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Colorimetric Logic Gates based on Aptamer-Crosslinked Hydrogels

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SUPPORTING INFORMATION

Reagents. DNA synthesis reagents were purchased from Glen Research (Sterling, VA, USA). The oligonucleotides and acrydite-modified oligonucleotides were synthesized using an ABI 3400 DNA/RNA synthesizer (sequences see Table S1). Gold nanoparticles of 13 nm were prepared by the citrate reduction of $HAuCl_4$ according to a reported literature method.[1] Bovine serum albumin (BSA), adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP), uridine triphosphate (UTP) and thymidine triphosphate (TTP), cocaine, benzoylecgonine (BE) and ecgonine methyl ester (EME) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All solutions were prepared with ultrapure water (18.2 MΩ·cm) from a Millipore Milli-Q system (Bedford, MA).

Table S1. Sequences of DNAs used in this work.

Name	Sequence*
S1	**5'- acrydite-AAA*ACTCATCTGTGAAAGAACCTGGG*GGAGTATTGCGGAGGAAGGT-3'**
S2	**5'- acrydite-AAA*CCCAGGTTCTTCTAGAGGGAGAC*-3'**
L1	**5'- GGGAGACAAGGATAAATCCTTCAATGAAGTGG*GTCTCCCTCTA*C*TCACAGATGAGT*-3'**
S3	**5'- acrydite-AAA*CCCAGGTTCTCT*-3'**
S4	**5'- acrydite-AAA*TGAGAGGGAGAC*-3'**
L2	**5'-GGGAGACAAGGATAAATCCTTCAATGAAGTGG*GTCTCCCTCTCAAGAGAACCTGGG*G GAGTATTGCGGAGGAAGGT-3'**

* The ATP aptamer fragment and cocaine aptamer fragment are represented in underlined portions. The complementary sequences are represented in italic portions in the same color.

Instrumentation. DNA synthesis was carried out with the ABI 3400 DNA/RNA synthesizer (Applied Bio-systems). DNA purification was performed with a ProStar HPLC (Varian) equipped with a C18 column (Econosil, 5U, 250 × 4.6 mm) from Alltech Associates. The eluent was 100mM triethylamine-acetic acid buffer (TEAA, pH 7.5) and acetonitrile. A Cary Bio 100 UV/vis spectrometer (Varian, Walnut Creek, CA, USA) was employed for DNA quantitation and UV-Vis measurements.

Synthesis of acrylic phosphoramidite. The acrylic phosphoramidite was synthesized in our lab by two steps according to the established protocol.[2] First, 6-amino-1-hexanol (9.32 g, 0.08 mol) and TEA (16.16 g, 0.16 mol) in 100 mL dichloromethane was cooled to 0°C. Methacryloyl chloride (10 g, 0.0957 mol) was added slowly, and the reaction was stirred at 0°C for 2 hr, after which water (100 mL) was added to terminate the reaction. Then the organic layer was washed with 5% HCl and dried by evaporation of all solvent to obtain the crude 6-hydroxyhexyl methacrylamide. Second, 6-hydroxyhexyl methacrylamide (2 g, 10.8 mmol) was dissolved in anhydrous CH_3CN (40 mL) at 0°C and N, N' diisopropylethylamine (DIPEA) (3.9 g, 30.0 mmol) was added over 15 min. Then 2-cyanoethyl diisopropyl chlorophosphoramidite (2.9 mL, 13 mmol) was added dropwise with stirring at 0 °C for 5 hr. After removing the solvent, the residue was dissolved in ethyl acetate, and the organic phase was washed with $NaHCO_3$ solution and NaCl solution and dried over anhydrous magnesium sulfate. The solvent was evaporated, and the residue was purified by column chromatography (ethyl acetate/hexane/triethylamine 40:60:3) and dried to afford the title compound (3.33 g, 8.64 mmol, 80%) as an colorless oil. ^{1}H NMR ($CDCl_3$): δ 5.92 (br, 1H), 5.63 (m, 1H), 5.27 (m. 1H), 3.86-3.72 (m, 2H), 3.66-3.49 (m, 4H), 3.30-3.23 (m, 2H), 2.61 (t, 2H), 1.92 (m, 3H), 1.58-1.50 (m, 4H) 1.37-1.32 (m, 4H) 1.17-1.13 (m, 12H). 13C NMR ($CDCl_3$): δ 168.6, 140.4, 119.3, 118.0, 63.8, 63.6, 58.6, 58.3, 43.2, 43.1, 39.8, 31.3, 29.7, 26.8, 25.8, 24.9, 24.8, 24.7, 19.0. 31P ($CDCl_3$): δ 148.

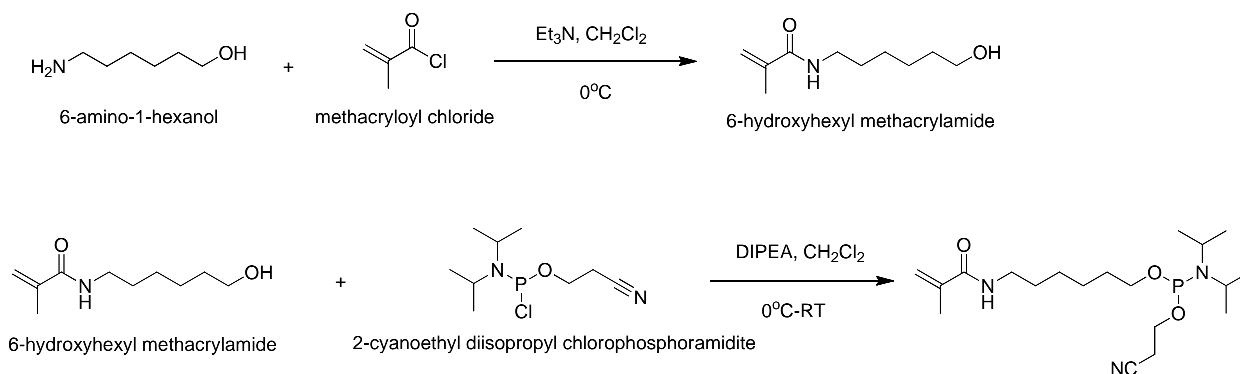

Figure S1. Synthesis of acrylic phosphoramidite.

DNA synthesis and purification. The DNA sequences were synthesized on the ABI 3400 DNA synthesizer according to our reported method. Briefly, the DNA sequences were first uploaded into a DNA synthesizer online. The synthesis protocol was designed according to the requirements specified by the reagents' manufacturers. After synthesis, the DNA product was deprotected and cleaved from CPG by incubating with ammonium hydroxide for 20 min in a 65°C water bath. Then

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the cleaved supernatant DNA product was transferred to a 15 mL centrifuge tube and mixed with 250 μL 3.0 M NaCl and 6.25 mL ethanol. Subsequently, the tube was placed in a freezer at -20°C for 30 min. Then, the DNA product was centrifuged at 4000 rpm at 4°C for 30 min. Following removal of the supernatant, the precipitated DNA product was dissolved in 500 μL 0.2 M triethylammmonium acetate for HPLC purification. The HPLC purification was performed on a Varian Prostar HPLC machine with a cleaned C18 column. The last peak in the chromatogram in Figure S1 was collected as the product. The collected DNA product was vacuum-dried and detritylated by incubating in 200 μL 80% acetic acid for 20 min. Then the detritylated DNA product was mixed with 20 μL 3.0 M NaCl and 400 μL ethanol for ethanol precipitation, and frozen at -20°C for 30 min. Finally, the supernatant reagent was removed by centrifugation at 14,000 rpm for 5 minutes. The purified DNA product was dissolved in ultrapure water and quantified by determining the UV absorption at 260 nm.

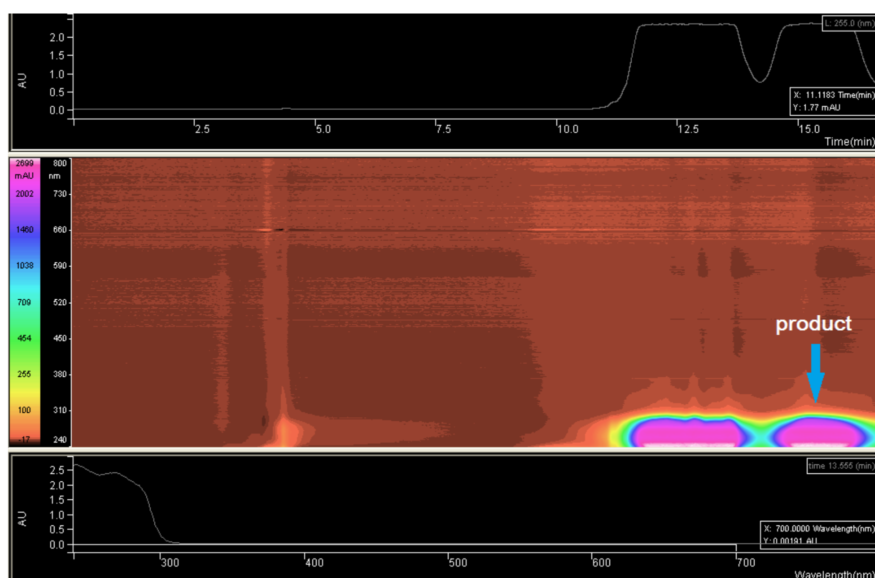

Figure S2. An original HPLC chromatogram with two elution bands. The second peak was collected as the product.

Hydrogel formation. The polymer strand/DNA solution was prepared by mixing 20 μL 4 mM acrydite-modified DNA, 40 μL 20% acrylamide, 100 μL 20 mM Tris-HCl (pH 8.0)/400 mM NaCl buffer, 40 μL H_2O. Then the solution was kept in a desiccator equipped with vacuum pump for 5 min to remove the air. Subsequently, 5.6 μL freshly prepared initiator consisting of 10% (w/v) ammonium persulfate and 10% (v/v) TEMED was added, and the mixture was put in the desiccator

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for another 20 min to polymerize. Finally, the mixture became a hydrogel by mixing polymer strand DNAs with corresponding linker DNA for 10 min at room temperature.

Logic gate fabrication. A concentrated gold nanoparticle (GNPs) solution was incubated with BSA solution to obtain the BSA-modified GNPs (BSA-GNPs) to avoid aggregation under high ionic strength conditions. In the "AND" logic gate experiment, polymer strand S1 (P-S1) and polymer strand S2 (P-S2) were mixed with BSA-GNPs (120 nM). After introduction of L1, a homogenous red hydrogel formed with GNPs trapped inside and distributed well. In the "OR" logic gate experiment, polymer strand S3 (P-S3) and polymer strand S4 (P-S4) were mixed with BSA-GNPs (120 nM). Then L2 was added to the mixture of P-S3 and P-S4 to form a homogenous red hydrogel. After washing with buffer solution (25 mM Tris-HCl (pH 8.2)/300 mM NaCl) to remove surface-bound BSA-GNPs, approximately 20 μL of the resulting BSA-GNPs-trapped hydrogel (for the AND gate or the OR gate) were placed on the bottom of a tube containing 100 μL Tris buffer (25 mM Tris-HCl, 300 mM NaCl, pH 8.2). Then the tested stimulus was added to these tubes containing the fabricated hydrogels. A Cary Bio-300 UV spectrometer was employed to monitor BSA-GNP release process in the presence of different chemical stimuli.

Study of the kinetic behavior of dissolution of aptamer-crosslinked hydrogel. We studied the kinetic behavior of dissolution of aptamer-crosslinked hydrogels by quantitatively monitoring BSA-GNP absorption at 520 nm as a function of time due to the release of BSA-GNPs to the buffer solution. The aptamer-crosslinked hydrogel was prepared with encapsulated BSA-GNPs and placed at the bottom of a quartz microcell with a buffer solution on top. The crosslinked hydrogel was monitored for 36 min after the addition of target in the different logic gate designs. As shown in Figure S3, no increase in absorbance at 520 nm due to the tight trapping of the BSA-GNPs in the gel matrix was detected when one target was added in AND gate design. After the addition of both targets, an increase of absorbance in the solution could be observed within 20 min, which indicated the release of the trapped BSA-GNPs and dissolution of the hydrogel triggered by the specific targets.

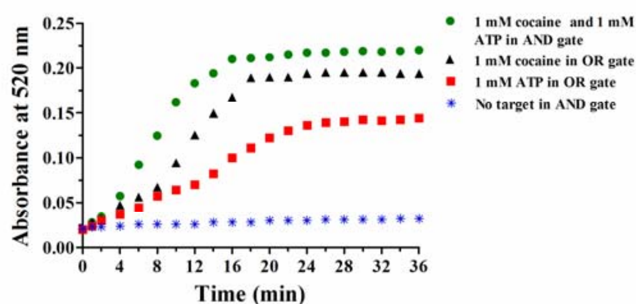

Figure S3. Kinetics of the BSA-GNP release after no target was added in AND gate design, and after the addition of 1 mM cocaine and 1 mM ATP in AND gate design, 1 mM ATP in OR gate design, and 1 mM cocaine in OR gate design, respectively.

Sensitivity of cocaine and ATP detection. The aptamer-crosslinked hydrogel response to cocaine and ATP in OR gate was investigated respectively. A series of OR logical gates were fabricated following the above description. Then, different concentrations of cocaine or ATP were introduced into the upper solution. A Cary Bio-300 UV spectrometer was employed to monitor the upper solution. As shown in Figure S4, the detection limits of cocaine and ATP in our system were 20 μM and 50 μM, respectively.

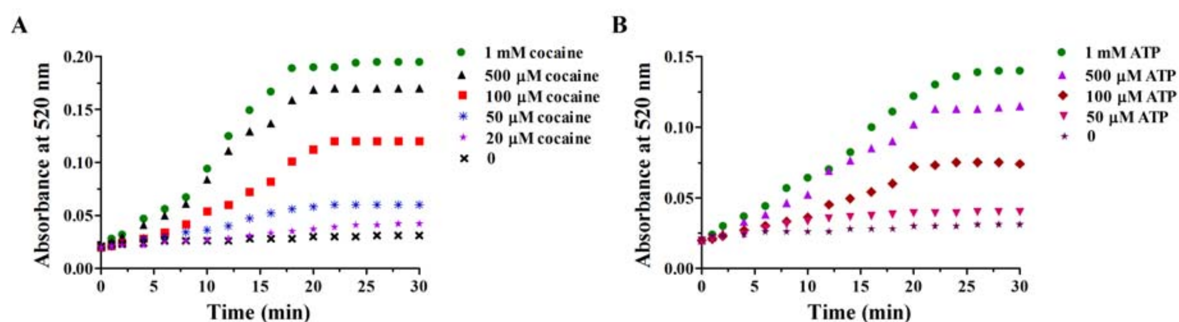

Figure S4. Kinetics of gold nanoparticle release for various concentrations of targets in OR gate design.

References

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

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