Supplementary Data

Highly Specific Triple-Fragment Aptamer for Optical Detection of Cocaine

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Materials and Methods

Materials. Trisodium citrate dehydrate and hydrogen tetrachloroaurate trihydrate (HAuCl4·3H2O) were purchased from Sigma-Aldrich and used as received. Oligonucleotides listed in Table 1 were synthesized and purified by Sangon Biotech Co., Ltd. (Shanghai, China). Cocaine, ecogonine methyl ester (EME) and benzoyl ecgonine (BE) were bought from State Food and Drug Administration (Beijing, China). Buffer solutions were all prepared from reagent grade chemicals with Millipore ultrapure H_2O.

Isothermal Titration Calorimetry. Isothermal titration calorimetry (ITC) was performed using a MicroCal ITC 200 instrument, and the data were analyzed using the packaged Origin software and fit to a one-site binding model. Samples were degassed before use with the MicroCal Thermo Vac unit. All experiments were corrected for the heat of dilution of the titrant. Cocaine and aptamer solutions were prepared in a buffer of 25 mM Tris (pH 8.0), 150 mM NaCl. All aptamer samples were heated in a boiling water bath for 5 min and cooled on ice prior to use in a binding experiment. When the monolithic aptamer (MA) or the double-fragment aptamer (DFA) was tested, binding experiments were typically performed with aptamer solutions of 20 μM using cocaine concentrations of 1 mM at 25 ℃ or 37℃. The process of binding experiments includes 20 successive 2 μL injections of cocaine sample every 120 s to a final molar ratio of 10:1 (cocaine : aptamer). When the triple-fragment aptamer (TFA) was measured, binding experiments were typically performed with aptamer solutions of 200 μM using cocaine concentrations of 10 mM at 4 ℃. The process of binding experiments includes 19 successive 2 μL injections of cocaine sample every 120 s with a first injection of 1 μL to a final molar ratio of 20:1 (cocaine : aptamer). Three different NaCl concentrations were used for ITC measurements of TFA-cocaine binding: 0 mM, 150 mM, and 500 mM.

Preparation of 13 nm unmodified AuNPs. A solution of 13 nm AuNPs was prepared by following previously published protocols.[31, 32] Briefly, an aqueous solution of $HAuCl_4$ (1 mM, 250 mL) was heated and refluxed for 10 min. A solution of trisodium citrate (38.8 mM, 25 mL) was quickly added to the refluxed $HAuCl_4$ solution, resulting in rapid color change from pale yellow to deep red. After continuous reflux for an additional 15 min, the solution was slowly cooled to room temperature and filtered through a 0.22 μm cellulose nitrate filter. The solution was stored at 4 °C until needed. The quality of the particles was monitored using UV/Vis absorption spectroscopy and transmission electron microscopy (TEM). The size of the particles was 13 nm and the concentration of the solution was 12±1 nM.

Cocaine detection using TFA. Probe 3 (1 μL, 100 μM), probe 4 (1 μL, 100 μM) and probe 5 (1 μL, 100 μM) were mixed with 2 × cocaine binding buffer (50 mM Tris-HCl + 0.3 M NaCl, pH 8.0, 3 μL). The probe solution (1 μL) was then mixed with a cocaine sample (4.5 μL) prepared in 1×cocaine binding buffer at various concentrations. After a 15 min incubation at 4℃, an AuNP solution (100 μL) was added into 2 μL of the cocaine/aptamer solution. The mixed solution was further diluted with PBS solution (10 mM phosphate sodium + 0.5 M NaCl, pH 7.4, 8 μL) after a 3 min incubation at 4 ℃. UV/Vis absorption measurements were conducted using a V600 UV/Vis Spectrophotometer (JASCO) with a 1 nm resolution and the photos were taken within 5 min. The experiments for testing the selectivity of TFA were conducted under the same conditions except replacing cocaine with its analogues (EME and BE), or replacing probe 5 by probe 5-1, probe 5-2, probe 5-3, or probe 5-4, respectively, or mixing cocaine in equal or ten times more concentrated cocaine analogues BE and EME.

Application. Both urine and serum samples were used to confirm the feasibility of this sensor for analysis of real-world sample. Urine samples were harvested from a member of our laboratory, filtered

through 0.22 μM membranes and directly used in cocaine detections. The serum samples were obtained from the hospital, either directly used or filtered through the NAP-5 column in cocaine detection. Specifically, the urine sample (or serum sample) were diluted 100-fold with 1 X binding buffer containing 100 μM cocaine, and followed by other steps as described above.

Improved stabilization of AuNPs with shorter fragments of an aptamer: An AuNP solution (300 μL) was mixed with TFA (probe 3 (1 μL, 200 μM), probe 4 (1 μL, 200 μM), probe 5 (1 μL, 200 μM)) and 2 × cocaine binding buffer (3 μL)), with DFA (probe 2 (1 μL, 200 μM), probe 3 (1 μL, 200 μM)), 2 × cocaine binding buffer (3 μL) and water (1 μL)), or with MA (probe 1 (1 μL, 200 μM)), 2 × cocaine binding buffer (3 μL) and water (2 μL)), respectively, followed by the further dilution with PBS (10 mM phosphate sodium + 0.6 M NaCl, pH 7.4, 10 μL). The image was taken and the UV/Vis spectra were recorded within five minutes.

ATP detection using TFA. Equal amounts (2 μL, 200 μM) of three fragments (5'-ACCTGGGGG-3'; 5'-AGTATTGCG-3'; 5'-GAG GAA GGT-3') were mixed with 2 × ATP binding buffer (6 μL, 50 mM Tris-Hcl + 0.6M Nacl, pH 8.2). The probe solution (0.5 μL) was then mixed with a ATP sample (4.5 μL) at various concentrations. All ATP samples were prepared with 1× ATP binding buffer. After a 5 min incubation at room temperature, an AuNP solution (100 μL) was added into 2 μL of the ATP/aptamer solution. The mixed solution was further diluted with PBS solution (12 μL) after a 2 min incubation at room temperature. UV/Vis absorption measurements were conducted and photos were taken immediately after adding the PBS solution.

Transmission electron microscopy (TEM) was performed on a Hitachi H-7650 transmission electron microscope. A typical sample was prepared by dropping 10 μL of the nanoparticle solution onto a holey carbon TEM grid, followed by a process that wicked the solution away. The grid was subsequently dried in air and imaged.

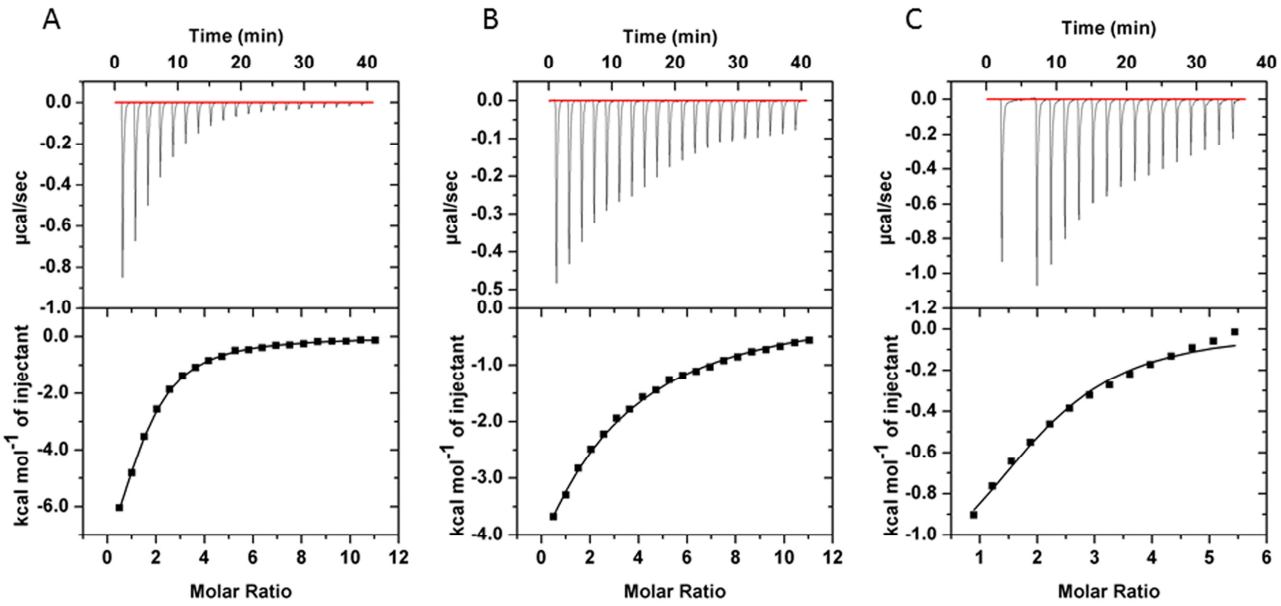

Fig. S1 Analysis of cocaine binding with the monolithic aptamer (A), the double-fragment aptamer (B), and the triple-fragment aptamer (C) using isothermal titration calorimetry. Shown are titrations of cocaine into aptamer solutions in 150 mM NaCl, 25 mM Tris, pH 8.0 at 25 ºC (A,B) or 4 ºC (C). On top is the raw titration data showing the heat resulting from each injection of cocaine into the aptamer solution. On bottom are the integrated heats after correcting for the heat of dilution. Binding affinities are 18.0 ± 0.6 µM (A), 120.6 ± 9.8 µM (B), and 111.2 ± 19.1µM (C), respectively.

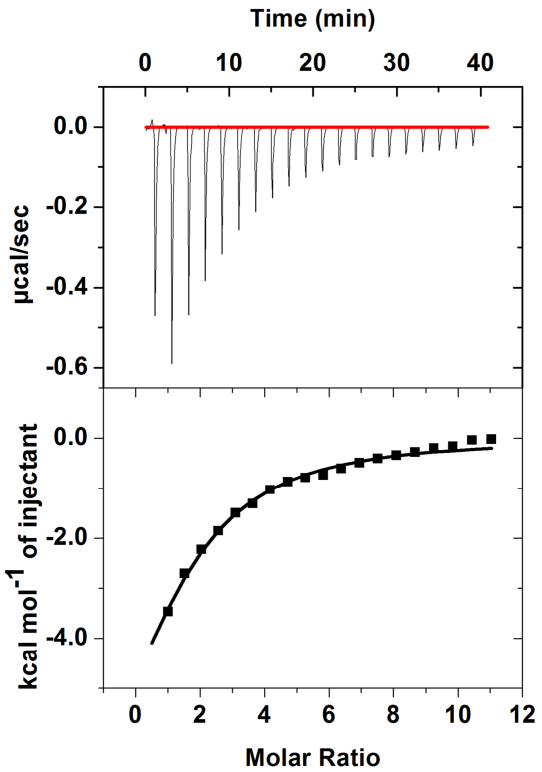

Fig. S2 Analysis of cocaine binding with the monolithic aptamer using isothermal titration calorimetry. Shown are titrations of cocaine into aptamer solutions in 150 mM NaCl, 25 mM Tris, pH 8.0 at 37 ºC On top is the raw titration data showing the heat resulting from each injection of cocaine into the aptamer solution. On bottom are the integrated heats after correcting for the heat of dilution. Binding affinities are 35.4 ± 0.7 µM (A).

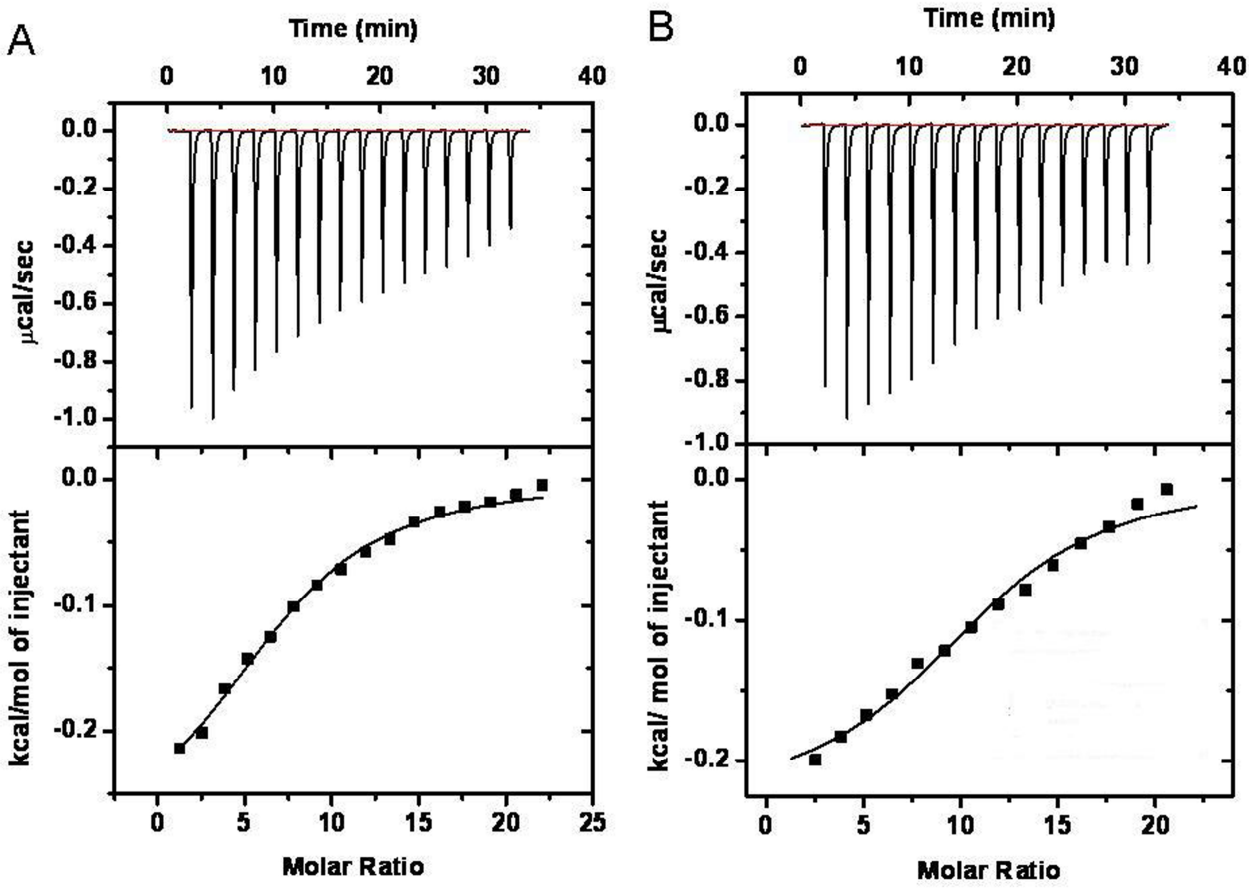

Fig. S3 Analysis of cocaine binding with the triple-fragment aptamer using isothermal titration calorimetry. Shown are titrations of cocaine into aptamer solutions in 25 mM Tris, pH 8.0, 500 mM (A) or 0 mM (B) NaCl at 4 ºC. On top is the raw titration data showing the heat resulting from each injection of cocaine into the aptamer solution. On bottom are the integrated heats after correcting for the heat of dilution. Binding affinities are 238.1 ± 3.0 µM (A) and 150.4 ± 13.5 µM (B), respectively.

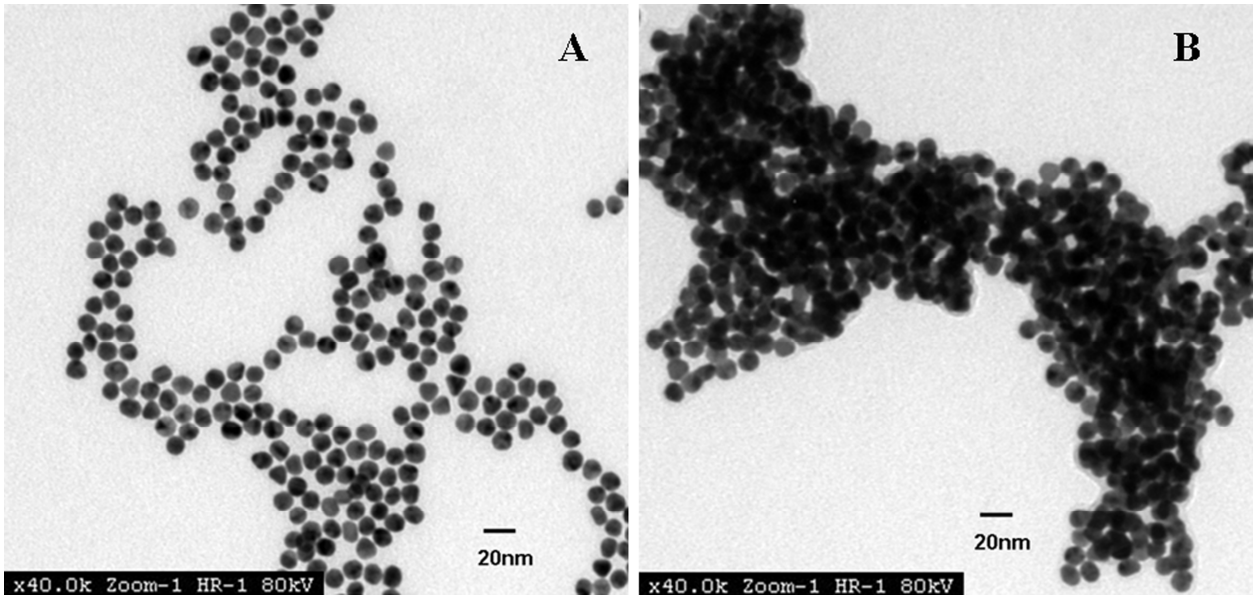

Fig. S4 TEM images of AuNPs with (right) and without (left) 100 μM cocaine.

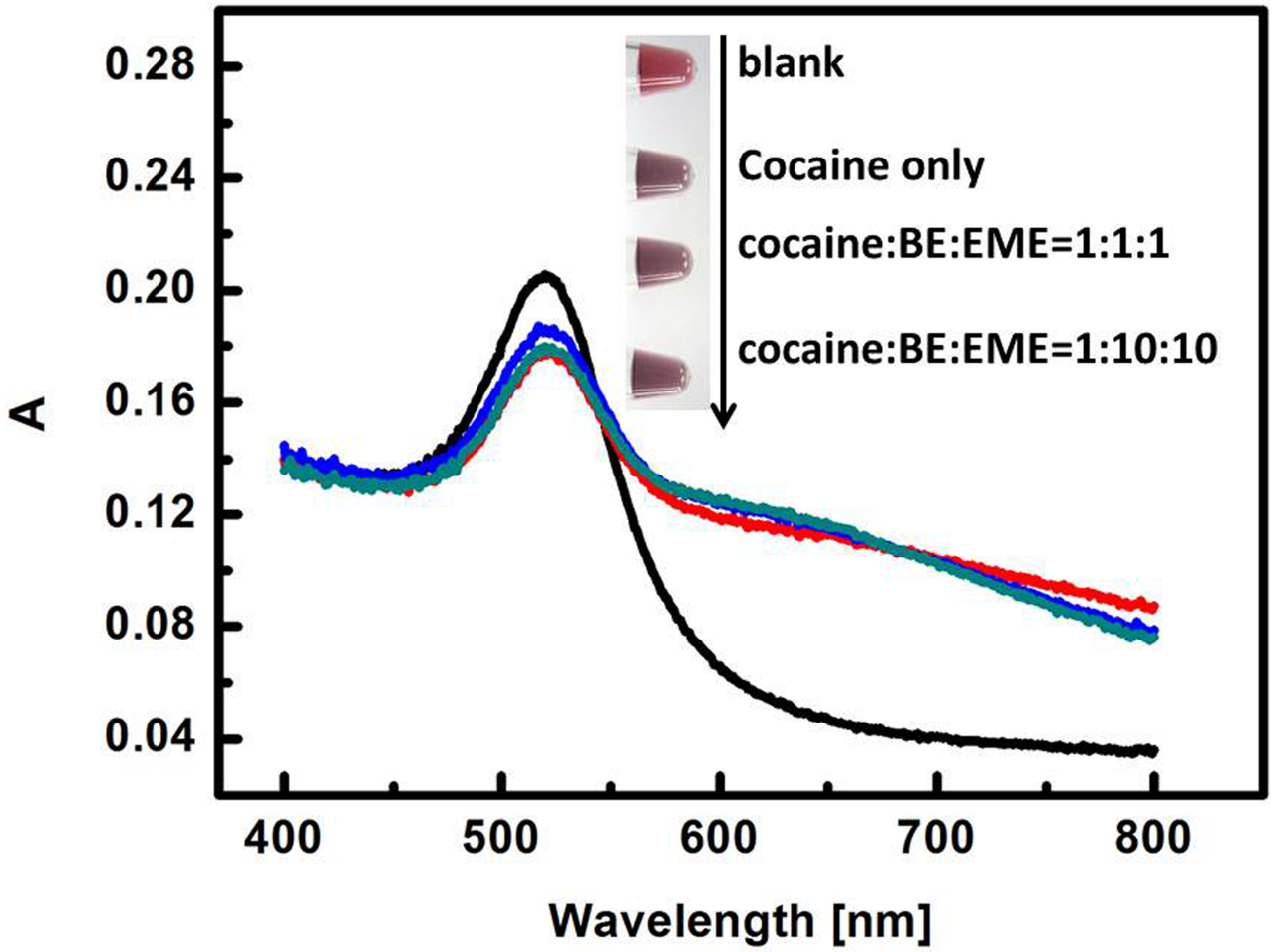

Fig. S5 The UV/Vis spectra and visual detection of 100 μM cocaine using TFA. The cocaine was mixed with equal or ten times more concentrated cocaine analogues BE and EME.

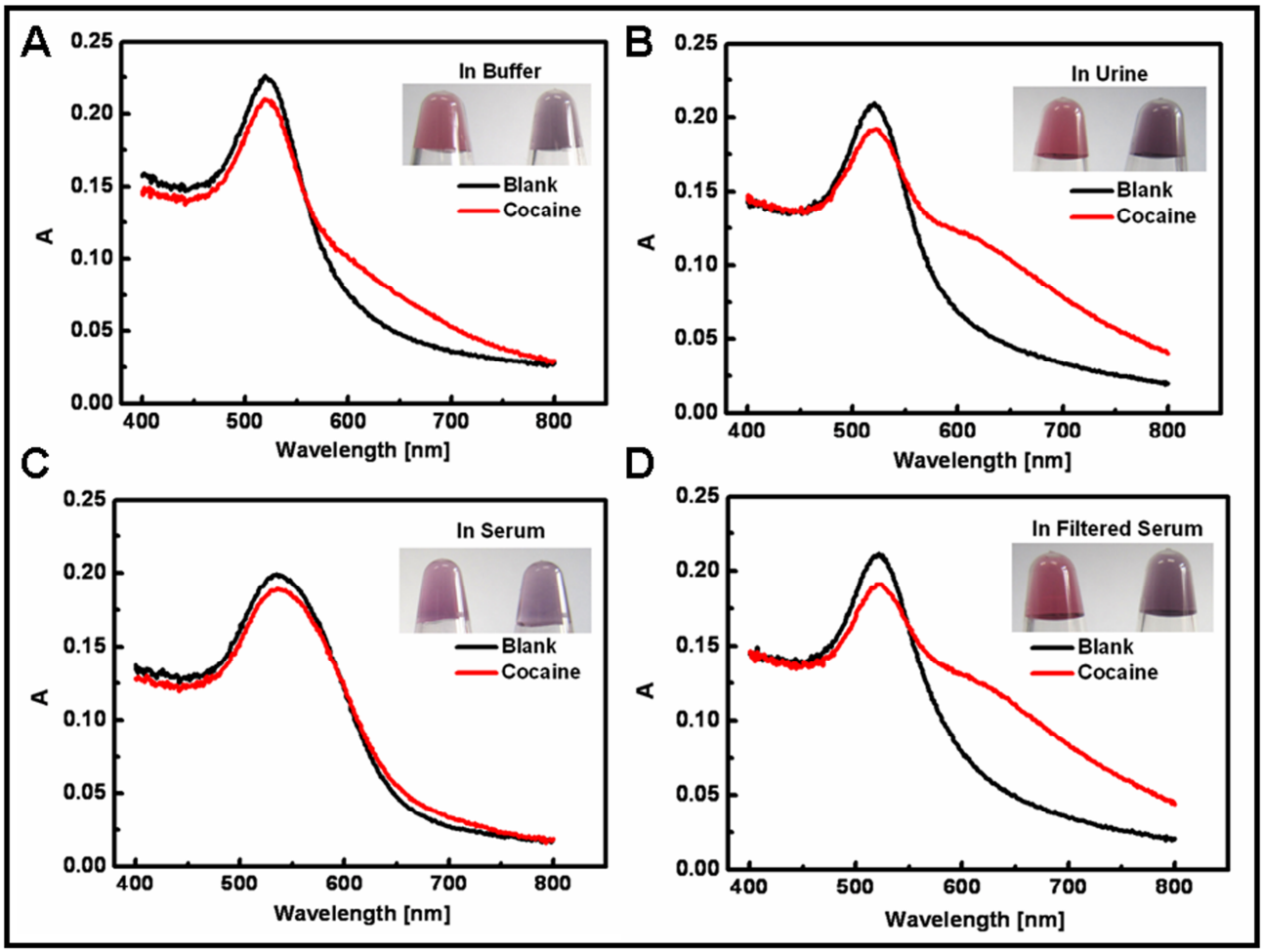

Fig. S6 The UV/Vis spectra and visual detection of 100 μM cocaine using TFA in buffer (A), in urine (B), in untreated serum (C), and in filtered serum (D).

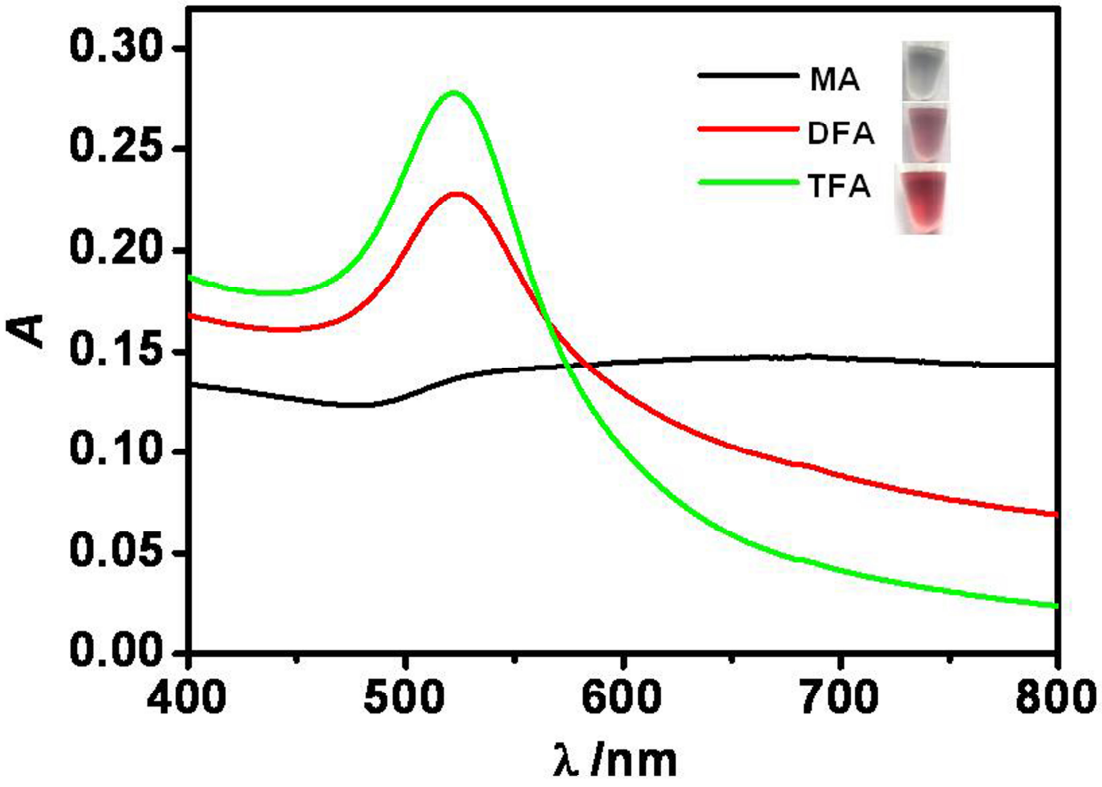

Fig. S7 The UV/Vis spectra of gold colloid mixed with the monolithic aptamer (MA，probe 1), the double-fragment aptamer (DFA, probe 2 and 3), and the triple-fragment aptamer (TFA, probe 3, 4, and 5) at equal molar concentrations, respectively. The insert images were taken 5 min after adding the salt solution. The AuNP solution showed gray (MA), purple (DFA), and red (TFA) after adding the same amount of salt solution, respectively.

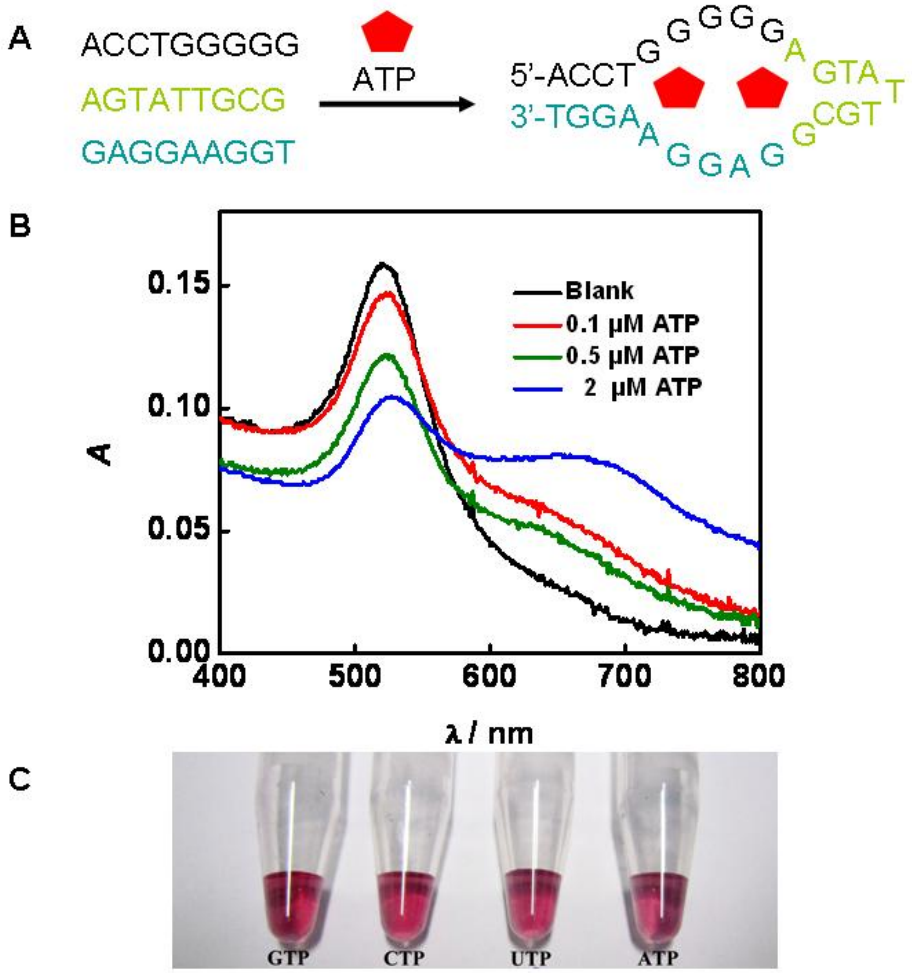

Fig. S8. Schematic drawing **(A)** of the probe design for ATP detection in triple-fragment sensor (TFA); UV/Vis spectra (B) and visual detection (C) of ATP using AuNP based TFA. (C) A good specificity of the sensor was obtained when challenging with ATP, GTP, CTP, and UTP, all at 2 μM.