Supplementary Information

A novel electrochemical biosensor for ultrasensitive Hg^{2+} detection via a triple signal amplification strategy

Wang He[a], Bin Qiao[b], Fengzhen Li[a], Lisha Pan[a], Delun Chen[a,b], Yang Cao[a,d], Jinchun Tu[a], Xiaohong Wang[a,*], Chuanzhu Lv[c,*], Qiang Wu[b,*]

[a] *State Key Laboratory of Marine Resource Utilization in South China Sea, Hainan University, Haikou, 570228, China*

[b] *School of Tropical Medicine and Laboratory Medicine, Key Laboratory of Emergency and Trauma of Ministry of Education, Research Unit of Island Emergency Medicine, Chinese Academy of Medical Sciences (No. 2019RU013), Hainan Medical University, Haikou, 571199, China*

[c] *Department of Emergency, Hainan Clinical Research Center for Acute and Critical Diseases, The Second Affiliated Hospital of Hainan Medical University; Emergency and Trauma College, Hainan Medical University, Haikou, 571199, China*

[d] *Qiongtai Normal University, Haikou, 571127, China*

[*] *Corresponding author. Tel: +86 898 66259764; fax: +86 898 66259764.*
E-mail address: wangxiaohong@hainu.edu.cn
[*] *Corresponding author. Tel: +86 898 66989196; fax: +86 898 66989196.*
E-mail address: lvchuanzhu677@126.com
[*]*Corresponding author. Tel: +86 898 66984599; fax: +86 898 66984599.*
E-mail address: wuqiang001001@aliyun.com

Experiment

Materials and reagents

All oligonucleotides used in this study were obtained from Sangon Biotech. Co., Ltd. (Shanghai, China). Their base sequences are shown in Table S3.

Exo III was purchased from Sangon Biotechnology Co. Ltd. (Shanghai, China). Hexanethiol (HT), aurochtorohydric acid, trisodium citrate, Tris (hydroxymethyl) aminomethane hydrochloride (Tris) and Tris (2-carboxyethyl) phosphine (TCEP) were obtained from Sigma (St. Louis, USA). Mercury (II) Nitrate ($Hg(NO_3)_2$) was obtained from Xiya Reagent (Shandong, China). Sodium chlorid (NaCl), ethylenediaminetetraacetic acid (EDTA), magnesium chloride ($MgCl_2$), copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), disodium hydrogen phosphate (Na_2HPO_4) and sodium dihydrogen phosphate (NaH_2PO_4) were procured from Macklin Scientific (Shanghai, China). Ultrapure water was used throughout the experiment.

Preparation of AuNPs

AuNPs were synthesized by the citrate-mediated reduction of $HAuCl_4$ on basis of literature with slight modification.[1] Briefly, 1.25 mL of aurochtorohydric acid (1% *w*/*v*) was added to 100 mL of ultrapure water and heated on a hot-plate with continuous stirring until it slightly boiled. Then, 2.6 mL of trisodium citrate solution (1%) was added to the prepared solution quickly during continuous heating and stirring to keep the liquid

in a slightly boiling state. Upon heating for 5 min, the color of the mixture changed from yellow to wine red. Finally, the AuNPs solution was cooled to room temperature and stored at 4 ℃ for the next experiment.

Preparation of RDNA

RDNA was prepared by adding 30 μL of HP1 (5 μM), 4 U of Exo III and 30 μL of different concentrations of Hg^{2+} solutions to 40 μL of Tris-HCl buffer (pH 7.4) containing 20 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA and 5 mM $MgCl_2$. The mixture was heated to 30 ℃ for 60 min. Then the solution was heated to 80 ℃ for 10 min and cooled to room temperature. Finally, the RDNA was stored at 4 ℃ for further use.

Synthesis of c-probe/AuNPs/$Cu_3(PO_4)_2$ HNFs

$Cu_3(PO_4)_2$ HNFs were prepared according to literature[30] with some modifications. In brief, 6.7 μL of $CuSO_4 \cdot 5H_2O$ (0.12 M) and 20 μL of BSA (0.5 mg mL^{-1}) were added to 1 mL of phosphate buffer (0.03 M, pH 7.4) and reacted at 25 ℃ for 72 h. Then, the mixture was centrifuged at 5,000 rpm for 5 min and washed thrice with deionized water. The precipitate was stored at 4 ℃ for the next experiment.

Exactly 1 mL of AuNPs was added to the prepared $Cu_3(PO_4)_2$ HNFs and stirred for 6 h at room temperature. The compound was centrifuged and washed thrice with phosphate buffer (10 mM, pH 7.0) to remove excessive AuNPs. Next, c-probe (50 μL, 5 μM) was added to the mixture and stored at 4 ℃ for 12 h. Then, the precipitate of c-

probe/AuNPs/$Cu_3(PO_4)_2$ HNFs was centrifuged and washed twice with phosphate buffer (10 mM, pH 7.0). The sediment was dispersed in 1 mL of phosphate buffer (10 mM, pH 7.0).

Preparation of R-probe/AuNPs

R-probe/AuNPs were prepared by centrifugation of 1 mL of AuNPs (12,000 rpm, 30 min) and subsequent dispersion in 1 mL of phosphate buffer (10 mM, pH 7.0). Next, 100 μL of R-probe (0.10 mM r-probe 1, 1 mM r-probe 2, 10 mM TCEP) was added into 1 mL of AuNPs solution and the sample was incubated away from light at 4 °C for 16 h. The compound was centrifuged and washed thrice with phosphate buffer (10 mM, pH 7.0) to remove excessive R-probe. Then, the red oily deposits was dispersed in 1 mL of phosphate buffer (10 mM, pH 7.0).

Fabrication of biosensor

The GCE (Φ = 3 mm) was polished to a mirror using alumina slurry and washed with ultrapure water before use. Then, the electrode was electrodeposited a layer of AuNPs with 1% $HAuCl_4$ at −0.2 V for 30 s. Exactly 20 μL of HP2 (2.5 μM) was added onto the gold surface of the electrode and the sample was incubated for 16 h at room temperature. Afterward, 10 μL of HT (1 mM) was added to the electrode surface to block the specific binding sites. Subsequently, 20 μL of the prepared RDNA was added to the electrode surface. After incubation for 1 h, 20 μL of HP3 (2.5 μM) was modified on the electrode surface for another 1 h. Next, 20

μL of c-probe/AuNPs/$Cu_3(PO_4)_2$ HNFs was dropped to the electrode, and the samples were incubated at 37 ℃ for 1 h. After washing with phosphate buffer (10 mM, pH 7.0), 20 μL of R-probe/AuNPs was incubated on the electrode at 37 ℃ for 2 h. The present biosensor was fabricated and the signal was recorded by square wave voltammetry (SWV).

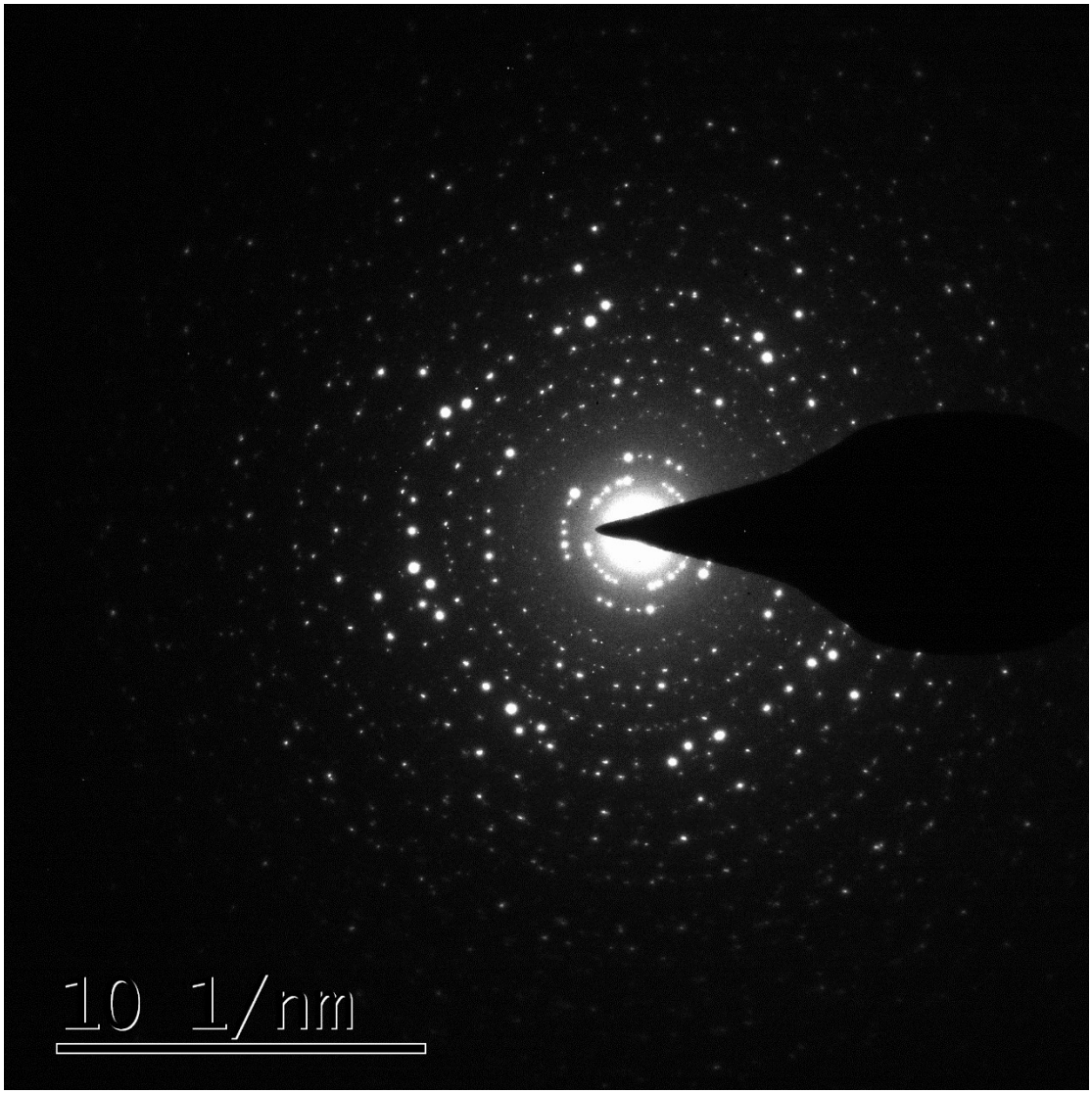
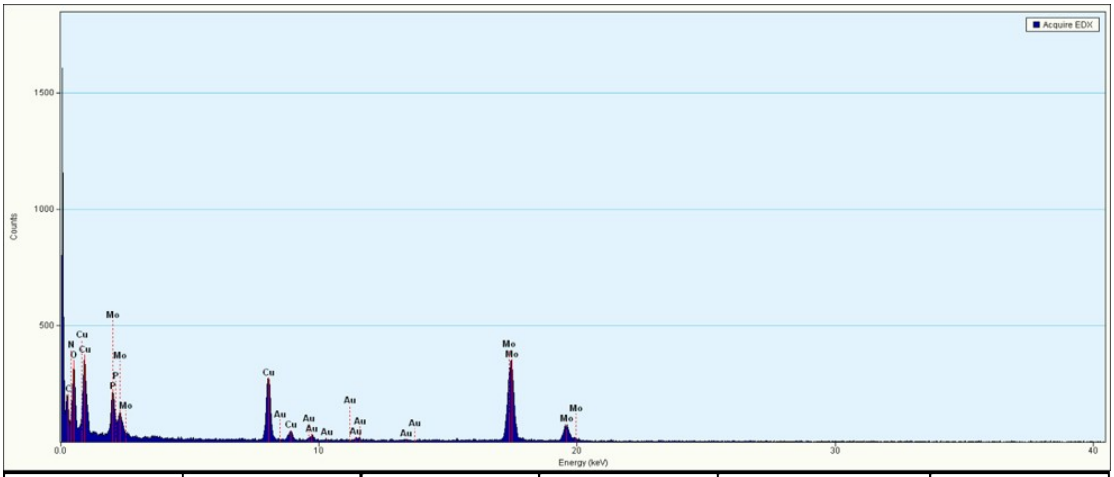


Fig. S1 SAED pattern of the c-probe/AuNPs/$Cu_3(PO_4)_2$ HNFs.



Element	Weight %	Atomic %	Uncert. %	Detector Correction	k-Factor
C(K)	21.25	37.37	0.25	0.28	3.685
N(K)	5.19	7.84	0.15	0.28	3.536
O(K)	27.48	36.28	0.18	0.51	1.921
P(K)	10.87	7.41	0.08	0.9	1.064
Cu(K)	32.59	10.83	0.16	0.99	1.598
Au(L)	2.61	0.27	0.09	0.99	4.037

Fig. S2 EDX pattern of the c-probe/AuNPs/$Cu_3(PO_4)_2$ HNFs.

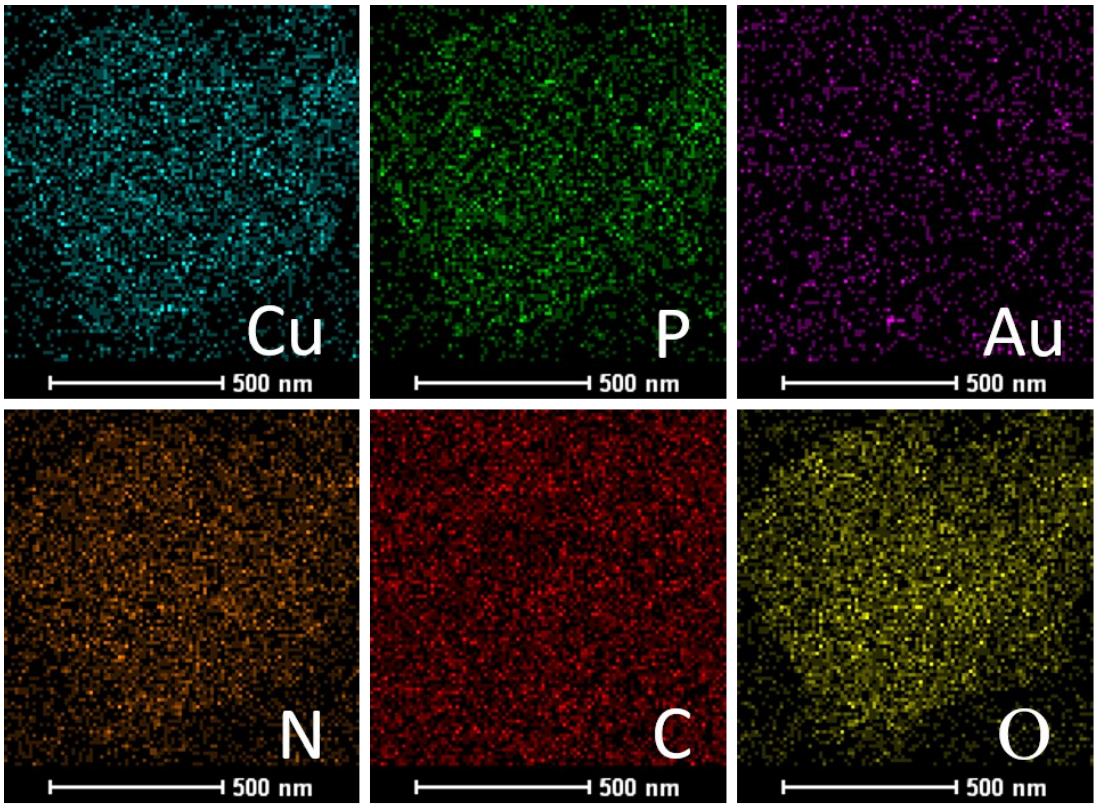


Fig. S3 STEM-EDX mapping analyses of the c-probe/AuNPs/$Cu_3(PO_4)_2$ HNFs.

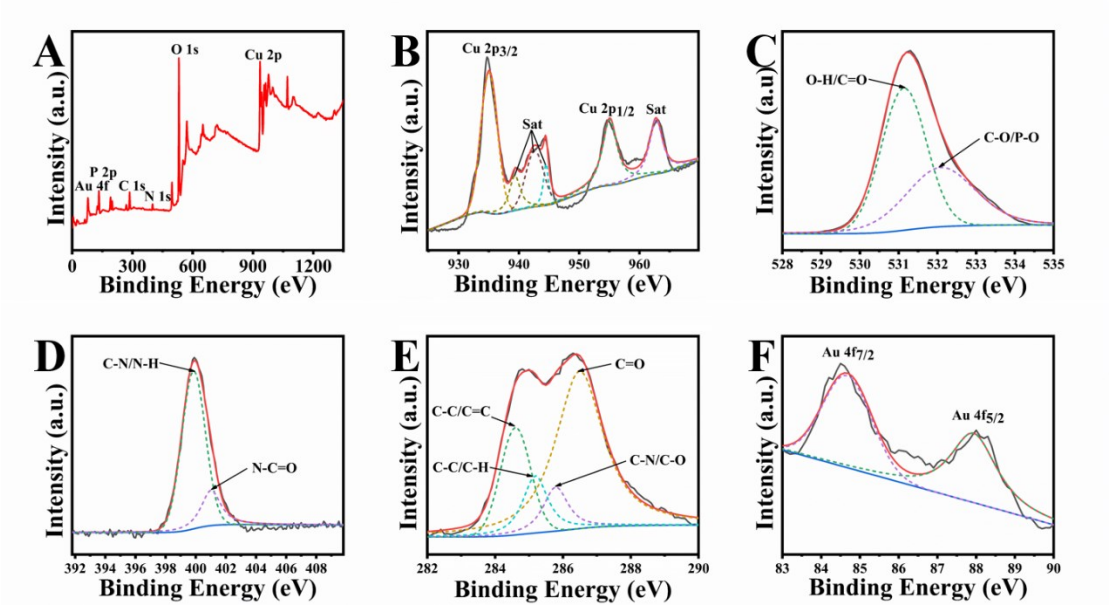


Fig. S4 XPS survey spectra of c-probe/AuNPs/$Cu_3(PO_4)_2$ HNFs (**A**) survey spectrum, (**B**) C 1s, (**C**) Au 4f, (**D**) Cu 2p, (**E**) N 1s and (**F**) O 1s.

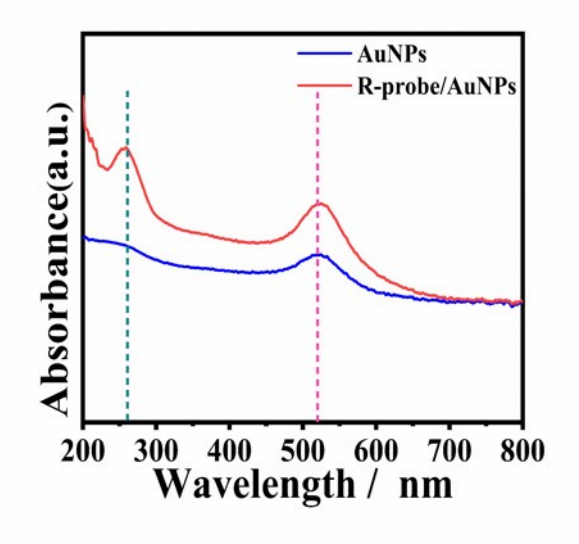


Fig. S5 UV-vis absorbance spectrum of AuNPs and R-probe/AuNPs.

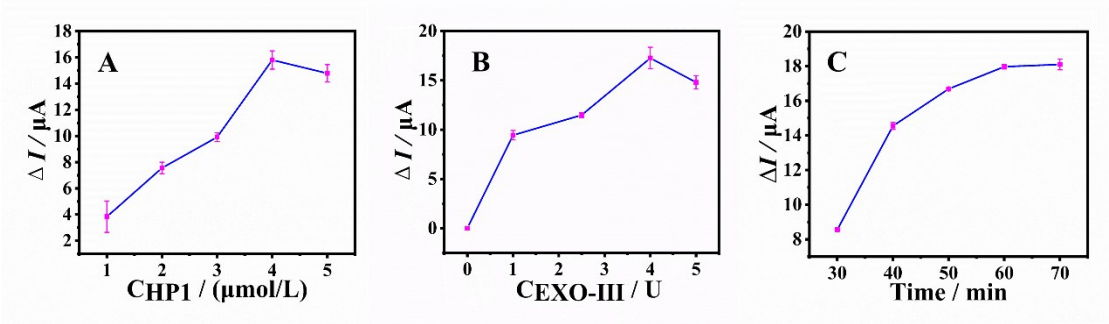


Fig. S6 (**A**) Effects of different concentrations of HP1, (**B**) Different concentrations of Exo III and (**C**) reaction time of Exo III. The concentration of Hg^{2+} was 10.00 pM.

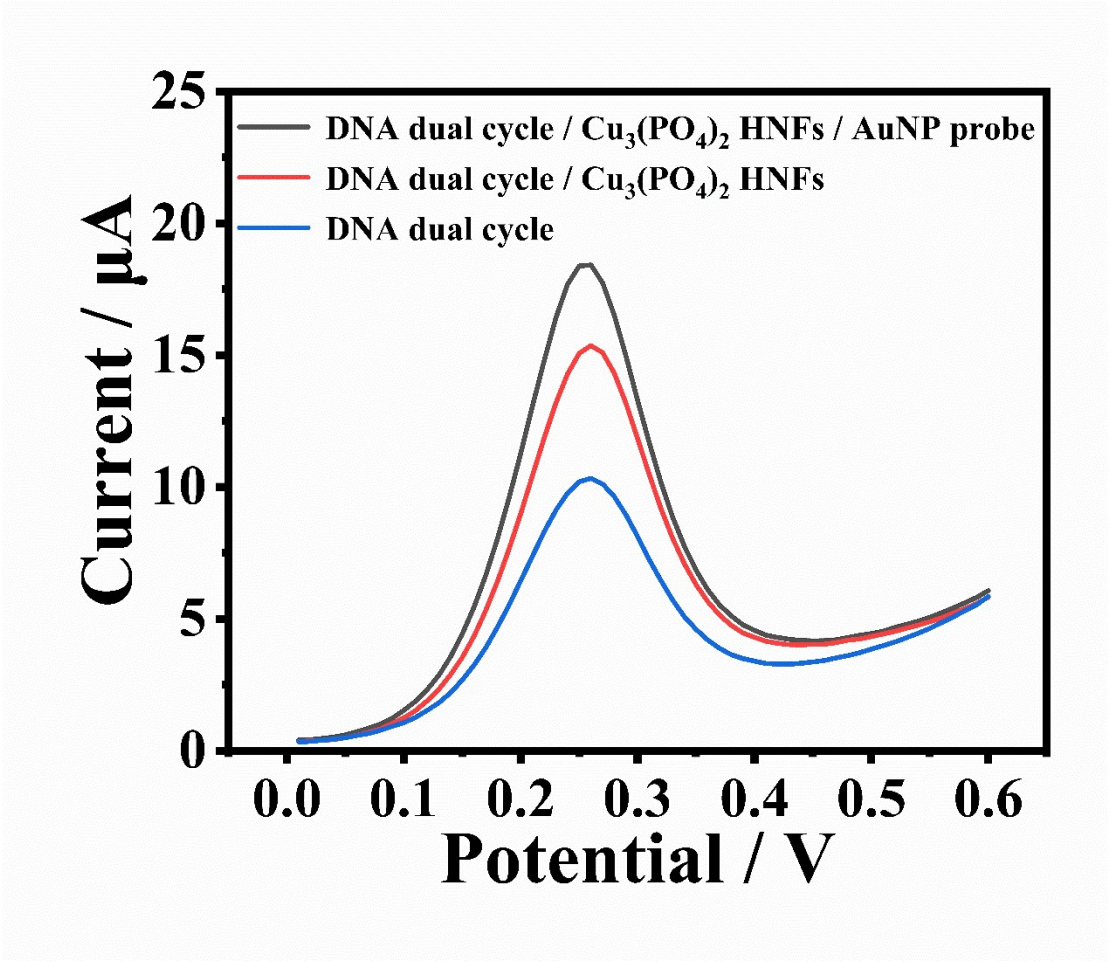


Fig. S7 SWV response of different capture probes. The concentration of Hg^{2+} was 10.00 pM.

Table. S1 Comparison of different Hg^{2+} detection methods based on T-Hg^{2+}-T.

Method	Dynamic range	Detection limit	References
Surface-initiated enzymatic polymerization	0.05-100 nM	0.024 nM	2
Colorimetric method	0.001-10 μM	0.90 nM	3
Fluorescence	0.10-10 μM	7.70 nM	4
SERS and fluorescence	0.03-3 μM	5.00 nM	5
Electrochemiluminescence	10 pM-1 mM	3.10 pM	6
Electrochemical biosensor	1 fM-10 nM	0.19 fM	This work

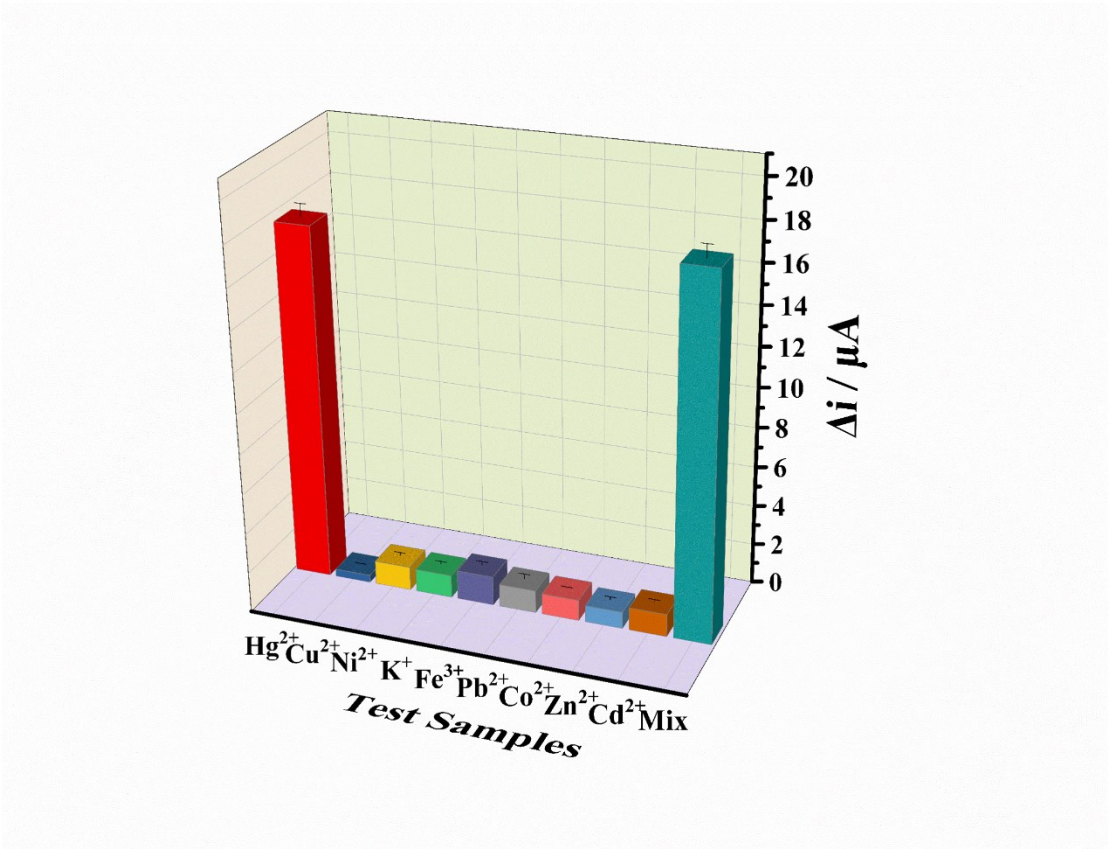


Fig. S8 Selectivity of the biosensor against other metal ions. The concentrations of Hg^{2+} and other metal ions were 10 pM and 10 nM respectively.

Table. S2 Recovery experiments of Hg^{2+} in drinking water

sample	Added Hg^{2+} (pM)	Detected concentration [a] (pM)	Relative standard deviation (RSD, %)	Recovery rate (%)
1	0.01	0.00943	4.35	94.30
2	0.20	0.21382	4.55	106.91
3	10.00	10.34245	4.40	103.42
4	500.00	475.05262	3.59	95.01

[a] Data were obtained from three independent experiments.

Table. S3 Sequences for nucleic acid used in this study.

name	Oligonucleotides sequences (5'−3')
HP1	CTTTAGGGTGGGGAGGGTGGGGCCCCACCCTTTTG-SH$(CH_2)_6$-
HP2	CCCCACCCTCCCCACCCTAAAGATGCCTCTAACCTAGCCTTTAGGGT GGGG
HP3	GTTATTAATGTGTGATGTCCTAAAGGCTAGGTTAGAGGCATCTTTA GGGTGGGGAGGCCTCTAACCTAGCC
c-probe	ACA TCA CAC ATT AAT AAC-SH$(CH_2)_6$-
r-probe 1	AAT GTG TGA TGT-SH$(CH_2)_6$-
r-probe 2	-SH$(CH_2)_6$-AAT GTG TGA TGT-Ferrocene

References

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