SUPPORTING INFORMATION

DNA Based Signal Amplified Molecularly Imprinted Polymers Electrochemical Sensor for Multiplex Detection

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Table S1 DNA sequence Probes and Targets

Name	**Sequence (5'-3')**
Probe 1	TTG TTC GTT TGT TGT TCC CCT TCT TCT TTC GTT CTT-NH_2
Probe 2	TTA AAG GAC CAG CAG GCG CAA CTA AAT TCA TGG-NH_2
Target DNA	CCA TGA ATT TAG TTG CGC CTG GTC CTT TAA
Target DNA a (Ta, onebase mismatched with Probe 1)	CCA TGA ATT TAG TTA CGC CTG GTC CTT TAA
Target DNA b (Tb, two bases mismatched with Probe 1)	CCA TGA ATT TAG TTA TGC CTG GTC CTT TAA

Materials and Reagents

Tris (hydroxymethyl) aminomethane (Tris), ExonucleaseIII (Exo III), 1-ethyl-3-(3-dimethyl-aminopro-pyl) carbodiimide hydrochloride (EDC), N-hydroxysulfo-succinimide (Sulfo-NHS), folateand other reagents were obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Folate receptor (FR), bovine hemoglobin (BHb), chronic viral hepatitis (CVH), bovine serum albumin (BSA), lysozyme (LZM), horseradish peroxidase (HRP), thrombin (Th), carcinoembryonic antigen (CEA), immunoglobulin G (IgG), Vitamins B_1, B_2, B_6, Vitamin C, tryptophan, cystein, histidine, amine terminated poly N-(iso-propyl acrylamide) (NH-PNIPAAm, average Mn = 2500), *o*-Phenylenediamine (*o*-PD, ≥ 98%), N, N' -Methylenebisacrylamide (MBA ≥ 98%) were purchased from Sigma–Aldrich and used as received. Ultrapure water (18.2 MΩ•cm) was purified with PSDK2-10-C (Beijing, China) and used throughout the experiments. The oligonucleotides were

used as provided and diluted in 20 mM Tris-HCl buffer solution (pH 7.4, containing 2 mM $MgCl_2$, 20 mM KCl and 100 mM NaCl) to obtain stock solutions with the concentration of 10 μM for further use.Hg^{2+} assay solution: 500 μL of 0.05 M Tris-HCl buffer (pH 7.4)containing 5 μM Hg^{2+}. FA-assay solution: 500 μL of 0.05 M Tris-HCl buffer (pH 7.4) solution containing the mixed reaction solution of 5 μM FA-P2 and 5 μM target DNA. Before use, each oligonucleotide was heated to 95 °C for 10 min, and then slowly cooled to 30 °C to remove aggregates. To evaluate the practicality of the proposed sensor for assay Hg^{2+}, five samples were collected at five sewage outfalls along the Qingyi River (Wuhu, China). Before use, these samples were diluted with equal volume of 0.05 M Tris-HCl (pH 7.4) followed by filtration through a column (packed with an anionic-exchange resin) to remove oils and other organic impurities. And to test the practicality of the proposed sensor for detection of target DNA, human serum samples were collected as an example. Human serum samples were kindly obtained from the Yijishan Hospital (Wuhu, China). Before use, human serum samples were centrifuged at 10,000 rpm for 15 min, following, the supernatant was diluted with Tris-HCl.

Apparatus

The pH values of phosphate buffer solution (PBS), Tris-HCl buffer solution and acetate buffer were measured with a glass electrode connected to a PHS-3C pH meter (Shanghai, China). Centrifugation was performed using a HERMLEZ 36 HK apparatus (Wehingen, Germany). Scanning electron microscopy (SEM) images of the prepared molecularly imprinted polymers electrode were obtained using Hitachi S-4800 SEM (operated at 10 kV). The removal of template molecule was performed in a thermostat steam bath vibrator (Jite Instrument Co., Ltd., Jiangsu, China). Electrochemical measurements, including electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV), and differential pulse voltammetry (DPV) were performed using an electrochemical workstation (CHI 660B, Shanghai Chenhua Instrument Co. Ltd., Shanghai, China) connected to a personal computer with a conventional three-electrode system: a platinum wire electrode as counter electrode, a saturated calomel electrode (SCE) as reference electrode and a bare or modified gold electrode (GE, 2 mm diameter) as working electrode.

Preparation of imprinted electrode

Prior to electropolymerisation, the gold working electrode was first polished with 0.3 and 0.05 μm alumina powder respectively, and then ultrasonically cleaned with ethanol and ultrapure water for 3 min each. Then the electrode was electrochemically cleaned by continuous scanning in 0.1 M H_2SO_4 between -0.2 and 1.55 V at 100 mV s^{-1} for 20 circles until a characteristic cyclic voltammogram of a clean gold electrode was obtained. The prepared electrode was dried under pure nitrogen atmosphere prior to further use.

Electrochemical Measurements Procedure

Electrochemical measurements of the prepared sensor for characterization and target determination including CV and DPV, were performed in 5 mL PBS (0.05 M, pH 7.4) containing 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) and 0.1 M KCl. To characterize the molecularly imprinted film, EIS was performed in the above mentioned solution with the frequency ranging from 0.1 Hz to 100 kHz and 5 mV as the amplitude, at a polarization potential of -0.18 V. CV was recorded in the potential range 0.0 to 0.6 V vs. SCE using scan rate of 50 mV s^{-1}. DPV was applied to quantify each concentration of test analyte over a potential range of 0.0 to 0.6 V at a scan rate of 50 mV s^{-1} and pulse amplitude of 25 mV.

Cyclic voltammogram of Electropolymerisation for Preparation of MIPs/GE

In this paper, the electrochemical sensors were based on molecularly imprinted polymers electrode. Cyclic voltammogram of electropolymerisation for preparation of MIPs/GE at gold electrode was shown as Figure S1. A typical cyclic voltammogram was recorded during the electropolymerisation of *o*-PD in the acetate buffer solution (pH 5.8) containing MBA, PNIPAAm and FA. A decrease in the anodic peak was observed during the continuous cycling scanning. This indicates that a process of irreversible monomer oxidation occurred on the electrode surface.[S1] The nonconductive film, which suppressed the anodic oxidation of monomer, was formed on the electrode surface.

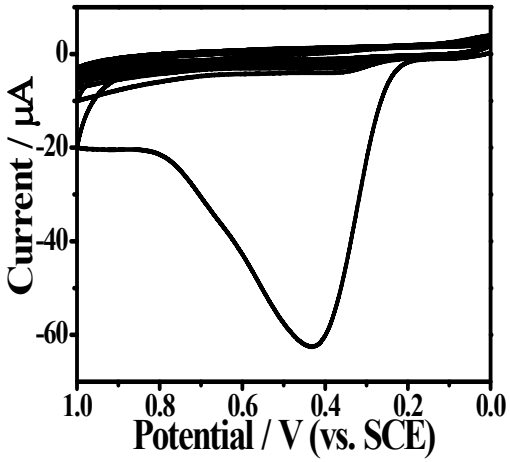

Figure S1. Cyclic voltammogram of electropolymerisation for preparation of MIPs/GE at goldelectrode.

EIS Characterization

Considering that EIS is a powerful tool for probing the features of a surface-modified electrode, EIS measurements were also used to characterize the surface change of the MIPs/GE. In the electrochemical impedance spectroscopy, the

semicircle portion at higher frequencies corresponds to the electron-transfer-limited process and the linear portion at lower frequencies may be ascribed to diffusion-controlled process.[S2, S3] Figure S2 illustrates the impedance spectra in the form of Nyquist diagrams. The semicircle diameter is equal to the electron-transfer resistance (R_{et}), which depends on the dielectric and insulating features at the electrode/electrolyte interface.[S4] The bare GE shows an almost straight line (curve a of Figure S2), which is a characteristic of a mass diffusion-limiting electron transfer process.[S5] The results show fast electron-transfer kinetics of $[Fe(CN)_6]^{3-/4-}$ on the bare GE. However, after the polymer membranes were electrodeposited onto the electrode surface (curve b of Figure S2), the impedances significantly enlarged and the semicircle diameters of the imprinted electrode were about 160 KΩ. The result indicates that the polymers membranes have larger blocking effect, resulting in a decreasing electron-transfer rate or increasing resistance to the flow of electrons. As shown in curve c of Figure S2, the diameter of semicircle significantly reduced at MIPs/GE. It is possible that the formation of imprinted sites on the electrode surface after the extraction of template FA provided channels for the diffusion of $[Fe(CN)_6]^{3-/4-}$ toward the electrode surface. After the MIPs/GE was immersed in Tris-HCl buffer solution containing 8×10^{-6} M FA, an increase of the semicircle diameter was observed (curve d of Figure S2). This may originate from the rebinding of FA in imprinted cavities, hindering the arrival of $[Fe(CN)_6]^{3-/4-}$ on the electrode surface. As compared to the MIPs/GE, the NIPs/GE shows a small change of resistance before (curve e of Figure S2) and after (curve f of Figure S2) it was immersed in the same FA solution, indicating the lack of imprinted cavities in the NIPs. According to the EIS results shown in Figure S2, the modification of the MIPs/GE was successfully performed and the prepared MIPs/GE had the capacity to selectively adsorb template FA.

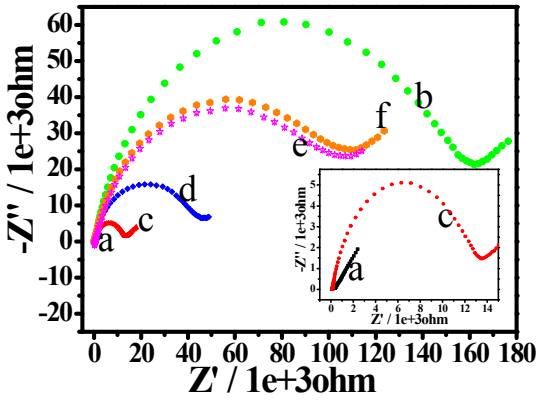

Figure S2. EIS measurements in PBS (pH=7.4) containing 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) and 0.1 M KCl for different modified electrodes: bare GE (a), polymers/GE (b), MIPs/GE (c), MIPs/GE after incubation in 8×10^{-6} M FA solution (d), NIPs/GE before (e) and after incubation in 8×10^{-6} M FA solution (f).

Optimization of External Conditions

As is well known that the amount of the imprinted polymers on the electrode surface strikingly influences the level of template molecules embedded into the polymers.[S6] The amount of polymers membranes can easily be adjusted by controlling the number of cycles during the electropolymerization process. Figure S3A showed the effect of different scanning cycles in the imprinting electropolymerization by the electrochemical response on MIPs/GE after incubation in FA (8×10-6 M) solution. As we can see from figure S3A, the current response obviously decreased from 2 to 20 cycles. While with a further increase of cycle number the current decreased negligibly. Therefore, 20 cycles can obtain a suitable membrane thickness to provide the highest sensitivity to FA analyte and was used in the subsequent experiments. Following the discussion of the effect of electropolymerization scan cycles for the electrochemicalamplification, the incubation pH was also an important factor for the rebinding of FA into the cavities, which further influenced the electrochemical response. In order to study the incubation pH effect on the signal, the current response of MIPs/GE to $[Fe(CN)_6]^{3-/4-}$ in Tris-HCl solution was recorded. The MIPs/GE was immersed in 0.05 M Tris-HCl with the pH value increased in the range of 4.0-9.5 in the presence of constant concentration of FA (8×10^{-6} M). The current responses of these electrodes inTris-HCl solution containing $[Fe(CN)_6]^{3-/4-}$ were shown as Figure S3B. It is obvious that the current values decreased with the pH value changing in the range of 4.0-7.5, which meant more FA binding in the cavities. Considering the rebinding conditions, 0.05 M Tris-HCl (pH 7.4) was used in the subsequent experiments. In addition, the time of rebinding of FA on the MIPs/GE was an important factor for sensitive detection of FA. The rebinding dynamics experiment was performed by recording the DPV response of $[Fe(CN)_6]^{3-/4-}$on the MIPs/GE incubated in FA solution (8×10^{-6} M) for different periods. As shown in Figure S3C, when the incubation time was more than 30 min, the current reached about 7.366 μA indicating that the rebinding may be saturated within almost 30 min. This demonstrates that the obtained sensor possessed a fast response time for the site accessibility.

Prior to the detection of FR, the effect of the specifically binding time of FR with FA on the current response was studied. As shown in Figure S4D, the responses increased with the binding time. When the specifically binding time was more than 45 min, a slight increase was observed, suggesting that the reaction of FR with FA was fulfilled within ~45 min. Therefore, 45 min was chosen as the binding time.

Prior to the detection of Hg^{2+} and target DNA, the effect of incubation time between the sensor and Hg^{2+}on the electrochemical signal of the sensor was investigated (1×10^{-4} M $Hg^{2+}$was used in this case), and the effects of Exo III concentration and digestion time on the electrochemical responses were also conducted (the conditions described earlier in supplemental information, shown as Figure S1). As indicated in Figure S3E, the responses decreased with the increment of incubation time, and reached a plateau after 40 min. Longer incubation time did not obviously improve the electrochemical response. Hence, an incubation time of 40 min

was selected for sensitive detection of Hg^{2+}at an acceptable throughput. Besides the digestion effect of ExoIII, another role of it is the signal amplification. As we could observe from Figure S4F, the responses decreased when the concentration of Exo III increased in the range of 0 to 300 U mL^{-1}. Figure S4G shows the current response in the presence of 200 U mL^{-1}Exo IIIin Tris-HCl buffer (pH 7.4) for a period ranging from 0 to 50 min. After that, the MIPs electrodes were incubated in the assay solution. The electrochemical signal was measured in probe solution and plotted against the time of digestion. It was obvious that (Figure S3G), the currents reduced gradually with the time of digestion before reaching saturation after 30 min. The continuously decreasing change of current signal indicates that the designed Exo III-assisted autocatalytic target recycling indeed took place. While the signal saturation at 30 min suggests the Hg^{2+} were almost liberated from the duplex DNA probe. Therefore, 200 U mL^{-1} of Exo III and 30 min digestion time were chosen as the optimized conditions in the subsequent experiment.

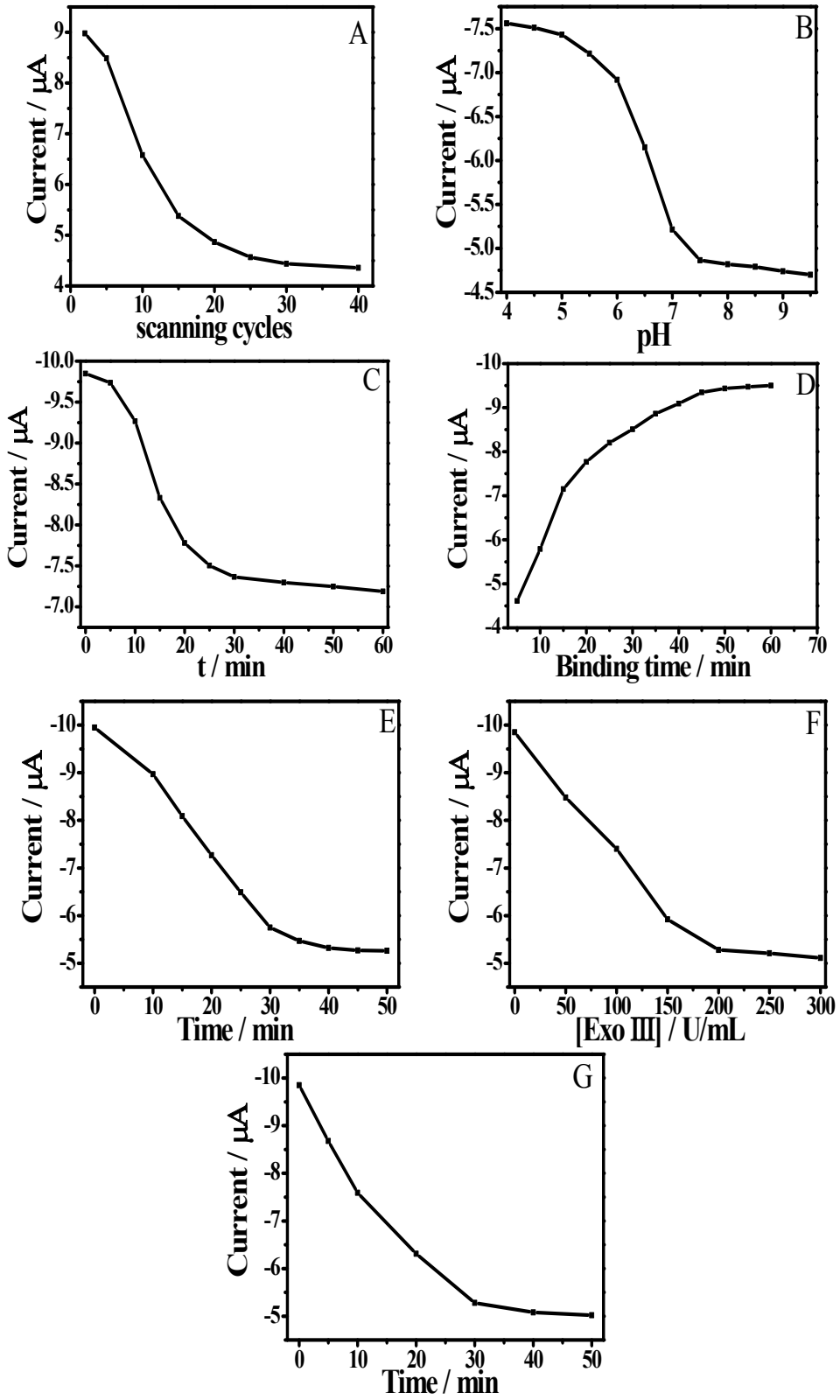

Figure S3. Optimization of experimental conditions: (A) electropolymerization scan cycles, (B) pH of rebinding solution and (C) the time of rebinding of FA (8×10^{-6} M), (D) the effect of the specifically binding time of FR with FA on the current response, the effect of different incubating time in 1×10^{-4} M Hg^{2+}solution (E), the concentration (F) of Exo III and digestion time (G) on the current responses.

Detection of FA

Based on the current response of $[Fe(CN)_6]^{3-/4-}$ on the MIPs/GE in Figure 2C and under the discussed optimized conditions (the conditions described earlier in supplemental information, shown as Figure S3), the MIPs/GE was incubated in solutions containing different concentrations of FA for its detection.

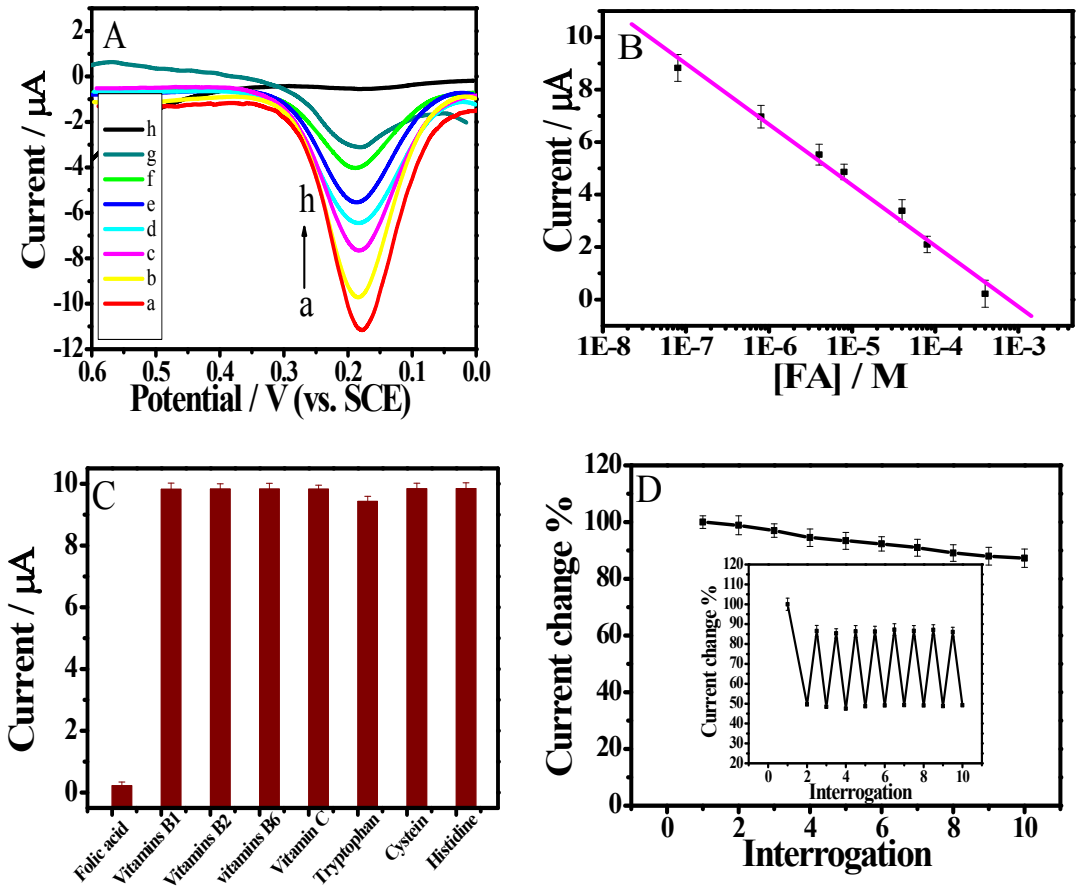

Figure S4. (A) DPV of FA: (a–h) 0; 8×10^{-8}; 8×10^{-7}; 4×10^{-6}; 8×10^{-6}; 4×10^{-5}; 8×10^{-5}; 4×10^{-4} M in Tris-HCl buffer, (B) calibration curve of current vs. the concentration of FA, (C) Selectivity of FA analysis in Tris-HCl buffer (4×10^{-4} FA or equal concentration of different potential interfering substances as example), (D) CV responses of MIPs/GE for 10 cycles and Sensor interrogation-regeneration plot (inset).

The Gel-Electrophoresis Characterization

We used agarose gel electrophoresis to confirm the degradation process of probe DNA by Exo III. As shown in Figure S5,Usually, it takes some time to form the T–Hg^{2+}–T coordination. there were bright bands on the lanes where FA-linked probe 1 was subjected to Exo III treatment or not (lane 1 and 2). However, when FA-linked probe 1 was folded into a duplex structure by been incubated in Hg^{2+} solution, the formed conjugates without Exo III treatment moved significantly slower than FA-linked probe 1 (lane 3) and bands for the Exo III-treated the formed conjugates appeared as continuous smears with very weak visible (lane 4).This proved that degradation of the duplex DNA by Exo III had occurred because the digested DNA probes would migrate out of the gel, which provided immediate evidence for the postulated mechanism of our Exo III-mediated conformational switch converted the T-rich probe for assay.

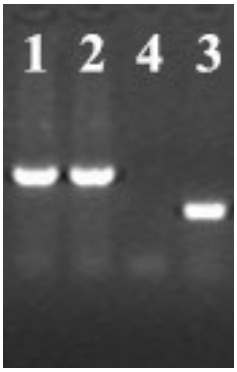

Figure S5. Agarose gel electrophoresis image for Hg^{2+} assay: lane 1, 5 μMFA-linked probe 1; lane 2, 5 μM FA-linked probe 1 digested by 200 U mL^{-1} of Exo III for 30 min; lane 3, 5 μMFA-linked probe 1 incubated in 10^{-6} M Hg^{2+} for 40 min; lane 4, 5 μM FA-linked probe 1 incubated in 10^{-6} M Hg^{2+} for 40 min and digested by 200 U mL^{-1} of Exo III for 30 min.

Table S2. Performance of MIPs sensor in comparison with other sensors for Hg^{2+} detection

Number of analytes	Linear range	Detection limit	References
One	--	10 nM	Ref. 41
One	0.5 nM to 2 μM	0.5 nM	Ref. 42
One	10-2000 nM	4.5 nM	Ref. 43
One	0.1 nM to 5 μM	0.06 nM	Ref. 44
One	10 - 800 nM	10 nM	Ref. 45
Four	10^{-10}-10^{-4} M	3.45 pM	This paper

Table S3. Determination of Hg^{2+} added in the Qingyi River.

Number	Added (nM)	Founded (n=3; nM)	Recovery
1	0	1.51±0.11	-
2	5	6.20±0.70	0.938
3	10	10.76±0.81	0.925
4	15	16.01±2.96	0.967
5	30	32.71±3.01	1.040
6	50	50.39±7.95	0.976

Table S4. Determination of target DNA added in diluted human serum samples.

Number	Added (μM)	Found (n=3; μM)	Recovery
1	1.0	0.925±0.08	0.925
2	1.5	1.39±0.07	0.926
3	2.0	1.91±0.2	0.955
4	3.0	3.12 ±0.3	1.040
5	5.0	4.88± 0.5	0.976

References

(S1) Karimian, N.; Zavar, M. H. A.; Chamsaz, M.; Turner, A. P. F.; Tiwari, A. On/off-switchable electrochemical folic acid sensor based on molecularly imprinted polymer electrode. *Electrochem. Commun.* **2013,** *36*, 92-95.

(S2) Chen, X. H.; Guo, H.; Yi, J.; Hu, J. Q. Fabrication of AucoreCo3O4shell/PAA/HRP Composite Film for Direct Electrochemistry and Hydrogen Peroxide Sensor Applications. *Sens. Mater.* **2009,** *21* (8), 433-444.

(S3) Pei, R.; Cheng, Z.; Wang, E.; Yang, X. Amplification of antigen–antibody interactions based on biotin labeled protein–streptavidin network complex using impedance spectroscopy. *Biosens. & Bioelectron.***2001,** *16* (6), 355-361.

(S4) Han, J.; Zhuo, Y.; Chai, Y.; Gui, G.; Zhao, M.; Zhu, Q.; Yuan, R. A signal amplification strategy using the cascade catalysis of gold nanoclusters and glucose dehydrogenase for ultrasensitive detection of thrombin. *Biosens. & Bioelectron.* **2013,** *50*, 161-166.

(S5) Ren, Y.; Deng, H.; Shen, W.; Gao, Z. A highly sensitive and selective electrochemical biosensor for direct detection of microRNAs in serum. *Anal. Chem.* **2013,** *85* (9), 4784-4789.

(S6) Liu, Y.; Zhu, L.; Zhang, Y.; Tang, H. Electrochemical sensoring of 2,4-dinitrophenol by using composites of graphene oxide with surface molecular imprinted polymer. *Sens. & Actuat. B: Chem.* **2012,** *171-172*, 1151-1158.