Supporting Information

Experimental Sections

Reagents and Apparatus

Hydrogen tetrachloroaurate(III) ($HAuCl_4.4H_2O$) was obtained from by Sangon Inc. (Shanghai, China). Adenosine, thrombin, cocaine and their analogues were purchased from Sigma (St. Louis, MO, USA). The working solution of the oligonucleotide was obtained by diluting the stock solution with a 30 mM Tris-HCl buffer (pH 7.4), which contains 100mM NaCl. All other chemicals were of analytical reagent grade and used without further purifying, while twice-distilled water was used throughout the whole process. Cary Eclipse Fluorescence Spectrophotometer (Varian, USA) was used for monitoring adsorption spectra. The apparatus parameters were set as follows: $\lambda_{ex(AMCA)}$= 353 nm (slit 10 nm), λ_{em}= 400-600 nm (slit 10 nm), $\lambda_{ex(FAM)}$= 490 nm (slit 10 nm), λ_{em}= 500-600 nm (slit 10 nm). $\lambda_{ex(ROX)}$= 580 nm (slit 10 nm), and λ_{em}= 590-700 nm (slit 10 nm). UV/visible (UV/vis) adsorption spectra were recorded on a Hitachi U-3900H UV/vis spectrophotometer (Kyoto, Japan) at room temperature. The TEM (Transmission electron microscopy）images of nanoparticles were obtained on a transmission microscope (Tecnai G2 F20 S-TWIN 200KV).

The engineered aptamers used herein were synthesized and purified by Sangon Inc. (Shanghai, China). The sequences of the involved oligonucleotides are listed below.

Anticocaine aptamer chips: 5’-AMCA-AGACAAGGAAAA-3’ (C_1)

5’-TCCTTCAATGAAGTGGGTCG-3’ (C_2)

Antiadensoine aptamer chips: 5'-ROX-AGCGGAGGAAGG-3' (A_1)

5'-ACCTGGGGGAGTAT-3' (A_2)

Antithrombin aptamer: 5'-FAM-GGTTGGTGTGGTTGG-3' (T_1)

Synthesis of AuNPs

AuNPs (~15nm in diameter) were synthesized according to the previous literature[S1]. In brief, 50 mL of 2.5×10^{-4} M $HAuCl_4$ solution was boiled and stirred vigorously; then, it was followed by the adding of 1.75 mL of 1% sodium citrate solution into the boiling solution; the color change from light yellow to red in the solution was recorded after 30 min boiling, 15 min stirring. Subsequently it was cooled to room temperature and stored in a fridge at 4 C for further use. The concentration of Au-NPs was about 1.4×10^{15} particles L^{-1} based on completely reaction estimation.

Performance of Multiplex Analyte Detection

10 μL three dye-modified nucleosides (C_1, A_1, T_1) and two unmodified (C_2, A_2) sequences were mixed and added to a solution (150 μL, 5 nM) of unmodified AuNPs with a concentration of 0.5 μM each. The mixture was then relaxed allowing for 10 min reaction and sufficient absorption at room temperature. In order to detect multiplex analytes, a mixture of cocaine, thrombin, and adenosine (5 μL) was added to above solution for 4 h incubation time. Specifically, the emission of Rox-labeled antiadenosine aptamer-chip by adenosine appears at 607 nm, the emission of FAM-labeled G-quartet by thrombin at 520 nm, and the emission of AMCA- labeled anti-cocaine aptamer- chip by cocaine at 353 nm.

Cross-Reaction Analysis

In order to prove that these fluorescent dyes don't have cross interference with each other, an AMCA solution, a FAM solution, a ROX solution and a mixed solution containing AMCA, FAM and ROX (the concentration of each in the mixed solution is the same with that of pure solution) were analyzed by Fluorescent Spectrometry. The apparatus parameters are set as follows: λ_{ex}(AMCA)= 353 nm, λ_{em}= 400-800 nm, λ_{ex}(FAM)= 490 nm, λ_{em}= 500-800 nm. λ_{ex}(ROX)= 580 nm, and λ_{em}= 590-800 nm. The results were shown in Figure S1, line a, b and c is the fluorescence signal of AMCA, FAM and ROX, respectively. The results showed that the wavelengths of the max fluorescence intensity were 454nm, 516nm, 606nm respectively, and there has little interference for each other.

Then, setting the apparatus parameters of mixed solution as follows: λ_{ex}= 353 nm, λ_{em}= 400-800 nm. As shown in line d,which is nearly the same as line a, but there is a very weak interference peak at about 600 nm. Followed by setting the apparatus parameters of mixed solution as follows: λ_{ex}= 490 nm, λ_{em}= 500-800 nm. The result was shown as line e, there is no significant difference with that of line b. These results indicated that is no obvious cross reaction among AMCA, FAM and ROX.

Reference

S1 A. Doron, E. Katz, I. Willner, *Langmuir*, 1995, **11**, 1313–1317.

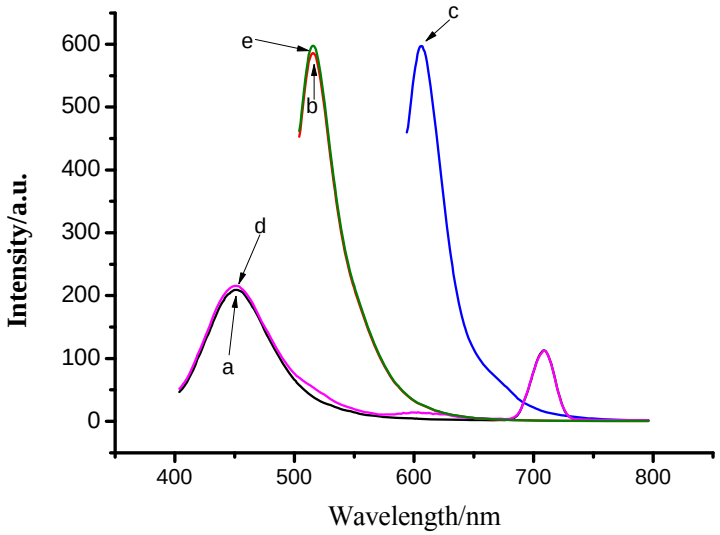


Figure S1 The fluorescence spectra of AMCA, FAM and ROX and the mixed solution (a: AMCA; b: FAM; c: ROX in pure solution, d: AMCA in mixed solution, e: FAM in mixed solution)

Table S1 The structures of the fluorescent dyes

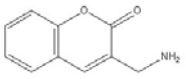
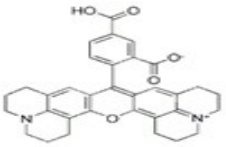
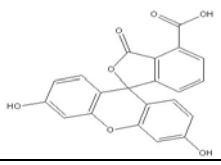
Fluorescent dye	Structure	Excitation wavelength (nm)	Emission wavelength (nm)
AMCA		353	442
ROX		588	608
FAM		495	520

Table S2 The related linear results of the proposed aptamer-based fluorescence biosensor

Targets	Concentration range (mol/L)	Regression equation	Regression coefficient R	Detection limit (mol/L)
Thrombin	5.0×10^{-8} ～ 2.5×10^{-6}	I/a.u=200.68+1.05×10^{8}C	0.9983	2.5×10^{-9}
Cocaine	1.0×10^{-7} ～ 2.0×10^{-6}	I/a.u=78.21+5.79×10^{7}C	0.9978	3.0×10^{-8}
Adenosine	1.0×10^{-8} ～ 1.0×10^{-6}	I/a.u=292.59+2.45×10^{8}C	0.9938	2.0×10^{-9}