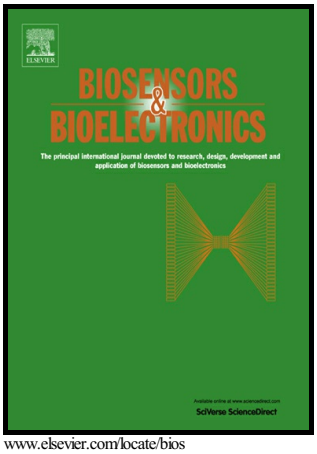

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Ultrasensitive electrochemical sensor for Hg^{2+} by using hybridization chain reaction coupled with Ag@Au core-shell nanoparticles

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ABSTRACT:

A novel electrochemical biosensor for Hg^{2+} detection was reported by using DNA-based hybridization chain reaction (HCR) coupled with positively charged Ag@Au core-shell nanoparticles ((+)Ag@Au CSNPs) amplification. To construct the sensor, capture probe (CP) was firstly immobilized onto the surface of glass carbon electrode (GCE). In the presence of Hg^{2+}, the sandwiched complex can be formed between the immobilized CP on the electrode surface and the detection probe (DP) modified on the gold nanoparticles (AuNPs) based on T-Hg^{2+}-T coordination chemistry. The carried DP then opened two ferrocene (Fc) modified hairpin DNA (H_1 and H_2) in sequence and propagated the happen of HCR to form a nicked double-helix. Numerous Fc molecules were formed on the neighboring probe and produced an obvious electrochemical signal. Moreover, (+)Ag@Au CSNPs were assembly onto such dsDNA polymers as electrochemical signal enhancer. Under optimal conditions, such sensor presents good electrochemical responses for Hg^{2+} detection with a detection limit of 3.6 pM. Importantly, the methodology has high selectivity for Hg^{2+} detection.

Keywords: Electrochemical sensor, Hg^{2+} detection, hybridization chain reaction, positively charged Ag@Au core-shell nanoparticles

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Introduction

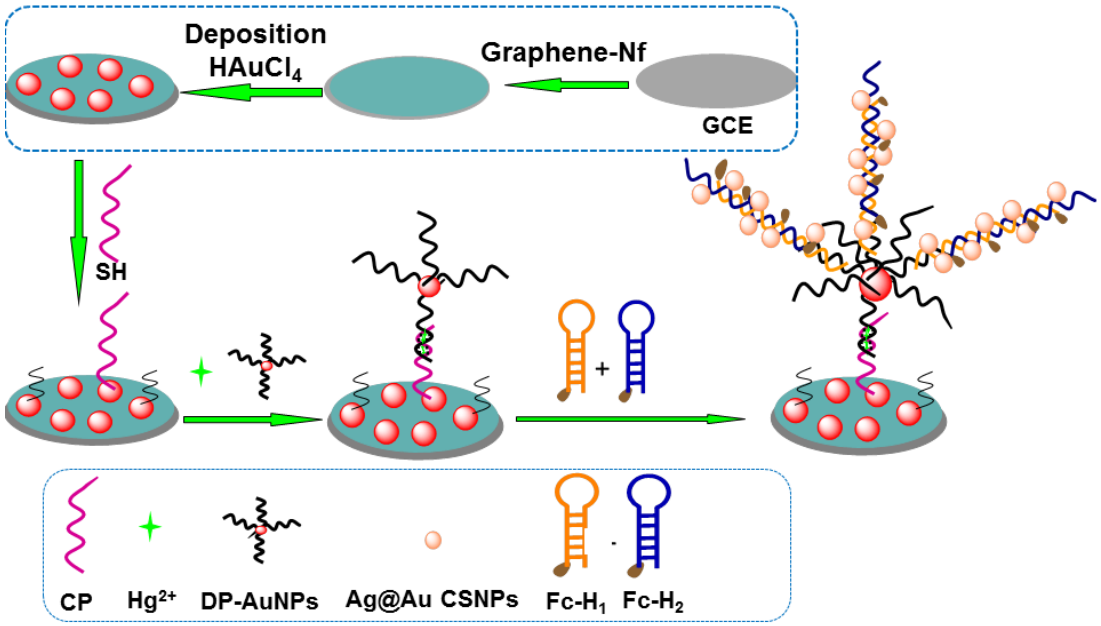
Hg^{2+} is one of the most dangerous metal ions with high toxicity, the emission of it will has adverse effects to environment, and the water sources pollution by Hg^{2+} is severe in many countries. A small amount of Hg^{2+} can also cause long-term damage to human organs such as brain, nervous system, kidney and liver (Wang et al., 2010, Nolan et al., 2008 and Zahir et al., 2005). Thus, developing facile, sensitive, and reliable methods for efficient detection of Hg^{2+} is essential.

Since Ono and co-workers reported the specific binding property of Hg^{2+} with T-T mismatched base pairs (One et al., 2004), oligonucleotides based T-Hg^{2+}-T structures have been widely used in colorimetric and fluorescent Hg^{2+} analysis coupling with advanced materials including gold nanoparticles (Chen et al., 2014; Huang et al., 2013), silver nanoclusters (Deng et al., 2011 and MacLean et al., 2013), quantum dots (Zheng et al., 2014; Ma et al., 2013), fluorescent probe (Li et al., 2013 and Fang et al., 2015), magnetic nanoparticles (Wang et al., 2014 and Zheng et al., 2013) and catalytic DNAzyme (Jia et al., 2011 and Liu et al., 2007). However, there remain some drawbacks including limited sensitivity or bad biocompatibility. Compared to other methods, the inherent advantages of the electrochemical methods, such as good portability, low cost, easy operation, high sensitivity and good selectivity, make them most attractive for fabricating Hg^{2+} sensors (Zhang et al., 2015; Xuan et al., 2013; Zhuang et al., 2013).

Nowadays, a number of amplification protocols such as nuclease-based target-recycling (Wang et al., 2014; Chen et al., 2014; Ma et al., 2013; Wang et al., 2014), rolling circle amplification (RCA) (Chen et al., 2015 and Bi et al., 2013) and hybridization chain reaction (HCR) have attracted great attention. Among these strategies, HCR based methods can realize the enzyme-free detection of Hg^{2+} with high sensitivity, which was driven by the self-assembly of two stable species of DNA hairpins (Huang et al., 2011) that have the high molecular-weight with a length corresponding to over 1000 base pairs (bp), structural flexibility with high intrinsic viscosity and rich negative charges. For instance, Huang and co-workers reported a

fluorescent biosensor for Hg^{2+} by using HCR coupled with graphene oxide amplification (Huang et al., 2014); Tang et al realized the Hg^{2+} detection based on HCR and silver nanowire amplification (Tang et al., 2015); Liu groups constructed a bio-inspired DNA sensor for Hg^{2+} (Xu et al., 2015). Moreover, HCR can also exhibit the advantages of low background, mild reaction, and PCR-like sensitivity (Ge et al., 2014 and Zhang et al., 2012).

Herein, a sensitive sensor for Hg^{2+} detection was reported by using DNA-based HCR coupled with positively charged Ag@Au core-shell nanoparticles ((+)Ag@Au CSNPs) amplification (Fig. 1). For constructing such a sensor, graphene-nafion (graphene-Nf) film was introduced to enlarge the electrode surface. Then gold nanoparticles (AuNPs) were electrodeposited on the modified electrode to provide a rough and stable surface for the immobilization of capture probe (CP). Subsequently, CP would hybridize with the detection probe (DP) that modified onto the surface of AuNPs based on T-Hg^{2+}-T coordination chemistry in the presence of Hg^{2+}. After that, the carried DP then opened two ferrocene (Fc) modified hairpin DNA (H_1 and H_2) in sequence and propagated the happen of HCR for the formation of extended dsDNA polymers. Furthermore, (+)Ag@Au CSNPs were adsorbed onto such dsDNA polymers surface to amplify the electrochemical signal. This work exhibits several merits: Firstly, HCR was used for Hg^{2+} detection to amplify electrochemical signal. In addition, (+)Ag@Au CSNPs that exhibit improved physical and chemical properties than individual AuNPs or AgNPs due to a localized electric field enhancement in the core-shell structure (Guha et al., 2011) was used to realize the dual signal amplification, which can electrostatically adsorbed onto the negatively charged surface of double-stranded DNA without any modification. Meantime, such CSNPs can make up for the deficiencies of both AuNPs and AgNPs (Wu et al., 2012 and Dong et al., 2013). Moreover, because of the highly specific T-Hg^{2+}-T coordination chemistry, such sensor showed high selectivity for Hg^{2+} detection. Thus, the current method holds promise as a great choice for sensitive and selective detection of Hg^{2+}.



2. Experimental

2.1. Materials

$Hg(Oc)_2 \cdot 2H_2O$, tri-(2-carboxyethyl) phosphine (TCEP), cetyltrimethyl ammonium bromide (CTAB), 6-mercapto-1-hexanol (MCH), chloroauric acid ($HAuCl_4 \cdot 3H_2O$), nafion (Nf) and sodium borohydride ($NaBH_4$) were purchased from Aladdin Biotech CO. Ltd. (Beijing, China). Graphene was purchased from Nanjing Xianfeng nano Co. (Nanjing, China). Ultra-pure water was obtained from Heal Force Smart-Nultra-pure water system and used for all of the experiments. 10.0 mM of tris-buffer (pH 7.5) was used throughout every experiment. All other chemicals were analytical grade and used without further purification. All oligonucleotides were purchased from Shanghai Sangon Biotech Co. Ltd, and the sequences of the oligonucleotide were as follows:

Capture probe (CP): 5'- TTATCCCTCGCCGTTTCCTTGTCT-$(CH_2)_6$-SH-3'

Detection probe (DP): 5'-AGTCTAGGATTCGGCGTGGGTTAA-SH-3'

H_1:5'-TTAACCCACGCCGAATCCTAGACTCAAAGTAGTCTAGGATTCGGC GTG-$(CH_2)_6$-Fc-3'

H_2:5'-AGTCTAGGATTCGGCGTGGGTTAACACGCCGAATCCTAGACTACT TTG-$(CH_2)_6$-Fc-3'

*2.2. Preparation of (+)Ag@*Au CSNPs

(+)Ag@Au CSNPs were prepared by deposition of Au on the preformed Ag nanoparticles (about 22 nm). The initial Ag colloid was prepared following the literature (Wei et al., 2005). Briefly, 5 mL of freshly prepared aqueous sodium borohydride solution (1%) was added to 30 mL of silver nitrate (5 mM) aqueous solution under vigorous stirring until the color of such colloids changed to yellow-green. Afterward, (+)Ag@Au CSNPs were prepared according to the literature with a slight modification (Cui et al., 2006): Firstly, 3.0 mL of $HAuCl_4$ (1.0 mM), 3.0 of mL NH_2OH•HCl (1.0 mM) and 2 mL of CTAB were added to 15 mL of Ag colloid dropwise by three separate pipets upon vigorous stirring, and then the mixture was allowed to stirring continually for 45 min. The average size of such CSNPs estimated from TEM was about 32 nm (Fig. S1A). Meantime, zeta potential analysis illustrated that (+)Ag@Au CSNPs was positively charged (Fig. S1D).

2.3. Fabrication of AuNPs/graphene-Nf modified electrode

Firstly, the glass carbon electrodes (GCE, φ=3 mm, CHI) were polished successively with 1.0, 0.3, 0.05 μm alumina slurry to obtain a mirror surface and sonicated in an ethanol/water bath for 5 min. Then, AuNPs/graphene-Nf modified electrode surface was obtained according to the literature (Gui et al., 2014). Subsequently, the AuNPs/graphene-Nf modified electrode was immersed into 3.0 μM of thiolated CP and incubated overnight at room temperature (before modification, the disulfide bond at the 3' end of CP was cleaved by using Tri-(2-carboxyethyl) phosphine (TCEP)). Finally, 10 μL of 6-mercapto-1-hexanol (MCH, 2.0 mM) was drop-coated onto the modified electrode surface and incubated for 60 min to block the nonspecific binding sites to obtain CP/AuNPs/graphene-Nf modified electrode.

2.4. Preparation of DP modified AuNPs

AuNPs that used for the preparation of DP-AuNPs probes were prepared according to the literature (Jin et al., 2003), 1.0 mM of $HAuCl_4$ (20 mL) was reduced using 2 mL of 38.8 mM sodium citrate under vigorous stirring. The average sizes of them

estimated from transmission electron microscopy (TEM) analysis were about 16 nm. Then, the DP modification onto AuNPs was constructed as follows: Firstly, 5.0 OD of the DP was dissolved in 50 mL of acetic acid buffer (pH 5.0, 10 mM) and deprotected for 2.0 h by using 4.0 mL of TCEP (20 mM). Then, the above DNA solution was added into 4.0 mL of AuNPs (10.6 nM) solution and stored for 16 h at room temperature, followed by another 44 h incubation, during which 0.1 M of NaCl was added by a stepwise manner. After that, excess reagents were removed by centrifugation at 13,500 rpm for three times (the red oily precipitate that contained DP-AuNPs was washed by using 10 mM PBS each time). Finally, the red colloid solution was dissolved in 10 mM of PBS prior to use (300 nM).

2.5. Electrochemical measurement

The proposed sensor was immersed into DP modified AuNPs solution containing different concentration of Hg^{2+} and incubated for 30 min at 37 °C. Then, HCR happened by immersing above sensor into the mixture containing 3.0 μM of H_1 and H_2 (1:1 ratio). Subsequently, 10 μL of (+)Ag@Au CSNPs homogeneous solution was drop-coated onto the modified electrode surface and incubated for 50 min. Fig. 1 illustrated the fabrication process of the proposed sensor. The resultant electrode was rinsed with tris-buffer (pH 7.5) thoroughly after each step of modification. Finally, the electrochemical characteristics of the sensor were investigated in tris-buffer by using differential pulse experiments (DPV) from 0.0 mV to 500 mV at room temperature.

3. Results and discussion

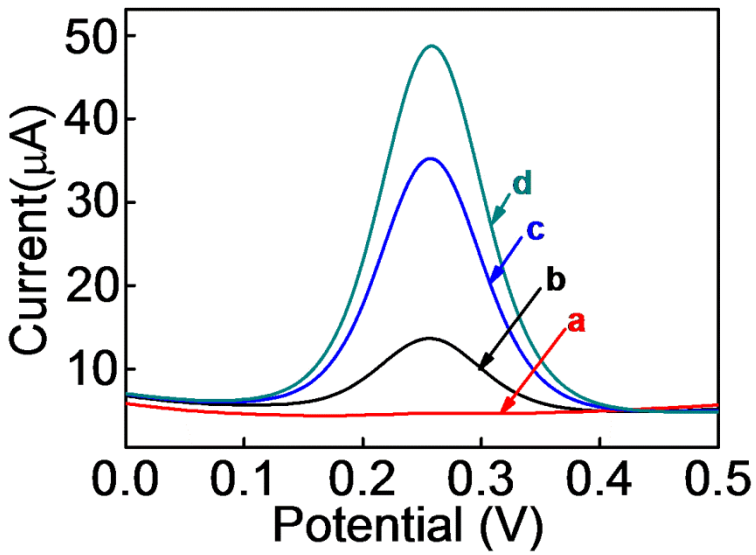
3.1. Characterization of Ag@Au CSNPs

The morphology of (+)Ag@Au CSNPs was characterized by TEM. The typical TEM image demonstrated that the average size of such CSNPs was about 32 nm with a central bright and outer dark core-shell nanostructure (Fig. S1A), which was in accord with literature reports (Guha et al., 2011). Meantime, from uv/vis absorption spectra in Fig. S1C it could be seen that a characteristic absorption peak of pure AgNPs was appeared at 393 nm (curve a) while there were two absorption peaks of

AgNPs and AuNPs appeared after the formation of (+)Ag@Au CSNPs (curve b). Moreover, zeta potential analysis was also constructed to testify the positively charged properties of Ag@Au CSNPs (Fig. S1D).

3.2. The electrochemical characterization of the sensor

In order to confirm the successful fabrication of the proposed sensor, the electrochemical behavior of the sensor was characterized by DPV. As seen from curve a in Fig. 2, no redox peak was observed about the CP and DP modified electrode in the presence of 1.0 nM of Hg^{2+}. After H_1 hybridized with DP, an obvious redox peak was observed, which attributes to the high conductivity of ferrocene that modified on H_1 (curve b). When HCR happened between H_1 and H_2, the redox peak increased, owing to the formation of numerous Fc on the electrode surface (curve c). After the assembly of (+)Ag@Au CSNPs on the surface of dsDNA polymer, the redox peak increased again, because of the properties that such nanoparticles can promote the electron transfer (curve d). Moreover, SEM images provided more information about the formation of (+)Ag@Au CSNPs-DNA complex on the electrode surface (Fig. S1B).



3.3. Optimization of experimental conditions

In this work, detection of Hg^{2+} could be realized by estimating the current change,

which was accompanied by the incubation of Hg^{2+} with CP and DP. So, the incubation time of 1.0 nM Hg^{2+} with two DNA probes was investigated in Fig. S2A, the current intensity increased along with the increase of the incubation time in the range of 5-30 min, and then reached a platform between 30-60 min. Such results revealed that Hg^{2+} could react effectively with two DNA probes within 30 min. Thus, 30 min was selected for the research.

The temperature that may affect the formation of T-Hg^{2+}-T chemistry was studied in Fig. S2B, the current intensity was increased linearly when the incubation temperature between CP and DP in the presence of 1.0 nM of Hg^{2+} increased from 15 °C to 37 °C. To realize the optimal respond performance of such sensor, we selected 37 °C as the incubation temperature for the formation of T-Hg^{2+}-T chemistry.

The degree of HCR formation mainly based on the concentration of H_1 and H_2 strands. Thus, the sensitivity of the sensor could be affected by the concentration of H_1 and H_2. As shown in Fig. S2C, the current intensity was proportional to the H_1 and H_2 concentration between 0.1 μM and 3.0 μM, then reached a plateau when the H_1 and H_2 concentration was higher than 3.0 μM. So, 3.0 μM was used in the experiments of this work.

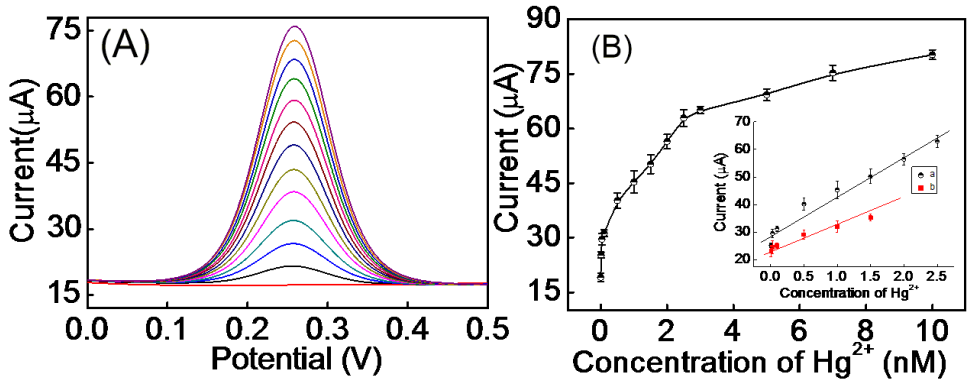
Hybridization time between H_1 and H_2 strands has an important role on the analytical performance of the proposed sensor. As shown in Fig. S2D, the current intensity enhanced with increasing hybridization time between H_1 and H_2 in the presence of 1.0 nM Hg^{2+}. When the hybridization time reached 40 min, the current intensity increased slightly. Thus, 40 min was selected as the optimal hybridization time for HCR.

Considering the adsorption of (+)Ag@Au CSNPs to negatively charged dsDNA polymers was another important factor, we also investigated the effect of the adsorption time of (+)Ag@Au CSNPs onto dsDNA polymers upon addition of 1.0 nM Hg^{2+}. From Fig. S2E we could see that the current signal increased gradually with increasing adsorption time of (+)Ag@Au CSNPs between 5 to 70 min. However, it should be noted that when the adsorption time was higher than 50 min, the current intensity increased slightly. Thus, the results indicated that the optimum adsorption

time of (+)Ag@Au CSNPs was 50 min.

3.4. Sensitivity of the sensor

Under the above optimal experimental conditions, the sensitivity of the proposed method was examined upon addition of different concentrations of Hg^{2+}. From the results shown in Fig. 3A and B, it could be seen that the electrochemical current increased accordingly as the concentration of Hg^{2+} is varied from 10 pM to 2.5 nM (inset of Fig. 3B, linear a), with a detection limit of 3.6 pM (3σ/slope). The regression equation is I=27.85+14.69 c (c: nM, R^2=0.992), with an acceptable relative standard deviations (RSD) of 2.93% (n=5). The results demonstrated that the proposed method could be used to detect Hg^{2+} concentration quantitatively. For comparison, the current response of the proposed sensor was recorded without the adsorption of (+)Ag@Au CSNPs, and the sensor showed a linear range to Hg^{2+} from 30 pM to 1.5 nM (inset of Fig. 3B, linear b, relative standard deviations (RSD) was 2.56 % (n=5)). The higher sensitivity and wider linear range of the proposed immunoassay should be attributed to the dual signal amplification of HCR coupled with (+)Ag@Au CSNPs.



3.5. Stability, Repeatability and Selectivity of the sensor.

To investigate the stability of the sensor, melting temperature experiments were constructed for double-stranded DNA (dsDNA) formed between CP and DP in the absence and presence of 1.0 nM Hg^{2+}. As shown in Fig. S3A, the melting temperature (T_m) was 60 °C for dsDNA in the presence of Hg^{2+}, while it was 41 °C for dsDNA in the absence of Hg^{2+}. These results obviously revealed that the stability of dsDNA

greatly improved due to the formation of highly stable T-Hg^{2+}-T base pairs. To further study the stability of the sensor, cyclic voltammetry (CV) experiments were executed by using one sensor for scanning 15 cycles continuously. As shown in Fig. S3B, the relative standard deviation (RSD) of the CV was 0.67% after continuous CV scans for 15 cycles, such result indicated that the proposed sensor had excellent stability. Meantime, the repeatability of the sensor was evaluated with four proposed sensors, and such four sensors exhibited close DPV response to 1.0 nM of Hg^{2+} with a RSD of 2.36%, which demonstrated that the proposed sensor was acceptable for Hg^{2+} detection.

The selectivity of the proposed sensor was evaluated based on detecting the current intensity change (ΔD) in the presence of 1.0 nM of Hg^{2+} upon addition of 10.0 nM, 100.0 nM and 1.0 μM of different metal ions including Hg^{2+}, Ca^{2+}, Mg^{2+}, Al^{3+}, Ba^{2+}, Cd^{2+}, Cu^{2+}, Cr^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+} respectively. As shown in Fig. S4, the interference of other ions to Hg^{2+} was less than 10.0 %. These results demonstrated the good ion selectivity of the assay, such high selectivity of the sensor mainly because of the highly specificity of T-Hg^{2+}-T coordination chemistry.

3.6. Hg^{2+} detection in real samples.

To investigate the effectiveness of this Hg^{2+} sensor in practical application, Hg^{2+} detection in water samples that collected from laboratory tap water and drinking water was constructed, and no Hg^{2+} was founded. Then the water samples were diluted 50-fold with tris-buffer and spiked with 50 pM, 100 pM and 500 pM of Hg^{2+}, a good recovery ranging from 98.9% to 112.3% and a RSD between 1.63% and 3.12% were obtained by using the addition and recovery experiments (each sample was parallel detected 3 times, Table S1). These results demonstrate that our detection platform was applicable for Hg^{2+} analysis in natural water samples.

4. Conclusion

In the present study, a novel and sensitive electrochemical sensor based upon the dual signal amplification of HCR and (+)Ag@Au CSNPs was developed for Hg^{2+} detection, such detection was realized based on monitoring the current change after the incubation of Hg^{2+} with CP and DP. The sensor utilized HCR as one of the amplification strategies, which is an isothermal amplification method and has significant amplification ability without needing protease. In addition, the application of graphene-Nf greatly increasing the electrode surface for DNA immobilization. Moreover, (+)Au@Ag CSNPs can adsorb onto the negatively charged surface of dsDNA polymers based on electrostatic adsorption, which greatly avoid the complicated label procedure. On basis of their excellent performance, this sensor achieved a detection limit of 3.6 pM and exhibited excellent selectivity. Moreover, satisfactory results were obtained for Hg^{2+} detection in real sample because of the highly specific T-Hg^{2+}-T coordination chemistry.

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Figure Captions

Fig. 1. Scheme of the electrochemical sensor for Hg^{2+} detection.

Fig. 2. DPV of the different modified electrodes in 10 mM tris-buffer solution (pH=7.5): (a) bare GCE; (b) H1/DP/CP/graphene-Nf/GCE; (c) DP/CP/graphene-Nf/GCE after the happen of HCR; (d) HCR based DP/CP/graphene-Nf/GCE after the adsorption of (+)Ag@Au CSNPs (b, c and d were detected in the presence of 1.0 nM Hg^{2+}).

Fig. 3. DPV experiments for Hg^{2+} detection in 10 mM tris-buffer solution (pH 7.5). (Inset: Calibration curve for two sensors: Linear a is HCR based DP/CP/graphene-Nf/GCE with the adsorption of (+)Ag@Au CSNPs and linear is HCR based DP/CP/graphene-Nf/GCE).

*Highlights

A novel sensor for the sensitive detection of Hg^{2+} was developed with several merits:

1. Hybridization chain reaction was used to improve the sensitivity of Hg^{2+} detection.
2. Positively charged Ag@Au core-shell nanoparticles were introduced to further improve sensor sensitivity.
3. A low detection limit of 3.6 pM was obtained for Hg^{2+} detection.
4. AuNPs/graphene-Nf film was used to enlarge the electrode surface for DNA immobilization.
5. The proposed method showed good selectivity for Hg^{2+} detection due to T-Hg^{2+}-T chemistry.