Electronic Supplementary Material (ESI) for Analyst.

A DNAzyme-mediated logic gate system based on Ag(I)-Cysteine

Xun Zhang[1], Qiang Zhang[1]*, Yuan Liu[1], Xiaopeng Wei[1]

[1] School of Computer Science and Technology, Dalian University of Technology, Dalian 116024, China

Correspondence: zhangq@dlut.edu.cn (Q.Z.); Tel: +86 0411 87402106;

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Table S1 DNA Sequences Design and Modifications

The DNA used in the article for the electrophoresis and FRET experiments was ordered from Sangon Biotech, and DNA sequence is shown below:

Oligonucleotides	Sequences (5 '-3 ')
E6-01	AATTTATTTACTCTTCAGCGATGACTGTTTTTTTCAGTCCACCCATGTTTCTAC
E6-S-01	TTTTTTTTTTTTGTAGAAT/rA/GGAAGAGTATATTTTTTTTT
Ag10c-01	CGCCATCTTTAGGTGATTTCCACGATTATGCGGAAACAGGGCAGCGTATAGTGAG
Ag10c-S-01	TTTTTTCTCACTAT/rA/GGAAGATGGCGTTTT
E6-02	TATACTCTTCAGCGATGACTGTTTTTTTCAGTCCACCCATGTTTCTACCT
E6-S-02	5'BHQ2-AGGTAGAAT/rA/GGAAGAGTATA-3'6-FAM
Ag10c-02	CGCCATCTTTAGGTGATTTCCACGATTATGCGGAAACAGGGCAGCGTATAGTGAG
Ag10c-S-02	5'BHQ2-CTCACTAT/rA/GGAAGATGGCG-3'ROX
E6-01-P	TATACTCTTCAGCGATGACTGTTTTTTTCAGTCCACPCATGTTTCTACCT
E6-S-03	GTAGAAT/rA/GGAAGATGGCG
E6-S-part1	TTTTTTTTTTTTGTAGAATA
E6-S-part2	GGAAGAGTATATTTTTTTTT
E6-S-03-part1	GTAGAATA
E6-S-03-part2	GGAAGATGGCG
Ag10c-S-part1	TTTTTTCTCACTATA
Ag10c-S-part2	GGAAGATGGCGTTTT

Note: the underline portion represents DNAzyme conservative domain, the **P** represent the pyrrole-dC modification.

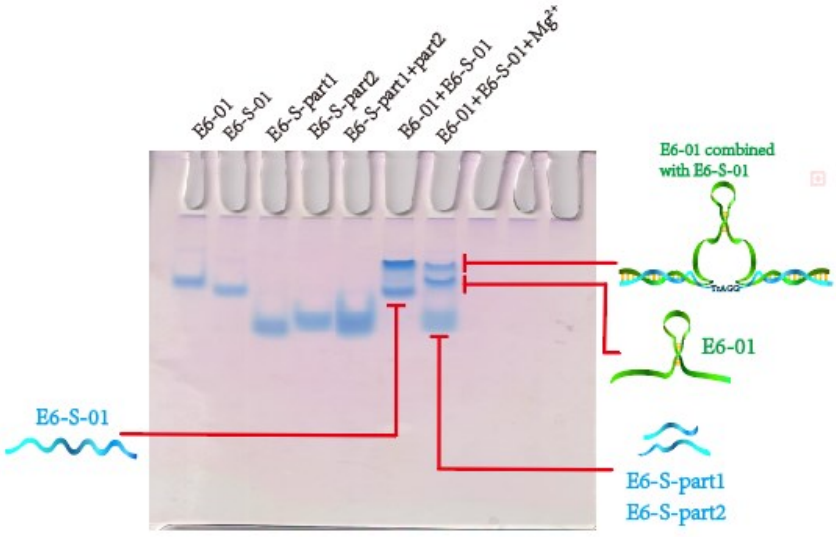
Text S1 Experimental Method and Apparatus

DYY-6D electrophoresis apparatus used in this article was manufactured by Beijing Liuyi Company. In the electrophoresis experiment, the amount of DNAzyme substance was 30 pmol, the corresponding reaction substrate was 80 pmol, and the volume of solution reaction system was 20 μl. All reactant mixed in Mops buffer (50mM MOPS, 200mM $NaNO_3$, NaOH adjust PH to 7.5). First, the corresponding DNAzyme and reaction substrates were added into the reaction system, incubated for 30 min at room temperature, and then metal ions corresponding to each group of experiments were added as input and placed in a constant temperature oscillations chamber to reaction for 6h. 12% of PAGE gel was configured, and electrophoresed at constant voltage 65V for 160min, and dyed by Stains- All solution.

The instrument used in FRET experiment was Tecan Microplate Reader made in Australia, mode Spark. In FRET experiment, the amount of DNAzyme substances was 20 pmol, and corresponding substrate was 100 pmol, the solution of reaction system was 100μl. All reactant dissolved in MOPS buffer (50 mM MOPS, 200 mM $NaNO_3$, NaOH adjust PH to 7.5). First, the corresponding DNAzyme and reaction substrates were added into the reaction system, incubated for 30min at room temperature, and then metal ions corresponding to each group of experiments were added as input and temperature of Microplate Reader was adjusted to 28.5 centigrade recording variation of fluorescence for 2 h.

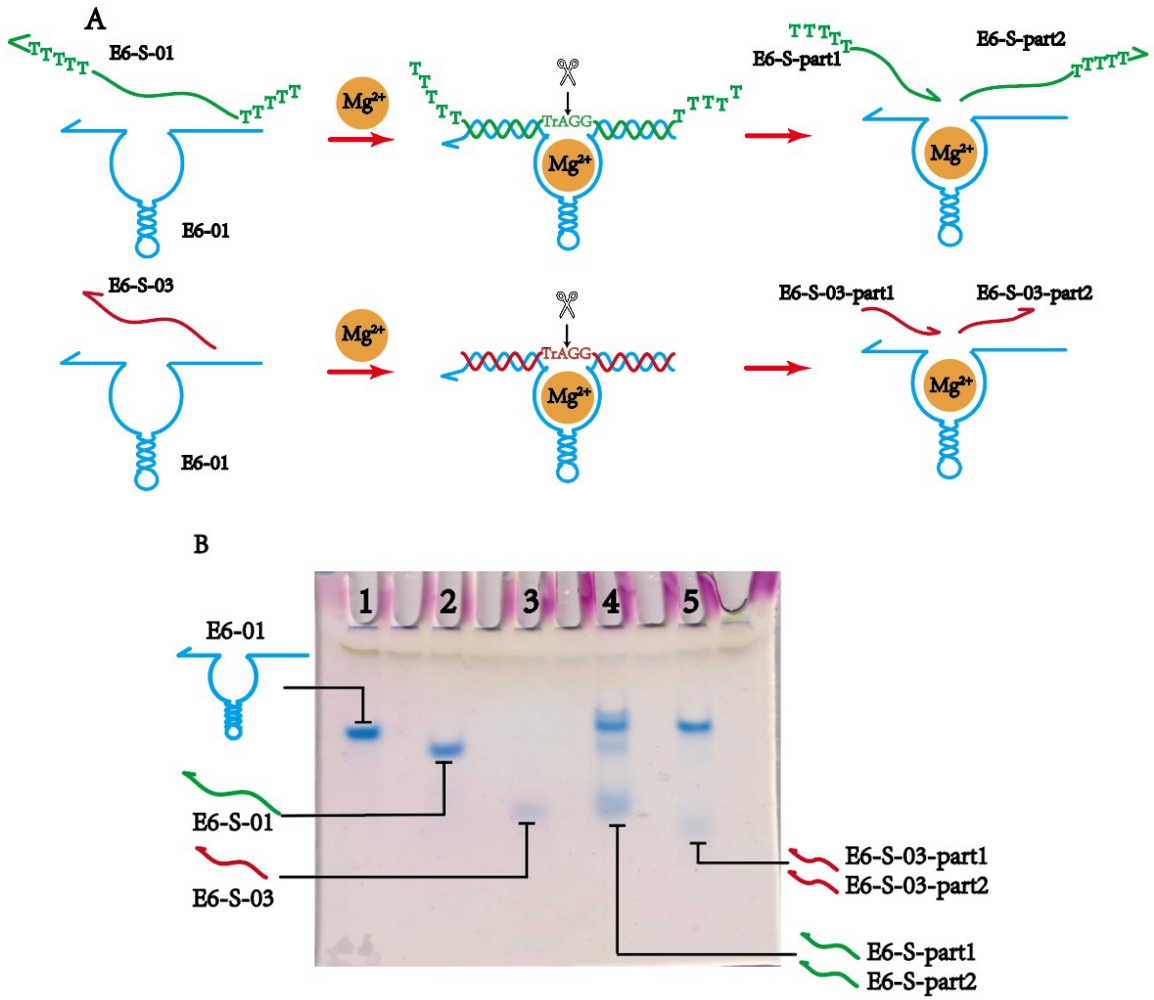
Agarose gel electrophoresis was preformed by Beijing Liuyi DYY-6D electrophoresis analyzer(Liuyi, China, http://www.ly.com.cn/?s=DYY-6D&lang=zh). The fluorescent responses were measured by Tecan Microplate Reader Spark 20M (Tecan, Australia, http://www.lifesci.com.cn/prdview.asp?id=3228).

Figure S1 E6 DNAzyme digestion reaction electrophoresis in refrigerator



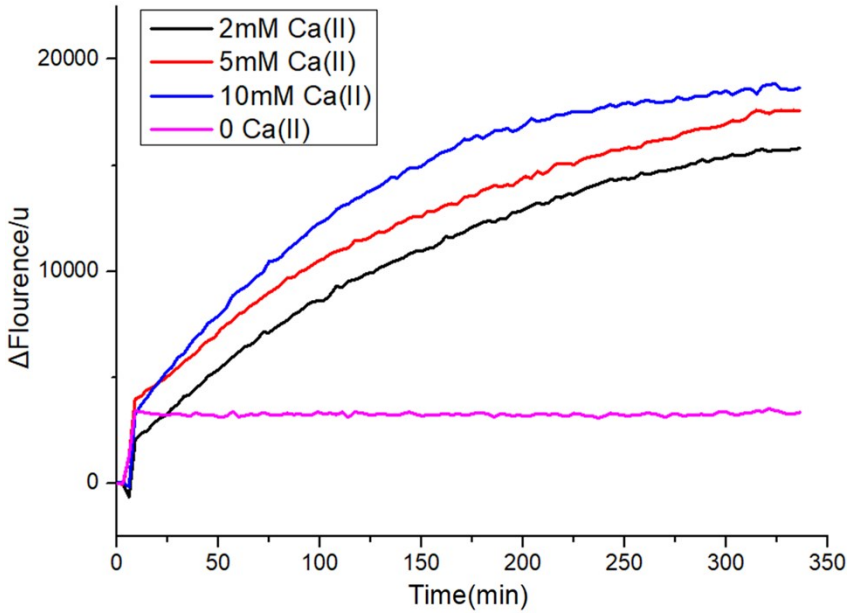
E6 DNAzyme digestion reaction electrophoresis conducted in room temperature can result to the gel picture in Figure1c and Figure2c. Because of that, in process of electrophoresis, the temperature may increase, E6-01 and E6-S-01 would not bond together. If we conduct the electrophoresis in refrigerator under the circumstance of 4 centigrade, gel picture shows above, and the E6-01 and E6-S-01 would bond together.

Figure S2 Electrophoresis effect of different bases number of E6 DNAzyme substrate



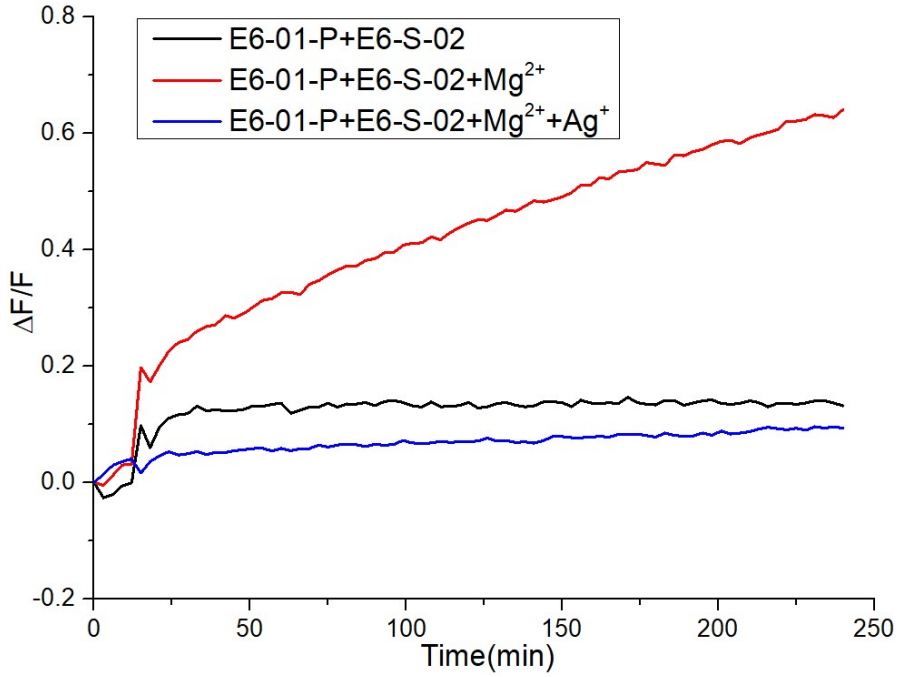
(A). Schematic diagram of two kinds of substrate, E6-S-01 and E6-S-03 with different length. (B). Lane1:E6 DNAzyme E6-01, Lane2: E6 DNAzyme substrate with thymine decorated in both side of it (E6-S-01). Lane3: E6 DNAzyme substrate without thymine decorated in both side of it (E6-S-03). Lane4: E6-S-01 combined with E6-01 (E6-S-01:E6-01=4:1.5) in the Mops buffer with 2 mM Mg^{2+} circumstance. After 2 h reaction, the production of substrate shows clearly in gel. Lane 5: E6-S-03 combined with E6-01(E6-S-03:E6-01=4:1.5) in the Mops buffer with 2 mM Mg^{2+} circumstance. After 2 h reaction, the production of substrate shows dim in the gel. So the substrate of E6 DNAzyme with thymine decorated is better than the other one.

Figure S3 Ca^{2+} gradient FRET experiment for E6 DNAzyme



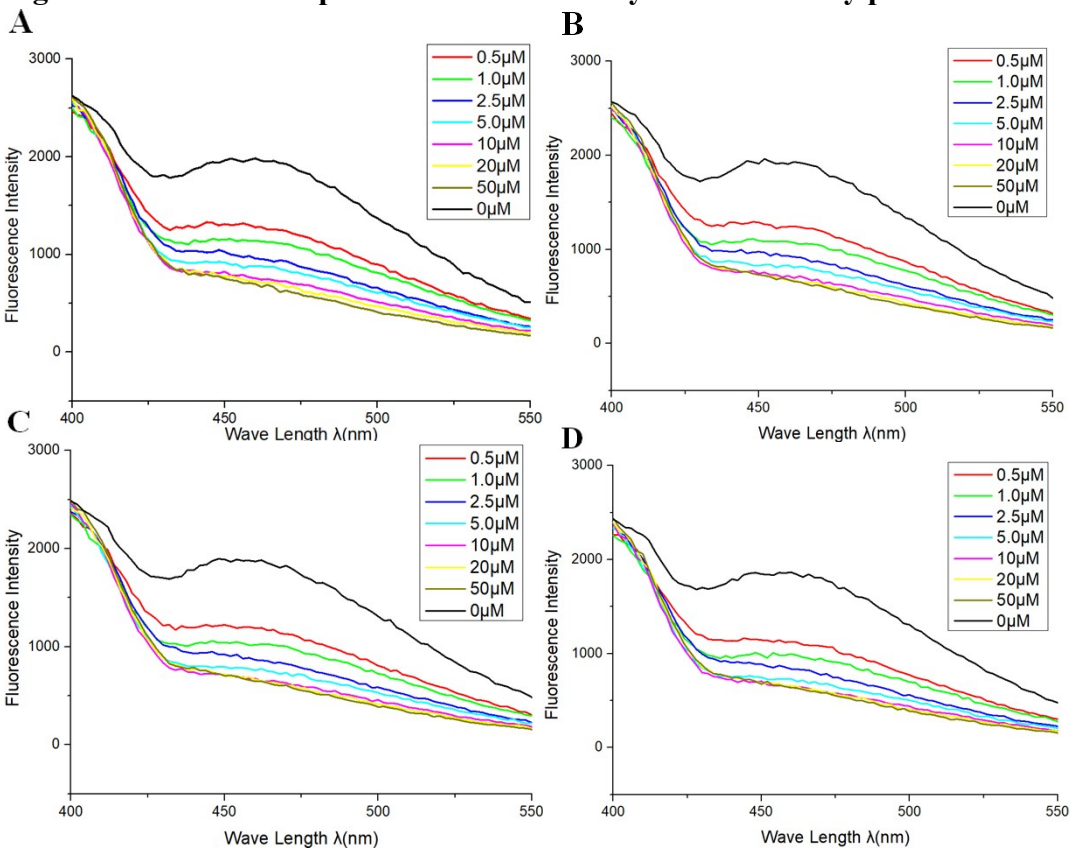
Ca^{2+} can also activate the E6 DNAzyme, with the concentration increasing of Ca^{2+}, the fluorescence increasing rapidly, but the catalytic rate is lower than Mg^{2+}. The four experiment groups include 30 pmol E6-01 and 80 pmol E6-S-01 added into 100 μl Mops buffer with different Ca^{2+} concentration shows in the figure.

Figure S4 Digestion effect of E6 DNAzyme modified with PdC



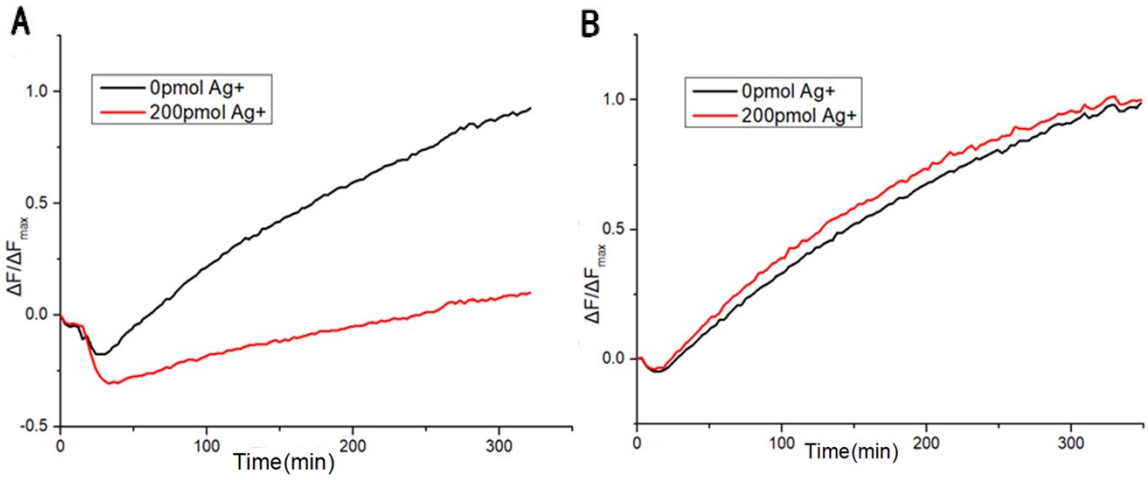
40 pmol PdC modified E6 DNAzyme and 100 pmol E6-S-02 has been added into the buffer and incubated for 15 min. Then added 200 μmol Mg^{2+} to group2 and added 200 pmol Ag^{+} to group3, monitoring fluoresce variation in these three groups. We can see that E6 DNAzyme modified by PdC also has the property to digest E6-S-02 substrate which is similar to E6 itself and the PdC modified operation did not infect the inherent character of E6.

Figure S5 the FRET experiment for E6 DNAzyme Modified by pdC



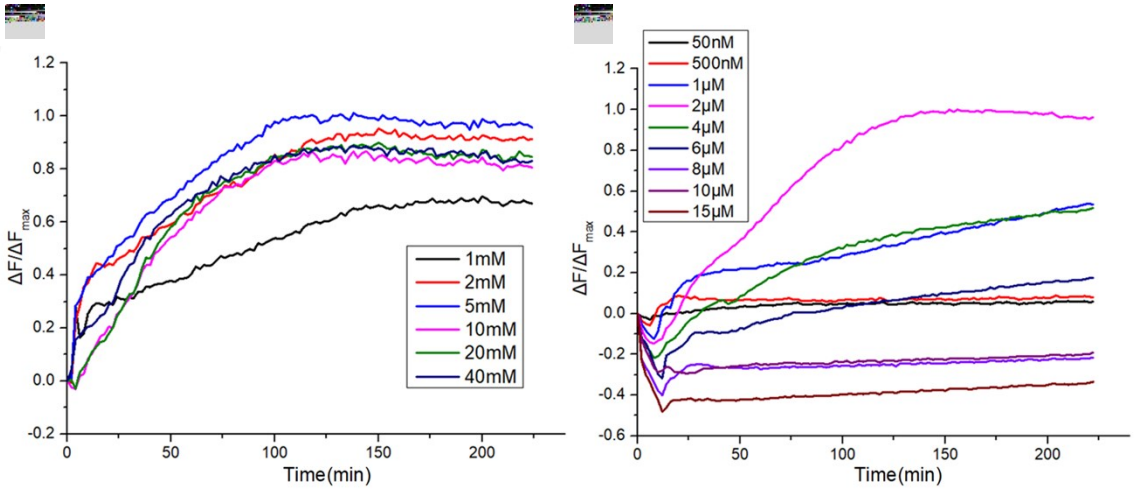
(A). Fluorescence variation after gradient concentration Ag^{+} is added into E6 DNAzyme modified by PdC for 200 s. (B). for 400 s. (C). for 800 s. (D). for 1200s.

Figure S6 the influence of Ca^{2+} and Ag^{+} to E6 DNAzyme



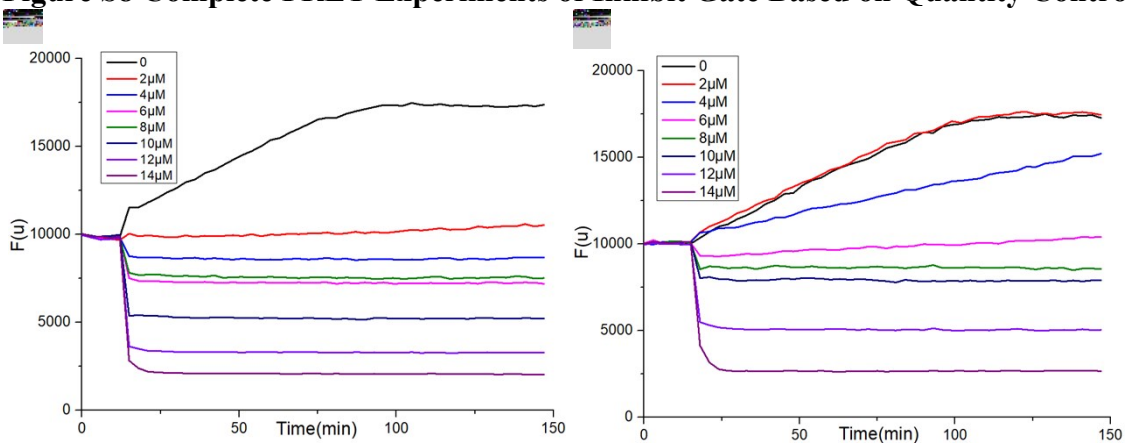
(A). 40 pmol E6-01 and 100 pmol E6-S-02 were mixed in 100 μl Mops buffer with 5 mM Ca^{2+} circumstance, black line represent the reaction with no Ag^{+} in the buffer, which have rapid fluorescence enhancement, but if 200 pmol Ag^{+} existed in the buffer, the fluorescence intensity would be suppressed which is shown by the red line. (B). With the same condition, if there was 500 pmol Cys existed in the buffer, however, fluorescence intensity would have a great enhancement also.

Figure S7 Complete FRET Experiments of Yes Gate Part



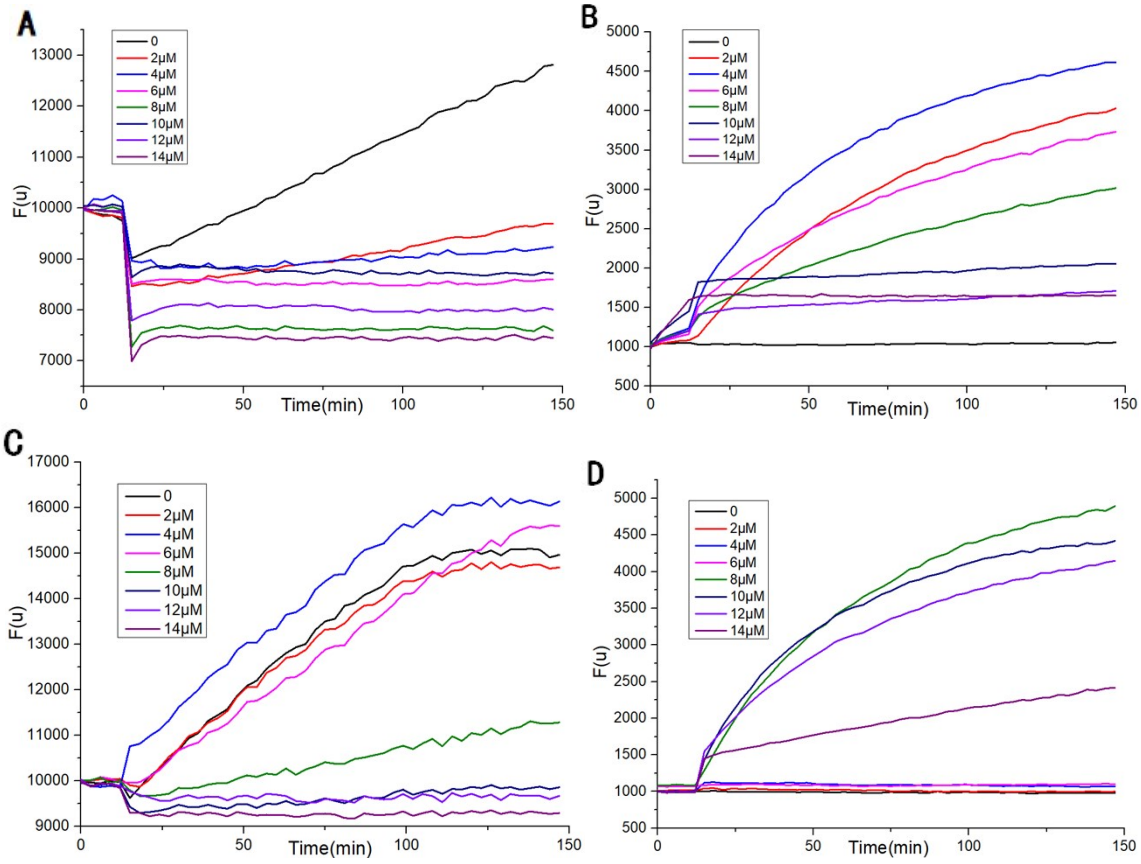
(A). 40 pmol E6-01 and 100 pmol E6-S-02 were mixed in 100 μl Mops buffer with circumstance of gradient concentration of Mg^{2+}, the variation of fluorescence intensity is shown in the graph. (B). 40 pmol Ag10c-01 and 100 pmol Ag10c-S-02 were mixed in 100 μl Mops buffer with circumstance of gradient concentration of Ag^{+}, the variation of fluorescence intensity is shown in the graph.

Figure S8 Complete FRET Experiments of Inhibit Gate Based on Quantity Control



(A). 40 pmol E6-02 and 100 pmol E6-S-02 were mixed in 100 μl Mops buffer. Ag^+ concentration gradient was tested for E6 activity without Cys by FRET. (B). At the same condition, Ag^+ concentration gradient was tested for E6 activity with 500 pmol Cys by FRET

Figure S9 Complete FRET Experiments of switchable logic circuit



(A). Conjugate control system based on the E6 enzyme and the Ag10c enzyme. Every experimental group contained 40 pmol E6-02, 40 pmol Ag10c-02, 100 pmol E6-S-02 and 100 pmol Ag10c-S-02. Mg^{2+} and Ag^{+} were added into the solution, reaction system volume was 100 μl at last. FAM fluorescence information was recorded. (B). ROX fluorescence information was recorded with the same condition. (C). Conjugate control system based on the E6 enzyme and the Ag10c enzyme. Every experimental group contained 40 pmol E6-02, 40 pmol Ag10c-02, 100 pmol E6-S-02, 100 pmol Ag10c-S-02 and 500 pmol Cys. Mg^{2+} and Ag^{+} were added into the solution, reaction system volume was 100 μl at last. FAM fluorescence information was recorded. (D). ROX fluorescence information was recorded with the same condition.