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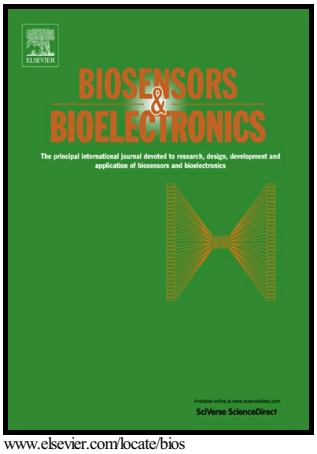

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Highly sensitive electrochemical biosensor for streptavidin detection based on CdSe quantum dots

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ABSTRACT

An electrochemical biosensor was developed based on a steric hindrance hybridization assay to allow the highly sensitive detection of streptavidin. In the steric hindrance hybridization assay, the signaling strand DNA (sig-DNA) was labeled at the 3' end with CdSe quantum dots (QDs) and at the 5' end with biotin, and capturing strand DNA (the complementary strand of sig-DNA) was labeled at the 5' end with thiol. The steric hindrance effect generated by streptavidin which was bound with the signaling DNA strand. The streptavidin limited the ability of the sig-DNA to hybridize with the cap-DNA, which were linked on the surface of a gold electrode. Therefore, the concentration of streptavidin was detected indirectly based on the concentration of CdSe QDs on the electrode surface. The concentration of CdSe QDs on the electrode surface was detected by differential pulse anodic stripping voltammetry. Under optimal conditions, the streptavidin detection range using the as-prepared biosensor was 1.96 pg/mL to 1.96 μg/mL and the detection limit was 0.65 pg/mL. The experimental results showed that the electrochemical biosensor could detect streptavidin rapidly and accurately.

Keywords: differential pulse anodic stripping voltammetry; CdSe QDs; steric hindrance hybridization assay; streptavidin

1. Introduction

Quantum dots (QDs) are spherical semiconductor nanomaterials (Jie et al., 2013),

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which are employed widely in biological sensing because of their controllable size, emission wavelength, high-yield photoluminescence, and high chemical stability (Wang et al., 2012). Due to the presence of amidogen and carboxyl groups, the QDs could facilely link with DNA or nanomaterials and enhancing the application area. For example, QDs can combine with graphene oxide to obtain an electrogenerated chemiluminescence sensor (Li et al., 2012; Wang et al., 2009). Jie et al. (Jie et al., 2012) found that QDs modified by magnetic nanoparticles could fabricate a high selectivity biosensor for the detection of carcinoembryonic antigen (CEA) and thrombin. In addition, QDs@MOF has been applied in light traps, photocatalysis, and biosensors (Liu et al., 2017; Zhu et al., 2014). Due to the high chemical stability and electrochemical properties of CdSe QDs, we employed them as signal element to connect with signaling DNA .

The steric hindrance hybridization assay was developed by Mahshid (Mahshid et al., 2015). When the assay is applied in an electrochemical format, the multiplexed, quantitative, one-step detection of various macromolecules is possible in the low molar range. In recent years, DNA has been employed widely as a useful tool in biotechnology and nanotechnology because of its ability to hybridize specifically with complementary sequences (Ravana et al., 2014). This excellent property of DNA plays a critical role in biology, genotyping diagnostics, and a variety of molecular biology techniques (Harrison et al., 2013; Yin et al., 2011; Zhang et al., 2012). Most DNA sensor systems based on the hybridization between a DNA strand and its complementary probe have been described. These DNA sensor systems can be based on fluorescence, electrochemiluminescence, surface Plasmon resonance and electrochemical detection. For instance, Krul group reported that a self-contained fluorescent fiber optic DNA biosensor detect PCR product (Wang et al., 2005). An electrochemiluminescence DNA biosensor was developed and possessed excellent analytical performance in the detection of the DNA binging protein (Wang et al., 2013. In our previous work, a novel electrochemical DNAzyme sensor was developed for Ni^{2+}detection (Yang et al., 2016). This biosensor exhibited high sensitivity and fast response to Ni^{2+}. In the present study, we selected oligonucleotides to combine with

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biotin and then each oligonucleotide linked with its complementary strand to assemble on the surface of an electrode. When the streptavidin was presence in this system, there would generate steric hindrance to influence the conjugation of the DNA. Thus, we combined steric hindrance and DNA biosensor to construct a sensitive and highly selective sensor for the detection of streptavidin.

Electrochemical detection methods have received much attention because of their significant advantages in terms of accuracy, sensitivity, simplicity, and portability (Jelen et al., 1997). These analytical methods are used widely for the detection of DNA because they provide simple, accurate platforms (Rasheed et al., 2017). In addition, electrochemical DNA biosensors also can provide electronic signals directly without the need for expensive signal transduction equipment (Grabowska et al., 2014; Liu et al., 2008).

In this study, we developed a novel electrochemical biosensor for detecting streptavidin selectively and in a sensitive manner. The probe comprised biotin, sig-DNA strand with an amino group, CdSe QDs, and its substrate strand (capturing DNA strand) with a thiol group. Hybridization of the sig-DNA strand and capturing DNA strand (cap-DNA) was impeded in the presence of streptavidin due to steric hindrance, thereby decreased the amount of CdSe QDs on the surface of a gold electrode. The amount of CdSe QDs on the surface of the gold electrode was detected by differential pulse anodic stripping voltammetry. Under optimal conditions, the streptavidin detection range using the as-prepared sensor was from 1.96 pg/mL to 1.96 μg/mL and the detection limit was 0.65 pg/mL.

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2. Experimental

2.1. Chemical and materials

Bovine serum albumin (BSA). Cytochrome C (cyt-C). Streptavidin (60 KDa). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). N-hydroxysuccinimide (NHS) and DNA were purchased from Shanghai Sangon Biotech. Co. Ltd. (Shanghai, China). L-cysteine (98.5%). TE buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA) and tris-(2-carboxyethyl)-phosphine hydrochloride (TCEP) were purchased by Shanghai

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Chemical Reagent Co., Ltd. (Shanghai, China). Selenium powder (Se) was supplied from Aladdin Reagent Co., Ltd (Shanghai, China). $3CdSO_4 \cdot 8H_2O$ was purchased by Energy Chemical (Shanghai, China). Interleukin 6 (IL-6) and Carcinoembryonic antigen (CEA) were purchased from Beijing Biosynthesis biotechnology Co. Ltd (Beijing, China). All reagents were used without further purification. Phosphate buffer saline (PBS pH=7.4, 10 mM) was consisted of 1.9 mM NaH_2PO_4, 8.1mM Na_2HPO_4 and 0.9% NaCl. Electrochemical impedance spectroscopy (EIS) electrolyte solution was consisted of 5.0 mM $[Fe(CN)6]^{3-/4-}$ containing 0.1 M KCl. Ultrapure water was obtain from an AK Exceed-E-V lab pure water system and was used throughout this work (electrical resistivity ≥ 18 MΩ).

The sequences of two DNA were shown as follow:

cap-DNA: SH-5′- ACACTAGCACGCAGAGATAAGTTAAACTGATCCAAGACT

AAGCCG-3′

sig-DNA: Biotin -5′-CGGCTTAGTCTTGGATCAGTTTAACTTATCTCTGCGTG

CTAGTGT -3′-NH2

2.2. Apparatus

Transmission electron microscope (TEM, JEM-2100, JEOL, Japan, at 200 kV). UV–vis absorption spectrometer (UV-3600, Shimadzu, Japan). Photoluminescence spectrometer (PL, F-2500, Hitachi, Japan). Fourier transform infrared spectrometer (FTIR, NEXUS-870, Nicolet Instrument Co., USA). Electrochemical workstation (CHI 660D CH Instrument Co., China). Thales electrochemical workstation (Zahner-elektrik GmbH & Co., KG, Germany) with the three electrode system consisted of a platinum electrode, Ag/AgCl electrode, and the modified gold electrode.

2.3. The preparations of CdSe QDs

The preparations of water soluble CdSe QDs were using a previously reported process with slight modifications (Wang et al., 2012). Briefly, 2 mL 0.15 M $CdSO_4$ solution were poured into the boiling flask-3-neck which had 7.5 mL 1.0 M NaOH

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solution. Then the solution turned into milky white suspension. 0.3998 g L-cysteine (L-cys) was dissolved in the suspension and then the suspension turned clear immediately. After that the mixture solution was adjusted to approximately pH 10 by 0.5 M H_2SO_4, and diluted to 200 mL by ultra-pure water. The mixture was stirred tenderly at 40℃ for 30 min, then 0.75 mL of 0.1 M $NaSeSO_3$ was injected into the reaction liquid. The reaction solution was kept at 40℃ for 2.5 hours and the whole process was demonstrated under N_2 atmosphere. Finally, the bright yellow solution of CdSe QDs was successfully synthesized.

2.4. Preparation of CdSe QDs/sig DNA composites

The terminal carboxylic acid groups of the CdSe QDs were activated by addition 20 mg EDC and 10 mg NHS into 1 mL CdSe QDs solution and the mixture was incubated at 37℃ for 30 min. Then 20 μL 100 nM sig-DNA were injected into the activated CdSe QDs and incubated at 37℃ for 2 h. In order to remove the unlinked sig-DNA, the incubated solution was centrifuged by ultrafiltration tube (30KDa, Merck Millipore) at about 6000 rpm for 5min. The deposits were redistributed in 500μL TE buffer. Finally, we got one of the most important role of this electrochemical aptasensor, the signal and recognition component.

2.5. Fabrication of the electrochemical biosensor

2.5.1 Pretreatment of gold electrode

Firstly, the gold electrode (d=4 mm) was polished with 1.0, 0.3, and 0.05 μm α-Al_2O_3 powder on a polishing cloth respectively, and washed ultrasonically with ethanol and ultrapure water in turn. Then 20 μL piranha solution (98% H_2SO_4: 30% H_2O_2=7:3) was dropped on the gold surface in order to clean the electrode surface. Ten minutes later, the gold electrode was thoroughly flushed by ultrapure water. The cleaning process of using piranha solution was repeated three times. Next, the gold electrode was immersed in 0.5 M H_2SO_4 and scanned until a reproducible cyclic voltammogram (CV) was obtained. After that the gold electrode was flushed by ultrapure water and dried by N_2.

2.5.2. Fabrication of the electrochemical biosensor

<Scheme 1>

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The complete fabrication process for the electrochemical biosensor is illustrated in Scheme 1. First, cap-DNA strands were modified on the gold electrode surface, before their modification where the disulfide bonds in the cap-DNA stands were reduced in aqueous TCEP solution. 20 μL of the reduced cap-DNA strands (100 nM) was dripped onto the surface of the pretreated gold electrode, which was then incubated at 4°C for 18 h in order to form Au-S bonds between the gold electrode and cap-DNA strands. The gold electrode bound with cap-DNA strands was washed using TE buffer to remove any unbound DNA strands, before drying under N_2. To avoid nonspecific adsorption, the electrode was immersed in 500 μL of 2 wt% bovine serum albumin solution (BSA) and incubated at 37°C for 2 h. After 2 h, the gold electrode was taken out and washed with phosphate-buffered saline to remove any residual BSA. The gold electrode was then dried under N_2. Next, the gold electrode was immersed in 500 μL of buffer solution containing CdSe QDs-sig-DNA and incubated at 37°C for 2 h to hybridize the cap-DNA and sig-DNA. To detect streptavidin, we added a suitable amount of streptavidin to this buffer solution before inserting the gold electrode. The gold electrode was washed with TE buffer and stored at 4°C until use.

2.6. Electrochemical analysis

The amounts of CdSe QDs on the surface of the gold electrode were detected by differential pulse anodic stripping voltammetry, where the detection process was as follows. First, the modified gold electrode was washed with TE buffer to remove any unbound sig-DNA and dried under N_2. The gold electrode was immersed in 500 μL of 0.1 M HNO_3 solution for 2 h to dissolve the CdSe QDs on the surface of the gold electrode. The HNO_3 solution was then transferred to 4.5 mL of acetic acid-sodium acetate buffer solution (0.1 M pH = 7.4). A traditional three-electrode system was used to detect the amount of CdSe QDs in the buffer solution. The auxiliary electrode was platinum wire, the reference electrode was Ag/AgCl electrode (saturated KCl), and the working electrode was a glass carbon electrode. The glass carbon electrode had previously been deposited with a mercury film in 5 mL of 0.2 M acetate buffer containing 40 ppm mercury (II) at –0.8 V for 50 s. After deposition at –1.1 V for 6 min, the differential pulse voltammetry (DPV) response was recorded over the

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potential from –0.85 to –0.2 V, with a pulse amplitude of 25 mV, pulse frequency of 25 Hz, and potential steps of 4 mV in order to detect the CdSe QDs in the buffer solution.

3. Result and discussion

3.1. Characterization of CdSe QDs

The CdSe QDs were characterized morphologically by transmission electron microscopy. As shown in Figure S1A, the round CdSe QDs were distributed relatively evenly. The high-resolution transmission electron microscopy image (Figure S1B) showed that the CdSe QDs had a uniform size of about 5 nm. The ultraviolet-visible (UV-Vis) and photoluminescence (PL) spectra obtained for the CdSe QDs were depicted in Figure S1C. The UV-Vis absorption peak at 420 nm (curve a) and PL emission peak at 500 nm (curve b) indicated the quantum confinement effect (Jie et al., 2009). The Fourier transform-infrared (FTIR) spectra were shown in Figure S1D. In the FTIR spectra obtained for the CdSe QDs, the absorption peak at 3442 cm^{-1},1581-1431 cm^{-1},1133 cm^{-1} and 616 cm^{-1} were attributable to O-H stretching, carboxylate species stretching, C-O stretching, and vibration absorption of CH_2-NH-CH_2, respectively. The FTIR spectra obtained for the CdSe QDs showed that the carboxyl group was linked to the surface of the CdSe QDs.

3.2. Feasibility of the biosensor

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were used to monitor the assembly process for the electrochemical biosensor. The CV responses of different modified electrodes in the electrolyte (5 mM $Fe(CN)_6^{3-/4-}$ and 0.1 M KCl) were shown in Figure 1A. The bare gold electrode exhibited a relative high peak according to CV (curve a). After the cap-DNA was assembled on the electrode, the CV signal decreased to some extent (curve b). When BSA was immobilized on the electrode, the CV signal decreased further (curve c) due to the poor conductivity of the cap-DNA and BSA. After incubation with the sig-DNA, the peak current decreased dramatically because the sig-DNA hindered the electron transfer (curve d). These results indicate that the sig-DNA hybridized successfully with the cap-DNA. When the sig-DNA conjugated with CdSe QDs was modified on

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the electrode, the CV signal clearly increased because the CdSe QDs had better conductivity than the biomolecules, which could accelerate the transformation of electrons (curve e) (Shi et al., 2017). In order to support the opinion of steric-hindrance effect, the CV responses of the gold electrode which was modified with streptavidin/sig-DNA/CdSe QDs/BSA/cap-DNA were shown in curve f. Compared with curve e, the CV signal of curve f decreased obviously. This phenomenon may be caused by streptavidin hinder the connection of CdSe QDs on the gold electrode. On the other hand, streptavidin could enhance the effect of blocking the electron transfer. Hence, the CV signal of curve f decreased obviously.

EIS was a valid method for monitoring the changes on the surface of the electrode. The impedance spectra comprised a semicircular region and a linear region. The semicircular region corresponded to the electron transfer resistance and the linear region indicated the diffusion process. As shown in Figure 1B, the bare gold electrode produced a small semicircular region (curve a), which indicated the good conductivity of the gold electrode. Compared with the bare gold electrode, the diameter of the semicircular region was larger than the curve a after the immobilization of the cap-DNA (curve b). BSA was then assembled on the gold electrode and the diameter of the semicircular region increased further because BSA hindered the electron transfer (curve c). After the sig-DNA was modified on the surface of gold electrode, the diameter of the semicircular region was larger than curve c because the sig-DNA further prevented the transformation of electrons on the electrode surface (curve d). When the sig-DNA conjugated with CdSe QDs was modified on the electrode, the diameter of the semicircular region decreased dramatically, mainly because the CdSe QDs are semiconductors that can accelerate the transformation of electrons. As shown in the curve f, the diameter of the semicircular region increased after the streptavidin was modified on the gold electrode. This result further proved that streptavidin indeed hinder the hybridization of DNA.

<Figure 1>

3.3. Optimization of the experimental conditions

In order to acquire higher sensitivity for streptavidin detection, the experimental

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conditions were optimized including the concentration of cap-DNA, the duration of assembling the cap-DNA, the incubation time and temperature of streptavidin. This optimization results was shown in Figure S2 and a detailed description could be found in supplementary information.

3.4. Electrochemical detection

Under the optimized conditions, the as-prepared electrochemical biosensor was investigated by detecting a series of streptavidin concentrations. The concentration of streptavidin was related to the amount of CdSe QDs loaded on the gold electrode, and then the amount of CdSe QDs was detected by DPV. Therefore, the DPV response signal correlated with the concentration of streptavidin, where the signal decreased as the concentration increased. As shown in Figure 2A, the DPV response signal decreased as the concentration of streptavidin increased. The DPV response signal decreased in a linear manner with the logarithm of the streptavidin concentration within the range from 1.96 pg/mL to 1.96 μg/mL (Figure 2B), where the linear equation was: $I = 0.01716 - 0.00386\log C$, and the correlation coefficient was 0.992. In the equation, I represents the peak current value of the DPV response signal (μA) and C is the concentration of streptavidin (μg/mL). The limit of detection for streptavidin was 0.65 pg/mL. Compared with those developed in previous studies （Zhang et al., 2017; Divya et al., 2017）, our biosensor had higher sensitivity. These results indicate that we successfully developed a sensitive biosensor for the quantitative determination of streptavidin.

<Figure 2>

3.5. Selectivity, stability, and reproducibility of the biosensor

In order to study the selectivity and specificity of the biosensor, a series of standard interference biomolecules (i.e., IL-6, cyt-c, and CEA) were tested in the same conditions. Figure 3 shows the DPV response signals for blank sample, 19.6 ng/mL IL-6, 19.6 ng/mL cyt-c, 19.6 ng/mL CEA, 1.96 ng/mL streptavidin, a mixture (containing 19.6 ng/mL IL-6, 19.6 ng/mL cyt-c, 19.6 ng/mL CEA, and 1.96 ng/mL streptavidin). Clearly, the biosensor was sensitive to streptavidin and negligible

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changes were caused by the other interfering biomolecules. Thus, the proposed biosensor had high selectivity and specificity for streptavidin. To test the stability of the biosensor, it was stored for two weeks at 4°C but the DPV detection results changed very little. The reproducibility of the biosensor was investigated using four electrodes, which were prepared in the same conditions. These electrodes detected the same concentration of streptavidin (59.6 pg/mL) and the relative standard deviation was 3%. These results demonstrate that the biosensor exhibited high stability and reproducibility.

<Figure 3>

4. Conclusions

In this study, we successfully established a novel electrochemical biosensor for detecting streptavidin based on the steric hindrance hybridization assay. The experiment results demonstrate that this biosensor was achieved with a wide linear range from 1.96 pg/mL to 1.96 μg/mL and the detection limit was 0.65 pg/mL, and excellent anti-interferential capability. This electrochemical biosensor is a promising example for the development of other convenient and sensitive detection of other biomacromolecules by changing the recognition element based on steric hindrance hybridization assay. Thus, our strategy has a good potential in the disease diagnosis.

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Figure captions

Scheme 1. Schematic diagrams of the stepwise electrochemical biosensor.

Figure 1. (A) CV curve and (B) EIS of: (a) bare Au electrode; (b) cap-DNA/Au electrode; (c) BSA/cap-DNA/Au electrode; (d) sig-DNA/BSA/cap-DNA/Au electrode; (e) sig-DNA-CdSe QDs/BSA/cap-DNA/Au electrode; (f) streptavidin-sig-DNA-CdSe QDs/BSA/cap-DNA/Au electrode. CV curve was obtained in 5 mM/L of $Fe(CN)_6^{3-/4-}$ containing 0.1 M/L of KCl over a scan range of −0.2 to 0.6 V at a rate of 100 mV/s. EIS was measured in the frequency range between 0.01 Hz and 100 KHz and at a signal amplitude of 5 mV.

Figure 2. Differential pulse voltammetry response (A) and calibration curve (B) for different streptavidin level (from bottom to top: 1.96 pg/mL, 59.6 pg/mL, 1.96 ng/mL, 19.6 ng/mL, 196 ng/mL, and 1.96 μg/mL streptavidin, respectively).

Figure 3. Selectivity of the biosensor with different targets: (a) blank sample, (b) 19.6 ng/mL of IL-6, 19.6 ng/mL of Cyt-c, 19.6 ng/mL of CEA, 1.96 ng/mL of streptavidin, and a mixed sample (containing 19.6 ng/mL of IL-6, 19.6 ng/mL of cyt-c, 19.6 ng/mL of CEA, 1.96 ng/mL of streptavidin).

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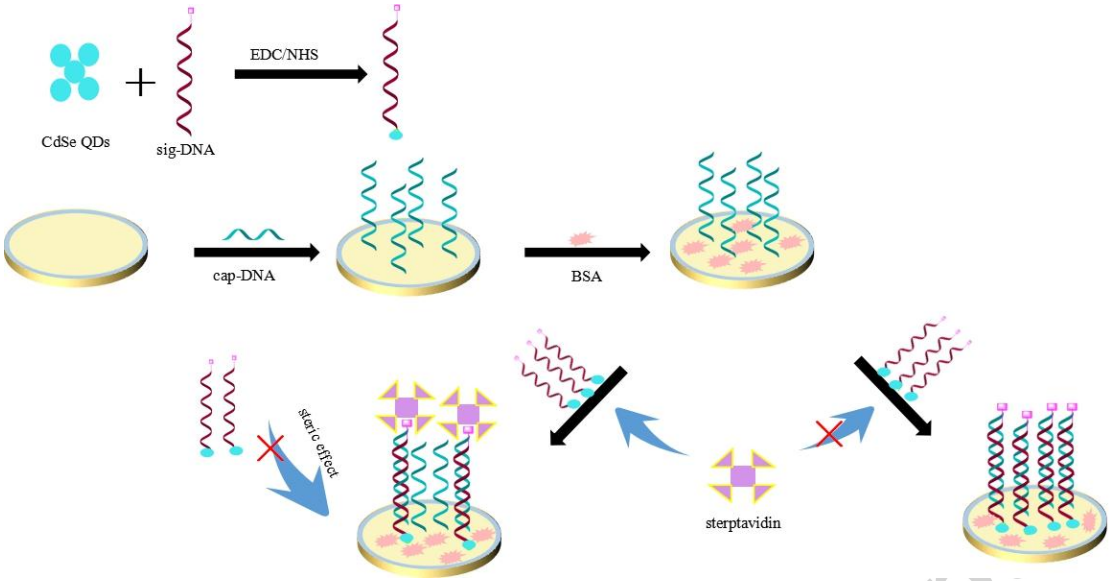

Scheme 1

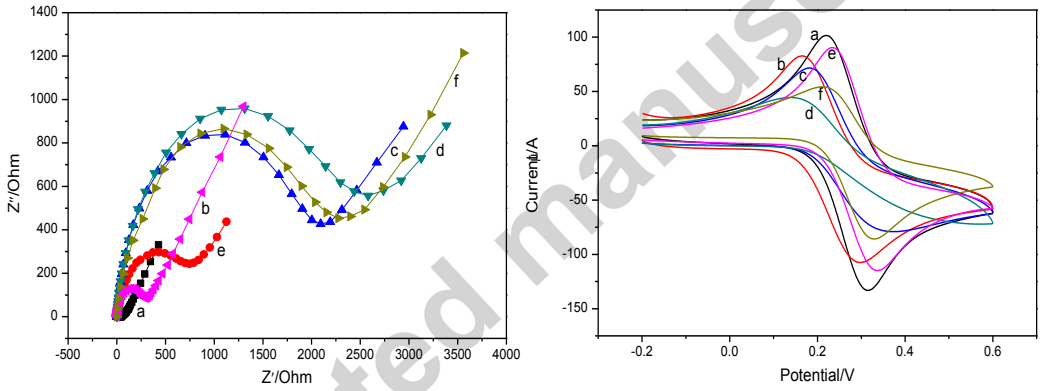

Figure 1

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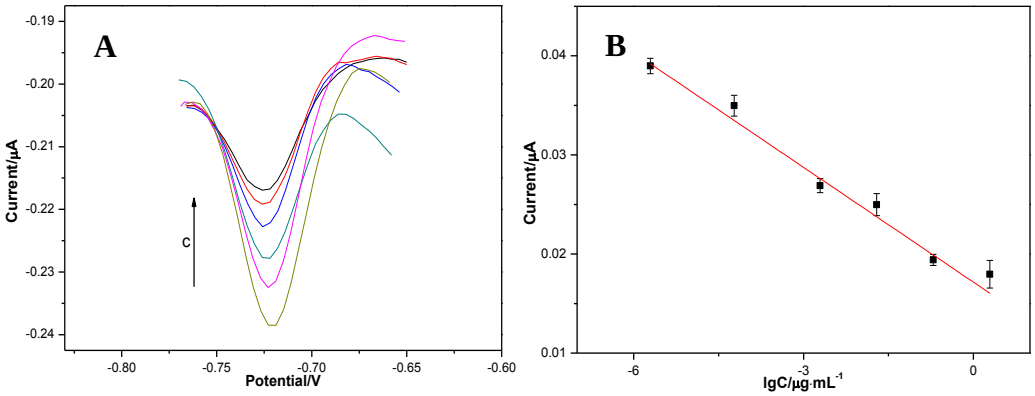

Figure 2

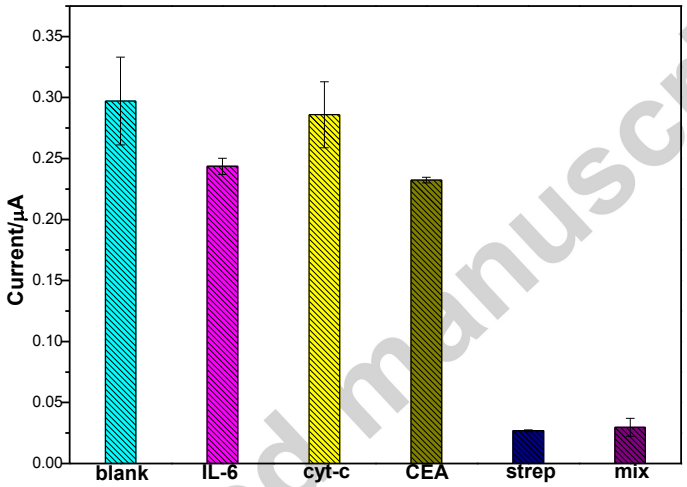

Figure 3

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> An electrochemical biosensor was developed based on a steric hindrance hybridization assay.

> The biosensor for streptavidin had a wide linear range.

> This biosensor could also be employed for the detection of other biomacromolecules by changing the recognition element.

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