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Supporting Information

Logic Circuit-Controlled Multi-Responsive Branched DNA Scaffolds

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Experiments details

Chemicals and Materials.

The PAGE-purified synthetic oligonucleotides (Table S1† in the supporting information), ATP, UTP, GTP and CTP were obtained from Shanghai Sangon Biotechnology Company (Shanghai, China). Before use, DNAs were prepared in TE buffer (40 mM Tris-HCl, 0.1 mM EDTA, pH 8.0) and quantified by UV–vis adsorption spectroscopy. All chemicals used in this study are of analytical grade and used without further purification. Millipore MilliQ (18 $M\Omega \cdot cm^{-1}$) water was used in all experiments.

Apparatus.

Agilent cary60 UV–vis spectrometer (Santa Clara, USA). Tanon 1600 Gel Imaging System (Shanghai, China). Fluorescence emission spectra were measured using the F-4600 fluorescence spectrophotometer (Hitachi, Japan).

Preparation of TWJ.

The component strands of TWJ were mixed at 1:1:1 molar ratio in TAE-Mg buffer (40 mM Tris-Ac, 5 mM $MgAc_2$, 0.1 mM EDTA, pH 8.0), heated to 90 ℃ for 10 min, then cooled to room temperature slowly to form TWJ structure. The formation of TWJ was finally verified by native PAGE.

Gel electrophoresis analysis.

Non-denaturing polyacrylamide gel (9%) was used and prepared in the TAE buffer (pH 8.0 or pH 5.0) containing 5 mM $MgAc_2$. Gel electrophoresis analysis was run in the identical buffer at 4°C for 5 h under a voltage of 8 V/cm. The gel was then stained in Gel Red solution for half an hour, finally photographed with a Tanon camera.

Fluorescence measurement.

All 2-AP fluorescence data were measured with an excitation wavelength at 300 nm at 4°C. When the DNA samples were excited at 300 nm, the emission was scanned from 300 to 550 nm in steps of 1 nm. All of the experiments were operated in the TAE buffer (pH 8.0 or 5.0) containing 5 mM $MgAc_2$. All Cy5 fluorescence data were measured with an excitation wavelength at 640 nm at 4°C. When the DNA samples were excited at 640 nm, the emission was scanned from 650 to 720 nm in steps of 1 nm. The slit widths (ex/em) for 2-AP is 5nm/5nm. The slit widths (ex/em) for Cy5 is 5nm/10nm. All of the experiments were operated in the TAE buffer (pH 8.0 or 5.0) containing 5 mM $MgAc_2$.The final concentrations of TWJ and HIV gene are 200 nM. The concentrations of ATP are 8 mM in the operations of the logic circuit-1 and logic circuit-2.

Cell Culture and Cell Lysates Methods.

MCF-7 cells were cultured in DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% FBS (Fetal Bovine Serum) in a 5% CO_2 incubator at 37 °C. For living cells disruption experiments, the culture medium surrounding the cells were first removed, and the cells were washed with PBS twice. Then the cells were incubated 1min in 1 mL trypsin, and washed with PBS twice. Finally, we obtained cell lysates by ultrasonic cell disruption system.

DNA sequences and Supplementary figures

Name	Sequence (5'-3')
HIV gene (H)	GCTAGAGATTTTCCACACTGACT
N5-1 (A)	GCTAGATGATTTTCCACACTCCTTCCTCCGCA
N5-2Mfl (B)	GTTAGGTTTAGGGAGTCAGTGTGGAAAATCATCTAGC
LG5-3ON (C)	ACCTGGGGGAGTATTGCGGAGGAAGGTTGACTCCCTAACCCTAACCCTAACCC
N5-1ctrl (A1)	CTGAAGTGTCTCAACCGTTCCCGTCATCGAC
N5-2mFLctrl (B1)	TGAGGTGTGAGTGATCTAACGGTTGAGACACTTCAC
LGN5-3ONctrl (C1)	TGCTGTAGGAGAGAGTCGATGACGGGTTAGATCACTCACACCTCACCTCACAC
HBV gene	AGTTACTCTCTTTTTTGCCTTCTGA
p53 gene	TTGAGGTGCGTGTTGTGCC
MicroDNA-21	TAGCTTATCAGACTGTTGA
Breast cancer gene	GAGAAACCCTATGTATGCTC
V-myc gene	GATCAGCCACCGGAACTGCA

Table S1. DNA sequences used in this study.

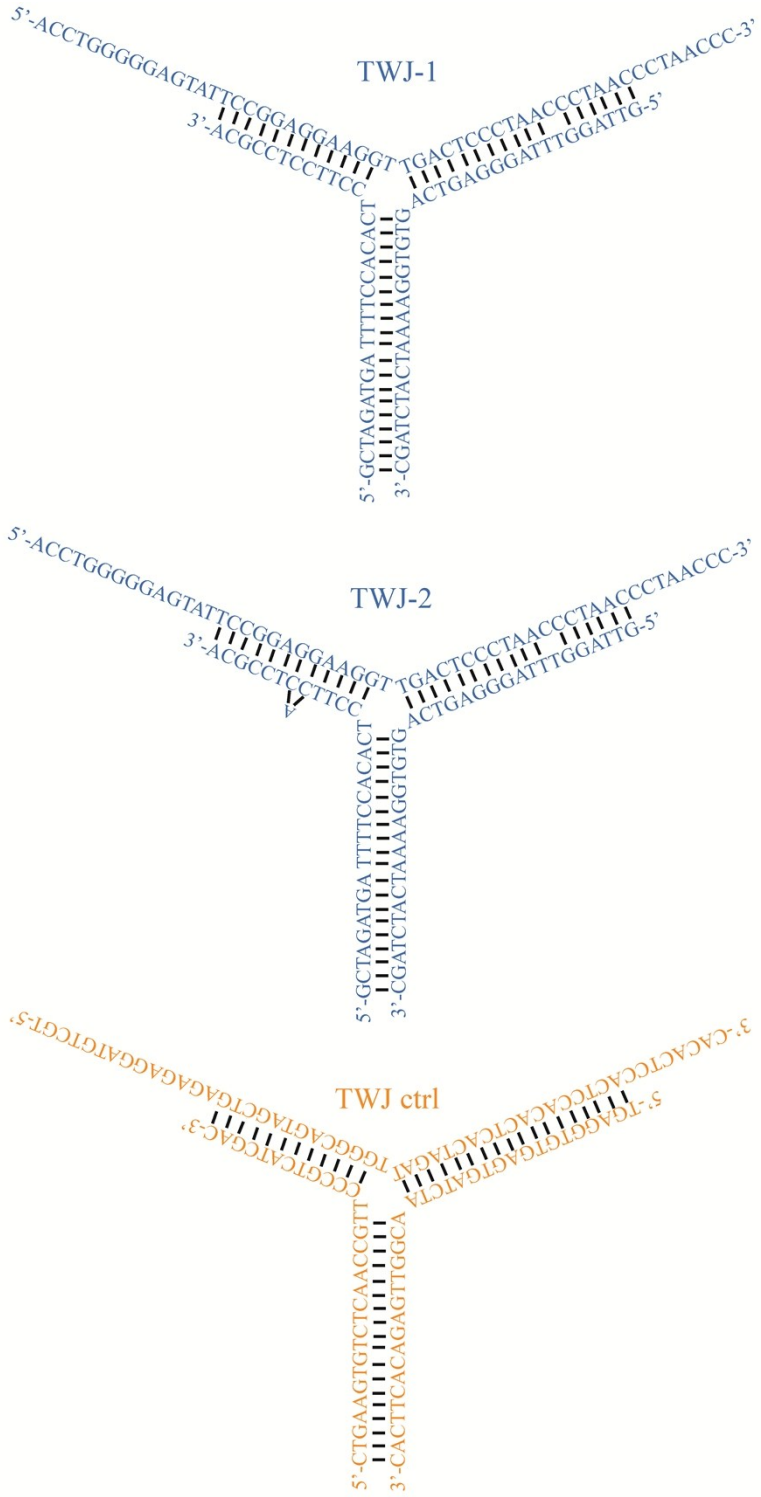


Fig. S1 Diagram of TWJ a, TWJ b and TWJ ctrl

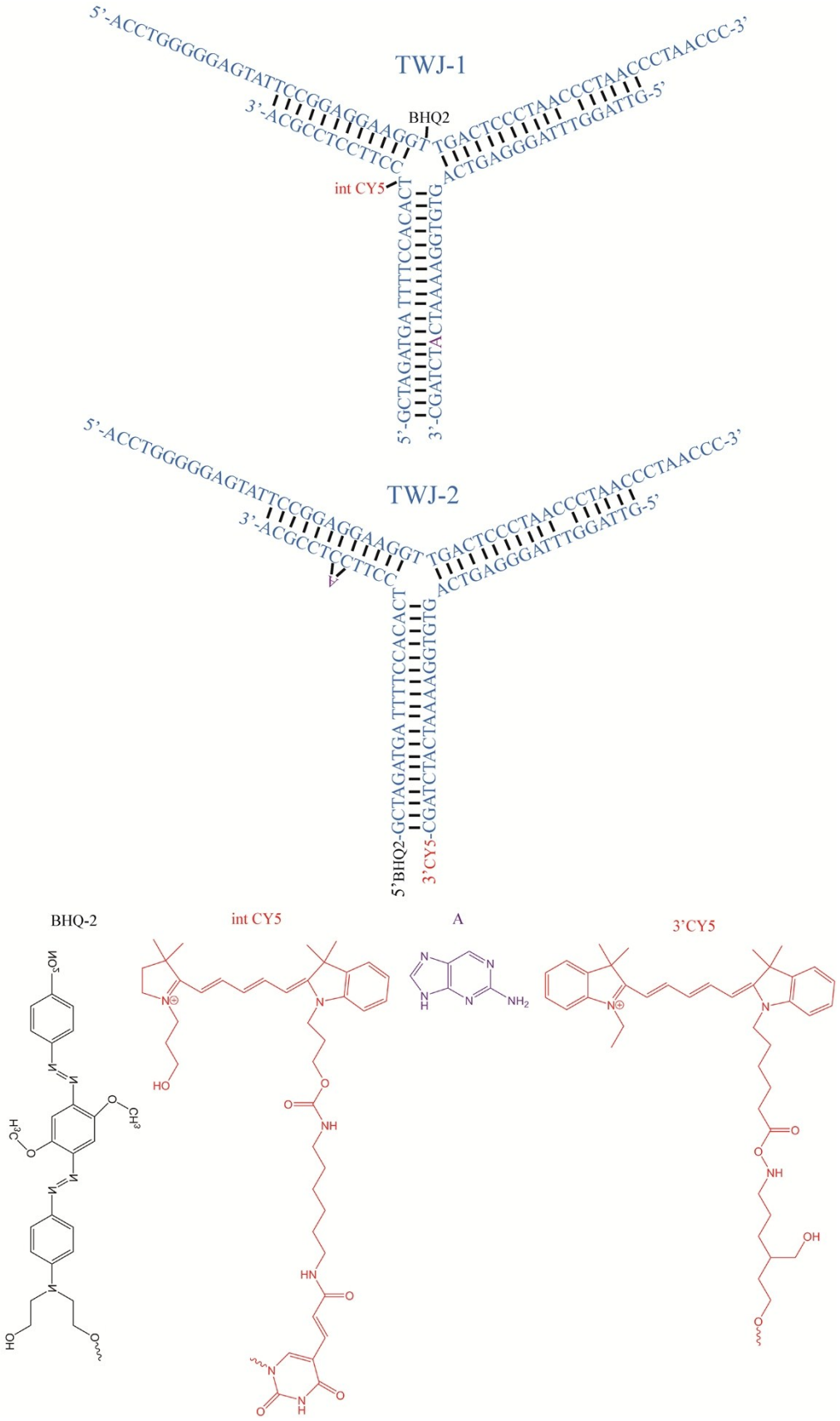


Fig. S2 Fluorophore/quencher-labeled DNA TWJ

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
A+B	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−
A+C	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−
B+C	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−
TWJ	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−
A1+B1	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−
A1+C1	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−
B1+C1	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−
TWJctrl	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	−
H	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	−	+	−
C	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
H+B	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−

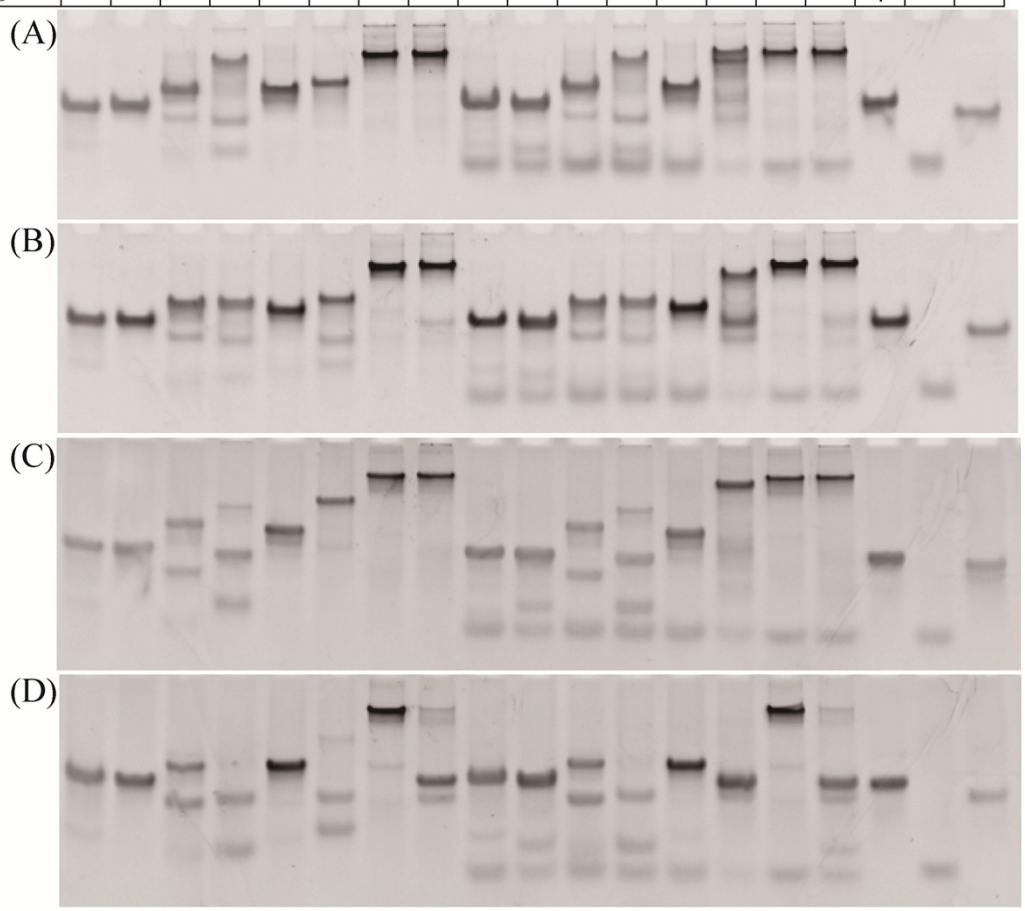


Fig. S3 Electrophoretograms of 0.5 µmol of oligonucleotides in 9% native gels under different conditions: (A) pH 8.0; (B) pH 8.0 with 8mM ATP; (C) pH 5.0 and (D) pH 5.0 with 8mM ATP. All of the experiments were performed in 40 mM Tris-Ac buffer containing 5 mM $MgAc_2$.

H^+	ATP	FI_{663}	HIV gene	FI_{370}
0	0	0	0	0
			1	0
0	1	0	0	0
			1	0
1	0	0	0	0
			1	0
1	1	1	0	0
			1	1

Fig. S4 The truth table for the logic circuit 1.

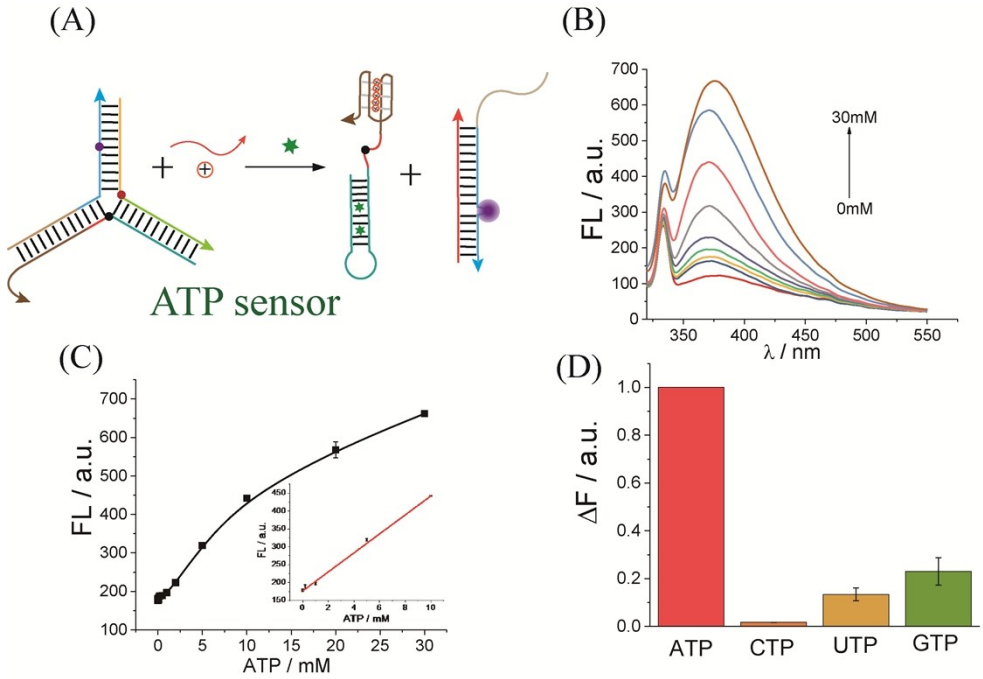


Fig. S5 (A) Schematic showing of the ATP detection. (B) Fluorescence spectra in response to varying concentrations of ATP. The ATP concentrations were as follows: 0 M, 0.05 mM, 0.2 mM, 0.5 mM, 2 mM, 5 mM, 10 mM , 20 mM and 30 mM. (C) Plot of the fluorescence intensity at 370 nm versus the concentrations of ATP. The inset shows a linear relationship in the concentration range from 0.05 to 10 mM. The R^2 value for the linear fit was 0.999. (D) Selectivity of ATP against CTP, UTP and GTP at 3 mM.

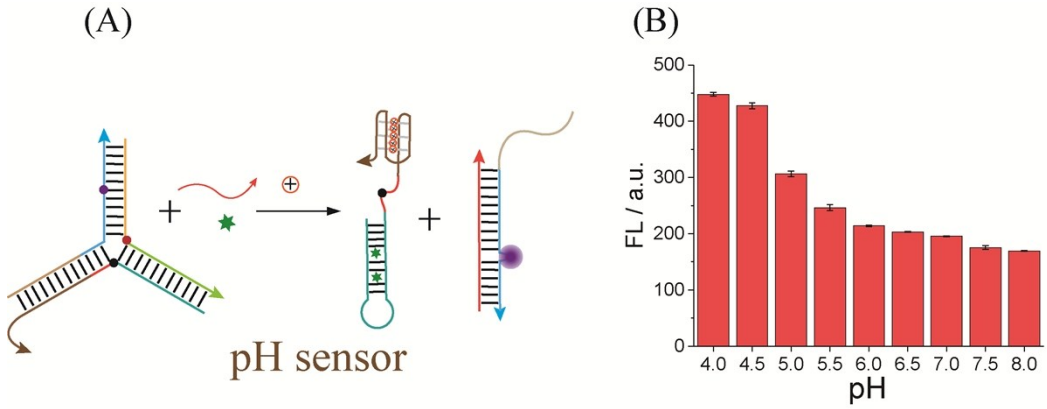


Fig. S6 (A) Illustration of the pH detector. (B) Fluorescence intensity at 370 nm in response to varying values of pH. The pH values were as follows: 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0.

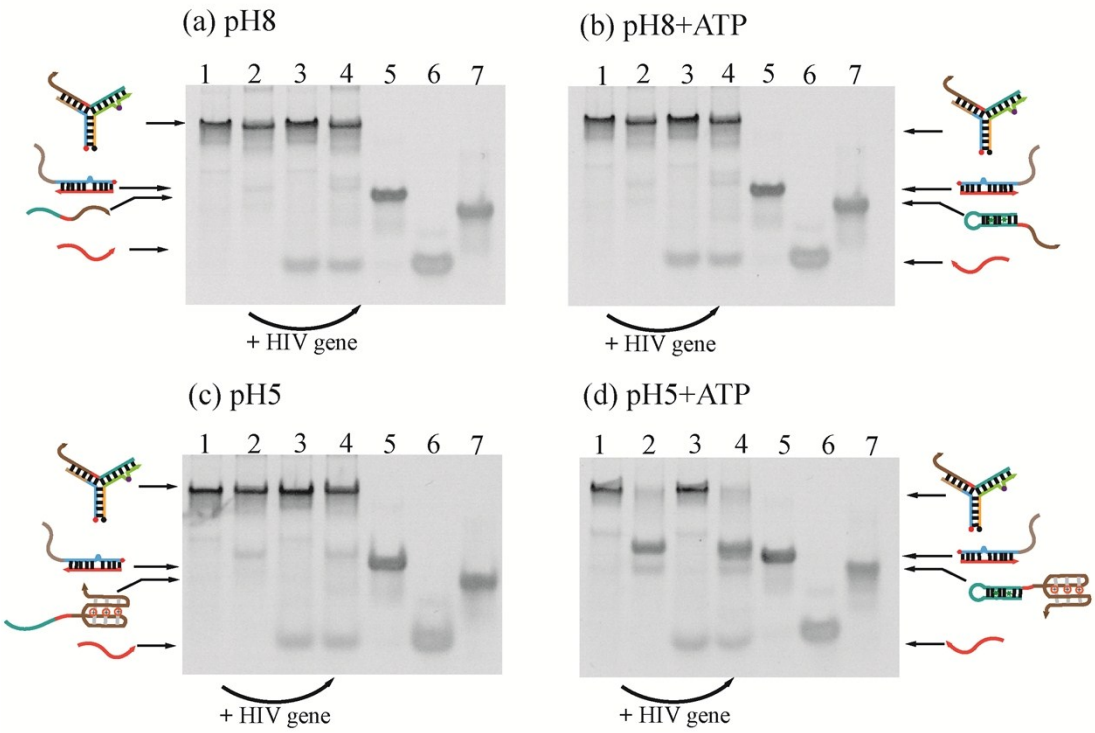
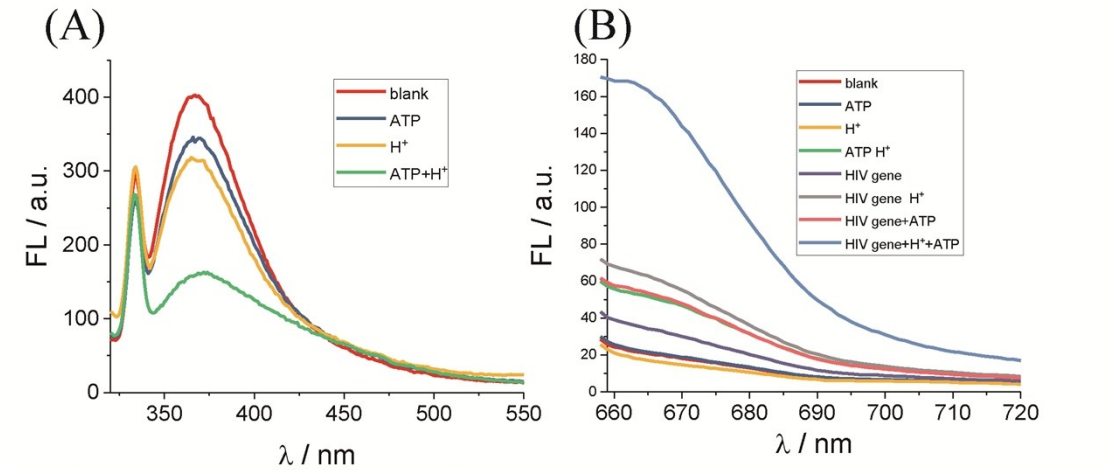


Fig. S7 Electrophoretograms of 0.5 μM of oligonucleotides in 9% native gels under different conditions. Lane 1, TWJ control; Lane 2, TWJ-2; Lane 3, TWJ control and HIV gene; Lane 4, TWJ-2 and HIV gene; Lane 5, duplex with a looped out 2-AP; Lane 6, HIV gene; and Lane 7, i-motif/ATP aptamer complex. (a) pH 8.0; (b) pH 8.0 with 8mM ATP; (c) pH 5.0 and (d) pH 5.0 with 8mM ATP.

Results of PAGE shows that TWJ-2 displays the same reaction features as those of TWJ-1. We found that the TWJ-2 can keeps stable upon addition of anyone or two targets among HIV gene, ATP, and proton (Fig S7, a-c, lanes 2, 4), except for the coexistence of ATP and proton (Fig S7, d, lane 2). It is observed that the band corresponding to TWJ-2 almost disappears in the presence of ATP and proton (Fig S7, d, lane 2), suggesting the disassembly of TWJ-2. Upon addition of HIV gene, the reporter structure is produced via TMSD reaction, indicated by a new band with a slightly slower mobility (Fig S7, d, lane 4). In contrast, it does not appear in the other cases (Fig S7, a-c, lane 4).



(C)

H^+	ATP	FI_{370}	HIV gene	FI_{663}
0	0	1	0	0
			1	0
0	1	1	0	0
			1	0
1	0	1	0	0
			1	0
1	1	0	0	0
			1	1

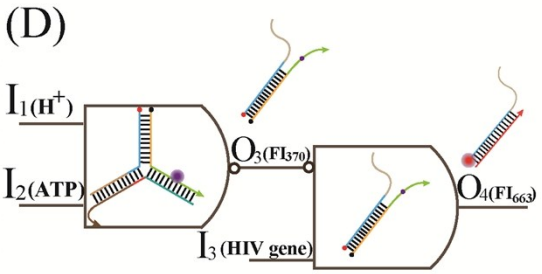


Fig. S8 Implementation of the logic circuit 2. (A) Fluorescence spectra of 200 nM oligonucleotides (each strand concentration) under different conditions for the first-order gate. (B) Fluorescence spectra of 200 nM oligonucleotides (each strand concentration) under different conditions for the second-order gate. (C) The truth table for the logic circuit 2. (D) For the NAND-INH logic circuit, Logic symbol are represented as schematic, where the presence of an input (I_1-H^+, I_2-ATP , I_3-HIV gene and O_3- FI_{370}) or output signal (O_3- FI_{370} and O_4- FI_{663}) is denoted by '1' and their absence is denoted by '0'.

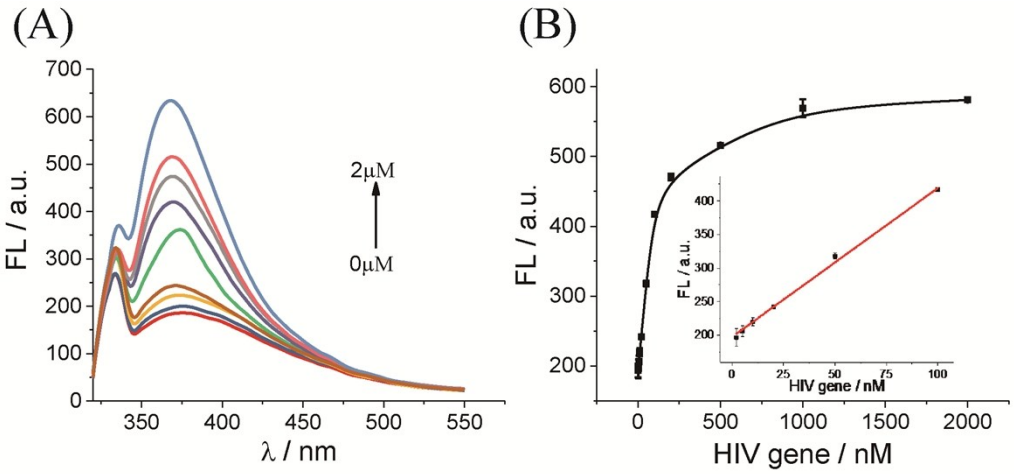


Fig.S9 Detection of serum samples by logic circuit-1 controlled biosensor. (A) Fluorescence spectra in response to varying concentrations of HIV gene in the serum. The HIV gene concentrations were as follows: 0 M, 2 nM, 10 nM, 20 nM, 50 nM, 100 nM, 200 nM, 1000nM and 2000 nM. (B) Plot of the fluorescence intensity at 370 nm versus the concentrations of HIV gene in the serum. The inset shows a linear relationship in the concentration range from 2 to 100 nM. The R^2 value for the linear fit was 0.996.

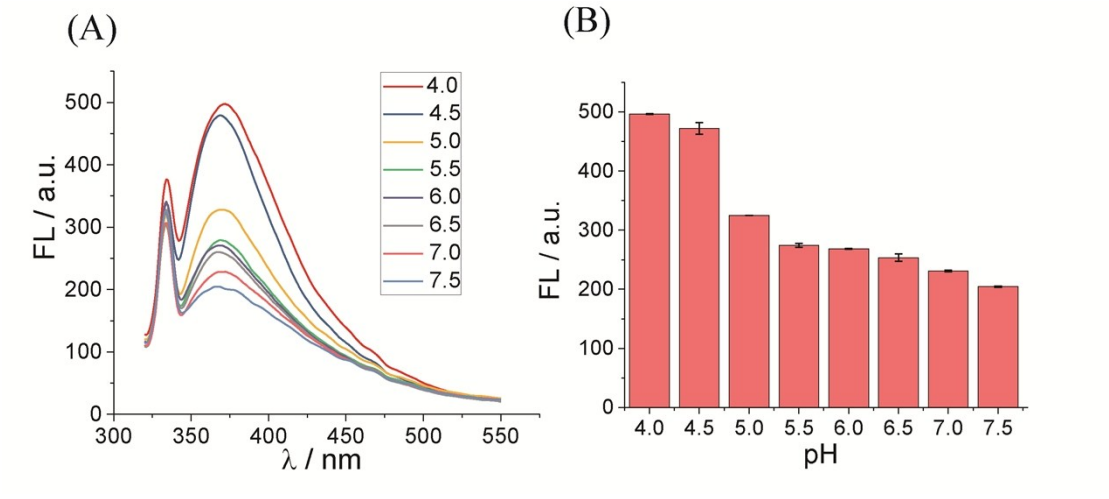


Fig. S10 (A) Fluorescence spectra in response to varying pH in serum. (B)Fluorescence intensity at 370nm in response to varying values of pH. The pH values were as follows: 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5.

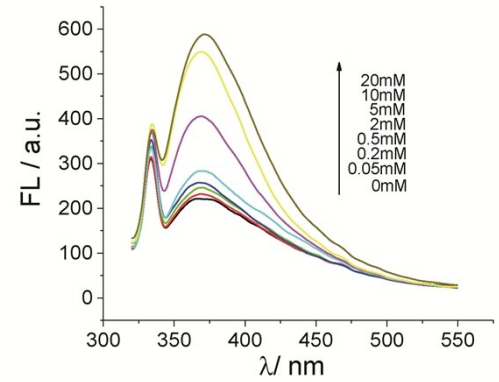


Fig.S11 Fluorescence emission spectra in response to varying concentrations of ATP in serum. fluorescence emission spectra in response to increasing concentrations of ATP. The concentrations of ATP were as follows: 0 M, 0.05 mM, 0.2 mM, 0.5 mM, 2 mM, 5 mM, 10 mM and 20 mM.

Table S2. Range of Response of Different Methods for pH. our developed sensing platform and previous counterparts listed the results below in the table, which shows that our platform has a comparable range of response for pH, as compared with other counterparts.

Analyte	Methods	Range of response
pH	Fluorescent method based on triplex DNA structure (Ricci, F. etl. *J. Am. Chem. Soc.***2014**, 136, 5836-5839.)	1.8-5.5 units
	Fluorescent method based on aggregation-induced emission by tetraphenylethene−cyanine adduct. (Tang, B. Z. etl. *J. Am. Chem. Soc.* **2014**, *136*, 11196-11196.)	3.5 units
	Fluorescent method based on DNA logic circuit (Our approach)	4.0 units

Table S3. Analytical Performances of Different Methods for Nucleic Acids or ATP Detection. our developed sensing platform and previous counterparts listed the results below in the table, which shows that our platform has a comparable sensitivity for targets, as compared with other counterparts.

Analyte	Methods	Limit of detection
Nucleic Acids	Fluorescent method based on CHA and HCR in buffer (Wang, K. M. etl. *Anal. Chem.* **2016**, *88*, 5857-5864.)	10 nM
	Fluorescent method based on DNA-Ag nanoclusters in buffer (Wang, E.etl. *Biosens. Bioelectron.* **2015**, 74, 318-321.)	10 nM
	Fluorescent method based on DNA-regulated silver nanoclusters in buffer (Liu, L.etl. *Chem. Commun.* **2014**, *50*, 13698-13701.)	10 nM
	Fluorescent method based on DNA logic circuit in cell lysates (Our approach)	2 nM
ATP	Electrochemical method based on Aptamer Biosensor (Yu, P.etl. *Anal. Chem.* **2015**, *87*, 1373-1380.)	10μM
	Fluorescent method based on DNA-templated silver nanoclusters (Zhang, M. etl. *Chem. Commun.* **2012**, *48*, 5488-5490.)	100μM
	Fluorescent method based on DNA logic circuit (Our approach)	50μM