Electronic Supplementary Information

for

Ultra-sensitive electrochemical detection of single nucleotide polymorphisms based on an electrically controllable magnetic gold electrode

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S1, The details on the experimental method

Reagents. All oligonucleotides were synthesized by TaKaRa biotechnology Co., Ltd. (Dalian, China), and their base sequences were illustrated in Table S1. The concentration of the stock standard DNA solution was accurately quantified by OD260 based on their individual absorption coefficients. More dilute standard solutions of DNA were prepared by serially diluting the stock standard solution (each diluted 10-fold) to the desired concentration with hybridization buffer solution. Streptavidin-modified MBs (1 μm, 10 mg/mL) were purchased from Dynal Biotech ASA. Streptavidin-HRP (horseradish peroxidase) was purchased from Sigma Company (Spain) and used as received. TMB (3,3',5,5'-tetramethylbenzidine sulfate)/H_2O_2 solution was purchased from Neogen Corporation (USA), and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tris-(hydroxymethyl) aminomethane(Tris) was purchased from Cxbio Biotechnology Co. Ltd. (Denmark). All chemicals were of analytical grade.

Stock solution of streptavidin-HRP (10 μg/mL) was prepared in a saline 0.01 M phosphate buffer (PB) solution in which containing 0.05% Tween 20, 0.138 M NaCl and 0.0027 M KCl (PBST, pH 7.4). DNA immobilization buffer was the mixture of 10 mM Tris, 1.0 mM EDTA, 0.01% Tween 20 and 1M NaCl (pH 7.5). Hybridization buffer was the mixture of 50mM Tris, 0.01% Tween 20 and 20 mM NaCl (pH 7.4). Washing buffer solution was the mixture of 0.1 M NaCl, 0.05% Tween 20 and 10 mM PB solution (pH 7.4). All solutions were prepared with MilliQ water (18.2 MΩ/cm).

Table S1: Details of the DNA sequences used in the experiment

Capture probe (Cp)	5'- biotin -ATG TGG AAA ATC TCT AGC AGT-3
complementary target (T)	5'- biotin -ACT GCT AGA GAT TTT CCA CAT-3'
one-base-mismatched target (MT1)	5'- biotin -ACT GCT AGA GTT TTT CCA CAT-3'
one-base-mismatched target (MT2)	5'- biotin -ACT GCT AGA GCT TTT CCA CAT-3'
one-base-mismatched target (MT3)	5'- biotin -ACT GCT AGA GGT TTT CCA CAT-3'
one-base-mismatched target (MT4)	5'- biotin -ACT GCT AGA GAT TTT CCA CTT-3'
one-base-mismatched target (MT5)	5'- biotin -TCT GCT AGA GAT TTT CCA CAT-3'
noncomplimentary target (NT)	5'- biotin -ATG TGG AAA ATC TCT AGC AGT-3'

The underlined letters indicate the mismatch position.

Fabrication and pretreatment of the electrically controllable magnetic gold working electrode. The electrically controllable magnetic gold working electrode (ECM-GE) used in the experiment was fabricated by ourselves. The structure of the working electrode was shown in Figure S1, a piece of gold circular plate (thickness 1mm, diameter 3 mm) was directly fixed on the end of a iron rod (length 5cm, diameter 3mm) by welding, and the iron rod was enlaced with varnished wire (internal diameter 0.10 mm) to form coil from the end with a height of 3 cm and a electric resistance of 22 Ω. The outside of the electrode was finally sealed with polytetrafluoroethylene. Two ends of the coil were then connected to the poles of a voltage controllable DC power supply (0-10 V). The magnetic force of the electrode can be precisely controlled by adjusting the voltage.

Before use, the electrode 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 working electrode was then electrochemically cleaned to remove any remaining impurities [1]. After drying with nitrogen, the electrode was immediately used for electrochemical measurements.

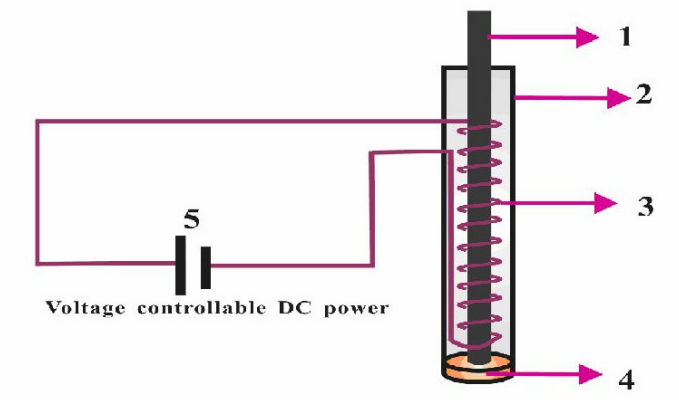

Figure S1: The structure of the electrically controllable magnetic gold electrode. (1), iron rod; (2), polytetrafluoroethylene tube; (3), the electrified coil; (4), gold plate; (5), voltage-controllable DC power.

Capture probe Self-Assembly and Hybridization on the surface of MBs. Capture probe self-assembly was carried out using a procedure based on that recommended by Dynal Biotech ASA (Oslo, Norway). A volume of 50 μL of streptavidin-modified MBs was added into a 2.0 mL centrifuge vial. Under the magnetic field, the MBs was washed three times (for 2 min each) with immobilization

buffer and was then re-suspended in 125 μL of the same buffer in which containing 2.5 μM of biotinylated capture probe DNA (C_p). The whole solution was incubated for 30 min under 37 ºC and gentle agitation to immobilize C_p on the surface of MBs. Subsequently, the capture probe-modified MBs (C_p-modified MBs) was washed three times (for 2 min each) with hybridization buffer to remove the excess C_p and re-suspended in 100 μL of the same buffer solution. In order to reduce the nonspecific interaction, the rest active sites of MBs were blocked with PB solution (pH 7.4) in which containing 0.1% BSA and 0.01% tween-20. Then, 40 μL of the hybridization solution in which containing target DNA was added, and the whole was incubated for 1 h at 37 ºC to hybridize target DNA with C_p. Then, the hybrid-attached MBs were extensively rinsed with washing buffer solution to remove the non-specific DNA adsorption under the magnetic field. After hybridization, 40μL of 10 μg/ml streptavidin-HRP was added to the hybrid-attached MBs with gentle mixing for 30 min at 37 ºC to tag HRP on the hybrid-attached MBs. Finally, the resulting HRP-tagged MBs were washed five times (for 5 min each) under the magnetic field with 100 μL of washing buffer solution, and the final HRP-tagged MBs were used for the electrochemical measurements in the TMB/H_2O_2 solution.

Electrochemical measurement. All electrochemical measurements were performed by using CHI-660D electrochemical workstation (CH Instrument, USA). The electrochemical system consisted of the electrically controllable magnetic gold working electrode, a platinum wire as the auxiliary electrode, and a reference electrode (Ag/AgCl). The electrochemical measurements were performed in the TMB/H_2O_2 solution. Firstly, the HRP-tagged MBs obtained above were adsorbed on the surface of controllable magnetic gold working electrode by adding a 2V voltage on the coil, then, the controllable magnetic gold working electrode was immersed in 600 μL of TMB/H_2O_2 solution. The CVs scanning was performed in the potential range of 0 ~ +0.8 V with a scanning rate of 100 mV/s, a sample interval of 1mV and a standing time of 2 s. The chronoamperometry was performed with incipient potential of +0.15 V, the sample interval of 0.1 s and the experimental time of 100 s. The surface temperature of the electrode was detected by infrared thermodetector (Fluke, USA) at the same time.

After electrochemical measurements, the C_p-modified MBs can be revived by dipping the controllable magnetic working electrode into de-ionized water and switching off the power supply of the coil. Without the magnetic field, the MBs were

dispersed in the solution again. Then, the solution was incubated at 60 ^{o}C for 5 min to dissociate target DNA on the surface of MBs. As a result, the C_p-modified MBs were revived and can be used for the next cycle of the hybridization and detection.

The preparation of artificial saliva sample. Un-stimulated saliva samples were collected between 9 a.m. and 10 a.m. with previously established protocols [2]. 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 603 g for 10 minutes at 4°C. Then, the 1mL of supernatant liquid was taken and diluted to 100mL with hybridization buffer solution. The different concentrations of target DNA were then added into the diluted saliva to prepare artificial saliva samples and the concentrations of target DNA in the artificial saliva samples were determined with our biosensor.

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S2, **Optimization of voltage applied to the coil of the electrically controllable magnetic gold electrode**

It is well known, the electrified coil can generate a magnetic field as well as the joule heat. We have studied the influence of the voltage on the magnetic field. We found the magnetic field is strong enough to collect all the functionalized MBs when the voltage is higher than 0.5 V. At the same time, the temperature of the surface of electrically controllable magnetic gold electrode (detected by FLUKE ST18 infrared thermometer, Fluke Corporation) increased when a voltage was added on the coil to generate magnetic field. The temperature of the surface of the working electrode will directly affect not only the hybridization efficiency of C_p and T but also the enzymatic activity of the HRP. Figure S2 showed the relationship between the temperature of the surface of gold electrode and the voltage applied to the coil. From Figure S2, it was clearly observed that the temperature of the surface of gold electrode rise with the increase of voltage. The temperature showed a good linear relationship with the square of voltage (see insert in Figure S2), which is the same as that observed in heated electrode [1]. When the applied voltage is 2V, the temperature of the electrode's surface is about 37◦C. By considering the fact that the theoretical hybridization temperature of C_p and T is about 40 ◦C [2] and the HRP has a highest enzymatic activity when the temperature in the range of 35-38◦C [3], 2V was selected as the optimum voltage.

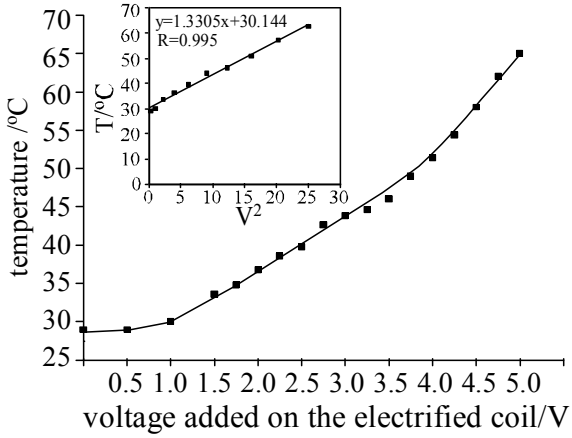

Figure S2: The relationship between the temperature of the surface of electrically magnetic-controllable gold electrode and the voltage added to the electrified coil.

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S3, Optimization of MBs amount

The HRP-tagged MBs amount on the surface of the controllable magnetic gold electrode directly affects the sensitivity of the method by influencing the amounts of HRP. In this study, the effect of the MBs amount on the sensitivity was investigated in detail in order to obtain the highest sensitivity. The experimental results showed that the catalytic current of the TMB-H_2O_2 increased with the increase of MBs amount in the range of 0-40 μg, then the catalytic current turn to decrease with the increase of MBs amount when the MBs amount is more than 40 μg. This is probably due to the bigger electron transfer resistance caused by over HRP-tagged MBs loadings. Therefore, 40 μg of MBs was used in the study.

S4, Cyclic voltammetry (CV) characterization at different stages of the biosensor preparation.

The cyclic voltammetry (CV) behavior of H_2O_2–TMB was investigated at different stages of the biosensor preparation to validate whether the biosensor actually works. The CVs of H_2O_2–TMB at different surfaces of the ECM-GE are shown in Fig. 3S. Two pairs of peaks corresponding to the reduction and oxidation of H_2O_2–TMB were observed at the bare ECM-GE (Fig. 3Sa) [1]. When the Cp-modified MBs were captured on the surface of the electrode, there was a small increase in the current signal. This was attributed to the fact that MBs have intrinsic peroxidase mimetic activity and can slightly catalyze the H_2O_2-mediated oxidation of TMB (Fig. 3Sb) [2]. After Cpmodified MBs were hybridized with target DNA to form a duplex structure and the duplex structure captured the streptavidin–HRP to form HRP-tagged MBs, a higher current signal was observed on the surface of the electrode, which adsorbed the HRP-tagged MBs (Fig. 3Sc). This is because HRP has high peroxidase activity and can effectively catalyze the H_2O_2-mediated oxidation of TMB. The results of the CVs indicate that our biosensor indeed works as we expected.

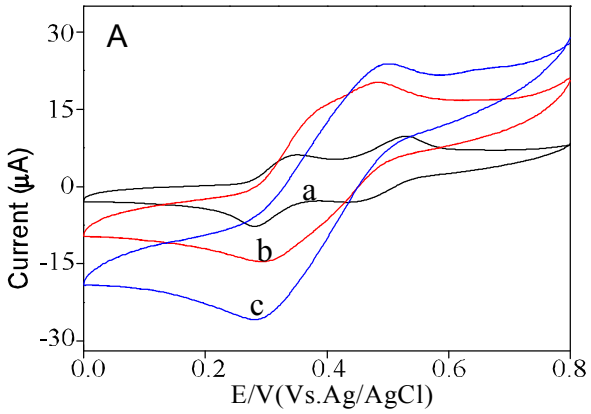

Fig. S3: The CVs of H_2O_2–TMB solution at different electrode surfaces. (a) Bare ECM-GE, (b) ECM-GE after the adsorption of Cp-modified MBs and (c) ECM-GE after the adsorption of HRP-tagged MBs.

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Talbe S1: Comparison on the analytical performance between several previous methods and this study.

Assay method	Detection limit	DF	RSD (%)	Ref.
Electrochemical biosensor based on ferrocene bearing zinc(II)-cyclen complexes with diethylamine	100fM	1.33	8.2	[1]
Fluorescent biosensor based on graphene oxide	14.3nM	1.57	1.13	[2]
Electrochemical biosensor based on junction-probe containing 2′-deoxyinosine	0.13pM	3.0	6.0	[3]
Electrochemical biosensor based on an electrically magnetic-controllable gold electrode	0.37 fM	2.63	6.0	this study
Electrochemical biosensor based on screen-printed electrodes and magnetic beads	50fM	3.7	7.1	[4]

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