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A sensitive electrochemical aptasensing platform based on exonuclease recycling amplification and host-guest recognition for detection of breast cancer biomarker HER2

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ABSTRACT

In this study, a novel and sensitive electrochemical aptasensing platform was fabricated for the detection of the breast cancer biomarker HER2. HER2 aptamer was firstly hybridized with ferrocene-labeled DNA/Au nanospheres (FcNS), and then bound with the target HER2. The released FcNS homogeneously hybridized with horseradish peroxidase-labeled DNA/Au nanospheres (HRPNS). Benefiting from the introduction of $RecJ_f$ exonuclease, HER2 was recycled as the degradation of aptamer and bound another aptamer connected on FcNS. Thus, FcNS/HRPNS in large amounts was generated and captured by the modified Au electrode through the host-guest recognition between beta-cyclodextrin (β-CD) and ferrocene (Fc). Horseradish peroxidase (HRP) catalyzed o-PD in presence of H_2O_2, producing a significantly amplified signal. Under the optimal conditions, the fabricated aptasensing platform showed an excellent sensitivity with a low detection limit of 4.9 ng ml^{-1} (S/N = 3), and high specificity towards HER2. Furthermore, this proposed strategy presented the good reliability and applicability in the analysis of human serum samples, showing great potential for applications in early diagnosis of breast cancer.

1. Introduction

Breast cancer is considered as one of the greatest causes of mortality in women [1–3], and early diagnosis could make a significant difference [4,5]. Thus, novel approaches for early diagnosis of breast cancer are highly desirable. HER2 (human epidermal growth factor receptor-2) is a transmembrane protein, which plays a major role in promoting breast cancer cell proliferation and malignant growth [6,7], leading to accelerated recurrence and death rates. A number of studies demonstrated that breast cancer patients have elevated concentrations of HER2 in their blood when compared to healthy subjects [8,9]. The concentration of HER2 from healthy individuals is between 2 and 15 ng/ml, while the patients with breast cancer show elevated levels of HER2 in a range of 15–75 ng/ml. Monitoring the HER2 levels in serum can realize the early diagnosis of breast cancer, decreasing the mortality rate [10–12].

In clinical diagnosis, four methods have already been approved by U.S. Food and Drug Administration (FDA) to determine a breast cancer's HER2 status, including immunohistochemistry (IHC), fluorescent in situ hybridization (FISH), subtraction probe technology chromogenic in situ hybridization (SPoT-Light CISH) and Inform Dual ISH in situ hybridization (Inform Dual ISH). IHC is the most common HER2 status test, which is widely available in preliminary HER2 protein detection. The others are more precise and reliable. However, these methods require invasive biopsy, specialized equipment and long turn-around time, which limit the extensive applications [13–15]. Thus, the development of a facile and sensitive method for the early diagnosis of breast cancer is urgently required. So far, some detection techniques have been reported, including fluorescence, chromatography, mass spectroscopy and electrochemistry [16–19]. The electrochemical methods are advantageous in terms of simplicity, quick response, high selectivity and superior sensitivity. To further increase the sensitivity, electrochemical detections were combined with signal amplification strategies, where the emergence of nuclease cleavage provide new analytical tools for sensitive detection of analytes in aptasensing, surmounting limitation of the traditional 1:1 binding ratio of the aptamer to analyte [20,21]. The introduction of the catalytic nuclease into the electrochemical sensing platforms achieved further recycling and reuse of target, thus amplifying the detection signal.

$RecJ_f$ exonuclease [22–25] is a single-stranded DNA specific exonuclease, which catalyzes the removal of deoxy-nucleotide monophosphates from DNA chain in the 5'–3' direction, leading

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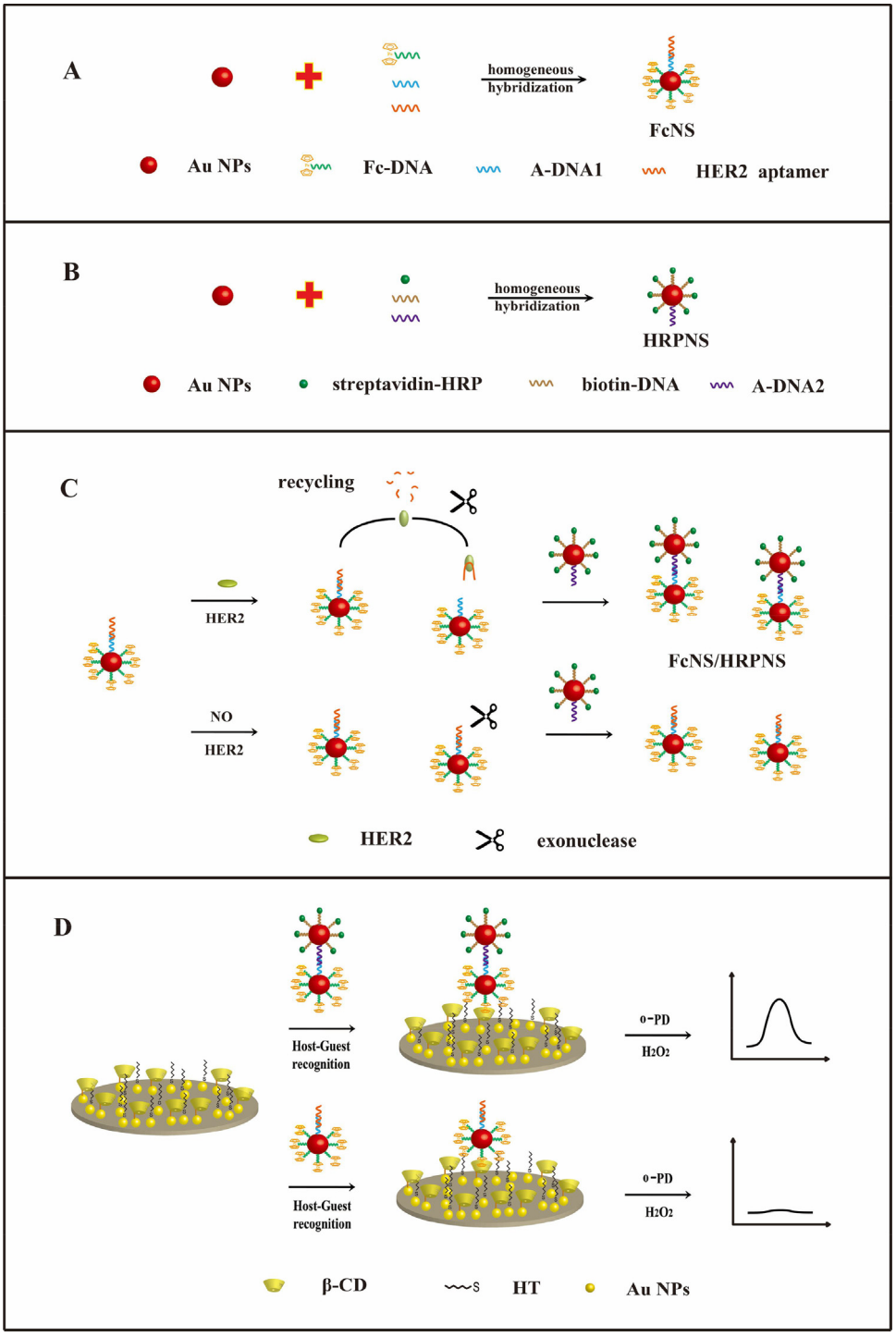
to degradation of DNA in the complex. It has been demonstrated as an effective signal amplifier in electrochemical detection of many biomolecules, such as ochratoxin A and thrombin, to yield relevant performances and improved sensitivities [26–29].

In this study, $RecJ_f$ exonuclease-catalyzed target recycling strategy was applied for the first time to develop an electrochemical HER2 aptasensing platform (Scheme 1). The gold nanoparticles (Au NPs) layer was electrochemically deposited on the Au electrode surface, followed by self-assembly of SH-β-CD to capture Fc labeled on Au nanospheres. Signal amplification was achieved through recycling between aptamer-HER2 complex formation and HER2 release from the complex using $RecJ_f$ exonuclease degradation, generating FcNS/HRPNS in large amounts and thus providing a more sensitive response. The fabricated aptasensing platform exhibited a low detection limit and wide linear range, providing an effective approach of HER2 detection in human serum for early diagnosis of breast cancers.

2. Experimental

2.1. Materials

$K_3Fe(CN)_6$, $K_4Fe(CN)_6$, KCl, Na_2HPO_4, NaH_2PO_4, HCl (aq), NaOH, hydrogen peroxide, glucose and trisodium citrate (Sinopharm,



Scheme 1. Schematic illustration of the electrochemical aptasensing platform for HER2 detection.

Shanghai, China); HER2, hexanethiol (HT), $HAuCl_4$ and o-phenylenediamine (o-PD) (Sigma-Aldrich, St.Louis, USA); SH-β-cyclodextrin (Tuokechem, Shanghai, China); $RecJ_f$ exonuclease with 1 × NEB buffer 2 (New England Biolabs, Beijing, China); Carcinoembryonic antigen (CEA) (Shuangliu Zhenglong, Chengdu, China); IgG (Dingguo Biotechnology, Beijing, China); Streptavidin-HRP (1 mg ml^{-1}) (Beyotime, Nantong, China); Bovine serum albumin (BSA) and DNA sequences (Sangon, Shanghai, China).

All the reagents were of analytical grade and all solutions were prepared with ultrapure water at a specific resistance of 18.2 MΩ cm.

Sequences information:

Fc-DNA: 5'-SH-(CH2)6- GCC ATT GGG TCT TGC-Fc-3'

Auxiliary DNA1 (A-DNA1): 5'-SH-(CH2)6-TTTTC AACAC TCCCC TCCCT ATCCC ATCCC GTGCT GATCA GTTCT TTTAC-3'

biotin-DNA: 5'-SH-(CH2)6-GCC ATT GGG TCT TGC-biotin-3'

Auxiliary DNA2 (A-DNA2): 5'-SH-$(CH_2)_6$-GTAAA AGAAC TGATC AGCAC GGGAT GGGAT AGGGA GGGGA GTGTT GAAAA-3'

HER2 aptamer: 5'-AAA GTAAA AGAAC TGATC AGCAC GGGAT GGGAT AGGGA GGGGA GTGTT GAAAA-3'

2.2. Apparatus

All the electrochemical experiments were performed using a CHI 660C electrochemical analyzer with a three-electrode configuration (Chenhua, Shanghai, China). A modified Au electrode, an Ag/AgCl with saturated KCl solution and a platinum wire were employed as the working, reference and counter electrode, respectively. The average sizes of the Au nanoparticles were characterized by transmission electron microscopy (TEM, JEOL JEM-2100, Japan). The pH values of 0.1 M PBS were measured by a PHS-3C pH meter (Leici Instrumental Corporation, Shanghai, China). The ultrapure water was obtained using a Millipore Milli-Q system (BIORISE, Shanghai, China).

2.3. Electrochemical aptasensing of HER2

2.3.1. Preparation of FcNS/HER2 aptamer and HRPNS

The Au nanoparticles (Au NPs) were prepared according to the literature [30]. 5 μl of 0.1 mM Fc-DNA and 5 μl of 0.1 mM A-DNA1 were mixed with 10 μl of Au nanoparticles for 12 h to form FcNS. Then, 5 μl of 0.1 mM HER2 aptamer was added to hybridize with A-DNA1 on FcNS for 1 h, and the formed FcNS/HER2 aptamer would be captured by β-CD immobilized on the electrode through supramolecular recognition. The preparation of HRPNS was achieved by incubating 10 μl of Au nanoparticles with 5 μl of 0.1 mM biotin-DNA and 5 μl of 0.1 mM A-DNA2 for 12 h, followed by incubating with 5 μl of 1 mg ml^{-1} streptavidin-HRP for 1 h to link HRP on Au NPs through the interaction between the biotin and streptavidin.

2.3.2. Binding recycling of HER2 by $RecJ_f$ exonuclease

HER2 at different concentrations was incubated with FcNS/HER2 aptamer. Meanwhile, 5 μl of $RecJ_f$ exonuclease was added for the continuous generation of FcNS for 1 h. After that, HRPNS was added into solution and the complex of FcNS/HRPNS was formed for 2 h.

2.3.3. Fabrication of electrochemical aptasensing platform

Firstly, the Au electrode was thoroughly rinsed with piranha solution (7:3 mixture of concentrated sulfuric acid with 30% hydrogen peroxide). The electrode was then cleaned by ultrasonication in ethanol and ultrapure water for 5 min, respectively. Next, the electrode was polished to a mirror-like surface using chamois leather with 0.3 μm and 0.05 μm alumina slurries. It was then rinsed with ultrapure water, followed by electrochemical cleaning using cyclic scan between −0.2 and 1.6 V (vs. Ag/AgCl) in 0.5 M H_2SO_4.

The resulting Au electrode was immersed in 10 ml of 2.5 mM $HAuCl_4$ solution to obtain Au nanoparticles modified electrode through electrochemical deposition at −0.2 V for 100 s. Next, SH-β-CD was self-assembled on the modified Au electrode through the Au-S bond by immersing the electrode in 1 mM SH-β-CD solution for 5 h at room temperature, followed by immobilization of HT (1 mM) to block the surface of Au electrode for 12 h. Then, the modified electrode was immersed into the FcNS/HRPNS solution to capture FcNS/HRPNS through β-CD on the surface for 5 h. The unbounded complexes were washed with ultrapure water, and then the modified electrode was dried at room temperature.

2.3.4. Electrochemical detection of aptasensing platform

The responses of differential pulse voltammetry (DPV) were recorded in 0.1 M PBS (pH 7.0) containing 50 mM o-PD and 50 mM H2O2 at room temperature in the potential range of −0.7 V to −0.3 V.

2.4. Preparation of serum samples

Human serum samples were obtained from Shanghai Electric Power Hospital and centrifuged with G value of 9990*g* before storage in the refrigerator at −20 °C. The supernatant was diluted to 2% with 0.1 M PBS and mixed with HER2 at various concentrations to yield 50 ng ml^{-1}, 100 ng ml^{-1} and 150 ng ml^{-1} spiked samples.

3. Results and discussion

3.1. Characterization of aptasensing platform

The synthesized Au NPs were characterized by TEM in Fig. S1, showing the homogeneous distribution and the average diameter of the Au NPs could be estimated to 15 nm. EIS was employed to characterize different modified electrodes [31,32]. Compared to bare Au (Fig. 1A, curve a) and Au/Au NPs electrode (Fig. 1A, curve b), the Au/Au NPs/β-CD electrode shows an enhanced electron transfer resistance (Fig. 1A, curve c), indicating the successful self-assembly of SH-β-CD on the electrode. HT, used to block the surface of Au, further raises the resistance (Fig. 1A, curve d). The capture of FcNS/HER2 aptamer (Fig. 1A, curve e) and FcNS/HRPNS (Fig. 1A, curve f) by β-CD result in the successive increase of the impedance value, indicating the successful immobilization of the complexes through host-guest interaction between Fc and β-CD.

The electrochemical performances of the fabricated aptasensing platform were evaluated by DPV as shown in Fig. 1B. The Au/Au NPs/β-CD/HT electrode (Fig. 1B, curve a) does not produce obvious electrochemical signals. The respective addition of HRPNS (Fig. 1B, curve b), FcNS/HER2 aptamer (Fig. 1B, curve c) and mixture of HRPNS and FcNS/HER2 aptamer (Fig. 1B, curve d) generate almost no signals in absence of HER2. This reveals that the captured Fc and physically absorbed HRP have no influence on the electrochemical determination of HER2. With the presence of HER2, FcNS/HRPNS complex is formed through the homogeneous hybridization and captured by the modified Au electrode, which contributes to the catalysis of o-PD oxidation in presence of H_2O_2 to generate a DPV signal (Fig. 1B, curve e). The addition of $RecJ_f$ exonuclease significantly increases the current signal resulting in an amplified response (Fig. 1B, curve f). Thus, the fabricated electrochemical aptasensing platform could be employed for the detection of HER2.

3.2. Self-assembly of SH-β-CD on Au NPs modified Au electrode

Au NPs was employed to boost the amount of SH-β-CD self-assembled on the Au electrode. To estimate the surface density

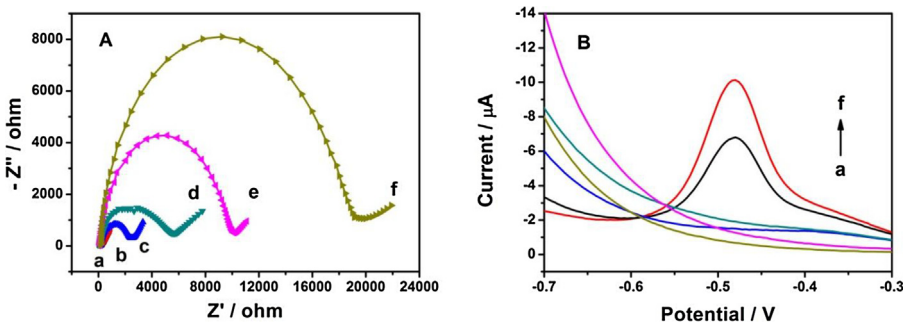


Fig. 1. (A) EIS of different modified electrodes in 10 mM $Fe(CN)_6^{3-/4-}$ containing 0.1 M KCl: (a) bare Au, (b) Au/Au NPs, (c) Au/Au NPs/β-CD, (d) Au/Au NPs/β-CD/HT, (e) Au/Au NPs/β-CD/HT/FcNS/HER2 aptamer and (f) Au/Au NPs/β-CD/HT/FcNS/HRPNS electrode. (B) DPV curves obtained from (a) Au/Au NPs/β-CD/HT (dark green line); (b) Au/Au NPs/β-CD/HT with HRPNS (magenta line); (c) Au/Au NPs/β-CD/HT with FcNS/HER2 aptamer (blue line); (d) Au/Au NPs/β-CD/HT with mixture of FcNS/HER2 aptamer and HRPNS (cyan line); (e) Au/Au NPs/β-CD/HT/FcNS/HRPNS without $RecJ_f$ exonuclease (black line); (f) Au/Au NPs/β-CD/HT/FcNS/HRPNS with $RecJ_f$ exonuclease (red line) in PBS (pH 7.0) containing 50 mM o-PD and 50 mM H_2O_2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

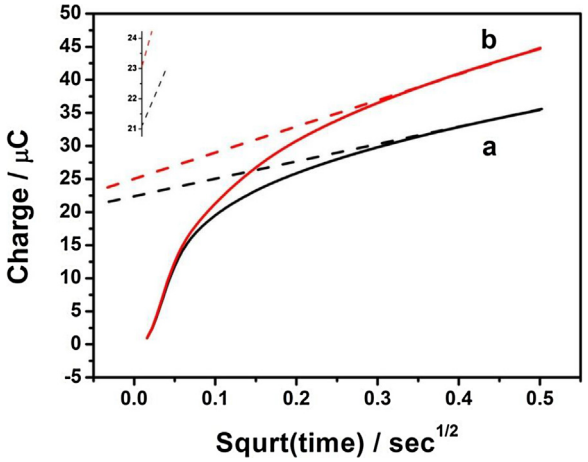


Fig. 2. Chronocoulometry curves of Au electrode before (a) and after (b) the immobilization of β-CD.

of β-CD, chronocoulometry measurements were performed before and after the self-assembly of SH-β-CD [33,34]. Clearly, the electric quantity increases with the Au-S bonds breakage detected in 0.1 M PBS (pH 10.5), since the reaction of R-S-Au + $e^- \leftrightarrows$ R-S^- + Au occurs during the breakage process (Fig. 2). The surface density of β-CD (Γ) on Au NPs modified Au electrode (Au/Au NPs) is calculated as 5.4×10^{-10} mol/cm^2 using the equation: $\Gamma = Q/nFA$ [35–37], in which, the area of Au/Au NPs electrode is obtained according to the Cottrell equation: $i(t) = \frac{nFAD_0^{1/2}C}{\pi^{1/2}t^{1/2}}$. When t equals to 10 s, the detected values of current in 50 mM ferrocene/PBS solution are 8.034×10^{-7} A and 9.285×10^{-7} A by Au electrode and Au/Au NPs electrode, respectively. The area of Au electrode is 0.0314 cm^2, thus calculating the value of Au/Au NPs electrode as 0.0363 cm^2. While, the theoretical value of β-CD monolayer on Au surface was 7.5×10^{-11} mol/cm^2 [38]. This indicates that the self-assembly efficiency of SH-β-CD is improved by the electrochemical deposition of Au NPs.

3.3. Optimization of experimental variables

To further improve the detection sensitivity, experimental variables including pH and capture time between immobilized β-CD and Fc were optimized. The pH value of PBS has a significant impact on the catalytic activity of HRP. A number of studies found that neutral pH was more appropriate for HRP sensing application, and the catalytic activity of HRP would be damaged in low or high pH media [39–41]. Herein, the effect of pH was investigated in the range of 5.0–9.0 and the results were shown in Fig. 3A. The data clearly demonstrates that the strong current signals could be obtained near pH 7.0. At pH below or above 7.0, the generated signals reduce in intensity. Besides, the normal pH of human blood is around 7.0. Thus, pH 7.0 was selected as the optimal condition.

The capture time between β-CD and Fc is another important factor affecting the HER2 detection, because the capture efficiency would directly influence the detection sensitivity. Thus, the capture time was optimized to bring maximum HRP to the electrode surface. As shown in Fig. 3B, the current signal increases from 1 h to 5 h. While, the peak currents decrease after 5 h, probably due to the declined activity of HRP. Therefore, 5 h was selected as the optimal capture time.

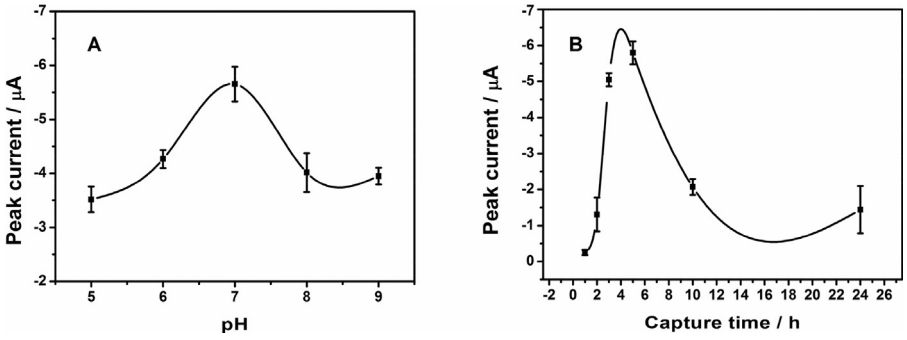


Fig. 3. Effects of (A) pH value and (B) capture time between β-CD and Fc on the peak current at room temperature.

Table 1
Determination of HER2 in the spiked human serum samples using the aptasensing platform and UV–vis spectrophotometry as a comparison.

Sample No.	Spiked value (ng ml^{-1})	Measured value (ng ml^{-1})		Recovery (%)		Reproducibility (RSD%)	
		Sensing	UV–vis	Sensing	UV–vis	Sensing	UV–vis
1	50.0	52.7	58.3	105.5	116.7	7.3	0.2
2	100.0	92.7	125.0	92.7	125.0	4.5	0.3
3	150.0	145.9	161.5	97.3	107.7	6.6	0.2

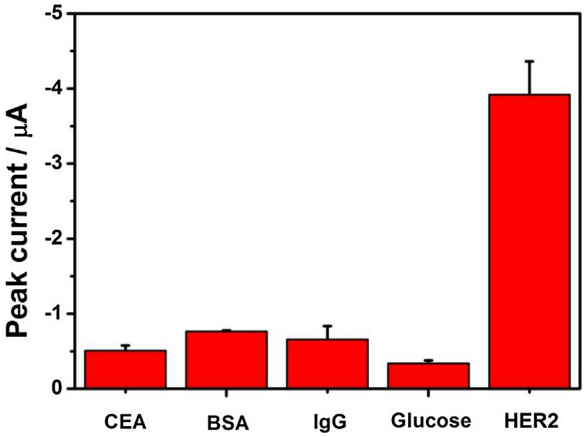


Fig. 4. Peak current of DPV responses at the aptasensing platform with HER2 (100 ng ml^{-1}), BSA (100 ng ml^{-1}), CEA (100 ng ml^{-1}), glucose (100 ng ml^{-1}) and IgG (1×10^4 ng ml^{-1}) in PBS (pH 7.0).

3.4. Specificity of aptasensing platform

To evaluate the specificity of the aptasensing platform, different analytes, including HER2 (100 ng ml^{-1}), BSA (100 ng ml^{-1}), CEA (100 ng ml^{-1}), glucose (100 ng ml^{-1}) and IgG (1×10^4 ng ml^{-1}) were respectively added to the detection solution. Under the optimal conditions, the highest peak current is observed in presence of HER2 (Fig. 4). On the other hand, the signal intensities obtained in presence of the interferences were less than 20% of the signal from HER2, demonstrating that the fabricated aptasensing platform possesses an excellent specificity towards HER2.

3.5. Quantitative analysis of HER2

The DPV responses of the aptasensing platform towards HER2 at various concentrations were gathered in Fig. 5A. The signal intensity increases as HER2 concentration rises from 10 ng ml^{-1} to 150 ng ml^{-1}. According to Fig. 5B, the detection sensitivity is deduced as -0.039 ± 0.001 μA (ng/ml)$^{-1}$ with a correlation coefficient of 0.993, and the detection limit is as low as 4.9 ± 0.2 ng ml^{-1} (S/N = 3). Compared to other strategies for HER2 detection (Table S1), the fabricated aptasensing platform presents a more excellent sensitivity and wider linear range.

3.6. Serum sample testing

The practical application of the fabricated aptasensing platform in clinical analysis was verified in human serum samples at the optimized conditions (Table 1). Using the standard addition method, the recoveries of HER2 detection in real samples are obtained in the range of 92.7%–105.5% (n = 3), demonstrating the reliability and applicability of the fabricated aptasensing platform for real human serum sample analysis.

4. Conclusions

A sensitive electrochemical aptasensing platform for HER2 detection was fabricated for the first time with signal amplification by RecJ_f exonuclease. HER2 could bond with its aptamer, releasing FcNS to homogeneously hybridize with HRPNS. Due to the degradation of HER2 aptamer by RecJ_f exonuclease, HER2 was recycled to bind with another HER2 aptamer, thus generating lots of FcNS/HRPNS. With the capture by Au/Au NPs/β-CD electrode based on the host-guest interaction between β-CD and Fc, a large number of HRP was brought to the electrode surface and then generated the significantly amplified oxidative current by the catalysis towards o-PD in presence of H_2O_2. Under the optimized conditions, this aptasensing platform exhibited a linear electrochemical responses of HER2 in a wide range of 10 ng ml^{-1}–150 ng ml^{-1}, a high detection sensitivity of -0.039 ± 0.001 μA (ng/ml)$^{-1}$ and a low detection limit of 4.9 ± 0.2 ng ml^{-1}. It also showed the impressive specificity towards HER2, and applicability in spiked human serum samples, indicating the potential of the fabricated aptasensing platform in early diagnosis of breast cancer.

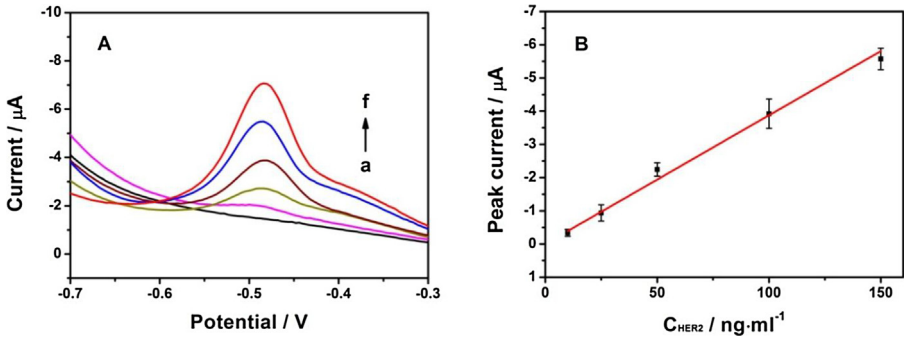


Fig. 5. (A) DPV responses at the aptasensing platform with HER2 at different concentrations: (a) 0 ng ml^{-1} (black line); (b) 10 ng ml^{-1} (magenta line); (c) 25 ng ml^{-1} (dark green line); (d) 50 ng ml^{-1} (brown line); (e) 100 ng ml^{-1} (blue line) and (f) 150 ng ml^{-1} (red line). (B) Calibration curve of peak currents towards the concentrations of HER2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.snb.2017.11.119.

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