

## Supporting Information

### **A Portable Visual Detection Method Based on Target-responsive DNA Hydrogel and Color Change of Gold Nanorods**

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## Experimental Section

### Materials and Reagents.

The reagents for DNA synthesis were purchased from Glen Research (Sterling, VA, U.S.A.). Glucoamylase (GA), glucose oxidase (GOx), *N,N,N',N'*-tetramethylethylenediamine (TEMED), ammonium persulfate (APS) and acrylamide were obtained from Sigma-Aldrich (St. Louis, MO, USA). Lead standard was purchased from Aladdin (Shanghai, China). Seawater was obtained from Baicheng Beach (Xiamen, China). Other reagents were supplied by Sinopharm Chemical Reagents (Shanghai, China). Lead reaction buffer for colorimetric detection of  $Pb^{2+}$  contained 10 mM Tris-acetic acid and 300 mM NaCl (pH 7.5). The cocaine buffer contained 77 mM  $Na_2HPO_4$ , 23 mM  $NaH_2PO_4$ , 50 mM NaCl, and 5 mM  $MgCl_2$  (pH 7.3).

### Synthesis of Acrydite Phosphoramidite.

The acrydite phosphoramidite was synthesized as described in a previous report.<sup>1</sup> 6-Amino-1-hexanol (585 mg, 5 mmol), 2-methylacrylic acid (440 mg, 5 mmol), *N,N'*-dicyclohexylcarbodiimide (DCC, 1356 mg, 6 mmol) and 1-hydroxybenzotriazole (HOBT, 810 mg, 6 mmol) were dissolved in 5 mL of DMF under nitrogen, and stirred at room temperature overnight. The crude product was purified by column chromatography to obtain acrylic-OH. The acrylic-OH (213 mg, 1.15 mmol) was dissolved in 2 mL of dry  $CH_2Cl_2$  under nitrogen in an ice bath. After slowly adding *N,N'*-diisopropylethylamine (DIPEA, 0.55 mL, 3.22 mmol), 2-cyanoethyl-*N,N'*-diisopropylchlorophosphoramidite (0.28 mL, 1.15 mmol) was added dropwise. Then the reaction mixture was stirred in an ice bath for 3 h. The product was obtained after isolating by silica gel column chromatography. The synthesized product was used to conjugate with normal DNA to obtain acrydite-DNA on a DNA synthesizer.

### Oligonucleotides Synthesis.

According to the standard DNA synthesis protocol, all oligonucleotides (**Table S1**) used in the work were synthesized on a PolyGen GmbH DNA synthesizer. Strand A and strand B were modified with acrydite phosphoramidite at the 5'-end. After DNA synthesis and modification, all the oligonucleotides were cleaved from the solid support, deprotected with ammonia and methylamine (1:1, v/v), and then purified by a LC300 semi-preparative high-performance liquid chromatography (HPLC) system with a C18 reverse-phase column. After desalting by NAP-5 and NAP-10, the DNA was quantified by a UV-Vis spectrometer and stored at -20 °C for later use. Substrate with the modification of adenosine triphosphate (rA) was purified on the basis of Glen

Research protocols. After purification by HPLC, the 2'-O-triisopropylsilyloxymethyl group was removed by incubating in a mixture of 100  $\mu$ L of DMSO and 125  $\mu$ L of triethylamine trihydrofluoride at 65  $^{\circ}$ C for 2.5 h. Then, 25  $\mu$ L of 3 M sodium acetate and 1.5 mL of n-butanol were added, and the solution was stored at -20  $^{\circ}$ C for 30 min to precipitate DNA. The DNA precipitate was dissolved by RNase-free water and desalted by 3K NMWL ultracentrifuge tubes several times. Finally, the substrate was stored at -20  $^{\circ}$ C for later use.

### **Preparation of Polyacrylamide–DNA Conjugates.**

Strands A and B (500  $\mu$ M, described in **Table S1**) modified with acrydite were mixed with 4% acrylamide, separately. 20  $\mu$ L of the solutions were placed in a vacuum desiccator for 10 min at 37  $^{\circ}$ C to remove air, and then 0.28  $\mu$ L freshly prepared APS (10%, w/w APS) and 0.56  $\mu$ L freshly prepared TEMED (5%, v/v) were added to the solutions immediately. The mixtures were placed in a vacuum desiccator at 37  $^{\circ}$ C for another 15 min for the polymerization reaction, producing polyacrylamide-DNA conjugates P-SA and P-SB. The product polymers P-SA and P-SB were purified by 100K NMWL ultracentrifuge tubes to eliminate the unpolymerized monomer.

### **Preparation of Target-Responsive Hydrogel.**

To prepare the target-responsive hydrogel, the solution of P-SA, P-SB and linker (molar ratio of 110:110:70) was mixed with 0.5 mg/mL GA for cocaine, while the molar ratio of P-SA/P-SB /DNAzyme/substrate was 100:100:100:55 for the lead hydrogel. The solution was shaken vigorously and heated to 50  $^{\circ}$ C for 5 min three times to ensure homogeneity of the hydrogel. The solution was then cooled to room temperature to produce target-responsive hydrogel.

### **Preparation of Gold Nanorods.**

Gold nanorods prepared using 5-bromosalicylic acid as an additive were synthesized following an improved method.<sup>2</sup> The seeds were produced by adding freshly prepared NaBH<sub>4</sub> (0.1 mL, 6 mM) to a solution of HAuCl<sub>4</sub> (0.5 mL, 0.5 mM) and CTAB (0.5 mL, 0.2 M). After vigorous shaking for 2 min, the seed solution was aged at room temperature for 30 min before use. The growth solution was prepared as follows: 0.9 g of CTAB and 0.11 g of 5-bromosalicylic acid were dissolved in 25 mL warm water. After adding AgNO<sub>3</sub> (1.2 mL 4.0 mM), the mixture was kept undisturbed for 15 min at 30  $^{\circ}$ C. Then HAuCl<sub>4</sub> (25 mL, 1 mM) was added. After 15 min of stirring, ascorbic acid (0.2 mL, 0.064 M) was added to obtain the growth solution. Then 80  $\mu$ L seed solution was injected into the growth solution. Gold nanorods were produced by incubating at 30  $^{\circ}$ C for 12 h. The synthesized AuNRs were centrifuged three times at 8500 rpm for 30 min each to remove excess growth solution, and then resuspended in 0.05 M CTAB.

Finally, AuNR solution containing 0.05 M CTAB was obtained.

#### **Hydrogen Peroxide Assay.**

Synthesized AuNR solution (75  $\mu\text{L}$ ) was mixed with 7.5  $\mu\text{L}$  of 20 mM  $\text{FeSO}_4$  and 11.25  $\mu\text{L}$  of 4 M HCl. Then 11.25  $\mu\text{L}$  of different concentrations of  $\text{H}_2\text{O}_2$  were added to the mixtures at 37  $^\circ\text{C}$  for 30 min. The absorbance of the solution was measured by UV-Vis spectrometer.

#### **Colorimetric Detection in Buffer.**

A series of concentrations of lead were diluted with GOx (0.08 mg/mL) and amylose (10 mg/mL). The solutions were added to 10  $\mu\text{L}$  of hydrogel in Eppendorf tubes. The tubes were then incubated at 25  $^\circ\text{C}$  for 1.5 h with gentle shaking for complete reaction. Finally, 15  $\mu\text{L}$  of the supernatant was added to a solution of 10  $\mu\text{L}$  of 20 mM  $\text{FeSO}_4$ , 15  $\mu\text{L}$  4 M HCl and 100  $\mu\text{L}$  as-synthesized AuNRs. After incubating at 37  $^\circ\text{C}$  for 1 h for the etching process to occur, the absorbance was measured by UV-Vis spectrometer and the samples were photographed.

The visual detection of cocaine was achieved by the same procedure. Different concentrations of cocaine were dissolved in GOx (0.08 mg/mL) and amylose (6 mg/mL). Then the solutions were added to the top of the hydrogel in Eppendorf tubes. The tubes were then incubated at 25  $^\circ\text{C}$  for 1.5 h with gentle shaking for complete reaction. Finally, the supernatant was added to the mixture of  $\text{FeSO}_4$ , HCl and AuNRs for the colorimetric reaction.

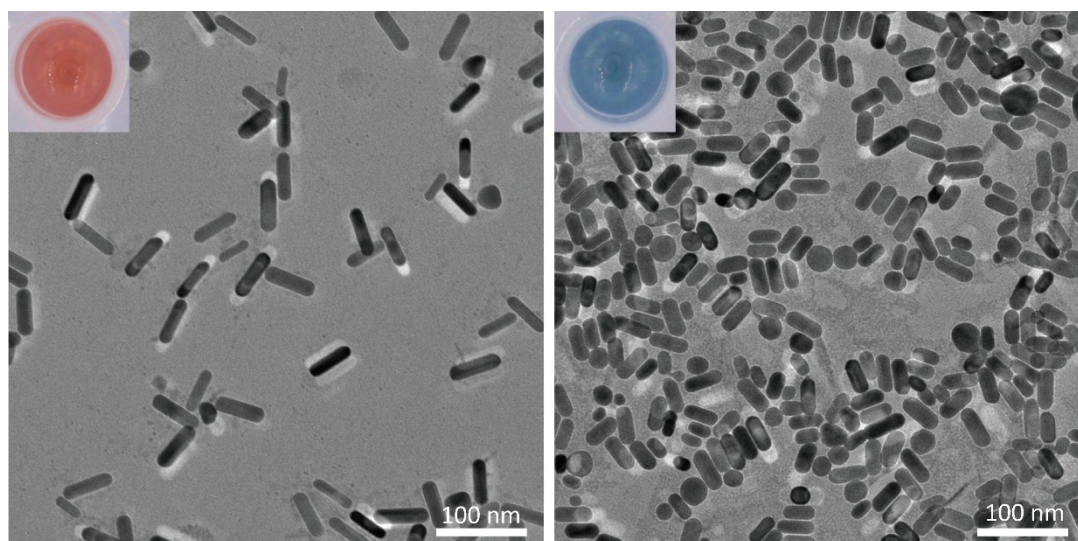
#### **Colorimetric Detection in Real Sample.**

A series of concentrations of lead were added to tap water and seawater. The solutions were mixed with GOx (0.08 mg/mL) and amylose (10 mg/mL). After incubating for 1.5 h with DNA hydrogel, the solutions were added to the mixture of 10  $\mu\text{L}$  of 20 mM  $\text{FeSO}_4$ , 15  $\mu\text{L}$  4 M HCl and 100  $\mu\text{L}$  AuNRs for chromogenic reaction. After completely etched, the AuNR solutions were measured by UV-Vis spectrometer and were photographed.

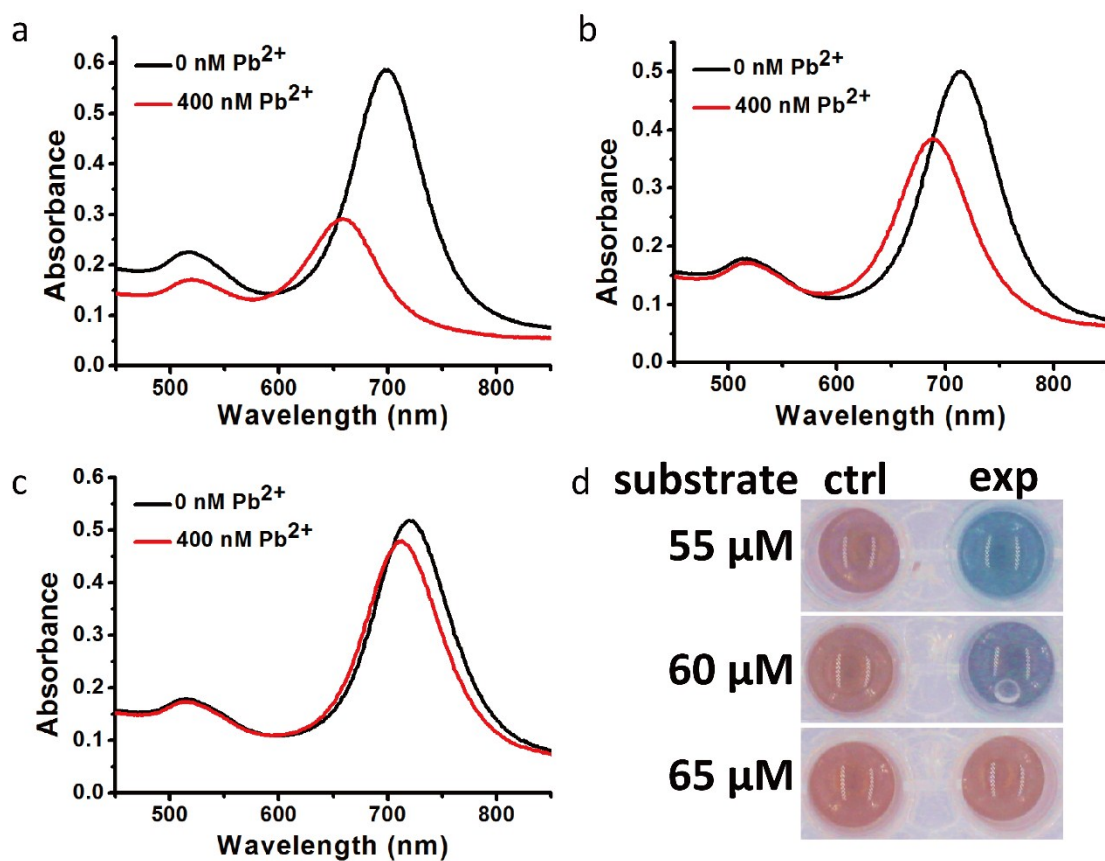
**Table S1.** Nucleotide sequences used in this work<sup>3, 4</sup>

Name	Sequence
Pb <sup>2+</sup> strand A (SA)	5' - Acrydite- CTG TGA AAA TGT GG -3'
Pb <sup>2+</sup> strand B (SB)	5' - Acrydite- ATG TGT TTT TGT AG -3'
Substrate strand	5' - CTA CAA AAA CAC ATA CTC ACT ATrA GGA AGA GAT GAT CCA CAT TTT CAC AG -3'
DNAzyme	5' - ATC TCT GAA GTA GCG CCG CCG TAT AGT -3'
Cocaine strand A (SA)	5' -Acrydite- AAA ATC ACA GAT GAG T -3'
Cocaine strand B (SB)	5'-Acrydite- AAA AGT CTC CCG AGA T-3'
Cocaine linker (linker)	5' - ACT CAT CTG TGA ATC TCG GGA GAC AAG GAT AAA TCC TTC AAT GAA GTG GGT CTC CC -3'

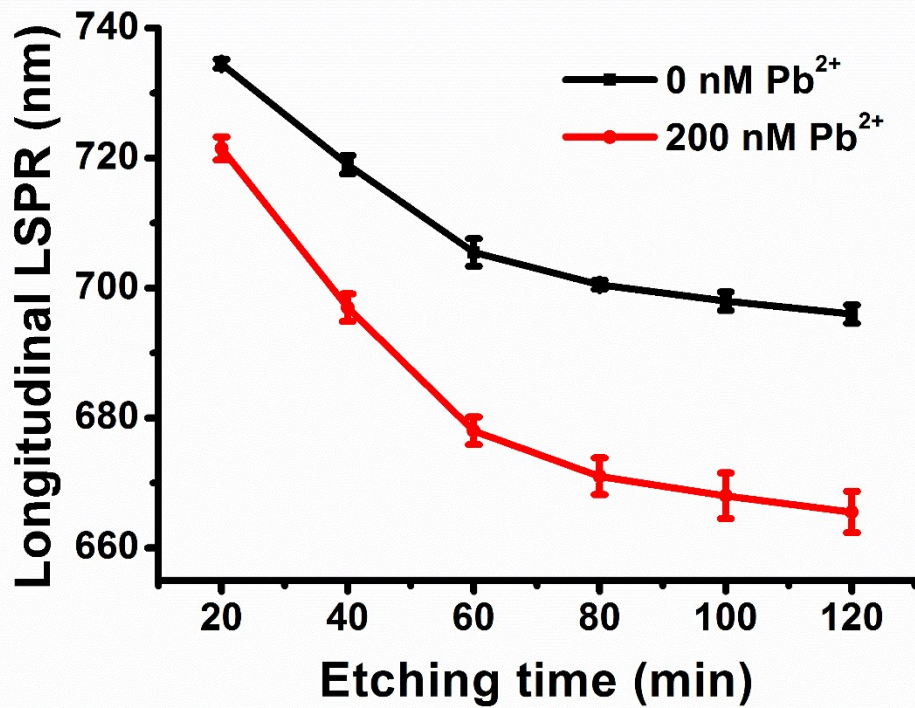
The sequences with same colors are the complementary parts.



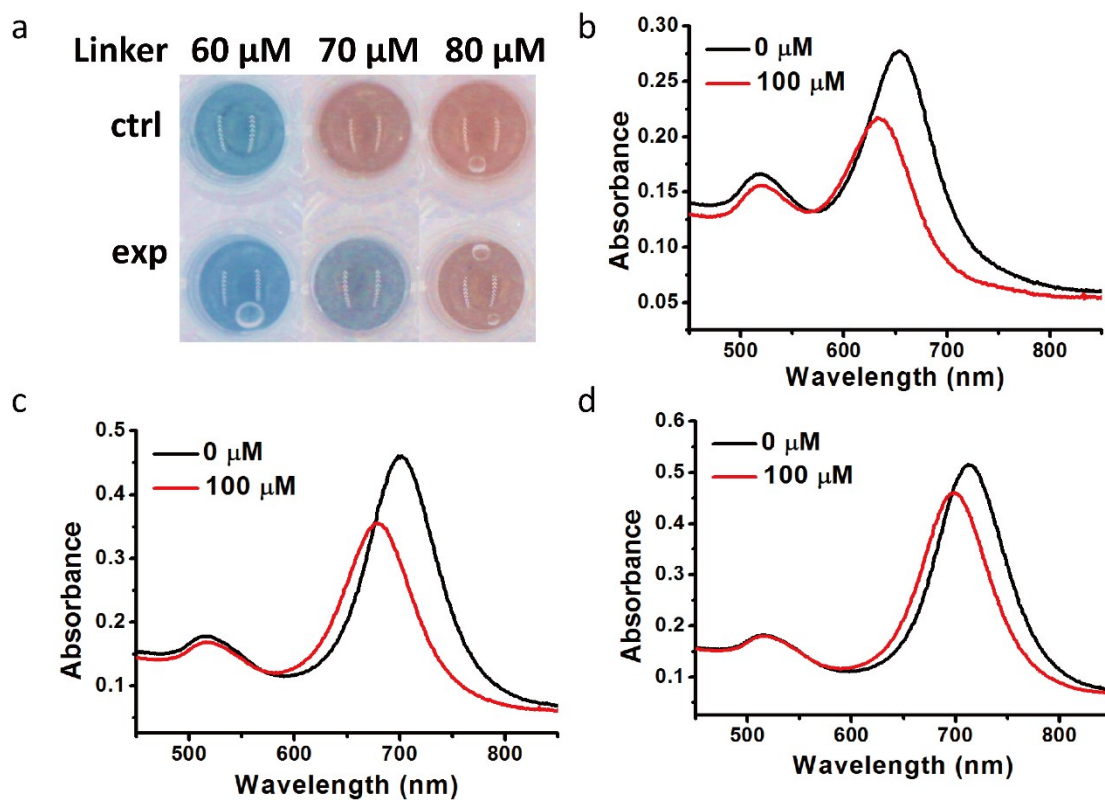
**Fig. S1.** TEM images of AuNRs before (left) and after (right) adding 2.0 mM H<sub>2</sub>O<sub>2</sub>. The scale bars are 100 nm.



**Fig. S2.** Optimization of the concentration of substrate for lead-responsive hydrogel. (a, b, c) The spectra of AuNRs for (a) 55, (b) 60, and (c) 65  $\mu\text{M}$  of substrate. (d) Corresponding images of AuNRs for different concentrations of substrates. “exp” means experimental sample containing 400 nM  $\text{Pb}^{2+}$ , while “ctrl” means control sample.

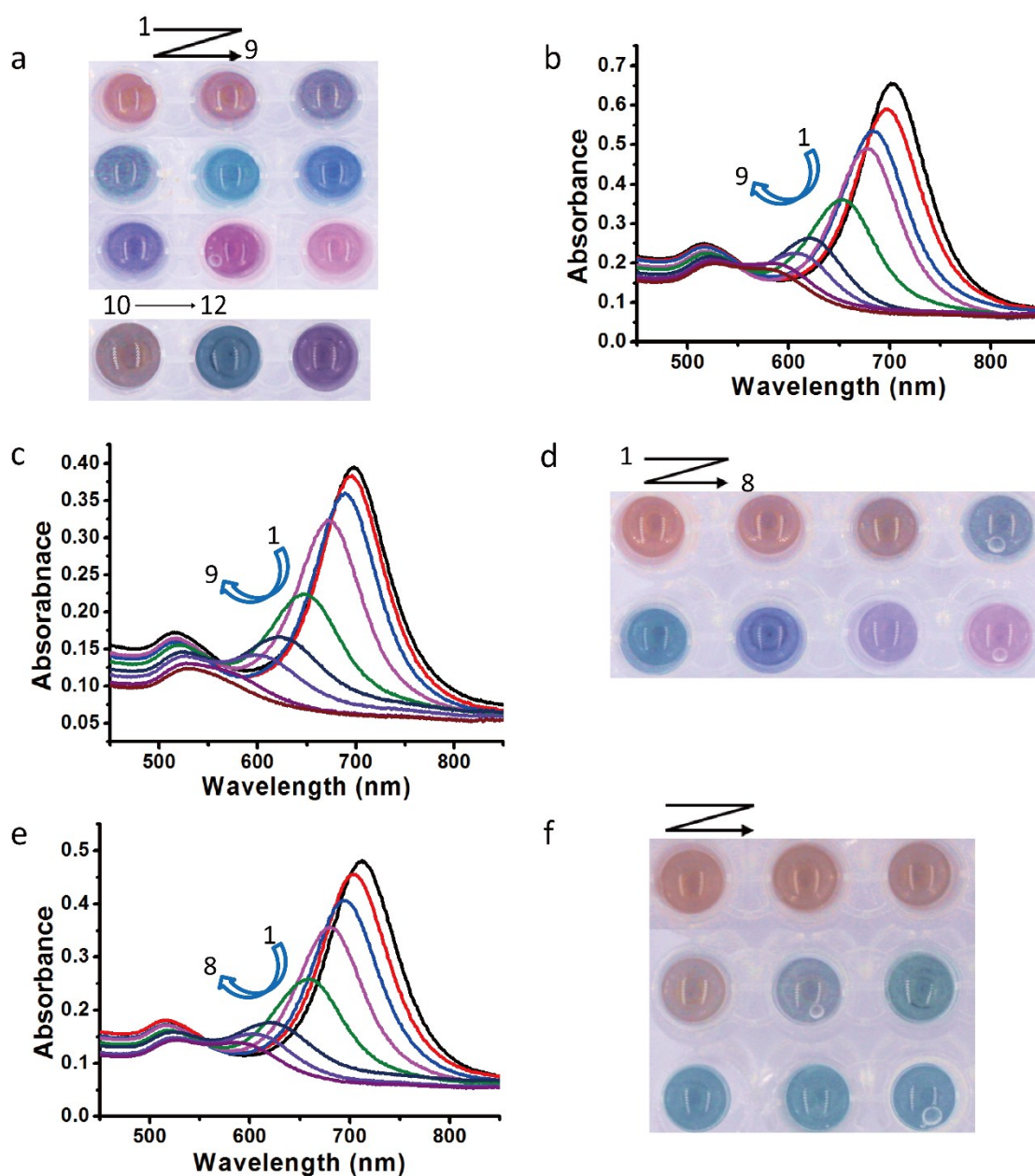


**Fig. S3.** Optimization of the etching time. The kinetics of etching AuNRs in response to 0 nM Pb<sup>2+</sup> (black solid line) and 200 nM Pb<sup>2+</sup> (red solid line).



**Fig. S4.** Optimization of the concentration of linker for cocaine-responsive hydrogel. (a) Images of AuNRs for different concentrations of linkers. “exp” means experimental sample containing 100  $\mu\text{M}$  cocaine, while “ctrl” means control sample. The corresponding spectra of AuNRs for (b) 60, (c) 70, and (d) 80  $\mu\text{M}$  of linker.





**Fig. S5.** (a) Calibration plot for different concentrations of Pb<sup>2+</sup> (1-9) in buffer (0, 50, 100, 200, 400, 700, 1000, 1600, 2000 nM). The concentrations of Pb<sup>2+</sup> (10-12) were 85 nM, 540 nM, and 1.25  $\mu$ M, respectively. (b) UV-Vis spectra of AuNRs for detecting different concentrations of Pb<sup>2+</sup> in buffer. (c) UV-Vis spectra of AuNRs for detecting different concentrations of Pb<sup>2+</sup> in seawater. (d) Images of AuNRs with different concentrations of Pb<sup>2+</sup> in tap water. The concentrations of Pb<sup>2+</sup> were 0, 50, 100, 200, 400, 750, 1000, 2000 nM, respectively. (e) Corresponding UV-Vis spectra of AuNRs for detecting different concentrations of Pb<sup>2+</sup> in tap water. (f) Images of AuNRs for cocaine assay in buffer (0, 15, 25, 50, 100, 200, 400, 800, 1600  $\mu$ M).

## References

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