Supporting Information

Experimental Section

Reagents. All oligonucleotides were synthesized by TaKaRa biotechnology Co., Ltd. (Dalian, China), and their base sequences were illustrated in Table S1. The concentrations were quantified by OD260 based on their individual absorption coefficients. The nicking enzyme (N.BstNB I) and NEBuffer 3 (50 mM Tris-HCl, 10 mM $MgCl_2$, 100 mM NaCl, 1 mM dithiothreitol, pH 7.9) were obtained from New England BioLabs. Tris-(hydroxymethyl) aminomethane was purchased from Cxbio Biotechnology Co. Ltd. (Denmark). Ethylenediaminetetraacetic acid (EDTA), Hexaammineruthenium (III) chloride (RuHex, 98 %), mercaptohexanol (MCH) and tris (2-carboxyethyl) phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich (USA). TMB/H_2O_2 and Hemin were purchased from Sigma-Aldrich (St. Louis, MO, USA), respectively. The stock solution of hemin (5 mM) was prepared in DMSO, stored in the dark at -20 ℃ and diluted to the required concentrations with Tris-HCl buffer solutions. All chemicals were of analytical grade.

Hybridization buffer was the mixture of 100 mM NaCl and 10 mM TE (pH 7.4). DNA immobilization buffer was the mixture of 10 mM TE, 10 mM TCEP and 1000 mM NaCl. Washing buffer solution was 100 mM NaCl and 10 mM PB solution (pH 7.4). All solutions were prepared with MilliQ water (18.2 MΩ).

Electrochemical measurements. All electrochemical measurements were performed by using CHI 660D Electrochemical Workstation (CH Instrument, USA). The electrochemical system consisted of a working electrode (a gold disk electrode modified with capture probe), a platinum wire as the auxiliary electrode, and a reference electrode (Ag/AgCl). Cyclic voltammetrys (CVs) were carried out at a scan rate of 100 mV/s. Amperometric detection was preformed with a fixed potential of 100 mV and the steady state was usually reached and recorded within 100 s. Electrochemical impedance experiments were performed in the presence of 10 mM Tris-HCl-10 mM $[Fe(CN)6]^{4-/3-}$ solution (pH 7.0), The biased potential was 0.21V (versus Ag/AgCl) and the amplitude was 5.0 mV. The electrochemical impedance

spectra (EIS) were recorded in the frequency range of 0.1 kHz~ 100 Hz with a sampling rate of 12 points per decade. A Nyquist plot (Z_{re} vs Z_{im}) was drawn to analyze the impedance results.

DNA self-assembly, Hybridization and the Nicking Reaction at gold electrode. The whole fabrication process of the biosensor is outlined in Figure 1. A gold disk electrode (2 mm diameter, GE) was firstly polished to obtain mirror surface with 0.05 μm alumina powder, followed by sonication in ethanol and water for 5 min respectively. The GE was electrochemically cleaned to remove any remaining impurities [1]. The surface area of gold electrode was 0.043cm^2 calculated by the integration of the cathodic peak in 0.1MH_2SO_4 [2, 3]. And the roughness factor of electrode, which was the ratio between the real surface area and the geometrical area of the electrode, was estimated to be 1.37. After drying with nitrogen, the electrode was immediately used for DNA immobilization. 5 μL of 2 μM Sp solution was first spread on the pre-cleaned gold electrode surface for 2 hours in the 100 % humidity. Next, this electrode was immersed in 1 mM MCH for 1 hour to remove the nonspecific DNA adsorption and optimize the orientation of the capture probes to make hybridization easier. The DNA surface density could be measured with chronocoulometry (CC) as previously described [4,5]. Then, 5 μL of the hybridization solution containing complementary T1, single-base mismatch T2-T5 or noncomplementary T6 was placed on the DNA SAM (self-assembly monolayer) of Sp modified GE for 1 h at room temperature, respectively. After hybridization, the GE was extensively rinsed with washing buffer solution and dried under a stream of nitrogen gas. Afterward, the sensor was incubated in a solution of N.BstNB I reaction buffer (10 units/Ul) in NEBuffer 3 at 55 °C (such temperature could facilitate faster hybridization and subsequent dissociation of DNA in a nicking reaction [6]). After a specified period of time, the electrodes were rinsed with wash buffer and dried under a stream of nitrogen gas. Finally, the above the electrodes were incubated in the hemin solution to form the G-Quadruplex-hemin DNAzyme at 25 ℃, and detected in the TMB/H_2O_2 solution by electrochemical measurement.

Electrochemical impedance spectra of different modified electrodes. Electrochemical impedance technique is employed to characterize the fabrication in whole process. Fig.S1 shows the EIS changes for surface-modified process. Compared with the bare Au electrode (Fig. S1a), the Sp modified Au electrode

(Sp/GE) shows a larger electron-transfer resistance (Ret) (Fig. S1f), mainly due to the electrostatic repulsion between negative charges of the DNA backbone and the $Fe(CN)_6^{3-/4-}$. The addition of the MCH results in a relatively small Ret (Fig. S1d). It is expected that MCH, as alkyl molecule, forms a self-assembled monolayer that contains many pinholes and the redox is sufficiently small to freely penetrate through these pinholes [7]. After treatment with MCH, the nonspecifically adsorbed probe is largely removed from the surface and only Sp is bound to the surface with all the relevant bases freely available for hybridization with target DNA. Such formation along with the less densely packed monolayer of MCH can account for the decrease in the value of Ret of the Sp/MCH modified electrode compared with the Sp/GE. Under optimal condition, the Sp can hybridize with target DNA T1 and become double-stranded DNA (Sp-T1/MCH/GE). At this time, the monolayer of DNA on the electrode surface becomes denser, and the negative charges on the electrode surface increase. Thus the value of Ret increases further (Fig. S1g). After the Sp-T1/MCH/GE is incubated in the nicking enzyme reaction buffer solution, the Ret sharply decrease (Fig. S1b). This is because that the nicking endonuclease can bind to and nick the double-stranded DNA. After nicking, the Sp-T1 hybrid becomes less stable, so the target and the target recognition fragment of the Sp dissociate from GE surface. The released target strand can then hybridize to another Sp and initiate the second cycle of cleavage. Eventually, the cleavage will result in the decreasing of negative charges of DNA on the GE surface. Thus, the electron-transfer ability is enhanced and the value of Ret decrease. Upon the incubation of the above electrode to the hemin solution (Fig. S1c), the value of Ret increases because the residual fragment of the Sp changed conformation to form compact quadruplex, which "switches off" the charge transport path of $Fe(CN)_6^{3-/4-}$ [8,9]. Hence, the penetration of $Fe(CN)_6^{3-/4-}$ through the DNA monolayer is hindered, which in turn leads to the increase of Ret. However, when the Sp/MCH/GE is directly treated with nicking enzyme, no significant difference of Ret can be observed (Fig. S1e). This is because that the Sp itself essentially does not contain the recognition site of nicking endonuclease. Therefore, it can not be cleaved by the enzyme. The results of CVs are consistent with that of EIS, indicating that our biosensor indeed works as our expected.

CVs of RuHex in the different stage of the electrochemical DNA biosensor. Previous studies have demonstrated that RuHex can bind to DNA by electrostatic interaction since the DNA backbone has negative charge. In order to further test whether indeed our biosensor works as expected, the CV behaviour of RuHex is investigated at different stages of the biosensor preparation. The CVs of RuHex at different GE surfaces is shown in Fig. S2. As shown in Fig. S2, a pair of peaks corresponding to the reduction and oxidation of RuHex can be observed at a relatively dilute concentration (5 μM) at Sp/MCH/GE (Fig. S2b), indicating that the electrontransfer reaction of RuHex at the modified electrode surface is a surface confined redox process since the peak separation is close to zero and peak currents are linearly proportional to scan rates. This pair of peaks is ascribed to RuHex electrostatically binding to the DNA surface, and reflects the amount of DNA on the electrode surface. When Sp/MCH/GE is incubated in the solutions containing target DNA (T1), the Sp hybridize with T1 to form a double helix structure. As a result, the amount of the RuHex electrostatically binding to the GE surface increases and finally results in a much higher current signal (Fig. S2a). After Sp-T1/MCH/GE being incubated in nicking enzyme (Fig. S2c), there is a sharp decrease in current signals. It can be attributed to the fact that the nicking enzyme binds to and nicks the DNA at the cleave site. Then the target DNA and the target recognition fragment of the cleaved probe dissociate from the GE surface. As a result, the binding amount of RuHex on GE surface decreases due to the decrease of negative charges on the GE surface. Upon the incubation of the above electrode in the hemin solution (Fig. S2d), a further decrease of current signals is observed because the conformation of quadruplex repelled the absorption of RuHex. The results of CVs are consistent with that of EIS, indicating that our biosensor indeed works as expected

Optimization of Experimental Conditions. The factors including buffer type, pH of the buffer, concentration of hemin, and reaction time between hemin and rudimental G-rich nucleic acid sequences were examined in detail. The results showed the DNAzyme displayed its maximum catalytic activity in PBS buffer at pH 6.4. The optimum concentration of hemin was 0.5 μM, and the optimum reaction time was 25 mins.

Detection of artificial saliva sample. In order to evaluate the applicability of the electrochemical DNA sensor in saliva, we compared its performance in pure buffer and in saliva. Saliva is complicated biological fluids containing interfering material such as cells, DNA, RNA and proteins. Unstimulated saliva samples were collected between 9 a.m. and 10 a.m. with previously established protocols [10]. The subjects were asked to refrain from eating, drinking, smoking, or oral hygiene procedures for at least 1 hour before the collection. Saliva samples were centrifuged at 2,600 rpm for 10 minutes at 4 °C. And the supernatant was taken 1 mL and diluted to 10 mL with buffer solution. Then different concentrations of target DNA T1 were added to the diluted saliva. We challenged the sensor with the artificial saliva sample to determined target DNA T1. The results are shown in Table S2. Significantly, we found that the electrochemical DNA sensor were highly resistant to saliva, with little alteration of the background noise and nearly the same hybridization signal, implying that our sensor can selectively detect target DNA in saliva with a simple dilution step.

References

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Table S1 Details of the DNA sequences*

Capture probe (Sp)	5'-AAAGAACGA**<u>GAGTC</u>**TTTC ↓ ***TG GG G TTG GGC GGG ATG GGT*** TTTT-HS-3'
complementary probe(T1)	5'-CAGAAAGACTCTCGTTCTTT-3'
single-base mismatched probe(T2)	5'-CAGAAAGACTCTCGTTCT**<u>G</u>**T-3'
single-base mismatched probe(T3)	5'-CAG**<u>G</u>**AAGACTCTCGTTCTTT-3'
single-base mismatched probe(T4)	5'-CAGAAAGACT**<u>G</u>**TCGTTCTTT-3'
single-base mismatched probe(T5)	5'-CAGAAAGA**<u>G</u>**TCTCGTTCTTT-3'
non-complementary probe(T6)	5'-GCTCCCTTCAGAGCAATCCC-3'

*The solid underlined bold letters are the recognition sequence of N.BstNB I, and the arrow indicates the nicking position. The italic bold letters represent the sequence of the DNAzyme. The underlined bold letters indicate the mismatched position.

Table S2 Determination of target DNA in artificial saliva sample (n=5)

target DNA added (fM)	Detected by this method (fM)	Recovery of this method (%)	R.S.D. (%)
1.00	1.02	102	6.4
2.00	1.97	98.5	6.2
5.00	4.86	97.2	5.1

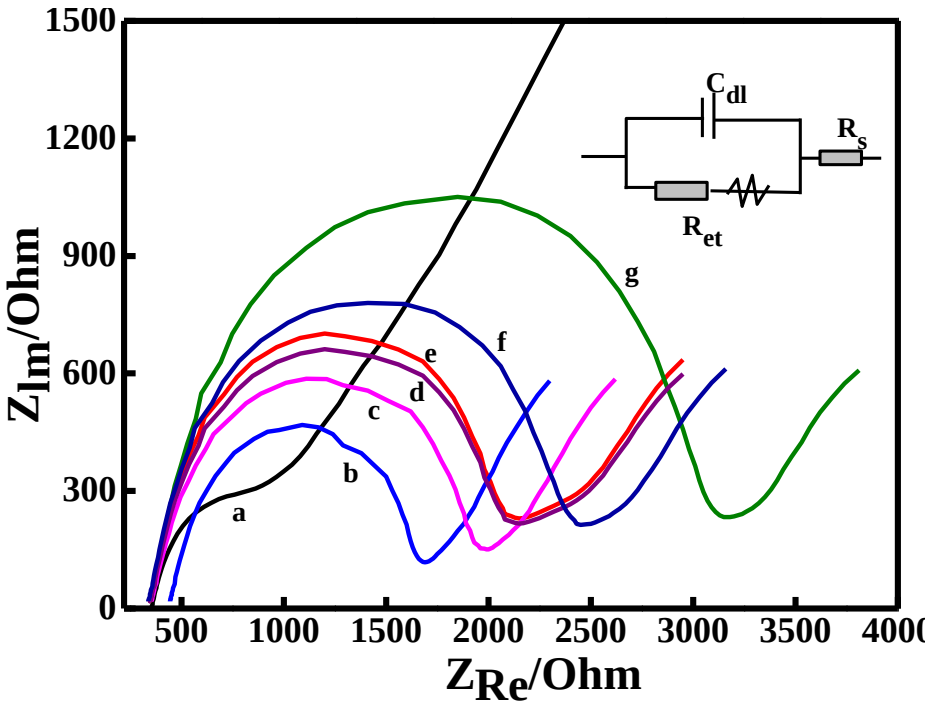

Fig. S1 Impedance spectra (Nyquist plot) of bare GE (a) the Sp-T1/MCH/GE after being incubated in the nicking enzyme buffer solution (b) DNA-Hemin(DNAzyme)/MCH/GE (c) Sp/MCH/GE (d), the Sp/MCH/GE after being incubated in the nicking enzyme buffer solution (e) the Sp/GE (f) and the Sp-T1/MCH/GE (g) in the presence of 10 mM $[Fe(CN)6]^{4-/3-}$. The biased potential was 0.21V (versus Ag/AgCl) in the frequency range of 0.1-10^5 Hz and the amplitude was 5.0 mV.

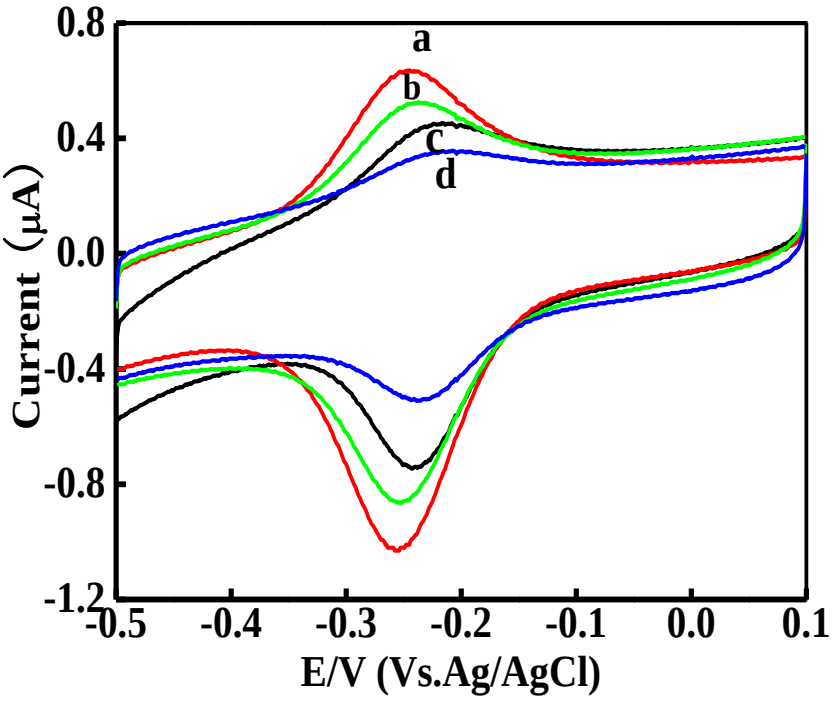

Fig. S2 CVs of RuHex on Sp-T1/MCH/GE (a) Sp/MCH/GE (b) Sp-T1/MCH /GE after being incubated in the nicking enzyme buffer solution (c) and DNA-Hemin(DNAzyme)/MCH/GE(d). The concentration of RuHex was 5 μM, data was obtained in 10 mM Tris-HCl buffer solution (pH 7.0), scan rates =400 mV/s.

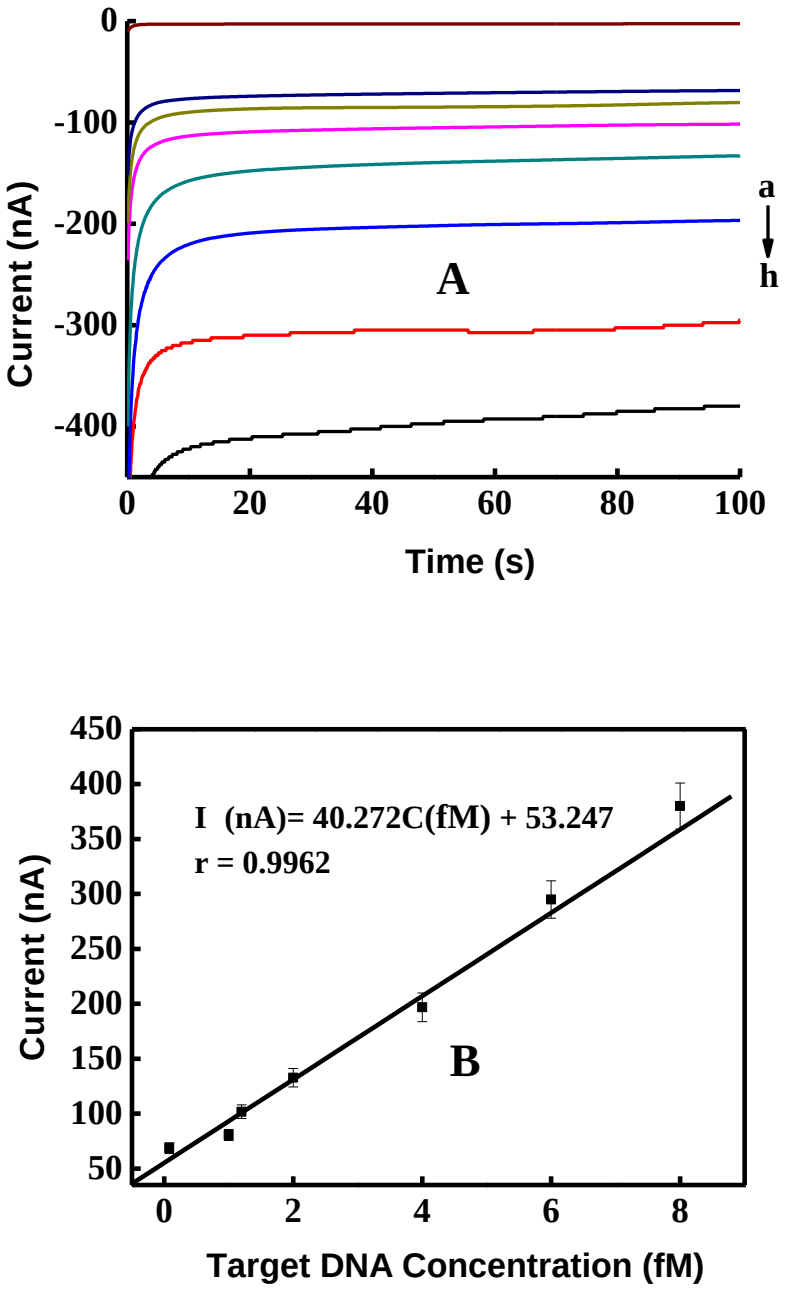

Fig. S3 A) Current-time curves of hybridization with different concentration of the target sequence. From a-h: 0 fM, 0.08 fM, 1 fM, 1.2 fM, 2 fM, 4 fM, 6 fM and 8 fM. B) A calibration curve demonstrating peak height versus target concentration.