Supporting Information

1. Experimental Section

1.1. Materials and chemicals

All oligonucleotides are synthesized by Sangon Inc. (Shanghai, China). Their sequences are shown below:

Capture DNA 1: 5'-PHOS- G ATT TTC TTC CTT TTG TTC-HS-3'

Capture DNA 2: 5'- G ATT TTC TTC CTT TTG TTC-HS-3

Target DNA (BRCA1 breast cancer gene): 5'- GAA CAA AAG GAA GAA AAT C -3'

Single-base mismatched DNA: 3'- GAA CAA AAG CAA GAA AAT C -5'

Random DNA: 3'- GGT CAG GTG GGG GGT ACG CCA GG -5'

3' end of capture DNA was thiolated which could be immobilized on the gold electrode. Target DNA could hybridize with capture DNA. Single-base mismatched DNA contained a mismatched single base (in underline).

Tris-(2-carboxyethyl) phosphine hydrochloride (TCEP) and 6-mercaptohexanol (MCH) were purchased from Sigma (St. Louis, MO) and used without further purification. Other chemicals employed were analytical reagent grade and double distilled water (Milli-Q, Millipore, resistance 18.2-MΩ) was used throughout the experiments.

DNA buffer solutions (the concentrations of oligonudeotides are 1 μM) were obtained by dissolving oligonudeotides in 40 mM Tris-acetate buffer solution (pH 7.4) containing 200 mM NaCl and 1 mM $MgCl_2$.

Lambda exonuclease (concentration, 10 units/μL) and its 10× reaction buffer (containing 670 mM glycine-KOH (pH 9.4), 25 mM $MgCl_2$, 0.1% (v/v) Triton X-100) were purchased from Sangon Inc. Storage buffer of lambda exonuclease contains 25 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 1 mM DTT, 50 mM NaCl, 0.1% (v/v) Triton X-100 and 50% (v/v) glycerol. The 10× reaction buffer consists of 670 mM glycine-KOH (pH 9.4), 25 mM $MgCl_2$, and 0.1% (v/v) Triton X-100.

5 mmol/L $[Fe(CN)_6]^{3-/4-}$ solution containing 0.1 mol/L KCl was prepared and used for electrochemical measurement.

1.2. Apparatus

All electrochemical measurements were carried out using CHI 660D electrochemical system (CH Instruments, Shanghai, China) at room temperature. A three-electrode electrochemical cell was used. Gold electrode (2 mm in diameter, CH Instruments, Shanghai, China) was used as the working electrode. Platinum wire and Ag/AgCl (saturated with KCl) were used as counter and reference electrodes, respectively.

1.3. Preparation of the sensor

A gold electrode was polished with aqueous slurries of 1.0 μm, 0.3 μm and 0.05 μm α-Al_2O_3 powders on a polishing microcloth and sonicated with deioned water, ethanol for 3 min, respectively. Finally, the gold electrode was rinsed with deioned water, and then electrochemically cleaned by consecutively cycling in the potential range of 0 ~ 1.6 V in 0.5 M sulfuric acid. Prior to attachment to the gold surface, thiolated capture DNA was incubated with 100 mM TCEP for 1 h to reduce disulfide

bonds and subsequently diluted to 0.1 μM with phosphate buffer. The clean gold electrode was then immersed into the reduced capture DNA solution for 60 min in the dark. The functionalized surface was subsequently passivated with 1 mM MCH for 2 h at room temperature. Thus, this capture DNA/MCH modified electrode was used for the following experiment.(Note: After each treatment, the electrode should be rinsed with deioned water or buffer solution to eliminate the physical adsorption)

1.4. DNA Target hybridization and exonuclease digestion.

The capture DNA/MCH modified electrode was then incubated in the reaction solution to allow the capture DNA to react with the DNA (target, single-base mismatched or uncomplimentary DNA, respectively) and exonuclease (0.2 units/μL) in solution. All reactions were performed for 80 min (except for the time-course study) in 37 ^{o}C water bath in the dark. After the reactions, the electrode was switched to phosphate buffer to equilibrate for at least 30 min prior to measurements.

1.5. Electrochemical detection

The above treated gold electrode, as the working electrode, was immersed into the electrochemical cell containing 5 mM $[Fe(CN)_6]^{3-/4-}$ solution containing 0.1 M KCl, and then EIS was performed. The parameter of EIS was set as follow: The based potential was 0.214 V (vs. Ag/AgCl) and the amplitude was 5.0 mV, and the electrochemical impedance spectra were recorded in the frequency range of 10 kHz to 1 Hz. The data are represented in Nyquist plots (Z'' vs. Z', Z''= imaginary part of impedance and Z'= real part of impedance). The impedance spectra are fitted to a Randles equivalent electrical circuit for this modified electrode, containing the

electrolyte solution resistance R_s, the surface electron transfer resistance R_{ct} (R_{ct} reflects the surface condition of the gold electrode surface), the Warburg impedance Z_w and the constant phase element related to double layer capacitance C_{dl}. From the regression, the charge-transfer resistance R_{ct} is obtained and used to quantified assay.

2. Optimization of Experimental Conditions

In order to obtain well assay result, some factors are investigated in detail. The surface density of capture DNA on the electrode is a crucial parameter contributing to signal gain, which can be controlled by varying the probe concentration during sensor fabrication. To optimize the signal gain, the performance of the sensors fabricated with different concentrations of capture DNA is studied under standard reaction conditions with 1 nM target DNA and 0.2 units/μL exonuclease. The change of R_{ct} ($\Delta R_{ct}=R_{ct0}-R_{ct1}$, where R_{ct0} and R_{ct1} represent charge-transfer resistances of capture DNA modified electrode and capture DNA-target DNA/MCH modified electrode after being immersed into the lambda exonuclease in reaction buffer solution, respectively) is used to characterize the change of electrode surface. As shown in Figure S1A, with increasing capture DNA concentration, the ΔR_{ct} enhances monotonically. When the concentration of capture DNA is 1.0 μM (a packing density of 1.34×10^{12} molecule/cm^2), a maximal ΔR_{ct} is observed. However, when the concentration of capture DNA exceeds 1.0 μM, the value of ΔR_{ct} gradually decreases. The reason may lie in that at the low molecular coverage capture DNA easily hybridize with target DNA to form dsDNA, thus the digestion can perform, resulting in large ΔR_{ct}. While at high surface density it is difficult to form dsDNA due to the

spatial restriction effects, which affects the digestion, resulting in the decrease of $\triangle R_{ct}$. Hence, 1.0 μM capture DNA is chosen as the optimal concentration for the following experiment.

Next, the digestion time of lambda exonuclease is checked. This time-course study is performed in the presence of 1.0 μM capture DNA, 0.2 units/μL lambda exonuclease, 1.0nM and 10nM target DNA, respectively. The result is shown in Figure S1B. It is found that within 80 min $\triangle R_{ct}$ enhances quickly, while digestion time is over 80 min, the increase of the $\triangle R_{ct}$ becomes slow (1.0nM target DNA, curve 1). To higher concentration target DNA (10nM, curve 2), when the digestion time reaches about 68 min, the increase of the $\triangle R_{ct}$ becomes slow. Hence, 80 min is used as the optimal digestion time in this experiment.

3. Efficiency of Signal Amplification

Under the above optimized condition, the absolute values of R_{ct} ($|\triangle R_{ct}|$) of the sensor between the absence and presence of lambda exonuclease are compared. The concentrations of capture DNA, target DNA and lambda exonuclease are 1.0 μM, 1.0 nM and 0.2units/μL, respectively. The results are shown in Figure S3. Firstly, in the absence of lambda exonuclease, after the hybridization a low $|\triangle R_{ct}|$ (130 Ω, histogram a) is obtained. In the presence of lambda exonuclease, $|\triangle R_{ct}|$ has 9-folds improvement (1130 Ω, histogram b) over the sensor without the amplification of exonuclease. Without exonuclease, one target DNA can just hybridize with one capture DNA, resulting in the slight $|\triangle R_{ct}|$. The presence of exonuclease digests capture DNA and releases target DNA, and then the released target DNA can hybridize with other

capture DNA to trigger a new cycle. The final result is that the negative charge decreases sharply, resulting in the distinct $|\Delta R_{ct}|$. Therefore, it is proved that the introduction of exonuclease can amplify the signal. Thus, combination of EIS and exonuclease, the amplified detection of target DNA can be achieved.

4. Specificity

The selectivity of the present biosensor was investigated by using the capture DNA to hybridize with the same concentration (1 nM) of complete complementary target DNA sequence, single-base mismatched DNA sequence and noncomplementary DNA sequence, respectively. As shown in Figure S4, after the digestion a large ΔR_{ct} is observed for the target DNA (histograms a). Whereas, there is a small ΔR_{ct} when the capture DNA hybridizated with noncomplementary DNA, which due to the no successful hybridization between the capture DNA and noncomplementary DNA (histogram c). Additionally, a small ΔR_{ct} (histogram b) is observed for single-base mismatched DNA, which indicates that much negative charge is still on the electrode surface due to the unaccomplished hybridization (attributed to the base mismatch). This result shows that this sensor has hybridization specificity. However, using this DNA sensor it is difficult to differentiate single base from low concentration matched DNA, one of the reasons is that the signal of the single base mismatch DNA may be blocked by that of matched DNA. Hence, improved strategies need to be developed to overcome this shortage in the future.

After the digestion, the modified electrodes are preformed EIS measurement at least 5 times. It is found that the relative standard deviations (RSD) of R_{ct} are in the

range of 1.2% to 1.8%. Moreover, after the test, the electrodes are polished and remodified. Under the same condition, 6.2% of R_{ct} change can be achieved compared with the previous measurement. These results show this target recycling DNA sensor has good stability and regeneration.

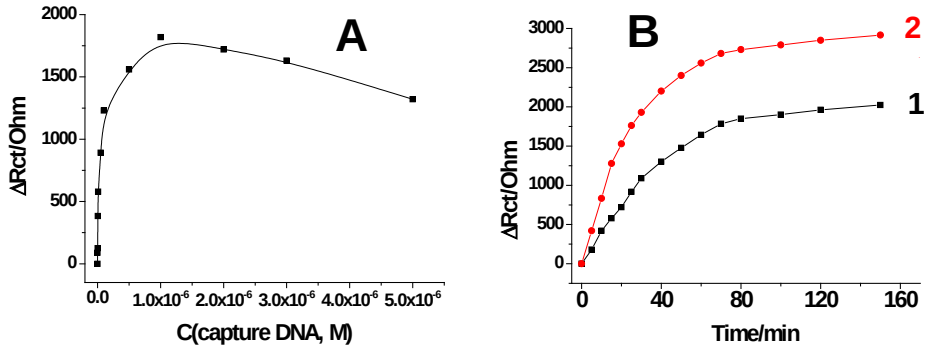
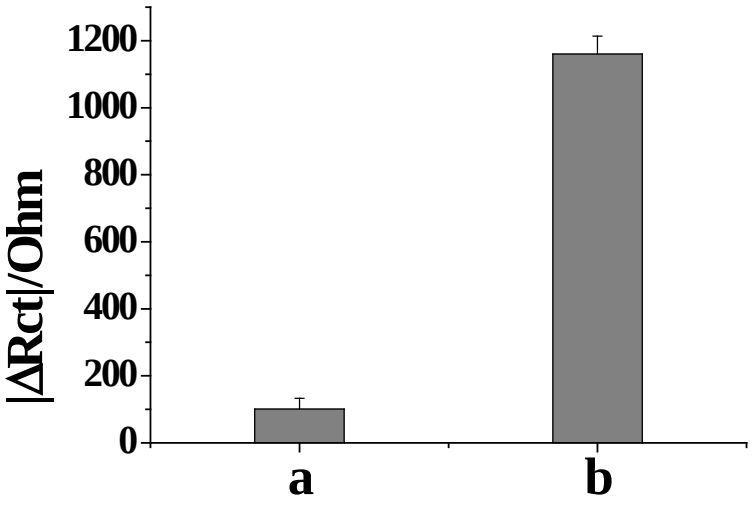


Figure S1 (A) The effect of capture DNA concentration. (B) The influence of the digestion time between dsDNA/MCH modified gold electrode and lambda exonuclease. Curve 1: 1.0 μM capture DNA, 0.2 units/μL lambda exonuclease and 1.0nM target DNA; Curve 2: 1.0 μM capture DNA, 0.2 units/μL lambda exonuclease and 10nM target DNA.



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165 **Figure S2** Changes of R_{ct} ($|\triangle R_{ct}|$) in the preasence of capture DNA with PHOS

166 (histogram a) and without PHOS (histogram b) in its terminal.

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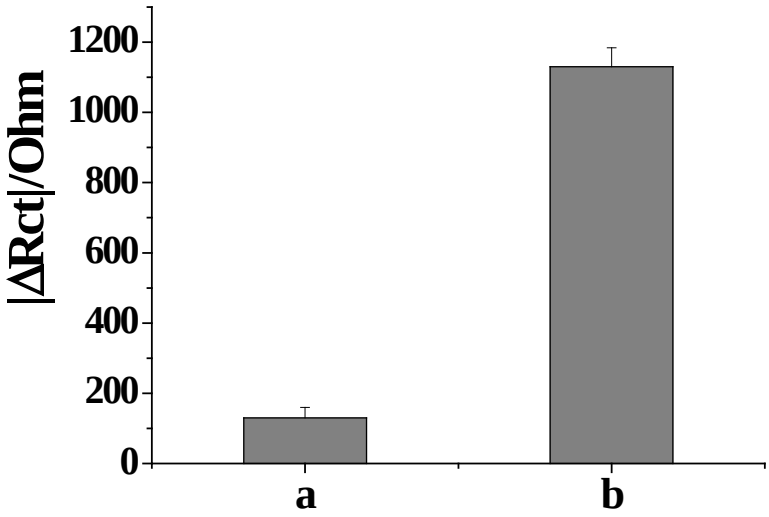

Figure S3 Changes of R_{ct} ($|\Delta R_{ct}|$) between the absence (histogram a) and presence (histogram b) of lambda exonuclease.

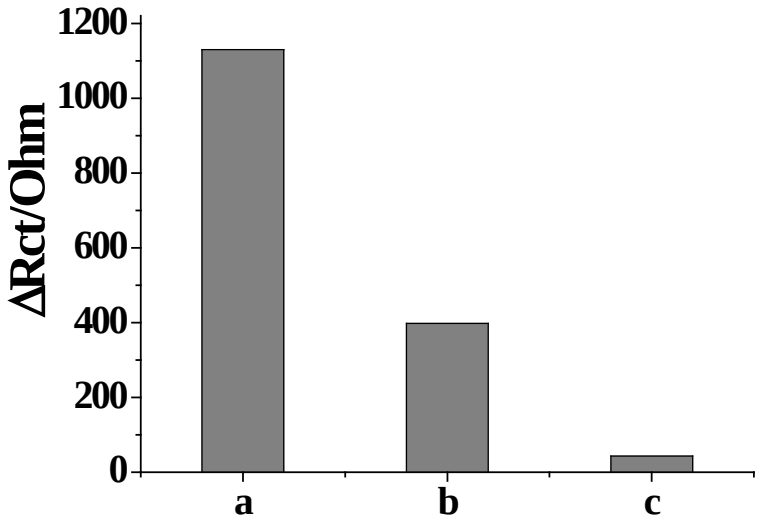

Figure S4 Histogram of R_{ct} change after hybridization with different kinds of target DNA: (a) complementary DNA; (b) single-base mismatched DNA sequence and (c) noncomplementary DNA sequence. Concentrations of three kinds of DNA are 1 nM.