Electronic Supplementary Information

for

Electrochemical genotyping and detection of single nucleotide polymorphisms based on junction-probe containing 2'-deoxyinosine

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S1. The details on the experimental method

Reagents: All oligonucleotides were synthesized by Shanghai Sangon Biological Engineering Technology Services Reagents Co., Ltd. (China). Their base sequences were illustrated in Table S1. Tris-(hydroxymethyl) aminomethane was purchased from Cxbio Biotechnology Co. Ltd. (Denmark). Ethylenediaminetetraacetic acid (EDTA), mercaptohexanol (MCH), Hexaammineruthenium (III) chloride (RuHex), tris (2-carboxyethyl) phosphine hydrochloride (TCEP) and potassium ferrocyanide ($K_4Fe(CN)_6$) were purchased from Sigma-Aldrich (USA). The buffer solutions are as follows: Hybridization buffer is the mixture of 0.1M NaCl and 10 mM TE (pH 7.4), electrochemical measurement buffer is 10 mM Tris-HCl solutions (pH 7.0), DNA immobilization buffer is the mixture of 10 mM TE, 10 mM TCEP and 1 M NaCl (pH 8.0), and washing buffer was the mixture of 0.1 M NaCl and 10 mM PB (pH 7.4). All solutions were prepared with Milli-Q water (18.2 MΩ/cm).

Table S1: Details of the DNA sequences.

Target DNA (T_1)	5′-GGA GAA GTT TTT GAA GAC GGC TGA-3′
Capture probe (Cp)	5′- AAAA TCA GCC GTC TTC AAA CTA G AAAA -3′-HS
dI probe (Dp)	5′- CTA GTT T AAA diAdi TT -3′
single-base mismatched DNA (T_2)	5′- GGA GAA GGT TTT GAA GAC GGC TGA -3′
single-base mismatched DNA (T_3)	5′- GGA GAA GCT TTT GAA GAC GGC TGA -3′
single-base mismatched DNA (T_4)	5′- GGA GAA GAT TTT GAA GAC GGC TGA -3′
non-complementary DNA (T_5)	5′- CGC AGA TGA GCG CGC TGA CGT CGA -3′

Electrochemical measurements: All electrochemical measurements were performed by using CHI-660D Electrochemical Workstation (CH Instrument, USA). The electrochemical system consisted of a gold working electrode (a gold disk electrode modified with DNA probe), a platinum wire as the auxiliary electrode and a

reference electrode (Ag/AgCl). Cyclic voltammetry (CV) and linear sweep voltammetry (LSV) were carried out in 10mM Tris-HCl-5μM RuHex solution (pH 7.0) at a scan rate of 100 mV/s in the potential range between -0.5 and +0.0 V. Electrochemical impedance spectra (EIS) was measured in 10mM Tris-HCl-10 mM $[Fe(CN)_6]^{4-/3-}$ solution (pH 7.0) in the frequency range of $0.1–10^5$ Hz.

DNA self-assembly and hybridization at gold electrode: A gold disk electrode (2mm diameter) was pretreated according to the method reported in our previous paper (1). Firstly, the gold electrode (GE) was polished to obtain mirror surface with 0.05 μm alumina powder, followed by sonication in ethanol and water for 5 min respectively. Then, the GE was electrochemically cleaned to remove any remaining impurities. After drying with nitrogen gas, the GE was immediately used for DNA immobilization. The GE was firstly modified with 5 μL of 2 μM capture probe (Cp) solution for 2 hours. Then, the Cp-modified GE was immersed in 1 mM MCH for 1 hour to cover the spare room of electrode surface and to optimize the orientation of the capture probes to make hybridization easier.

Genotyping of SNPs and determination of target DNA: Complementary DNA (T_1) used in this study is a salivary DNA of oral cancer. T_2, T_3 and T_4 are single-base mismatch DNA in which T base (red one) in T_1 was substituted by G, C and A base, respectively. T_5 is uncomplementary DNA. In the genotyping/detection experiment, target DNA was pre-annealed with dI probe (Dp, 2μM) at 37°C for 30 min in the hybridization buffer solution, and then 5 μL of the hybridization solution was placed on the DNA SAM (self-assembly monolayer) of Cp-modified GE for 1 h at room temperature. After hybridization, the GE was extensively rinsed with washing buffer solution and dried under a stream of nitrogen gas, and then was used for electrochemical measurements. After once measurement, the GE was then denatured

in ultra-pure water at 65 ^{o}C for 5 min (the melting temperature of "Y" Junction-type complex was about 47 ^{o}C) in order to revive the biosensor, and then the biosensor was used for the next cycle of hybridization and detection.

S2. The characterization of EIS at different stages of the electrochemical biosensor preparation

As shown in Figure S1, the Cp modified GE (Cp/GE) shows a larger electron-transfer resistance (*Ret*) (c) in comparison with bare GE (a), this is because the electrostatic repulsion between negative charges of the DNA backbone and the $Fe(CN)_6^{3-/4-}$. After treatment with MCH, Cp/MCH modified GE (Cp/MCH/GE) showed a little smaller *Ret* (b) in comparison with Cp/GE, indicating that the treatment of MCH has removed nonspecifically adsorbed probe from GE surface. When Cp/MCH/GE is incubated in the solution containing target DNA (T_1) and Dp, Cp-T_1-Dp/MCH modified GE (Cp-T_1-Dp/MCH/GE) showed a much bigger *Ret* (d). This is due to that Cp hybridized with T_1 and Dp to form "Y" junction structure, and the formation of "Y" junction structure resulted in a much denser negative charges on the GE surface and generated a much stronger electrostatic repulsion between GE surface and $Fe(CN)_6^{3-/4-}$.

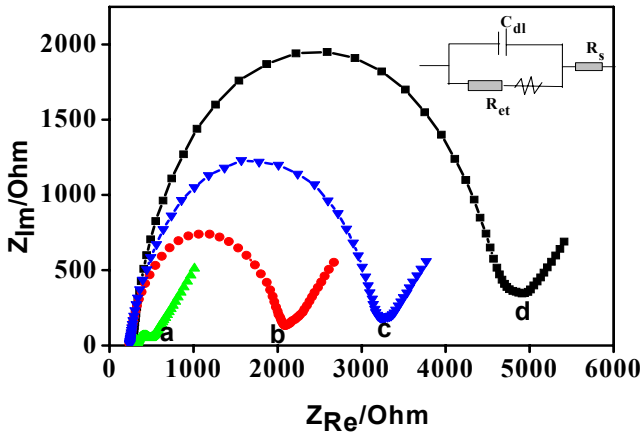

Figure S1: EIS (Nyquist plot) of bare GE (a), Cp/MCH/GE (b), Cp/GE (c), and Cp-T_1-Dp/MCH/GE (d). The concentration of target DNA, Cp and Dp was 2 pM, 2 μM and 2 μM, respectively. Data was obtained in 10mM Tris-HCl – 10mM $Fe(CN)_6^{3-/4-}$ solution (pH 7.0), the biased potential was 0.18V (versus Ag/AgCl), the frequency range is 0.1-10^5 Hz and the amplitude was 5.0 mV.

S3. Effect of surface density of Cp on the sensor's sensitivity

In general, DNA hybridization efficiency at electrode surfaces is sensitively dependent on the surface density of DNA. In this study, we varied the surface density of DNA by changing the capture probe concentration, self-assembly time and/or ionic strength in the immobilization buffer during capture probe self-assembly procedure, and measured the peak currents of RuHex at Cp/MCH/GE with different surfaces density. Our results showed that the control of DNA assembly was essential for the sensitivity. The sensor signal Δip (μA, $\Delta ip = I_0 - I_{after}$, where I_0 and I_{after} refer to the current obtained before and after Cp/MCH/GE was incubated in 50 pM of T_1 and dI probe solution, respectively) increased with the increase of surface density when the surface density is smaller than 1.5×10^{12} molecules/cm^2, then Δip decreased with the increase of surface density. This is because that the hybridization of target DNA, capture probe and dI probe is hindered due to spatial restriction in higher surface density. Our results showed that the ideal surface density for the sensor is 1.2×10^{12} – 1.8×10^{12} molecules/cm^2. In above range, there is a largest magnitude in the sensor signal (Δip).

REFERENCES

(1). Zhang,J., Chen,J.H., Chen,R.C., Chen,G.N. and Fu,F.F. (2009) Sequence-specific detection of trace DNA via a junction-probe electrochemical sensor employed template-enhanced hybridization strategy. *Biosens Bioelectron*, 25, 815-819.