Electronic Supplementary Information

Colorimetric logic gates for small molecules using split/integrated aptamers and unmodified gold nanoparticles

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Experimental Section

Materials. Hydrogen tetrachloroaurate (□) trihydrate ($HAuCl_4 \cdot 3H_2O$) was bought from Alfa Aesar. Sodium citrate was obtained from Aladdin Inc. (Shanghai, China). Tris Base was purchased from Calbiochem (Germany). Adenosine was purchased from Sigma. Cocaine hydrochloride was obtained from National Institute for the Control of Pharmaceutical and Biological Products (Changchun, China). Uridine was bought from Bio Basic Inc. (Markham, Canada). Sodium chloride and hydrochloric acid were obtained from Beijing Chemical Reagent Company (Beijing, China). The solutions of small molecules were prepared in Tris buffer (25 mM Tris-HCl, 300 mM NaCl, pH 8.2) and stored at 4 °C. Ultra-pure water (18.2 MΩ•cm) was used throughout the experiment.

DNA oligonucleotides were synthesized in Sangon Biotech Co., Ltd. (Shanghai, China). Their sequences are as follows:

1) OR1: 5'-*AGACAAGGAAAA***ACCTGGGGGAGTAT**-3',
2) OR2 (AND2): 5'-**TGCGGAGGAAGGT***TCCTTCAATGAAGTGGGTCG*-3',
3) AND1a: 5'-**ACCTGGGGGAGTAT**-3',
4) AND1b: 5'-*AGACAAGGAAAA*-3',
5) A33: 5'-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA-3'.

The italic letters in OR1 represent the sequence of one split cocaine-binding aptamer fragment, and the italic letters in OR2 represent the sequence of the other

split cocaine-binding aptamer fragment. The bold letters in OR1 correspond to the sequence of one split adenosine-binding aptamer fragment, and the bold letters in OR2 correspond to the sequence of the other split adenosine-binding aptamer fragment. AND1a and AND1b are partial sequences in OR1. A33 is a control oligonucleotide of OR2, which has the same length as OR2 with no self-folded structures. DNA solution was obtained by dissolving oligonucleotides in Tris buffer (25 mM Tris-HCl, 300 mM NaCl, pH 8.2) and was stored at 4 °C before use. The concentrations of oligonucleotides were determined according to the UV absorbance at 260 nm and the extinction coefficients were calculated by sum of extinction coefficients of individual bases in each sequence: ε(dA) = 15400 M^{-1} cm^{-1}, ε(dG) = 11500 M^{-1} cm^{-1}, ε(dC) = 7400 M^{-1} cm^{-1} and ε(dT) = 8700 M^{-1} cm^{-1}. *Note:* As reported previously by Lu's group,[1,2] the process of checking the exact concentration of DNA strands is very important, since a small amount of unassembled single-stranded DNA recognizing specifically to inputs could also stabilize AuNPs and thus influence the response of logic gates.

Instrumentation. UV-vis absorption spectra were recorded on a Cary 50 Scan UV-Visible spectrophotometer (Varian, USA) at room temperature. Transmission electron microscopy (TEM) measurements were made on a Hitachi H-800 transmission electron microscope operated at an accelerating voltage of 200 kV. The samples for TEM characterization were prepared by adding a drop of colloidal solution on a carbon-coated copper grid and allowed to dry at room temperature.

Synthesis of AuNPs. Gold nanoparticles (13 nm diameter) were synthesized by reduction of $HAuCl_4$ by sodium citrate.[3] Briefly, a solution of sodium citrate (10 mL, 38.8 mM) was rapidly added to a vigorously stirred boiling aqueous solution of $HAuCl_4$ (100 mL, 1 mM). After a continuous boiling for 10 min, the mixed solution was stirred for additional 15 min. The solution was then cooled to room temperature and stored in a refrigerator at 4 °C before use.

Logic gates preparation. For the "OR" logic gate, 4 μL of 35 μM OR1 solution was mixed with 4 μL of 35 μM OR2 solution, then 2 μL Tris buffer [input = (0, 0)], 1 mM adenosine [input = (1, 0)], 1 mM cocaine [input = (0, 1)], or 1 mM adenosine and

cocaine [input = (1, 1)] was added. The small volume of solution was pipetted in and out for several times to mix DNA and small molecules thoroughly, and the mixture was allowed to react for 5 min. 2 μL of the reaction mixture was added to 98 μL AuNPs, and after a gentle shake the solution was kept for another 5 min. Subsequently 100 μL of 194 mM NaCl was added, followed by either visual observation or UV-vis characterization.

For the "AND" logic gate, 2 μL of 70 μM AND1a solution, 2 μL of 70 μM AND1b solution and 4 μL of 35 μM AND2 solution were mixed together, and then 2 μL solution containing different inputs was added. Other procedures were done as the same as in the "OR" logic gate preparation.

To test the role of the short split oligonucleotides in protecting AuNPs in the "AND" logic gate, 2 μL Tris buffer was used instead of 2 μL of 70 μM AND1b solution to respond to the adenosine input (1, 0); 2 μL Tris buffer was used instead of 2 μL of 70 μM AND1a solution to respond to the cocaine input (0, 1).

To test the selectivity of the "OR" logic gate, 2 μL of 1 mM adenosine, cocaine, uridine and ecgonine were used as inputs, respectively. To test the selectivity of the "AND" logic gate, 2 μL of 1 mM adenosine and cocaine, adenosine and ecgonine, uridine and cocaine, and uridine and ecgonine were used as inputs, respectively.

To test the sequence specificity to targets, OR2 was replaced by A33 to prepare the "OR" logic gate to respond to the adenosine input (1, 0) and cocaine input (0, 1); AND2 was replaced by A33 to prepare the "AND" logic gate to respond to the both adenosine and cocaine inputs (1, 1).

Preparations and tests were performed in 0.5 mL microcentrifuge tubes at room temperature. Photographs were taken after each test had been done. Solution was diluted to a volume of 300 μL with water before UV-vis characterization. The reported values represented the results obtained from three independent parallel tests.

Additional Figures.

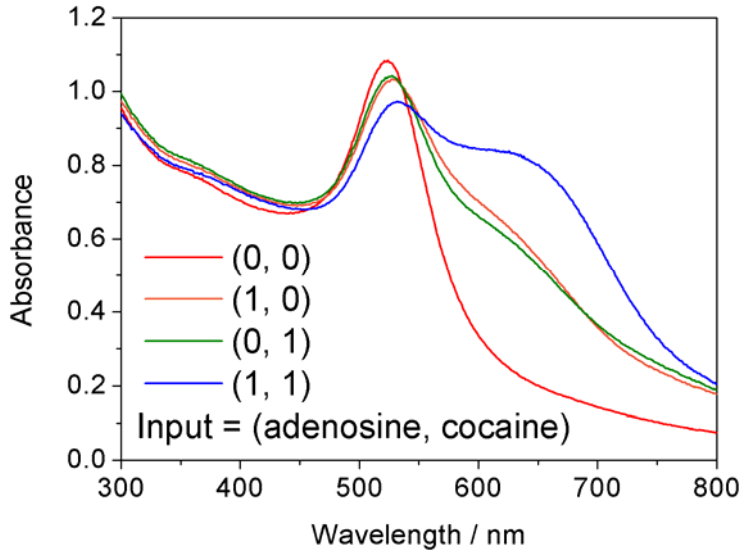

Fig. S1 UV-vis absorption spectra of the "OR" logic gate upon treatment with no adenosine and cocaine input (0, 0), adenosine input (1, 0), cocaine input (0, 1) and both adenosine and cocaine inputs (1, 1).

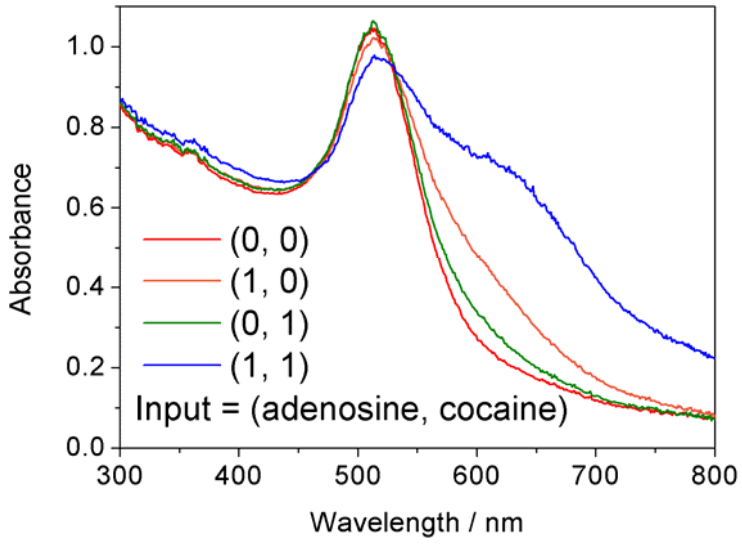

Fig. S2 UV-vis absorption spectra of the "AND" logic gate upon treatment with no adenosine and cocaine input (0, 0), adenosine input (1, 0), cocaine input (0, 1) and both adenosine and cocaine inputs (1, 1).

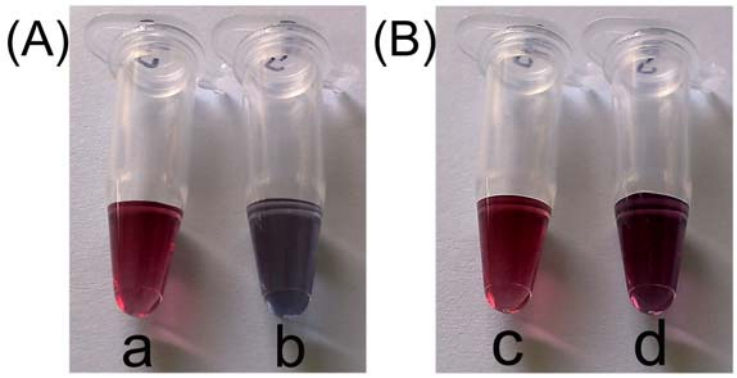

Fig. S3 Visual observations of (A) the case of (a) employing and (b) omitting AND1b oligonucleotides upon treatment with adenosine input (1, 0), and (B) the case of (c) employing and (d) omitting AND1a oligonucleotides upon treatment with cocaine input (0, 1) in the "AND" logic gate.

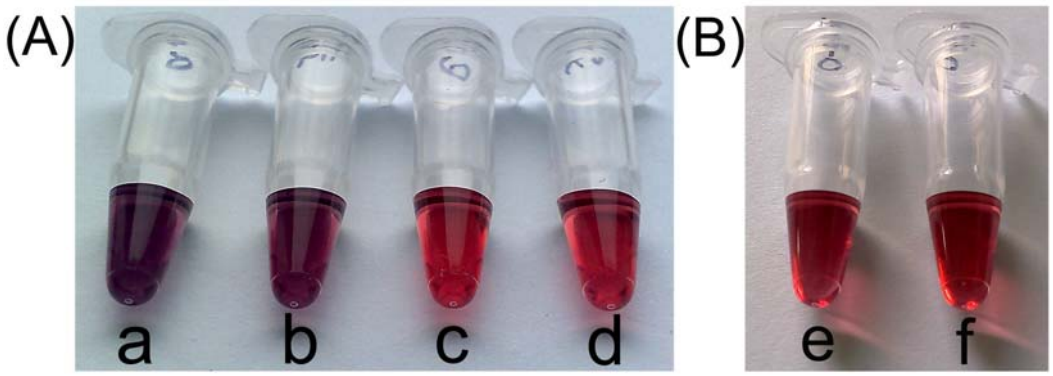

Fig. S4 Visual observations of (A) the "OR" logic gate upon treatment with (a) adenosine, (b) cocaine, (c) uridine and (d) ecgonine inputs, and (B) the case of substituting A33 for OR2 oligonucleotides upon treatment with (e) adenosine and (f) cocaine inputs in the "OR" logic gate.

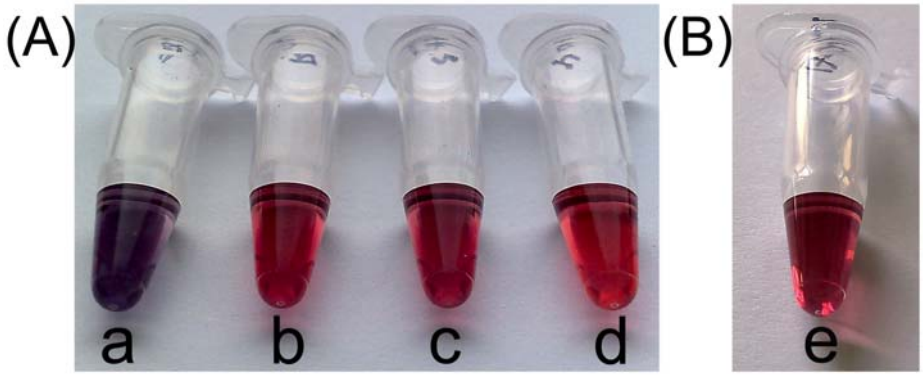

Fig. S5 Visual observations of (A) the "AND" logic gate upon treatment with (a) both adenosine and cocaine, (b) adenosine and ecgonine, (c) uridine and cocaine, and (d) uridine and ecgonine inputs, and (B) the case of substituting A33 for AND2 oligonucleotides upon treatment with (e) both adenosine and cocaine inputs in the "AND" logic gate.

Additional Notes.

In the logic gates preparation, the input concentrations for adenosine, cocaine, and both adenosine and cocaine are all 1 mM (original concentration of the 2 μL sample solution). Besides, we experimentally determined the sensitivity of the logic gates. The directly measured (not extrapolated) detection limits of the "OR" logic gate are 0.2 mM of adenosine input, 0.2 mM of cocaine input, and 0.1 mM of both adenosine and cocaine inputs, respectively, with the concentrations corresponding to the original concentrations of the sample solution; the directly measured detection limits of the "AND" logic gate are 0.4 mM of adenosine input, 0.6 mM of cocaine input, and 0.1 mM of both adenosine and cocaine inputs, respectively, with the concentrations corresponding to the original concentrations of the sample solution. Apparently, the "AND" logic gate has a lower sensitivity than the "OR" logic gate for adenosine or cocaine input, which results from the existence of the split short oligonucleotides with no recognition ability to adenosine (AND1b) or cocaine (AND1a).

References:

1 Z. D. Wang, J. H. Lee and Y. Lu, *Adv. Mater.*, 2008, **20**, 3263.

2 J. H. Lee, Z. D. Wang, J. W. Liu and Y. Lu, *J. Am. Chem. Soc.*, 2008, **130**, 14217.

3 K. C. Grabar, R. G. Freeman, M. B. Hommer and M. J. Natan, *Anal. Chem.*, 1995, **67**, 735.