Ultrasensitive detection of nucleic acids based on label-free triblock aptamer-triggered electrochemical signal amplification

ABSTRACT

Electrochemical aptasensor has been broadly advanced for nucleic acid detection. However, it is a long-term goal to design an aptasensor with high specificity, flexibility, and simplicity. In this work, we develop a strategy of triblock DNA probe, which consists of two DNA probes at both ends and ployA fragments in the middle as probe-polyA-probe. PolyA fragment has high affinity to gold electrode, so detection probes are able to be assembled on the electrode's surface via polyA instead of traditional Au-S bonds. When the target DNA simultaneously hybridize with the two capture probes, its hybridization stability improvs due to strong base stacking effect. $[Ru(NH_3)_6]^{3+}$ is electrostatically absorbed onto DNA strands with negative charges acting as signal probe. The limit for detection is obtained at 2.9 pM, with a linear range of (10 pM-10 μM). Our electrochemical aptasensor also shows good selectivity, repeatability, and stability. More importantly, the electrochemical sensor can successfully detect DNA in human serum samples, which proves its practical value and extensive applicability in complex environment.

Keywords:

Triblock DNA

Aptamer biosensor

Electrochemistry

Signal amplification

1. Introduction

DNA detection is essential in disease surveillance, molecular identification, and genetic disease diagnosis [1-4]. Therefore, an effective and sensitive detection strategy for DNA is desired. At present, many strategies for DNA detection have been widely applied, such as fluorescence [5-7], mass spectrometry [8], surface enhanced Raman scattering [9,10], colorimetry [11-13], polyacrylamide gel electrophoresis [14], etc. However, these methods have their own limitations, that is, time-consuming, high cost, and cumbersome operation [15]. As an alternative method, electrochemical aptasensors have attracted unique interest because of their low cost simple action, quick response, high precision, and high sensitivity [16-18].

So far, various electrochemical methods for sensitive DNA detection have been established [19-21]. Most electrochemical aptasensors were designed by fixing one end of the aptamer on the electrode surface and labeling the other end with electroactive signal molecules [22,23]. Chad et al. reported a DNA encapsulation analysis method, which used 3' terminal mercaptan fixed on the surface of gold electrode and 5' terminal ferrocene as marker, leading to DNA conformation and signal changes in presence of the target DNA [24]. Zhi group reported electrochemical aptasensors, consisting of methylene blue-modified probes and label-free capture probes. When target DNA was capture by the DNA, the labeled signal probes are released [25]. The above strategies had been to fix DNA on the exterior of gold electrode by using Au-S bonds. In contrast, it's mile greater vital to design label-free aptasensors for DNA detection.

Based on the above research, we propose a label-free electrochemical aptasensing approach primarily based on DNA probe to detect nucleic acid. In the improvement of aptasensor, DNA probe is an essential component that is frequently used in the construction of electrochemical biosensor [26,27]. DNA has specific affinity for a wide range of biological and chemical targets [28]. Therefore, it is far broadly used as an excellent material to build probes for various applications. In

this sensor structure, the DNA structure includes three parts: two DNA probes and polyA fragments. Two DNA probes are linked at each ends of polyA fragments. Different from the conventional SH-DNA probe, our label-free triblock DNA probe can be quickly anchored on the gold electrode through the covalent binding between the gold surface and adenine [29]. When adenine nucleotide fragments were integrated into the DNA strand, the similar competition of other nucleotides for the adsorption sites on the gold electrode causes saturation of adenine fragments on its surface, forcing the alternative probe sequences to shape an upright conformation [30] (Scheme 1). Two capture probes at both ends of the polyA strand would hybridize with the target DNA simultaneously; therefore, the hybridization stability is significantly increased due to the base accumulation effect [31]. Under optimum conditions, we successfully detected DNA in a wide concentration range (10 pM-10 μM). Furthermore, our electrochemical aptasensor shows excellent specificity, stability, and ability for application.

2. Experimental section

2.1 Reagent

The triblock DNA, target DNA, polyT, and polyC were synthesized by Sangon Biotech (Shanghai), and these DNA sequences are shown in Table 1. Tris(hydroxymethyl)methyl aminomethane (tris base), potassium ferricyanide, 6-mercaptohexanol (MCH), hexaammineruthenium (III) chloride, NaCl, $MgCl_2$, KCl, H_2SO_4, and HCl were purchased from Sigma-Aldrich. All chemical reagents were analytical grade. All concentrations of DNA were dissolved in 20 mM Tris-HCl buffer solution (pH=7.4, containing 20 mM KCl, 140 mM NaCl, and 20 mM $MgCl_2$).

Name	Sequences (5'-3')

Triblock DNA	GCG CTT TCC CAC CAA CAC ACA CAC AAA AAA AAA AAA AAA AAA AAA CAC ACA CAC ATT GCC CGG CTT TCT T
Target DNA	TGG TGG GAA AGC GCG TTA CAA GAA AGC CGG GCA A
Probe 1	GCG CTT TCC CAC CA
Probe 2	TTG CCC GGC TTT CT
PolyT	TTT TTT TTT TTT TTT
polyC	CCC CCC CCC CCC CCC

Table 1 The information of DNA oligonucleotides sequences.

2.2 Instrumentation

All electrochemical tests (cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and square wave voltammetry (SWV)) were conducted on an electrochemical workstation (CHI650e, China). The workstation has a three electrode system, which is composed of Au working electrode (2 mm in diameter), Pt wire auxiliary electrode, and Ag/AgCl reference electrode.

2.3 Pretreatment of the electrode

Firstly, the Au working electrode was washed with the newly prepared piranha solution (V_{H2SO4}:V_{H2O2}=3:1) for 6 min at 85 ℃. Then, 0.3 and 0.05 μm alumina powders were used respectively to polish the gold electrode. Subsequently, the electrode was sonicated again with ethanol, and deionized water, respectively. After that, the electrode was scanned in 0.5 M H_2SO_4, and continuous potential scanning was performed at a scanning rate of 0.2 V/s until a repeatable CV was obtained. Finally, the electrode was washed with deionized water and dried.

2.4 Pretreatment of the aptasesor

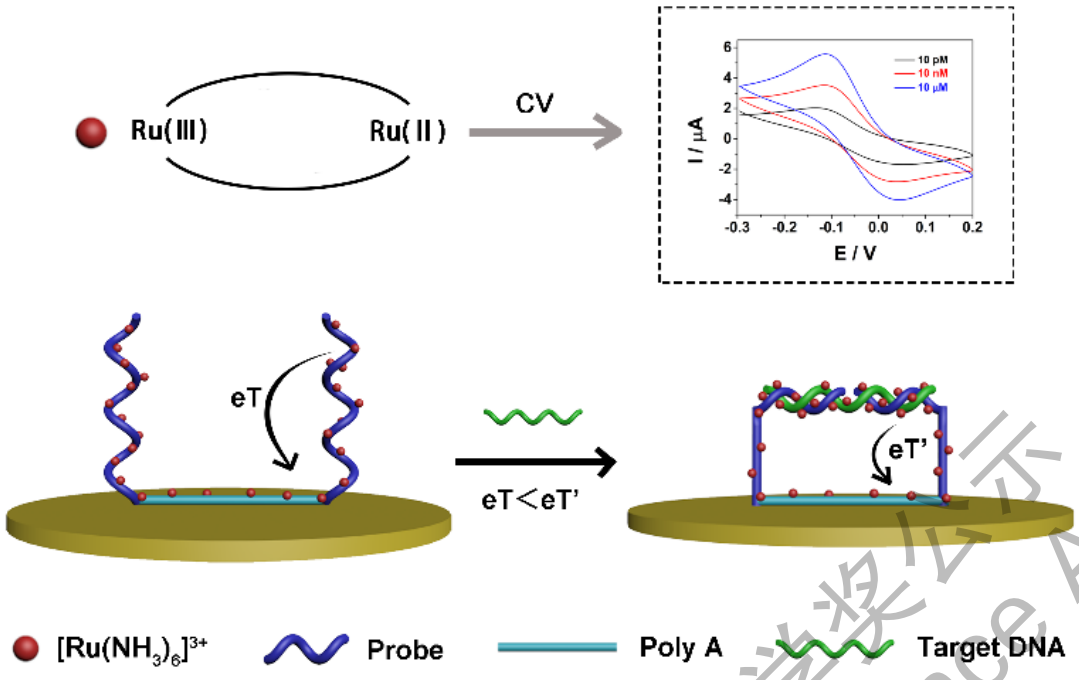
First, various DNA strands were centrifuged at 4000 rpm at room temperature for 1 min, and then prepared into 1 μM mother liquor. 1 μM triblock DNA was heated at 85 ℃ for 6 min and then

cooled to room temperature. 20 μL of triblock DNA solution was dropped onto the electrode and incubated at 37 ℃ overnight. To obtain a well-aligned triblock DNA-modified electrode, the DNA/Au electrode was incubated in 20 μL of 1 mM MCH for 30 min [32,33]. Then, the triblock DNA/Au electrode was rinsed with Tris-HCl buffer. Prior to capture of the target DNA, 20 μL of 20 mM $[Ru(NH_3)_6]^{3+}$ was dropped onto the electrode and incubated for 10 min to ensure $[Ru(NH_3)_6]^{3+}$ can be electrostatically adsorbed on the DNA. For DNA detection, 20 μL of the target DNA with various concentrations (10 pM-10 μM) was dropped on the triblock DNA/Au electrode for 1 hour at 37 ℃. Then, 20 μL of 20 mM $[Ru(NH_3)_6]^{3+}$ was incubated on the electrode for 10 min at 37 ℃ again to make sure that the$[Ru(NH_3)_6]^{3+}$ ions adsorbed on the DNA are saturated. All SWV measurements were carried out in 20 mM Tris-HCl buffer at room temperature.

3. Results and discussion

3.1 Principle of the aptasensor

In Scheme 1, we clearly describe the sensing mechanism of the aptasensor for DNA detection. Herein, we designed a triblock DNA, which connected two DNA probes with specific sequences through ployA fragments, and could be anchored on the gold electrode through ployA fragments. Wherein two DNA probes can apprehend and hybridize with the target DNA. As a signal indicator, $[Ru(NH_3)_6]^{3+}$ could be electrostatically adsorbed by negatively charged DNA phosphate skeleton [34]. With target DNA, $[Ru(NH_3)_6]^{3+}$ was adsorbed on the triblock DNA by electrostatic force. After the probe is hybridized with the target DNA, $[Ru(NH_3)_6]^{3+}$ could be electrostatically adsorbed not only on the polyA fragments and the two DNA probes, but also on the hybridized target DNA. Consequently, the rise in the amount of $[Ru(NH_3)_6]^{3+}$ adsorbed on the DNA resulted in an enhancement in SWV current. In this way, an effective electrochemical sensing strategy was well proposed.



Scheme 1 Illustration of the electrochemical aptamer biosensor for DNA sensing based on label-free triblock DNA.

3.2 Characterization of the electrochemical aptasensor

For the purposes of examing the feasibility of $[Ru(NH_3)_6]^{3+}$ adsorption strategy based on unlabeled probes capturing target DNA at various concentration, we measured the CVs of 10 pM, 10 nM, and 10 μM target DNA under the same conditions. As shown in Fig. 1A, the redox peak currents rise linearly with the increase of DNA concentration, testifying that the increase of the amount of target DNA hybridized increases the amount of $[Ru\ (NH_3)_6]^{3+}$ adsorbed on the DNA strand. In addition, EIS also was employed to characterize the preparation process of the aptasensor. As shown in Fig. 1B, the bare Au electrode exhibits a small semicircle (curve a). Compared with the bare Au electrode, in presence of triblock DNA, the diameter of the semicircle increases significantly (curve b) because of the increase of steric resistance. Also, the binding of the target DNA increases steric resistance on the electrode surface (curve c), which forms a larger semicircle.

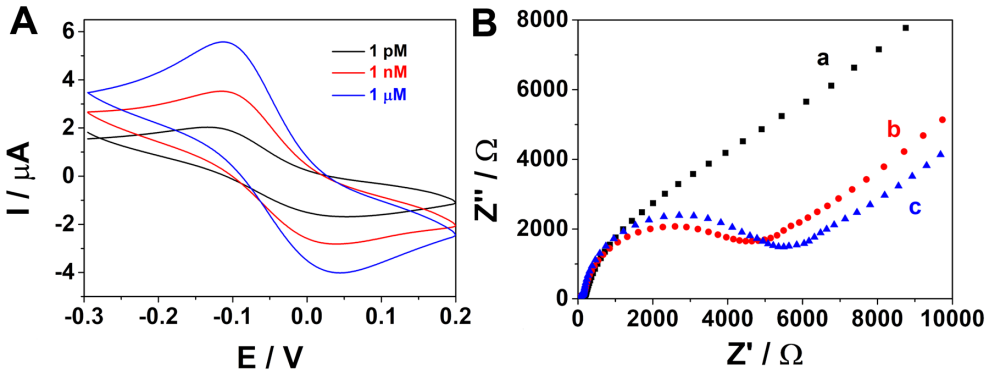


Fig. 1 (A) CV measurements for different concentrations of target DNA completed in Tris buffer solution. The scan rate is 200 mV/s. (B) Impedance diagrams of (a) bare Au electrode, (b) triblock DNA/Au electrode, and (c) 10 nM target DNA/ triblock DNA/Au electrode in 1 mM $[Fe(CN)_6]^{3-/4-}$ solution.

3.3. Sensing performance of the electrochemical sensor

The sensing performance of our electrochemical aptamer biosensor was evaluated in presence of target DNA with diverse concentrations in Tris-HCl buffer (pH 7.4). As shown in Fig. 2(A,B), When the target DNA concentration enhanced from 10 pM to 10 μM, the SWV current of the biosensor increased proportionally. A good linear relationship appears between the SWV peak current and the log of the DNA level (10 pM-10 μM) (Fig. 2C). The corresponding linear equation is y = 0.954 x + 14.46 with R^2 of 0.97. As shown in Fig. 2D, according to the 3σ rule, the limit of detection (LOD) was projected to be 2.9 pM.

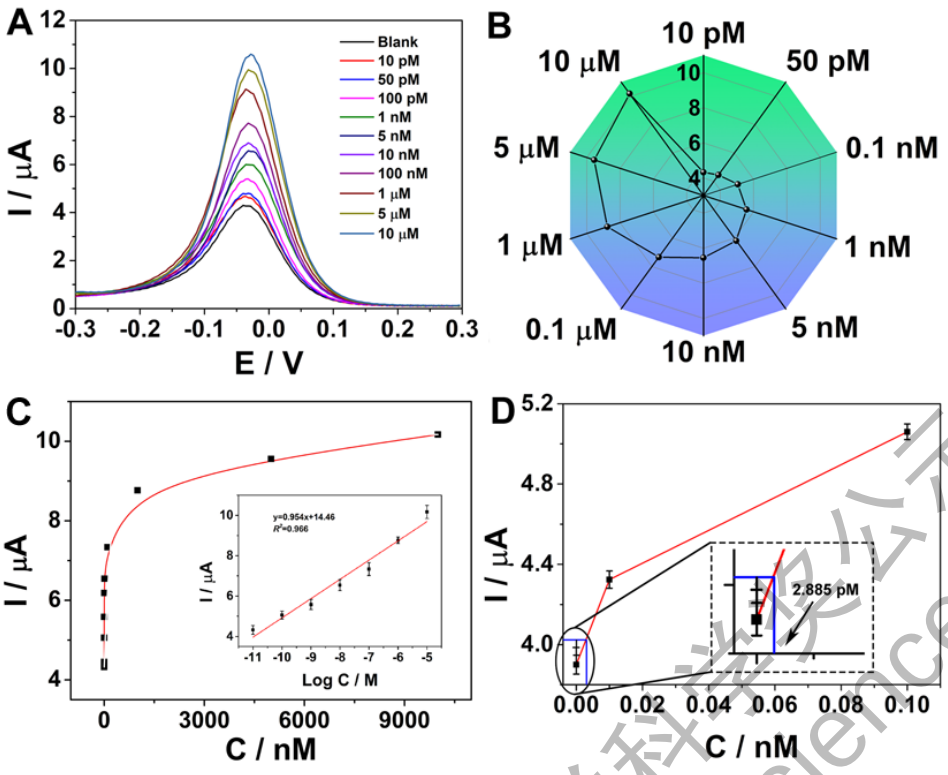


Fig. 2 (A) SWV curves and (B) radar chart of the electrochemical biosensor in presence of target DNA from 10 pM to 10 μM. (C) The SWV peak current-target DNA concentration (10 pM-10 μM) curve. The inset: the linear relationship. (D) The LOD plot of the aptasensor for DNA detection.

3.4. Reproducibility of the electrochemical aptasensor

It is well known that all sensing applications require good reproducibility. Thus, we test the reproducibility of the biosensor. Under the same conditions, the blank and target DNA at 1 nM were measured three times in parallel employing three Au electrodes. As depicted in Fig. 3A, the similar SWV currents of non-target DNA and 1 nM target DNA were obtained with 0.08% and 0.12% of the relative standard deviations (RSDs), respectively, indicating that the biosensor possessed excellent reproducibility. In addition, Fig. 3B shows the SWV current variations of blank, target DNA with 10 pM, 10 nM, and 10 μM in Tris buffer and human serum are negligible. Therefore, the electrochemical aptasensor has potential to be applied to practical samples for the detection of DNA.

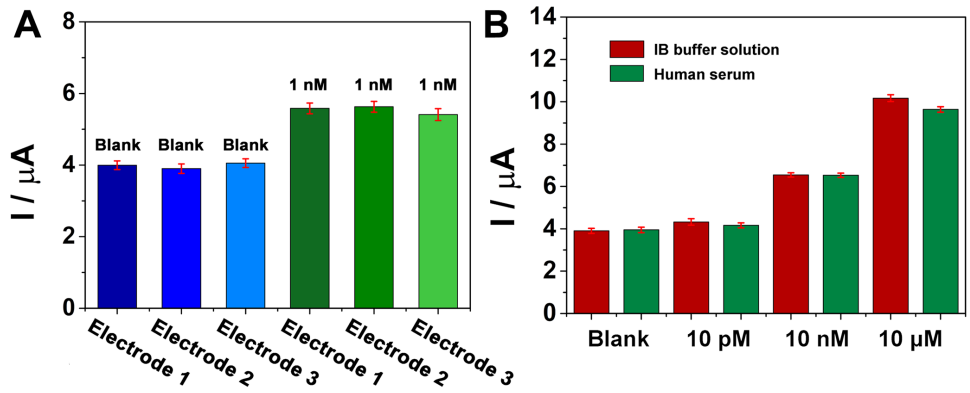


Fig. 3 (A) The reproducibility of the electrochemical aptasensor towards blank and 1 nM target DNA in parallel. (B) The SWV peak currents of the biosensor for detection of 0, 10 pM, 10 nM, and 10 μM target DNA in Tris-HCl buffer and serum.

3.5. Specificity of the electrochemical aptasensor

In order to test the selectivity of the aptasensor, polyT and polyC containing 15 base sequences were selected as interfering substances. As shown in Fig. 4, even if target DNA at 1 μM was 100-fold lower than that of those interferents, compared with the blank, the observed SWV signal of target DNA was significantly higher and the peak currents of the interferents were almost the same. This indicates that our electrochemical aptamer sensor had excellent specificity in detection for the target DNA.

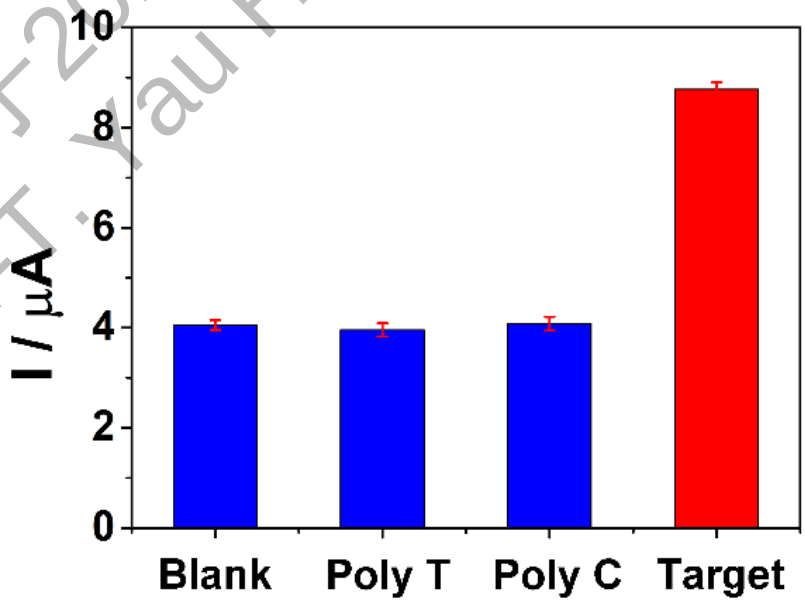


Fig. 4 Bar chart of SWV responses to the blank and three various DNA sequences. The signals

originated from the electrochemical sensor in the presence of 1 μM target DNA and interfering DNAs (polyT and polyC, each at 100 μM).

3.6. Stability of the electrochemical aptasensor

To evaluate the stability of the aptasensor, SWV peak currents were obtained at different storge times at 4 ℃ in refrigerator. Fig. 5 shows the peak current of SWV remained basically unchanged within 36 hour and kept 97.2% of the initial SWV signal. After storge for 36 h, the SWV peak current decreased significantly. After 84 h, the SWV current value remained at 87.2% and reached 71.9% of the initial SWV signal at 96 h. Based on the results above, it is concluded that the aptasensor demonstrates excellent stability.

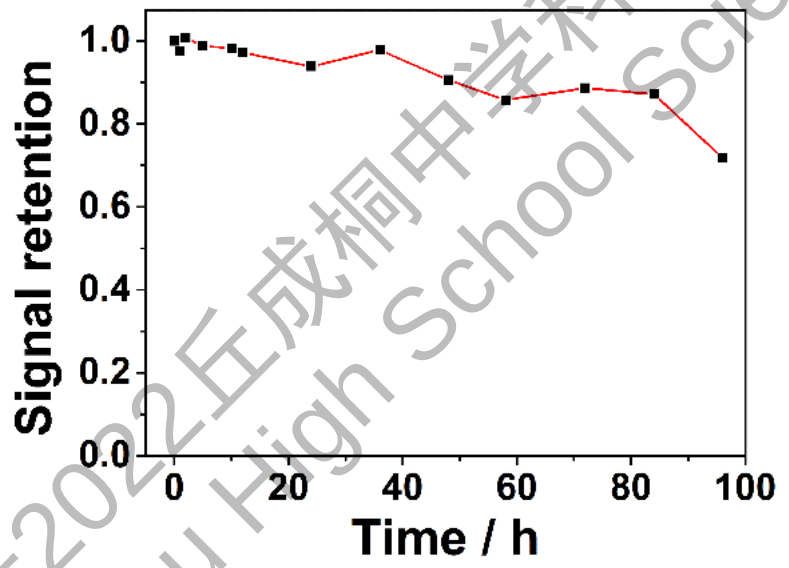


Fig. 5 The SWV signal retention-time curve for 1 nM target DNA

3.7. Application in human serum

To explore the practicality of the aptasensor, serum diluted 100 times was used as a sample for detection. As depicted in Fig. 6A, SWV current signal enhanced as the increasing DNA concentration ranging from 10 pM to 10 μM. The radar figure (Fig. 6B) clearly illustrates the change of SWV peak current to the gradient concentration of target DNA. Happily, as presented in Fig. 6C, a splendid linear relationship between the SWV current and logarithm of DNA level was acquired. The linear fitting equation is $I = 0.914 \lg C + 14.07$ with $R^2 = 0.98$. The LOD of 4.1 pM was

achieved (Fig. 6D). The above results not only show that our sensing strategy is possible to determine DNA in human serum analysis, but also prove the detection ability of our sensor.

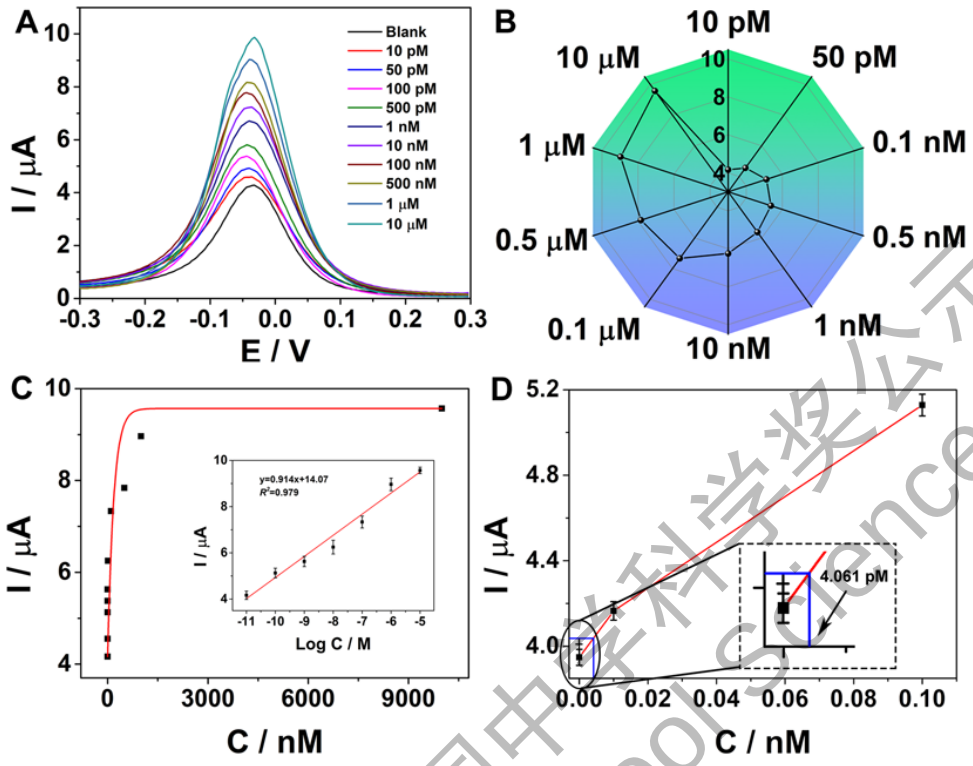


Fig. 6 (A) SWV responses and (B) radar chart of the electrochemical sensor after adding various levels of target DNA (10 pM-10 μM) in human serum. (C) SWV peak current for target DNA with different concentrations ranging from 10 pM to 10 μM in human serum. The inset illustrates the corresponding linear relationship. (D) The LOD of the biosensor for target DNA detection in serum.

4. Conclusions

To sum up, we have proposed a facile, label-free electrochemical aptamer biosensor that can sense nucleic acid. We used a type of unlabeled triblock DNA instead of traditional thiolate DNA to create the electrochemical aptasensor. Two DNA probes were designed to hybridize with the nucleotide sequences of the target DNA at the same time. As a signal converter, $[Ru(NH_3)_6]^{3+}$ could be absorbed onto the triblock DNA to obtain the blank signal through SWV measurement. A range of target DNA (10 pM-10 μM) hybridized with triblock DNA, which resulted in a proportional increase in SWV signal and LOD as low as 2.9 pM. Furthermore, the experiment showed that the reproducibility and

stability of the biosensor were extraordinary. What is more gratifying is that the biosensor can successfully detect DNA in serum samples.

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Acknowledgement

I sincerely thank my teacher for giving me supervision and help. I'm also thankful for my parents to support my interest in chemistry and encourages me to study chemistry in the future. I'm also thankful for S.T. Yau High School Science Award for an opportunity to learn more about what I'm interested in.