Supporting Information

DNA logic assembly powered by a triplex-helix molecular switch for extracellular pH imaging

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1. Experimental Section

Materials. All oligonucleotides used in this study were synthesized and purified by Sangon Biotechnology Co., Ltd. (Shanghai, China). Their sequences were listed in Table S1, and the secondary structures of the DNA hairpins were depicted in Fig. S1. The DNA oligonucleotides were suspended to a final concentration of 100 μM and stored in deionized ultrapure water at -20 °C. In all experiments, we used solutions of 1× TAE buffer (40 mM tris-base, 1 M glacial acid acetic, 2 mM EDTA-2Na, 15 mM $MgCl_2$, starting pH 7.0) with the pH adjusted with the addition of 1 M HCl or 1 M NaOH. Before experiment, the hairpin strands H1, H2 and duplex probes (DPs) were respectively diluted in 1× TAE buffer supplemented with 15 mM $MgCl_2$ (pH 7.0) and annealed on a thermal cycler before use (heat at 95 °C for 5 min, cool to 25 °C at a rate of 0.1 °C/s, and stand at 25 °C for 1.5 h at least). All the reagents were of analytical grade and used without further purification. Deionized ultrapure water was used during all the experiments.

Table S1. Oligonucleotides Sequence Used in This Work

Strand	Sequence (from 5' to 3')
initiator	TCCTCTTCTCCCTTCCTCAATTCC
H0	GGAATTGAGGAAGGGAGAAGAGGACATTACTCCTCTTCTCCCTTCCTC
H1	GTCGGTTGCTGGAATTGAGGAAGGGAGAAGAGGACATTACTCCTCTTCTCCC TTCCTCTCTCTATCATTATCTTCC
H2	TCCTCTTCTCCCTTCCTCAATTCCGAGGAAGGGAGAAGAGGAGTAATG
DNA (1)	TCTCTATCATTATCTTCC-BHQ1
DNA (2)	Alexa Fluor-488-GGAAGATAATGATAGAGAAGCAACCGAC
I-F	TCCTCTTCTCCCTTCCTCAATTCC-Alexa Fluor-488
H0-Q	GGAATTGAGGAAGGGAGAAGAGGACATTACTCCTCTTCTCCCTTCCTC-BHQ1
initiator'	AGTCTAGGATTCGGCGTGAATTCC
H0'	GGAATTCACGCCGAATCCTAGACTCATTACAGTCTAGGATTCGGCGTG
H1'	GTCGGTTGCTGGAATTCACGCCGAATCCTAGACTCATTACAGTCTAGGATTCG GCGTGTCTCTATCATTATCTTCC
H2'	AGTCTAGGATTCGGCGTGAATTCCCACGCCGAATCCTAGACTGTAATG

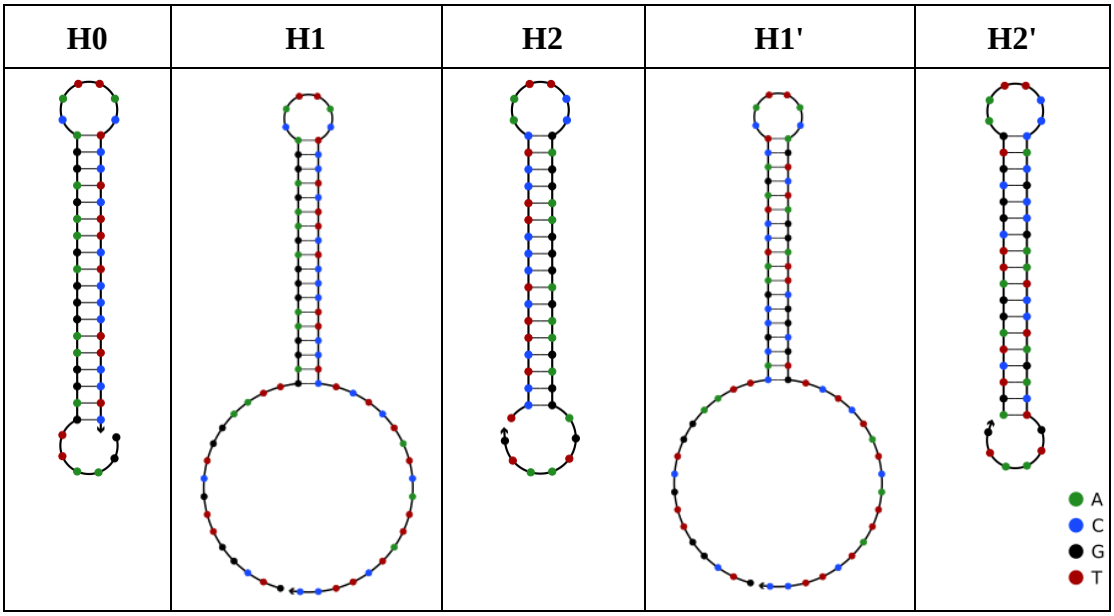

Fig. S1 Secondary structures of hairpin species used in this study, which are predicted using the NUPACK software (www.nupack.org).

Atomic Force Microscopy (AFM) Imaging. The assembled or unassembled products were directly visualized by AFM imaging. Before experiments, 1 μL of initiator and 1 μL of H0 (10^{-6} M for each, pH 7.0) were mixed in 20 μL of 1× TAE buffer with different pH values, respectively. After reacted at 25 °C for 30 min, 1 μL of H1 and 1 μL of H2 (10^{-5} M for each, pH 7.0) were added and reacted at 25 °C for 2 h. Before imaging, the reaction products were washed with 10 μL of deionized ultrapure water three times to remove free metal ions by centrifugation at 10000 rpm for 3 min. Then, 10 μL of each sample was deposited onto the surface of freshly cleaved mica and left to dry under ambience air. AFM imaging experiments were performed in air under the tapping mode using Being Nano-Instruments CSPM-4000 system (Benyuan, China), and the results were analyzed with CSPM Console software (Benyuan-CSPM4000, China).

Native Polyacrylamide Gel Electrophoresis (PAGE). Firstly, 8% non-denaturing PAGE was prepared by mixing 3.7 mL of 30% acrylamide/bis-acrylamide gel solution (29:1), 1 mL of 10×

TAE/Mg^{2+} buffer, 90 μL of 10% ammonium persulfate (APS), 10 μL of N,N,N',N'-tetramethylethylenediamine (TEMED), and 6.2 mL of deionized water. The gel was polymerized for about 30 min at room temperature and was soaked in 1× TAE/Mg^{2+} buffer (pH 8.0). And then, 10 μL of each sample was mixed with 2 μL of 10× loading buffer, which was added into the gel respectively. Electrophoresis was conducted at 170 V for 5 min and 110 V for 40 min. After staining in diluted 4S Red Plus solution (Sangon Biotech. Co., Ltd., China) for 40 min, the gel was scanned using a Tanon 2500R gel imaging system (Tanon Science & Technology Co., Ltd., China).

Real-Time Fluorescence Monitoring. Real-time fluorescent measurements were carried out to verify the kinetics of the self-assembly process at different pHs. Briefly, 1 μL of initiator and 1 μL of H0 (10^{-6} M for each, pH 7.0) were mixed with 45 μL of 1× TAE buffer with different pH values and reacted at 25 ^{o}C for 30 min. Then, 1 μL of hairpin H1, 1 μL of H2 and 1 μL of DPs (10^{-5} M for each, pH 7.0) were added and the mixture was immediately monitored on a LineGene 9600 Real-Time fluorescence detector (Hangzhou, China) with an interval of 30 s for 240 min totally (λ_{ex} = 470 nm and λ_{em} = 530 nm). The reaction temperature was set as 10 ^{o}C.

Fluorescence Measurements. Fluorescence measurements were conducted to record the fluorescence spectra of the downstream reaction at different pHs. The experiment procedures were the same as the real-time fluorescent measurements mentioned above except that when H1, H2 and DPs (10^{-5} M for each, pH 7.0) were added in solution, the mixture was reacted at 10 ^{o}C for 4 h. The fluorescence measurements were carried out on an FL-7000 spectrometer (Hitachi, Japan). The fluorescence emission spectra were recorded from 500 nm to 600 nm at room temperature with an excitation wavelength at 460 nm.

The reversibility study was performed using a mixture containing 1 μL of quencher-label initiator (I-Q) and 1 μL of fluorophore-labeled H0 (H0-F) (10^{-5} M for each) in 1× TAE-Mg^{2+} buffer (total volume of 50 μL). The pH of the buffer was changed between 5.0 and 8.0 by alternately adding 1 μL of HCl (1 M) and 1 μL of NaOH (1 M). The reaction temperature was 25 ^{o}C and the time interval was 30 min. The pH of the TAE/Mg^{2+} buffer was stable when it was at pH 5.0 (pK_a of acetic acid is 4.75) and pH 8.0 (pK_a of Tris is 8.3).

Operation of Logic Gates. For "AND" gate, the input A (pH 8.0) and input B (initiator/H0) with different combinations of (0,0), (0,1), (1,0) and (1,1) were introduced into the system containing 1 μL of initiator and 1μL of H0 (10^{-6} M for each, pH 7.0) in 45 μL of 1× TAE buffer (pH 8.0) and reacted at 25 ^{o}C for 30 min to form the initiator/H0 complex. Then, 1 μL of H1, 1 μL of H2 and 1 μL of DPs (10^{-5} M for each, pH 7.0) were added and the mixture was reacted at 10 ^{o}C for 4 h. The fluorescence measurement was carried out on the FL-7000 spectrometer (Hitachi, Japan) and the fluorescence emission spectra were recorded from 500 nm to 600 nm at room temperature with an excitation wavelength at 460 nm. For "INHIBIT" gate, the operation procedures were similar to the "AND" gate except that input A was defined as pH 5.0.

Cell Treatment and Extracellular pH imaging. The experiments were carried out according to Wang's work.[S1] First, the human breast adenocarcinoma cells (MCF-7 cells) were incubated with NHS-biotin (1 mM) in PBS (pH 7.4) for 30 min at room temperature. After washing three times, streptavidin solution (50 μg/mL) in PBS (pH 7.4) was added and reacted for another 30 min. Subsequently, biotin-modified initiator/H0 complex (200 nM) was introduced, followed by incubation for 1 h on ice. The cells were then washed three times using PBS to remove the excess DNA species.

For extracellular pH imaging, the initiator/H0-anchored MCF-7 cells were suspensed in different extracellular pH buffers and incubated for 30 min. followed by adding H1, H2 and DPs. After reaction at 10 ^{o}C for 1 h, the fluorescence images were recorded on a confocal laser microscope (Leica, TCS SP5 II)

2. Effect of Fluorophore

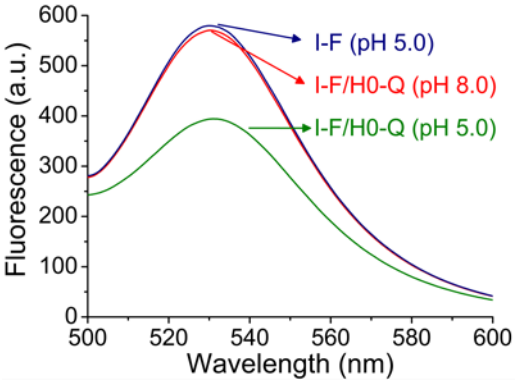

Fig. S2 The fluorescence emission spectra of Alexa-488 only at pH 5.0 (blue) and the pH-dependent substrate which is labelled with Alexa-488 and BHQ1 at 3' ends of I-F and H0-Q respectively at pH 5.0 (green) and 8.0 (red).

3. Effect of Temperature

The temperature of the downstream for HCR is an essential factor of the system. In principle, the fluorescence intensities should remain at a low level under the strong acidic conditions since the formation of triplex in the upstream can prohibit the initiation of HCR and no restored fluorescence signal can be observed. However, as shown in Fig. S3, as the temperature increases, the fluorescence signals are intensified at pH 4.0 and 5.0. This phenomenon can be attributed to the random hybridization of the extended regions of H1 and DNA (2) with the temperature

increasing, which thus could induce a high background. To ensure the accuracy of our method and improve the detection sensitivity, we choose 10 ºC as the optimal temperature to perform the downstream HCR process.

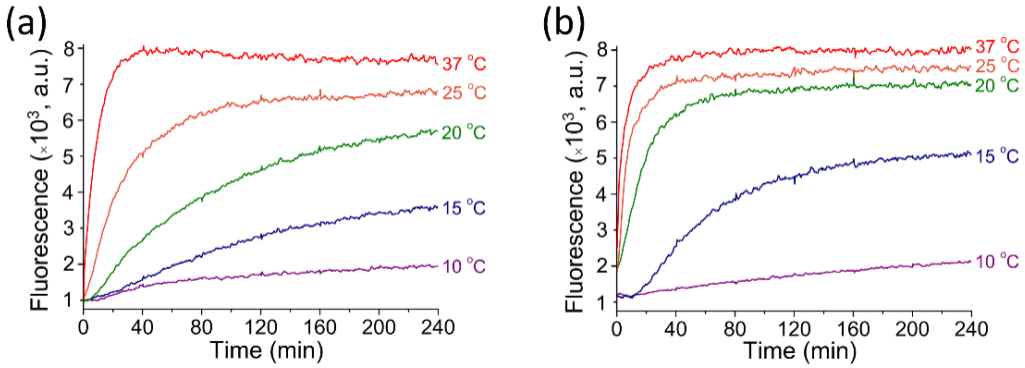

Fig. S3 Real-time monitoring of the fluorescence intensities at pH 4.0 (a) and 5.0 (b) under different temperatures. The reaction mixture contains initiator/H0 substrate, H1 and H2 with a ratio of 1:10:10. The concentration of the initiator/H0 substrate is 1 μM. The reactions are performed at 10 °C.

4. pH-Dependent Control Experiments.

The fluorescence spectra of the system at the downstream reaction time of 4 h under different pH from 4.0 to 9.0 are shown in Fig. S4. At pH 4.0 and 5.0, the fluorescence peak intensities at 530 nm are below 400, indicating the formation of reverse Hoogsteen interactions between the initiator and H0 under strong acidic conditions and the inhibition of the downstream HCR events. Thus, in the DPs the fluorophore on DNA (2) is in close proximity to the quencher on DNA (1), which results in a low fluorescence intensity. At pH 6.0-9.0, the fluorescence peak intensities at 530 nm are about 600. The enhanced fluorescence can be attributed to the destabilization of the reverse Hoogsteen interactions of initiator/H0 and the initiation of HCR between H1 and H2. The resulting overhangs in the HCR products separates the fluorophore labeled on DNA (2) from the

quencher on DNA (1) via strand displacement reaction, resulting in the intensified fluorescence readout. The plot of fluorescence intensities of the triplex-helix molecular switch triggered DNA assembly as a function of pH shows a sigmoid increase between pH 4.0 and 9.0 with a 1.8-fold ratio increase and a pK_a of 5.8 (Fig. S4b), which is consistent with that of the pH-dependent substrate shown in Fig. 2b.

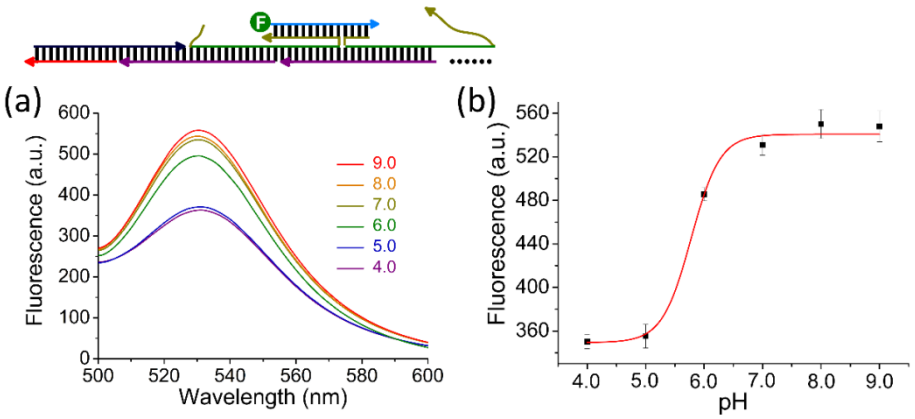

Fig. S4 (a) Fluorescence spectra of the system at different pHs. (b) pH calibration curve of the fluorescence intensities at 530 nm versus pH values. The reaction mixture contains initiator/H0 substrate, H1 and H2 with a ratio of 1:10:10. The concentration of the initiator/H0 substrate is 1 μM. The reactions are performed at 10 °C for 4 h.

The activation level can be regulated by changing the concentrations of initiator/H0 at different pHs (Fig. S5 and Fig. S6). When the substrates are at high concentrations (5 μM and 10 μM), we can only observe weak fluorescence intensities due to the formation of DNA nanowires with shorter length, which further confirms the fluorescence intensity is produced by the downstream HCR events and the separation of DPs.

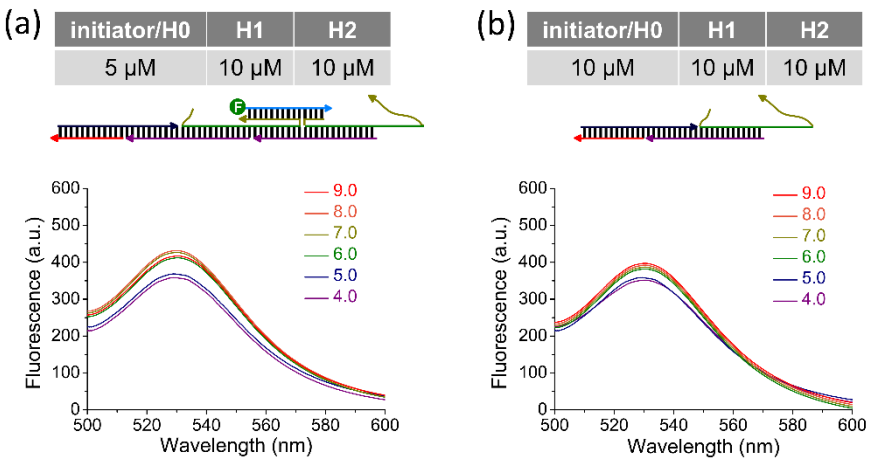

Fig. S5 pH-dependent control experiments. Fluorescence spectra obtained under different pHs with the ratios of initiator/H0 substrate, H1 and H2 are 1: 2: 2 (a) and 1: 1: 1 (b). The concentration of initiator/H0 substrate 1 μM. The reactions are performed at 10 °C for 4 h.

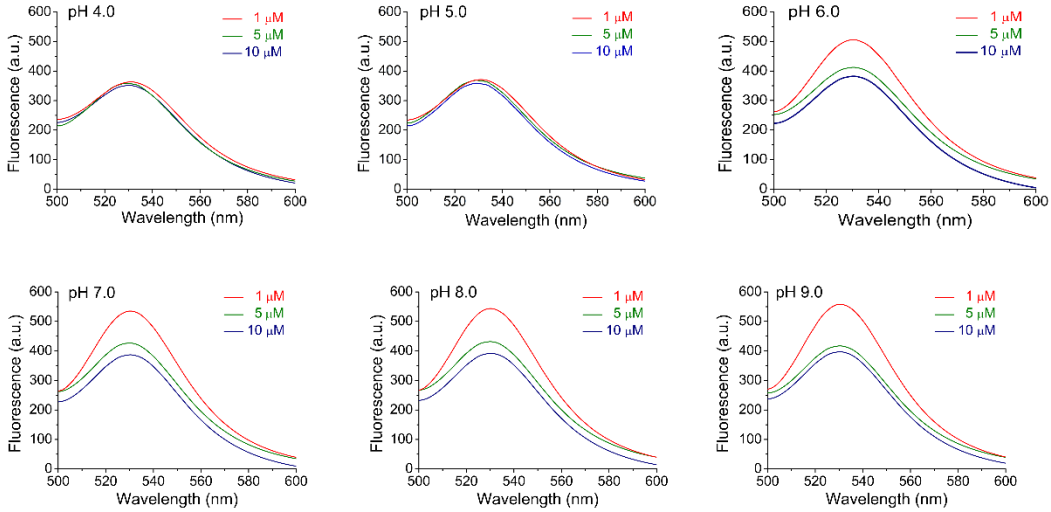

Fig. S6 pH-dependent control experiments. At pH 4.0 and 5.0, initiator/H0 with different concentrations leads to no significant fluorescence change, demonstrating the formation of sequence specific reverse Hoogsteen interactions under strong acidic conditions which thus inhibits the HCR. In contrast, at pH 6.0 to 9.0, the destabilization of the reverse Hoogsteen interactions allows the well performance of HCR. Thus, the fluorophore labeled on DNA (2) is separated from the quencher on DNA (1) and the fluorescence signal is restored.

5. pH-Independent Control Experiments

Control experiments containing the pH-independent substrates (initiator'/H0') are carried out, in which the triplex-forming domain in Scheme 1 is replaced with random sequences (Fig. S7). Thus, only duplex complex is formed over the pH from 4.0 to 9.0, which is independent of pH. No influence of pH is observed in the entire pH range investigated over a wide concentration range of initiator'/H0' substrate (1 μM, 5 μM and 10 μM). These results also demonstrate that the HCR process and the reaction between the HCR products and DPs are not sensitive to pH in our investigated range.

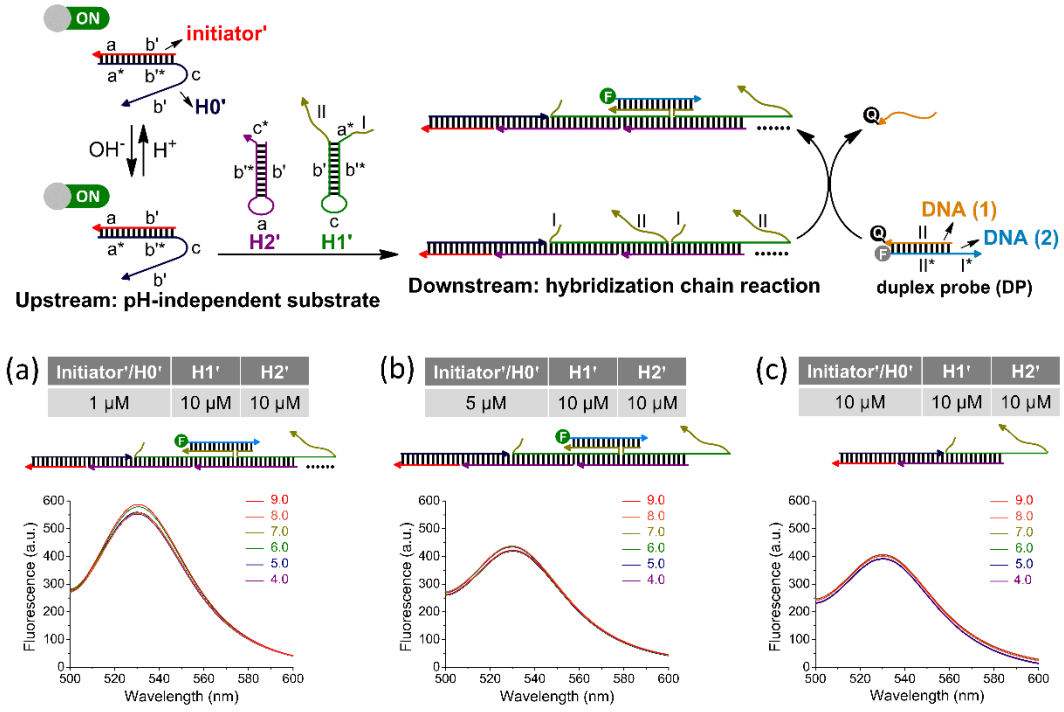

Fig. S7 pH-independent control experiments. The fluorescence spectra are obtained under different pHs. The ratios of initiator'/H0' substrate, H1' and H2' are 1: 10: 10 (a), 1: 2: 2 (b) and 1: 1: 1 (c), respectively. The concentrations of initiator'/H0' substrate in (a), (b) and (c) are 1 μM, 5 μM and 10 μM, respectively. The reactions are performed at 10 °C for 4 h.

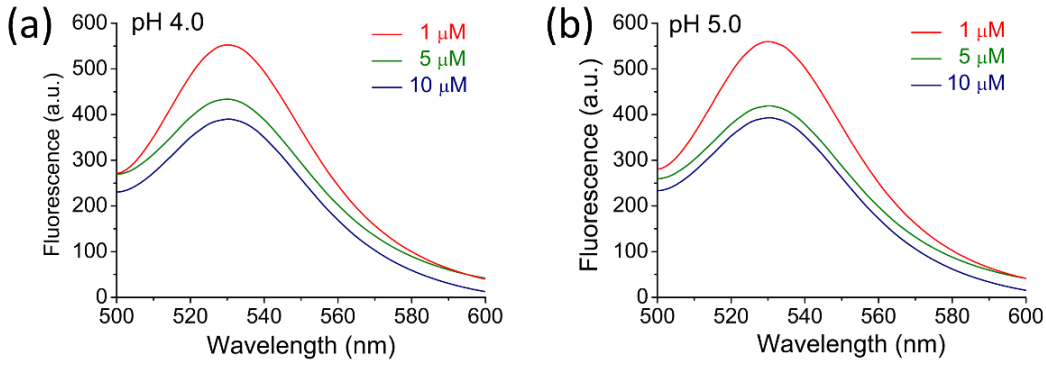

Fig. S8 pH-independent control experiments. At pH 4.0 and 5.0, initiator'/H0' with different concentrations leads to significant fluorescence signal change, thus demonstrating the occurrence of HCR in pH-independent system even under strong acidic conditions.

6. "INH" Gate

In ''INH'' gate (Fig. S9), we define the acidic condition (e.g. pH 5.0) and the presence of initiator/H0 as the positive input A and input B, respectively. Similar to the mechanisms mentioned in "AND" gate, for the input (0,0), or (1,0) or (1,1), no fluorescence signal above the threshold value of 400 a.u. is recorded (output 0). However, when the solution is basic (pH 8.0, input A= 0), the destabilization of the reverse Hoogsteen interactions leads to the performance of HCR in the presence of initiator/H0 (input B= 1) and following strand displacement reaction between the overhangs in HCR products and DPs, which ultimately generates a positive fluorescence readout above 400 a.u. (output 1). Thus, for ''INH'' gate, the fluorescence signal is generated ("1" output) only for the input (0, 1), that is, the solution is basic and the initiator/H0 is present. Otherwise, the fluorescence outputs are recorded as "0".

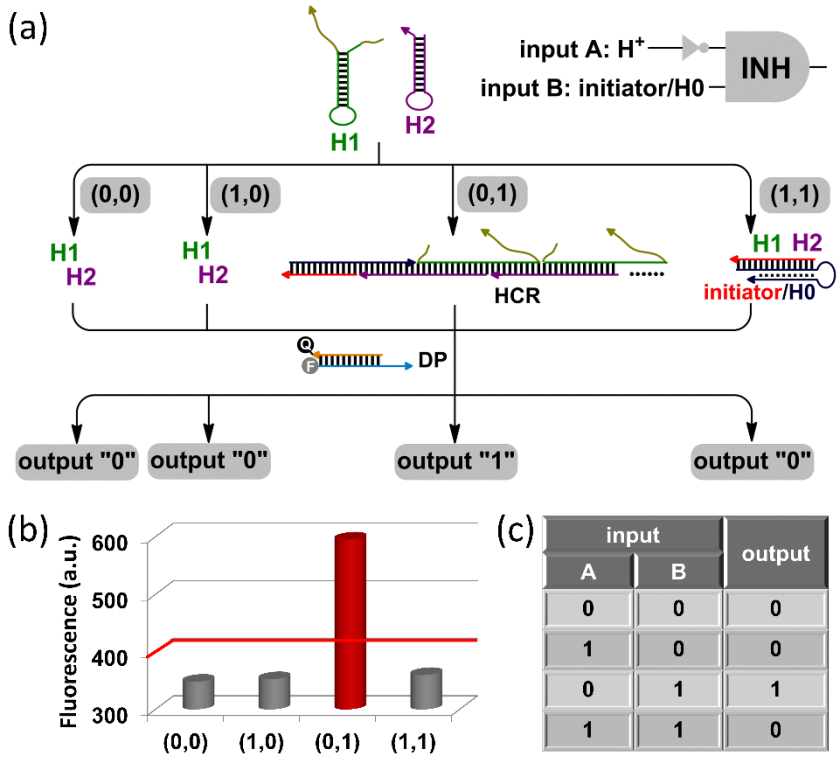

Fig. S9 "INH" logic gate. (a) Schematics, (b) column diagram of the fluorescence intensities, and (c) truth table of "INHIBIT" gate with different input combinations. The red solid line in (b) shows the threshold value as 400 a.u.

7. Cytotoxicity Study

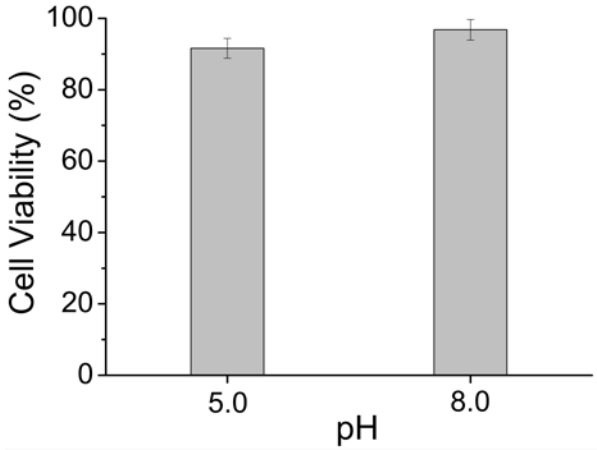

Fig. S10 CCK-8 assay to assess the cytotoxicity of MCF-7 cells treated with pH-programmable HCR at pH 5.0 and 8.0, respectively.

References

S1. Y. Le, N. Xie, Y. Yang, X. Yang, Q. Zhou, B. Yin, J. Huang and K. Wang, *Chem. Commun.*, 2016, **52**, 7818.