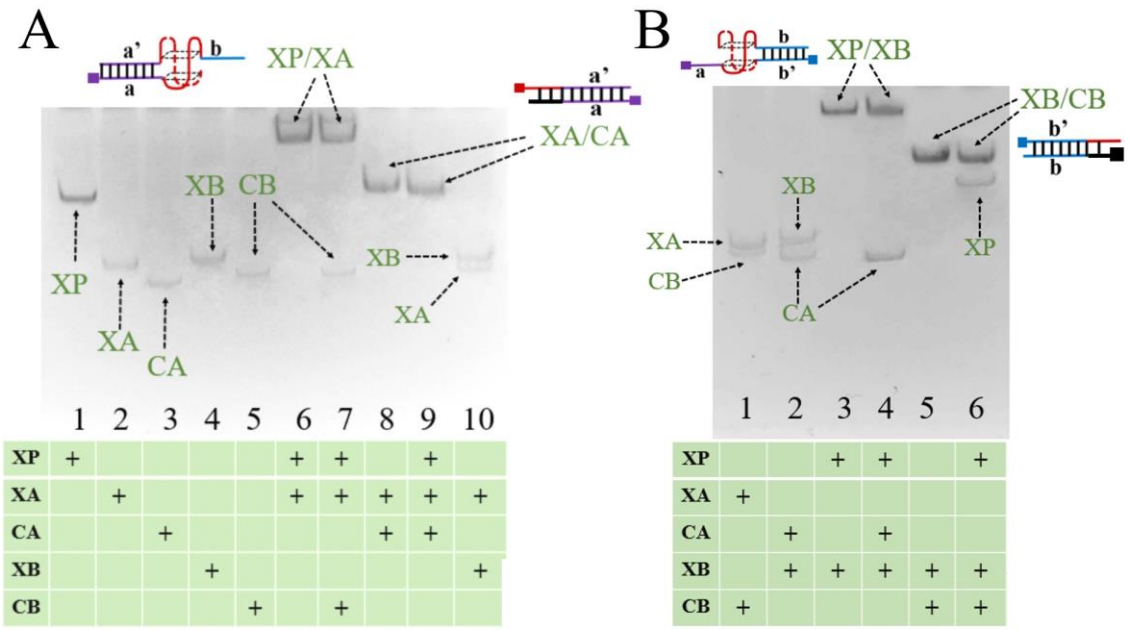


Figure S6. 15% PAGE analysis of the interactions between strands XP, XA, CA, XB and CB. The presence of strand was represented by "+", the formed duplexes were attached with corresponding graphics. For **(A)**, Lane 1 (XP), Lane 2 (XA), Lane 3 (CA), Lane 4 (XB), Lane 5 (CB), Lane 6 (XP, XA), Lane 7 (XP, XA, CB), Lane 8 (XA, CA), Lane 9 (XA and CA were premixed before the addition of XP), Lane 10 (XA, XB)； For **(B)**, Lane 1 (XA, CB), Lane 2 (XB, CA), Lane 3 (XP, XB), Lane 4 (XP, XB, CA), Lane 5 (XB, CB), Lane 6 (XB and CB were premixed before the addition of XP).

As can be seen in **Figure S6 (A)**, the bands of XP, XA, CA, XB and CB appeared at different positions from Lane 1 to Lane 5, respectively. While, after the mix of XP and XA, a new band appeared in Lane 6, indicating the formation of duplex XP/XA. Besides, in the presence of XP, XA and CB, two separate bands were observed in Lane 7, one band appeared at the same position with that of duplex XP/XA and another band was CB. This phenomenon proved that CB will not influence the G4 formation between XP and XA. Lane 8 showed the formation of duplex XA/CA. While, if XA and CA were premixed before the addition of XP, a band appeared at the same position with that of XA/CA was observed in Lane 9 and XP strand (the tail of band XA/CA in Lane 9) will not influence the formation of the duplex XA/CA. Finally, the two separate bands of XA, XB appeared in Lane 10 indicated that there is no interaction between XA and XB.

Figure S6 (B) presented other interactions of the five strands. For Lane 1, the two separate bands of XA, CB indicated that there is no interaction between XA and CB. Similarly, the bands in Lane 2 proved that XB will not interact with CA, either. For Lane 3, the new band appeared in it indicated the formation of duplex XP/XB. Besides, in the presence of XP, XB and CA, two separate bands were observed in Lane 4, one band appeared at the same position with that of duplex XP/XB and another band is CA. This phenomenon proved that CA will not influence the G4 formation between XP and XB. For Lane 5, the new band appeared in it indicated the formation of duplex XB/CB. While, if XB and CB were premixed before the addition of XP, a band appeared at the same position with that of XB/CB was observed in Lane 6 and another band was strand XP, indicating that XP will not influence the formation of the duplex XB/CB.

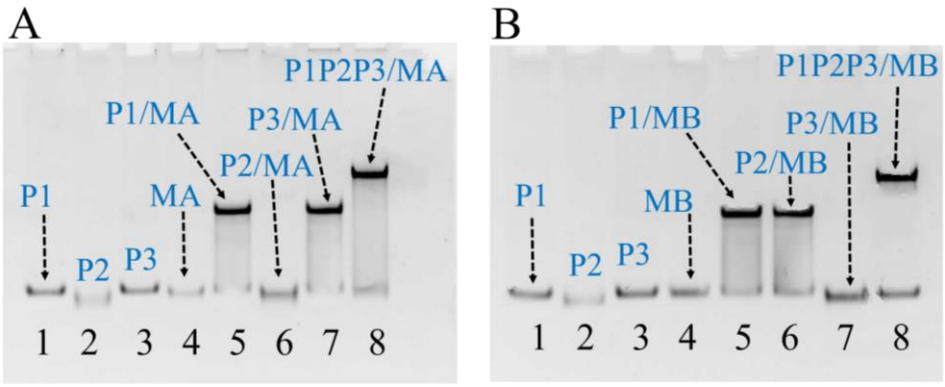


Figure S7. 15% native polyacrylamide gel analysis of the interaction between one input DNA strand and the platform DNA strands, P1, P2 and P3. **(A)** Interaction between MA and the platform DNA strands, P1, P2 and P3. Lane 1 (P1), Lane 2 (P2), Lane 3 (P3), Lane 4 (MA), Lane 5 (P1, MA), Lane 6 (P2, MA), Lane 7 (P3, MA), Lane 8 (addition of MA into the platform containing P1, P2 and P3). **(B)** Interaction between MB and the platform DNA strands. Lane 1 (P1), Lane 2 (P2), Lane 3 (P3), Lane 4 (MB), Lane 5 (P1, MB), Lane 6 (P2, MB), Lane 7 (P3, MB), Lane 8 (addition of MB into the platform containing P1, P2 and P3).

As presented in **Figure S7 (A)**, the DNA band of P1, P2, P3 and MA appeared at a similar position from Lane 1 to Lane 4. After adding MA into P1 or P3, new bands appeared in Lane 5 and Lane 7, respectively, indicating the formation of duplex P1/MA and P3/ MA. After adding MA into P2, no new band appeared in Lane 6, suggesting no hybridization between MA and P2. As shown in **Figure S7 (B)**, the DNA band of P1, P2, P3 and MB appeared at a similar position from Lane 1 to Lane 4. After adding MB into P1 or P2, new bands appeared in Lane 5 and Lane 6, respectively, indicating the formation of duplex P1/MB and P2/MB. After adding MB into P3, no new band appeared in Lane 7, suggesting no hybridization between MB and P3.

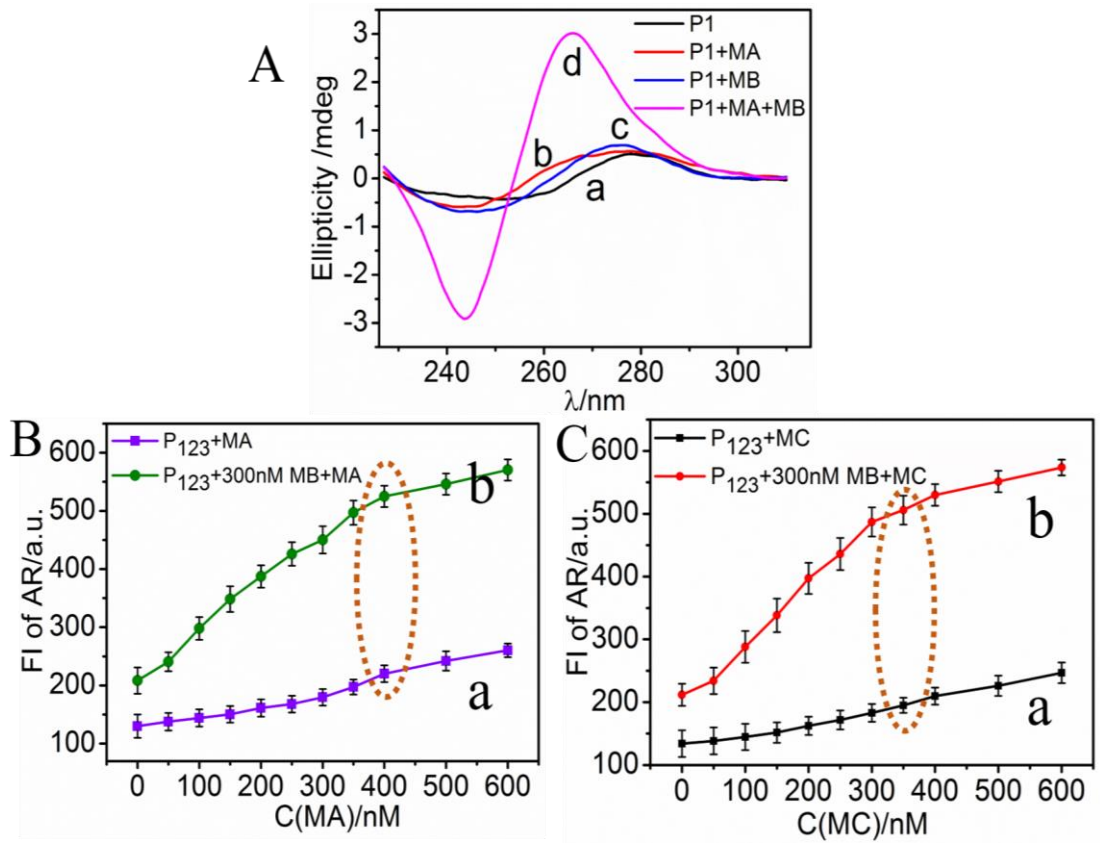


Figure S8. (A) CD spectra of P1 **(a)**, P1 and MA **(b)**, P1 and MB **(c)**, P1, MA and MB **(d)**; **(B)** Fluorescence changes with error bars of FI_{585} (AR-ox) in the presence of 300 nM P_{123} (P1+P2+P3) and increasing concentrations of MA without **(a)** and with 300 nM MB **(b); (C)** Fluorescence changes of FI_{585} (AR-ox) in the presence of 300 nM P_{123} and increasing concentrations of MC without **(a)** and with 300 nM MB **(b)** (The optimized concentrations of MA (400 nM) and MC (350 nM) were illustrated with circles).

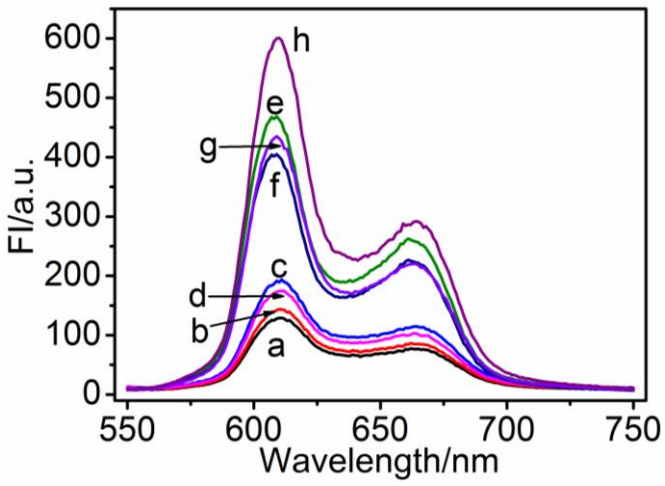


Figure S9. Fluorescence spectra of NMM of 300 nM P_{123} in the absence of any input **(a)**, in the presence of MA **(b)**, MB **(c)**, MC **(d)**, MA+MB **(e)**, MA+MC **(f)**, MB+MC **(g)** and MA+MB+MC **(h)**. (300 nM P_{123}, 400 MA, 300 nM MB and 350 nM MC were used).