Enhanced Charge Transfer by Gold Nanoparticle at DNA Modified Electrode and Its Application to Label-Free DNA Detection

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S Supporting Information

ABSTRACT: Rational utilization of nanomaterials to construct electrochemical nucleic acid sensors has attracted large attention in recent years. In this work, we systematically interrogate the interaction between gold nanoparticles (GNPs) and single-strand DNA (ssDNA) immobilized on an electrode surface and then take advantage of the ultrahigh charge-transfer efficiency of GNPs to develop a novel DNA sensing method. Specifically, ssDNA modified gold electrode can adsorb GNPs because of the interaction between gold and nitrogen-containing bases; thus, the negative electrochemical species $[Fe(CN)_6]^{3-/4-}$ may transfer electrons to electrode through adsorbed GNPs. In the presence of target DNA, the formed double-strand DNA (dsDNA) cannot capture GNPs onto the electrode surface and the dsDNA may result in a large charge-transfer resistance owing to the negatively charged phosphate backbones of DNA. So a simple but sensitive method for the detection of target DNA can be developed by using GNPs without any requirement of modification. Experimental results demonstrate that the electrochemical method we have proposed in this work can detect as low as 1 pM breast cancer gene *BRCA1* in a 10 μL sample volume without any signal amplification process or the involvement of other synthesized complex, which may provide an alternative for cancer DNA detection. This method may also be generalized for detecting a spectrum of targets using functional DNA (aptamer, metal-specific oligonucleotide, or DNAzyme) in the future.

KEYWORDS: biosensor, charge transfer, DNA, electrochemical impedance spectroscopy, gold nanoparticle
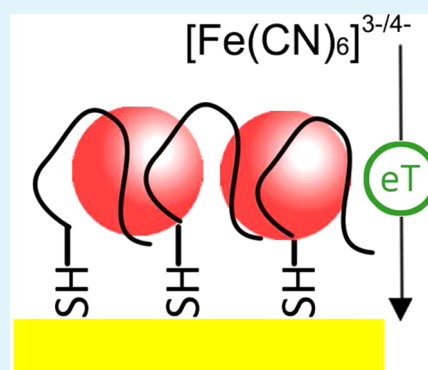
INTRODUCTION

The development of simple and sensitive methods for nucleic acid detection is a long-standing challenge for biochemists. Though polymerase chain reaction (PCR) has been viewed as the "gold standard" because of its high sensitivity,[1] the technique also meets with some difficulties in practical applications including complicated procedures, the need for highly trained operators, and easy false negative results. Electrochemistry-based biosensors may provide suitable alternatives to PCR-based methods for sensitive but cost-effective nucleic acids detection because these types of sensors mostly combine target-induced hybridization events with electrochemical transducers, thus providing rapid, user-friendly, and inexpensive signal readout.[2,3]

In recent years, to further improve the performance of electrochemical biosensors, the introduction of nanomaterials such as metal nanoparticles (Au and Pt),[4,5] carbon materials (carbon nanotubes, graphene, and carbon quantum dots),[6,7] and magnetic nanomaterials[8,9] to electrochemical assay has been a fashion. Among the nanomaterials, the gold nanoparticle (GNP) is a highly attractive candidate for constructing electrochemical biosensors because of its high surface-to-volume ratio, efficient electron transfer, and excellent biocompatibility.[10−13] To date, there are two main strategies to take advantage of GNPs in electrochemical DNA sensing. One is to adsorb amounts of GNPs onto the surface of electrode depending on chemical modification, thus largely increasing the surface area and electrical conductivity of an electrode.[14,15] Another is to construct functional GNPs that are used as labels to increase the loading of electroactive species such as small molecules and enzymes for signal amplification.[16] For these strategies, laborious functional electrode preparation, time-consuming synthesis of signal-triggered nanoparticles, and the modification of electrical molecules usually hamper the inherent properties of simplicity and low-cost of electrochemical methods. Hence, it is of considerable interest to better make use of the intrinsic property of GNPs to develop more

simple strategy, thereby circumventing the problems in electrochemical DNA detection.

On the other hand, it is reported that electrochemical impedance spectroscopy (EIS) is a simple, convenient, and sensitive approach for electrochemical DNA detection, since it bypasses the need to modify biomolecules with labels.[17,18] Moreover, although it is usually difficult to directly analyze the hybridization events of DNA using EIS, making EIS-based DNA detection less sensitive, the use of uncharged peptide nucleic acid (PNA) modified electrode seems to be an efficient way; thus, the change of charge-transfer resistance (R_{ct}) can be directly proportional to the hybridization of complementary DNA.[19,20] Nevertheless, the complicated and costly synthetic process of PNA may hamper its large-scale application.

To better address the above-mentioned problems, we present herein a simple electrochemical DNA sensing method based on the excellent electron-transfer ability of bare GNPs and its unique interaction with ssDNA immobilized on an electrode surface. As is well known, ssDNA can be adsorbed onto the surface of GNPs because of the noncovalent forces while dsDNA does not have this property.[21−26] Therefore, it is reasonable to consider that ssDNA modified electrode can "capture" amounts of GNPs and thus enhance the electron transfer. In this work, we have systematically studied the interaction between bare GNPs and ssDNA immobilized on a gold electrode surface and further taken the advantage to develop a novel nucleic acid detection method. As shown in Scheme 1, in this strategy, the presence of target DNA can

Scheme 1. Interaction between GNPs and DNA Immobilized on an Electrode Surface and Its Sensing Ability for Target DNA Detection
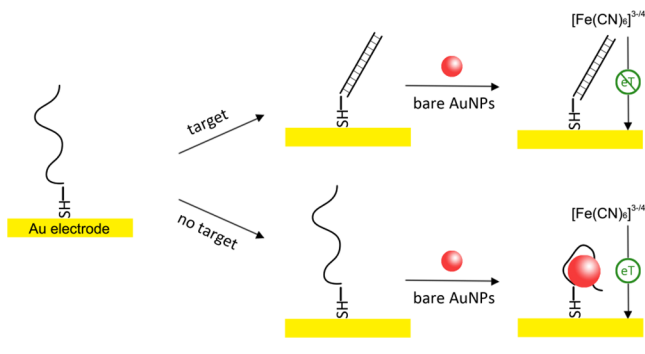
efficiently block the adsorption of GNPs onto the surface of electrode. Such blocking events cannot induce the enhancement of charge transfer between electrode and electrochemical species, resulting in a distinct alteration of the electrochemical response compared with control groups. So an electrochemical method for DNA detection can be developed. Moreover, the developed method is simple and the measurement is rapid without sacrificing sensitivity and selectivity of the measurement, since no requirement of modification of the nanoparticles is necessary; thus, bare GNPs can be directly used. Meanwhile, different from many previous reports that the substrate gold electrode has to be very complicatedly modified with many kinds of molecules, it is not necessary to further modify the substrate electrode, so preparation of the DNA sensor is very simple; thus, performance of the measurement is very convenient. We have also used this method to detect breast cancer gene *BRCA1* and obtained satisfactory results.

■ EXPERIMENTAL SECTION

Reagents. The DNA strands used in this work were synthesized by Sangon Biotechnology Co. Ltd. (Shanghai, China) (capture strand, 5′-GAGAAACCCTATGTATGCTC-(CH₂)₆-SH-3′; 5T, 5′-TTTTT-(CH₂)₆-SH-3′; 10T, 5′-TTTTTTTTTT-(CH₂)₆-SH-3′; 20T, TTTTTTTTTTTTTTTTTTTT-(CH₂)₆-SH-3′; 40T, TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-(CH₂)₆-SH-3′; target, 5′-GAGCATACATAGGGTTTCTC-3′; single-base mismatched target, 5′-GAGCATACATTGGGTTTCTC-3′). Trisodium citrate, tris(2-carboxyethyl)phosphine hydrochloride (TCEP), and mercaptohexanol (MCH) were purchased from Sigma. HAuCl₄·3H₂O was obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All other chemicals not mentioned here were of analytical reagent grade.

The buffer solutions used in this work are listed as follows. DNA immobilization buffer: 10 mM Tris-HCl, 1 mM EDTA, 10 mM TCEP, and 0.1 M NaCl (pH 7.4). Hybridization buffer: 10 mM Tris-HCl (pH 7.4) with 0.3 M NaCl. Electrode washing buffer: 10 mM Tris-HCl, 0.3 M NaCl, and 0.05% Tween-20 (pH 7.4). Buffer for cycle voltammetry (CV) and electrochemical impedance spectroscopy (EIS): 5 mM $[Fe(CN)_6]^{3-/4-}$ with 1 M KNO₃. Buffer for chronocoulometry (CC): 10 mM Tris-HCl containing 50 μM $[Ru(NH_3)_6]^{3+}$ (pH 7.4). The surface coverage of DNA as well as DNA hybridization efficiency is calculated using the relationship $\Gamma_{DNA} = \Gamma_0(z/m)N_A$, where Γ_{DNA} is the surface density of the DNA, Γ_0 is the surface coverage of $[Ru(NH_3)_6]^{3+}$, z is the charge of $[Ru(NH_3)_6]^{3+}$, m is the number of bases in the capture DNA, and N_A is Avogadro's number.[27] All the buffers were prepared with doubly distilled water, which was purified with a Milli-Q purification system (Barnstead, Bedford, MA) to a specific resistance of 18 MΩ cm.

Preparation of Gold Nanoparticles. Preparation of gold nanoparticles (GNPs) with an average diameter of 13 ± 2 nm was done according to our previous reports.[28] In short, 5 mL of trisodium citrate (38.8 mM) was added rapidly to a boiled solution of 50 mL of HAuCl₄ (0.01%) with vigorous stirring. The color of the solution changed from pale yellow to deep red within 1 min. Then the solution was boiled and stirred for another 30 min to ensure complete reaction and was slowly cooled to room temperature for use.

Immobilization of Capture Strand on the Gold Electrode. The capture strand was immobilized onto the gold electrode surface via gold−sulfur chemistry. The gold disk electrode was first cleaned with piranha solution (70% concentrated sulfuric acid, 30% H₂O₂) for 10 min followed by rinsing with doubly distilled water. Then the electrode was polished with 1 and 0.3 μm α-Al₂O₃ in sequence. Residual alumina powder was removed by sonicating the electrode successively in both ethanol and pure water, followed by soaking in nitric acid (50%) for 30 min and electrochemically cleaning with 0.5 M H₂SO₄ to remove any remaining impurities.

After being dried with nitrogen, the electrode should be immediately used for capture strand immobilization. Low-density surfaces were prepared by incubation of the electrode with 0.5 μM capture strand in the immobilization buffer for 1 h, followed by being rinsed with doubly distilled water. Medium-density and high-density surfaces were separately obtained by incubation of the electrode with 1 and 5 μM capture strand in the immobilization buffer with 1 M NaCl. Finally, the electrode was treated with 1 mM MCH for 2 h to obtain a well-aligned DNA monolayer.

Detection of Target DNA. In a typical DNA analysis, the prepared capture strand modified electrode was immersed in 10 μL of different concentrations of target DNA and reacted for 1.5 h, followed by washing with buffer. Thereafter, 4 μL of bare gold nanoparticles (10 nM) was placed on the electrode surface for 0.5 h. Cyclic voltammogram and electrochemical impedance spectra were recorded in 5 mM $[Fe(CN)_6]^{3-/4-}$ with 1 M KNO₃. The experimental parameters were as follows: bias potential, 0.225 V; amplitude, 5 mV; frequency range, 0.1−10 kHz. TEM images were obtained with a JEM-2100 transmission electron microscope operating at 120 kV (JEOL, Japan). AFM images were recorded with a 5500 AFM/SPM (Agilent

Instrument, USA) in tapping mode. Absorbance spectrum was collected using UV−vis spectroscopy (UV 2450, Shimadzu, Japan).

RESULTS AND DISCUSSION

To demonstrate the enhancement of electron transfer by GNPs on ssDNA modified electrode, cyclic voltammetry (CV) and EIS have been employed to study the electrochemical behavior of ssDNA modified electrode in the presence of $[Fe(CN)_6]^{3-/4-}$ redox probe. As shown in Figure 1A, the ssDNA
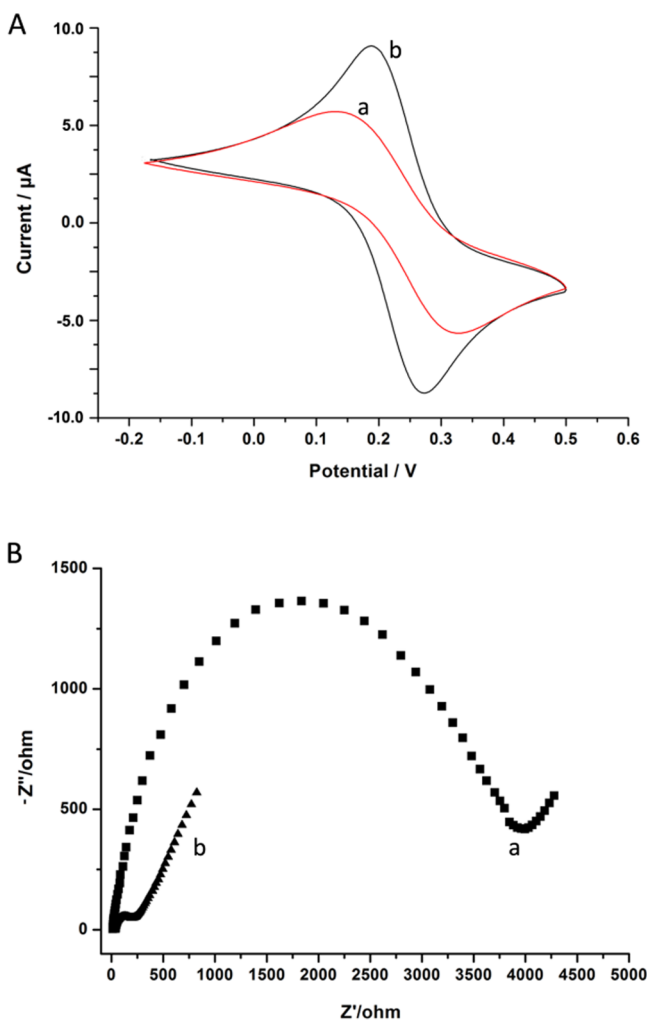
Figure 1. (A) CV and (B) EIS responses at the DNA modified electrode (a) before and (b) after its incubation with 10 nM GNPs for 30 min. Electrochemical species: 5 mM $[Fe(CN)_6]^{3-/4-}$ with 1 M KNO_3. Scan rate: 50 mV/s. Biasing potential: 0.225 V. Amplitude: 5 mV. Frequency range: 0.1 Hz to 10 kHz.

modified gold electrode shows a quasi-reversible redox cycle with a larger peak separation, while in the presence of GNPs, a reversible redox cycle with a peak separation of 50 mV is observed. So GNPs not only can be adsorbed onto the DNA modified electrode surface because of the strong noncovalent binding of nucleobases with GNPs but also can greatly promote the charge transfer at the interface.

EIS can provide more prolific information about the interface property change of the DNA modified electrode, since the increase in semicircle diameter of EIS spectrum is equal to the increase of the interfacial R_{ct}. So the capability of electron transfer at different conditions is further investigated by EIS

experiments. Figure 1B indicates the results of EIS experiments for the DNA modified electrode before and after its incubation with GNPs. Similar to the results of a CV study, R_{ct} decreases from 3213 to 199 Ω, about 93.8% signal reduction, after incubation of the DNA modified electrode with GNPs.

The formal potential $E°$ of $[Fe(CN)_6]^{3-/4-}$ at the bare electrode is found to be 225 mV according to the CV study, as shown in Figure 2A (red curve). $E°$ is estimated as $(E_{pc} + E_{pa})/2$, where $E_{pc} = 190$ mV is the cathodic peak potential and $E_{pa} = 260$ mV is the anodic peak potential. Interestingly, after incubation of the DNA modified electrode with GNPs, a subtly decreased peak separation and larger peak current can be observed, suggesting that the DNA modified electrode even has a better charge-transfer ability than bare gold electrode after it is loaded with GNPs. We attribute it to the direct interaction between electrode surface and amounts of GNPs, which enlarges the electroactive surface area and provides the conducting bridges for the electron transfer of $[Fe(CN)_6]^{3-/4-}$. To study the controlled factor of the electrochemical process on the electrode surface, we have also checked the effect of scan rate (ν) on the peak current in the cyclic voltammograms. At scan rates in the range of 10−250 mV/s, the reduction and oxidation peak currents increase linearly with the square root of the scan rate ($\nu^{1/2}$), indicating that the process is controlled by diffusion. Figure 2C shows the linear relationship between the peak currents and $\nu^{1/2}$, with the equation $y = 36.57x + 1.04$ ($R^2 = 0.997$) and $y = -39.86x - 0.65$ ($R^2 = 0.999$), where y and x stand for the peak current and $\nu^{1/2}$, respectively.

The length of DNA is a key factor that may influence the interaction between GNPs and the DNA immobilized on the electrode surface. So we have used 5T-40T DNA modified electrode for the comparison. Different from the homogenous system, it is observed that even the shortest DNA strand (5T) can efficiently capture GNPs and reduce the resistance (Figure 3). In addition, the GNPs/DNA modified electrode is found to be very stable because the peak currents remain almost unchanged after continuously cycling the electrode for 20 cycles. These results substantially indicate the strong polyvalent interaction between GNPs and ssDNA immobilized on the electrode surface.
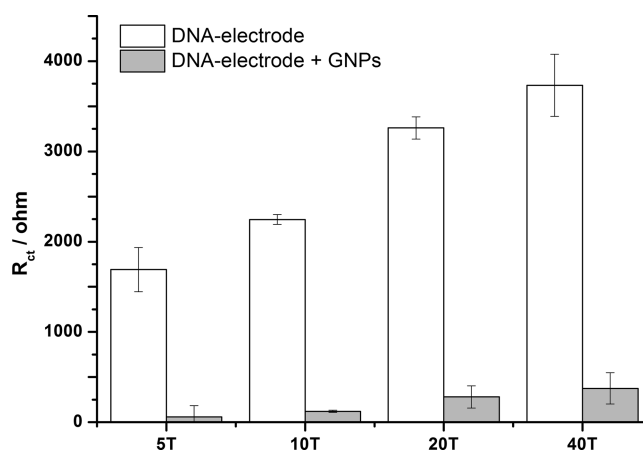
The above results are obtained at low density DNA modified electrode by preparing the modified electrode with 0.5 μM DNA. Since the surface coverage of DNA on gold electrode surface is an important factor for improving the performance of electrochemical DNA sensor, we have prepared two other DNA modified electrodes by using 1 and 5 μM DNA, namely, medium and high density DNA modified electrode. As shown in Figure 4, the degree of resistance decrease of the modified electrode $((R_{ct(before)} - R_{ct(after)})/R_{ct(before)})$ after its incubation with GNPs is $R_{ct(low)}$ (92.2%) $> R_{ct(medium)}$ (85.2%) $\gg R_{ct(high)}$ (37%). This result is reasonable because a lower density of DNA immobilization means a larger intermolecular distance, which may tolerate GNPs directly contacting with the substrate gold electrode, while high DNA density can block the interaction between GNPs and the substrate electrode. On the other hand, lower density surfaces often bring higher hybridization efficiency when it comes to solid-supported sensors.[29] In this work, we have used chronocoulometry to determine DNA hybridization at different density of DNA immobilization and have confirmed this issue. For instance, at a low density of 1.28×10^{12} molecules/cm^2, a high efficiency of 87.6% can be obtained, while a low efficiency of 11% will be obtained at a high density of 1.10×10^{13} molecules/cm^2. So we
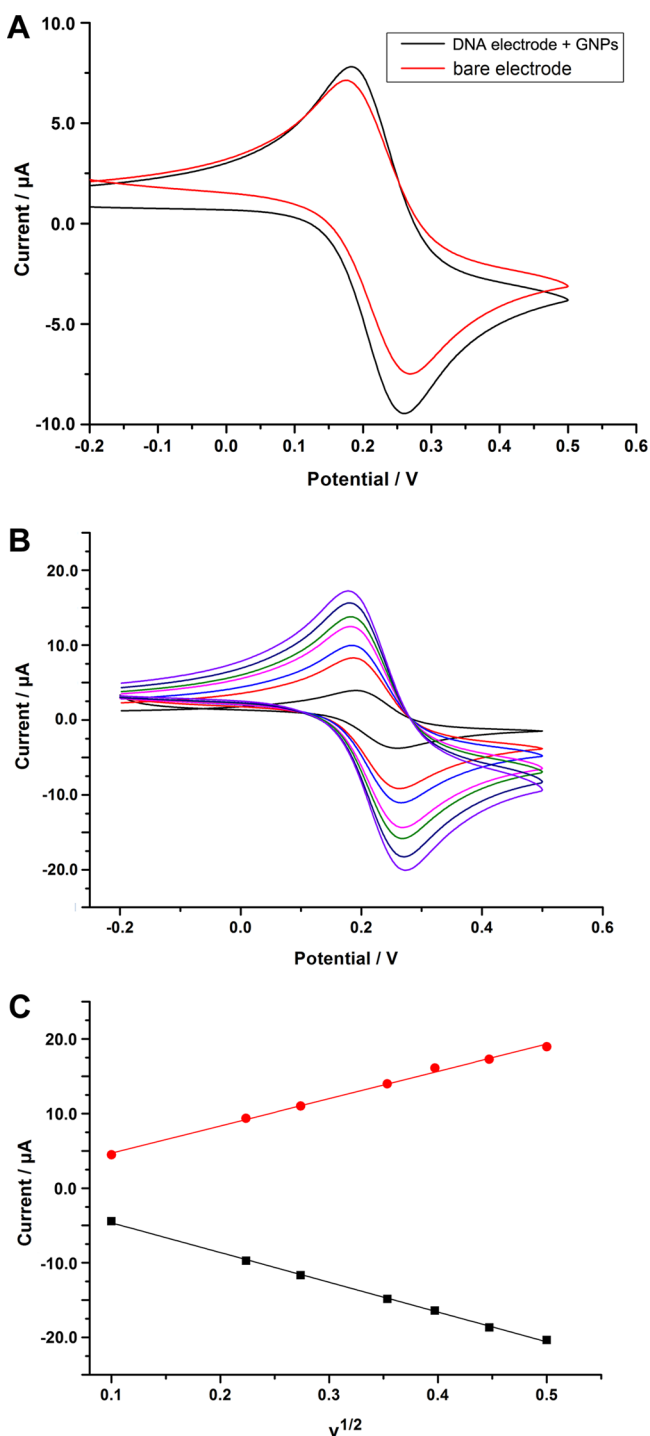
Figure 2. (A) Cyclic voltammograms of the bare electrode (red) and the DNA modified electrode after its incubation with 10 nM GNPs (black). Scan rate: 50 mV/s. (B) Cyclic voltammograms of the DNA modified electrode after its incubation with 10 nM GNPs recorded at various scan rates (10, 50, 75, 125, 150, 200, 250 mV/s from inner to outer). (C) Plots of reduction and oxidation peak currents vs the square root of scan rate ($\nu^{1/2}$). Electrochemical species: 5 mM $[Fe(CN)_6]^{3-/4-}$ with 1 M KNO$_3$.


Figure 3. Comparison of charge-transfer resistance before and after incubation of the DNA modified electrode with GNPs (10 nM) for 30 min by using different lengths of DNA. Error bars show the standard deviations of measurements taken from three independent experiments. Electrochemical species: 5 mM $[Fe(CN)_6]^{3-/4-}$ with 1 M KNO$_3$.
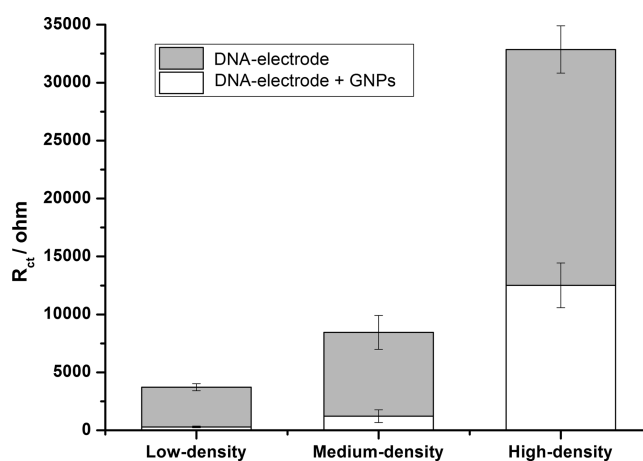
Figure 4. Comparison of the decrease of charge-transfer resistance of the DNA modified gold electrodes with different surface DNA densities after its incubation with GNPs. In the figure, low-density (1.28×10^{12} molecules/cm^2), medium-density (4.18×10^{12} molecules/cm^2), and high-density (1.10×10^{13} molecules/cm^2) surface coverage are calculated by chronocoulometry. Error bars show the standard deviations of measurements taken from three independent experiments.

have used a low density DNA modification on the substrate gold electrode.

After having proved the strong interaction between GNPs and DNA immobilized on an electrode surface and the possible application to DNA detection, we then tested the electro-

chemical assay for the detection of breast cancer gene *BRCA1*. As shown in Figure 5A, the cyclic voltammogram changes a little in the presence of 100 nM target DNA by using the DNA modified electrode without the involvement of GNPs. However, it can be observed that the change is very distinct if GNPs have been used for the test. What is more, the change can be simply and very sensitively illustrated by using the EIS technique. As is shown in Figure 5B, if the DNA modified electrode is treated with various concentrations of target DNA for 1.5 h followed by incubation with GNPs for 0.5 h, the R_{ct} can be intensified along with the increase of target concentration. By analysis of the R_{ct} value with various target concentrations, the linear detection range of this method between the R_{ct} value and the logarithm of the concentration of target DNA is known to be 1 pM to 500 nM. The linear equation is $y = 1913.2 + 511.7x$, where y is R_{ct} and where x is
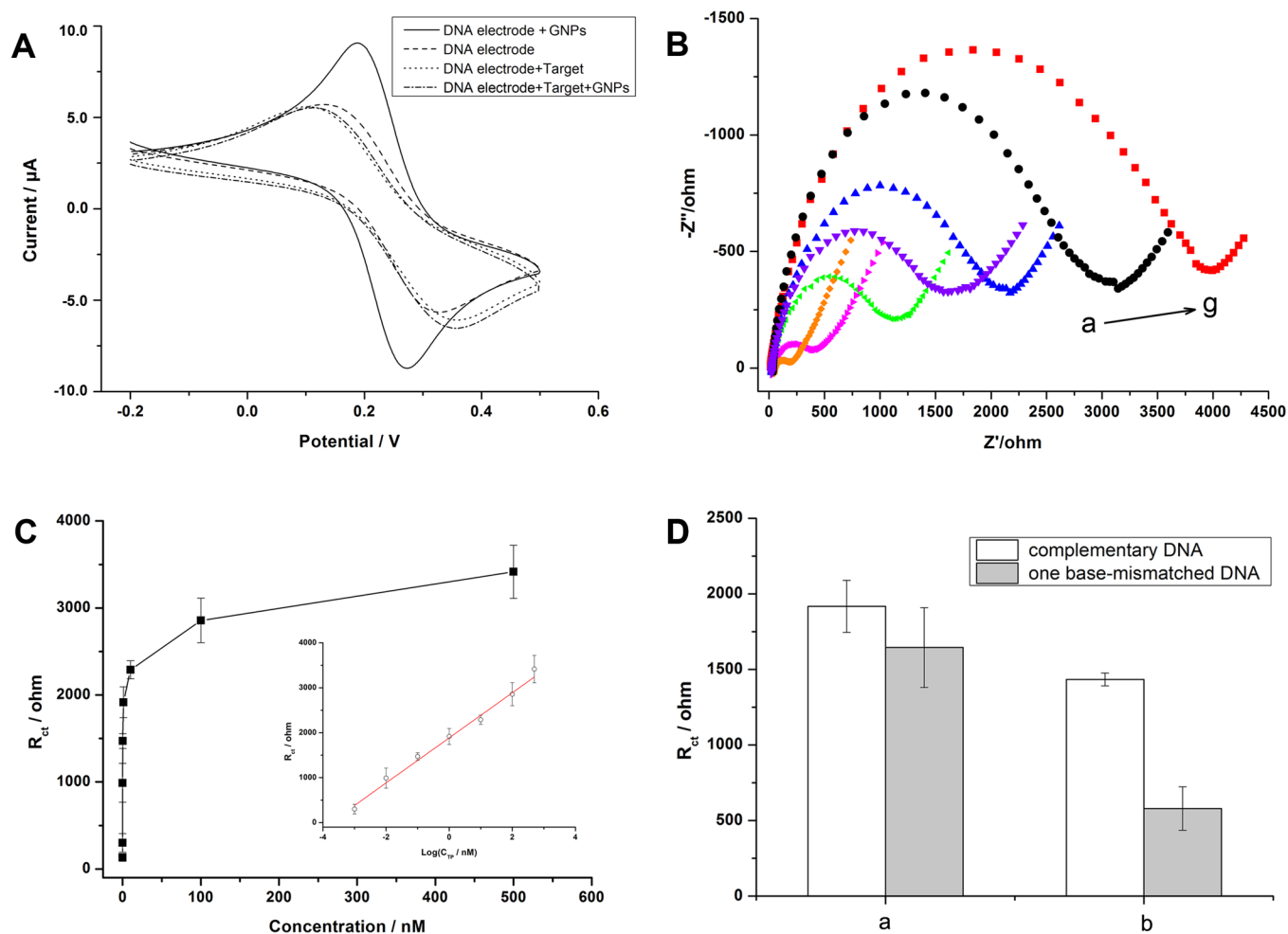
Figure 5. (A) Cyclic voltammograms obtained at the DNA modified electrode (dash), DNA modified electrode + GNPs (solid), DNA modified electrode + target DNA (dot), DNA modified electrode + target DNA + GNPs (dash dot). (B) Nyquist plots of impedance spectra obtained by the DNA sensor after incubation with different concentrations of target DNA for 1.5 h and 10 nM GNPs for another 0.5 h: (a) 1 pM; (b) 10 pM; (c) 100 pM; (d) 1 nM; (e) 10 nM; (f) 100 nM; (g) 500 nM. (C) Relationship between R_{ct} value and target DNA concentration. Inset shows the linear relationship between R_{ct} value and logarithm of the concentration of target DNA. (D) EIS responses of the DNA sensor to 1 nM complementary DNA and one base mismatched DNA at (a) 37 °C and (b) 65 °C.

the logarithm of the target concentration (regression coefficient $R^2 = 0.986$). The limit of detection is estimated to be 1 pM at a S/N of 3, and it allows the use of a small sample volume as low as 10 μL, equating to an absolute detection limit of 10 amol. In addition, at an elevated temperature around the melting temperature (65 °C) of capture strand/single-base mismatched DNA, the proposed sensor demonstrates a good selectivity towards complementary DNA (Figure 5D).

CONCLUSIONS

We have developed a simple, label-free, and sensitive electrochemical DNA sensor by making use of the excellent electron-transfer ability of GNPs and the interaction between GNPs and ssDNA immobilized on an electrode surface. The proposed method offers several notable advantages. (1) It is simple and convenient: label-free detection and no requirement of preparation of functional nanoparticles. (2) It is economical: tiny amounts of samples and reagents needed. (3) It is sensitive and versatile: detection of a large range of targets if using functional DNA structures such as aptamers. Therefore, the proposed method may present a promising strategy for

constructing high-performance electrochemical biosensors for analyte analysis in the future.

■ ASSOCIATED CONTENT

ⓢ Supporting Information
Additional figures showing UV−vis, AFM, and chronocoulometry results and equivalent circuit for electrochemical impedance spectroscopy. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions
The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes
The authors declare no competing financial interest.

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