Electronic Supplementary Material (ESI) for Chemical Communications

Label-free electrochemical assay for quantification of gene-specific methylation in nucleic acid sequence

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Experimental Details

Chemicals and Reagents. All of synthetic oligonucleotides were purchased from Sangon Biotech Co. Ltd. (Shanghai, China) with the following sequences: probe oligonucleotide: 5'-CAT TAT ACC GGA CTA GAG AT-3', unmethylated target DNA: 5'-ATC TCT AGT CCG GTA TAA TG-3', methylated target DNA: 5'-ATC TCT AGT C**m**CG GTA TAA TG-3', non-complementary (NC) DNA: 5'-ATC TCT AG**T** CCG GTA TAA TG-3'. Enzyme *Hpa*II and 10×Buffer Tango^{TM} were purchased from Fermentas Life Sciences (Shenzhen, China). 3-Mercaptopropionic acid (MPA) and N-(3-dimethylamino)propyl-N'-ethylcarbodiimide hydrochloride (EDAC) were obtained from Alfa Aesar (Tianjin, China). N-hydroxysulfosuccinimide (NHS) and methylene blue (MB) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Other chemicals were all of analytical grade. Ultrapure water was used throughout. Buffer solution used in this work was 40 mM Britton-Robinson (BR) buffer (pH 6.09) containing 25 mM NaCl. Washing solution was 40 mM BR buffer (pH 6.09) containing 0.1% sodium dodecyl sulfonate (SDS). *Hpa*II (0.125 u μL^{-1}) enzyme solution was prepared by the dilution of 1 μL of 10 u μL^{-1} *Hpa*II stock solution and 8 μL of 10×buffer Tango^{TM} to 80 μL with ultrapure water.

Preparation of DNA biosensor. Gold electrode (AuE, 2 mm in diameter) was treated with *piranha solution* (the *piranha solution* is a mixture of H_2SO_4 and 30% of H_2O_2 (3:1, v/v)) for 30 min (*safety note*: the *piranha solution* should be handled with extreme caution), and polished mechanically with 1.0, 0.3 and 0.05 μm alumina

slurries successively to mirror finish. After extensively rinsed with ultrapure water and ethanol, the AuE was cyclically scanned in 0 - 1.6 V in 1 M H_2SO_4 till achieving the characteristic Au peaks. The pretreated AuE was immersed into a 100 mM MPA ethanol solution for 1 h and then washed twice with ethanol. The resulting MPA/AuE was then incubated with 40 μL of EDAC/NHS solution (1/1 mg in 100 μL PBS buffer, pH 6.0) for 30 min. Removing the EDAC/NHS solution, a 40 μL of 40 μg mL^{-1} probe oligonucleotide (in 20 mM of PBS buffer, pH 8.6) was applied and incubated for 120 min. The resulting DNA biosensor was vigorously rinsed with washing solution to remove nonspecific adsorption.

Measurement process. Sample solution (10 μL) containing methylated and unmethylated target DNA was incubated with the fabricated DNA biosensor for 6 hrs at room temperature for selective hybridization. After thoroughly rinsed with washing solution to remove the unhybridized oligonucleotides, the resulting hybridized DNA biosensor was incubated with 0.125 u μL^{-1} *Hpa*II solution at 37 ^{o}C for 5 hrs, and then at 65 ^{o}C for 20 min. The resulting *Hpa*II digested DNA biosensor was rinsed thoroughly with washing solution. The DNA biosensor, hybridized DNA biosensor and *Hpa*II digested DNA biosensor were immersed in 15 μM MB in BR buffer (pH 6.09) containing 25 mM of NaCl for 15 min, and then measured by cyclic voltammetry.

Apparatus and Measurements. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were carried out on a CHI 760C electrochemical workstation (Chenhua, Shanghai, China) equipped with different DNA biosensor as

working electrode, a Ag/AgCl (3.0 M KCl) as reference electrode and a platinum wire as counter electrode.

Electrochemical behavior of MB on different DNA biosensor

Fig. S1 shows the electrochemical behaviors of 15 μM MB in BR buffer (pH 6.09) containing 25 mM NaCl on the fabricated DNA biosensor at different scan rate. A pair of reversible redox peak was found at the scan rate of 10 mV s^{-1}. The cathodic peak current (I_{pc}) and anodic peak current (I_{pa}) both increased linearly with the increase in scan rate from 10 to 100 mV s^{-1} (Fig. S1A). The linear regression equations were $I_{pc}=1.457\times10^{-8}v+7.394\times10^{-8}$ and $I_{pa}= -1.008\times10^{-8}v+7.645\times10^{-8}$ (Fig. S1B). The results indicate that MB undergoes a surface-controlled redox process on the DNA biosensor surface. The redox response mainly comes from the MB interacts with probe oligonucleotides on DNA biosensor, not the MB diffuses to the DNA biosensor.

Similarity, after the DNA biosensor was incubated with 5 μM unmethylated target, the I_{pc} and I_{pa} of the resulting hybridized DNA biosensor in 15 μM MB in BR buffer (pH 6.09) containing 25 mM NaCl also increased linearly with the increase in scan rate from 10 to 100 mV s^{-1} (Fig. S2A). The linear regression equations were $I_{pc}=1.186\times10^{-8}v+9.254\times10^{-8}$ and $I_{pa}=-9.413\times10^{-9}v+4.470\times10^{-8}$ (Fig. S2B). The results confirm that MB still undergoes surface-controlled redox process on hybridized DNA biosensor, and the redox response is from the MB interacts with double-stranded DNA (dsDNA).

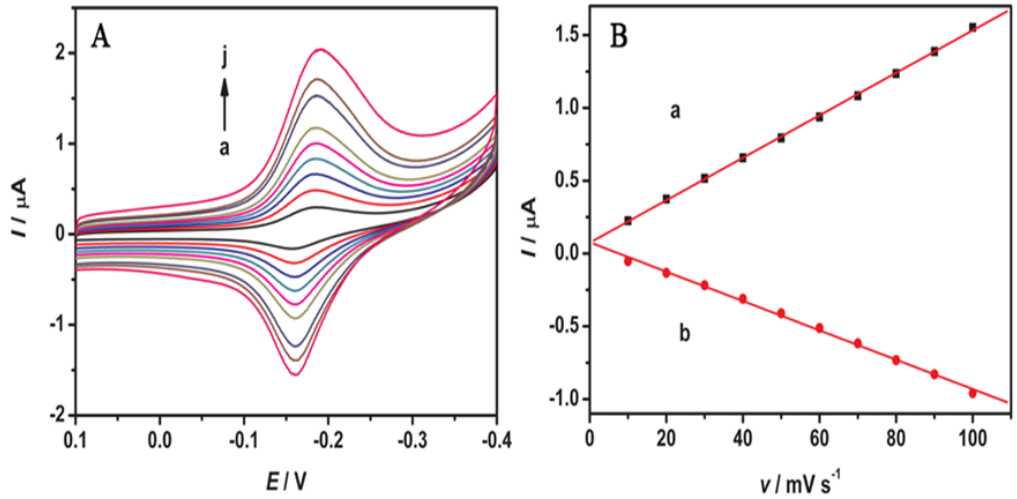

Fig. S1 (A) CVs of 15 μM MB in BR buffer (pH 6.09) containing 25 mM NaCl at DNA biosensor with different scan rate of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mV s^{-1} (from a to j). (B) Dependence of I_{pa} and I_{pc} of MB on scan rate.

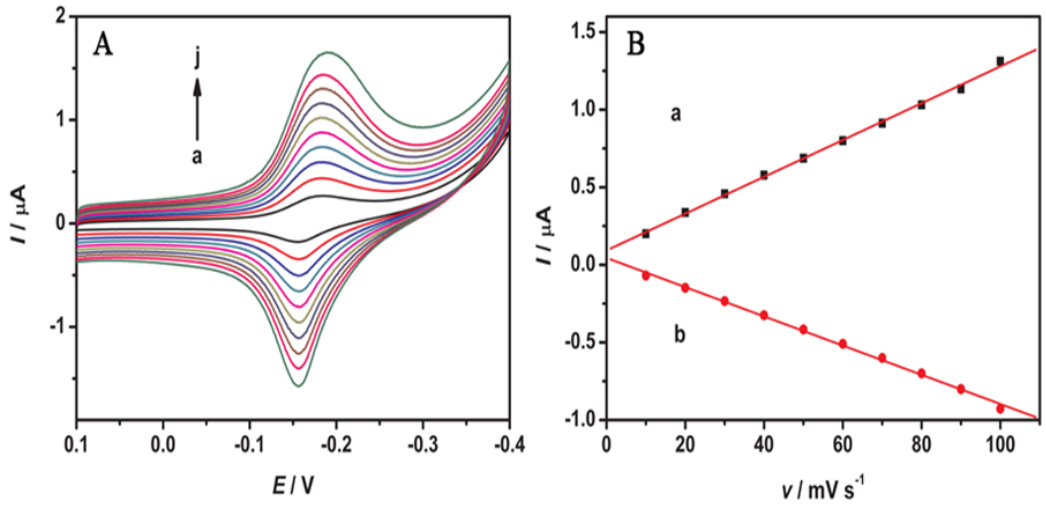

Fig. S2 (A) CVs of 15 μM MB in BR buffer (pH 6.09) containing 25 mM NaCl at hybridized DNA biosensor with different scan rate of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 mV s^{-1} (from a to j). (B) Dependence of I_{pa} and I_{pc} of MB on scan rate.

Optimization of determination conditions

The effect of the MB concentration on the difference of peak current of MB on DNA biosensor and hybridized DNA biosensor was investigated. It is known that MB interacts with single-stranded DNA (ssDNA) mainly through electrostatic interaction and specific interaction with exposed guanine residue. After the DNA biosensor was hybridized with target DNA, the formatted dsDNA improved the electrostatic interaction between MB and deoxyribose-phosphate backbone, and the intercalation of MB to dsDNA, while the specific interaction between MB and guanine residues was obstructed due to the package of guanine residues in dsDNA. Therefore, the voltammetric response of MB on ssDNA and dsDNA showed difference, which is relatived to the concentration of MB. As shown in Fig. S3, with the increase in MB concentration from 5 to 50 μM, the difference between the I_{pc} of MB on ssDNA and that on dsDNA (ΔI_{pc}) increased initially and then reduced. The maximal ΔI_{pc} was found when MB was 15 μM, indicating the highest discrimination. Therefore, 15 μM MB was chosen in the following experiments。

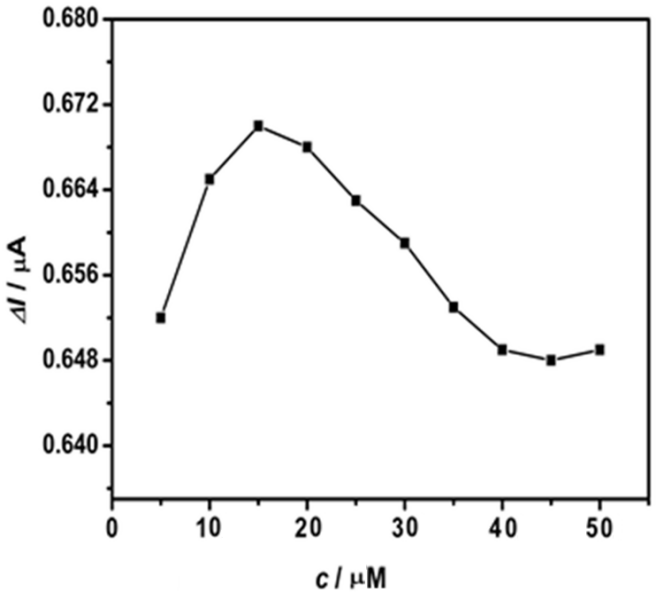

Fig. S3 Effect of MB concentration on the electrochemical behavior of MB in BR buffer (pH 6.09) containing 25 mM NaCl.

Because MB underwent different interaction modes with ssDNA and dsDNA, MB took different times for achieving adsorptive equilibrium on ssDNA and dsDNA. As shown in Fig. S4A, the I_{pc} of DNA biosensor in 15 μM MB in BR buffer (pH 6.09) containing 25 mM NaCl increased with the increase in immersing time from 0 to 14 min and leveled off after the immersing time was longer than 14 min, reflecting that MB on ssDNA reached adsorptive equilibrium. Similarity, the I_{pc} of the DNA biosensor after hybridized with 5 μM unmethylated target DNA increased with the increase in immersing time from 0 to 12 min and leveled off after the immersing time was longer than 12 min (Fig. S4B). In order to achieve adsorptive equilibrium of MB on both ssDNA and dsDNA, an immersing time of 15 min was selected in the following experiments.

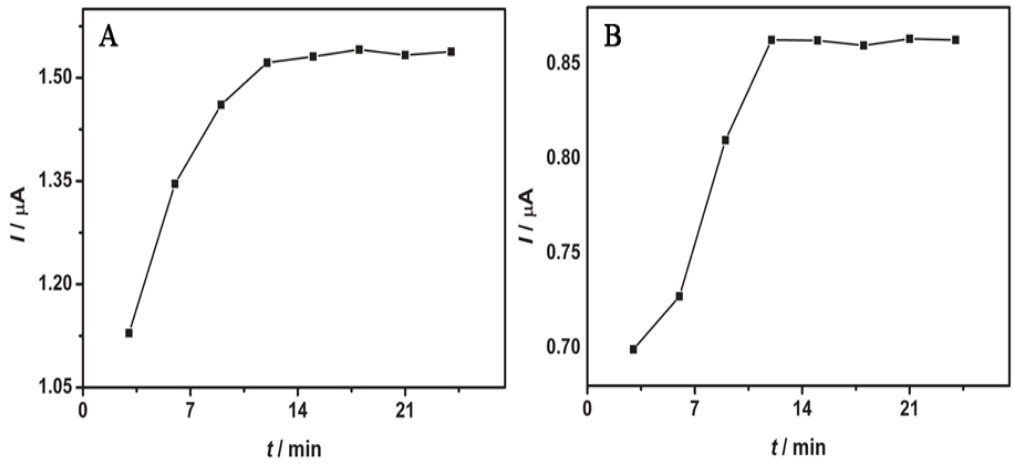

Fig. S4 Effect of quiet time on the peak current of 15 μM MB in B-R buffer (pH 6.09) containing 25 mM NaCl at ssDNA (A) and dsDNA (B).

Introduction of square-wave voltammetry

Square-wave voltammetry (SWV) is a technique beneficially used in analytical applications and in fundamental studies of electrode mechanisms. SWV is a further improvement of staircase voltammetry which is itself a derivative of linear sweep voltammetry. A square wave is superimposed on the potential staircase sweep as shown in Fig. S5. The current is measured at the end of each potential change, right before the next. Because the Faraday's current (i_f) is in proportion to $t^{(-1/2)}$, and the charging current (i_c) is proportion to $e^{(-t/RC)}$. The attenuation of i_f is slower than that of i_c. Therefore the contribution to the current signal from the i_c is minimized. The differential current is then plotted as a function of potential, and the reduction or oxidation of species is measured as a peak or trough. Due to the lesser contribution of charging current the detection limits for SWV are on the order of nanomolar concentrations.[1]

In our work, SWV was performed between -0.45 and 0.1 V with increasing potential, amplitude and frequency of 0.004 V, 0.025 V and 30 Hz, respectively.

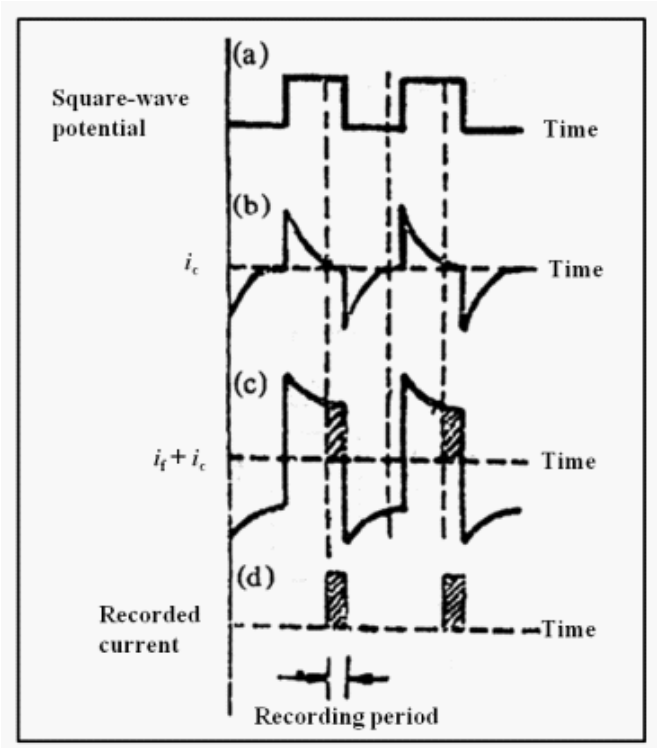

Fig. S5 The detection principle of square wave voltammetry. i_f, Faraday's current. i_c, charging current

[1] http://en.wikipedia.org/wiki/Squarewave_voltammetry

Investigation of the selectivity of proposed method

The selectivity of the proposed method has been verified by using a NC DNA with sequence of 5'-ATC TCT AG**T** CCG GTA TAA TG-3'. As shown in Fig. S6, the DNA biosensor in 15 µM MB in B-R buffer (pH 6.09) containing 25 mM NaCl showed an electrochemical response of MB at -0.19 V (vs. Ag/AgCl) with the peak current of 3.58 µA. After the DNA biosensor was incubated with 10 µM NC DNA at room temperature for 6 hrs and rinsed thoroughly with washing solution, the resulting DNA biosensor in 15 µM MB in B-R buffer (pH 6.09) containing 25 mM NaCl showed a peak current of 3.43 µA. The relative error (RE) was 4.2%. No obvious change of electrochemical response, indicating an excellent selectivity.

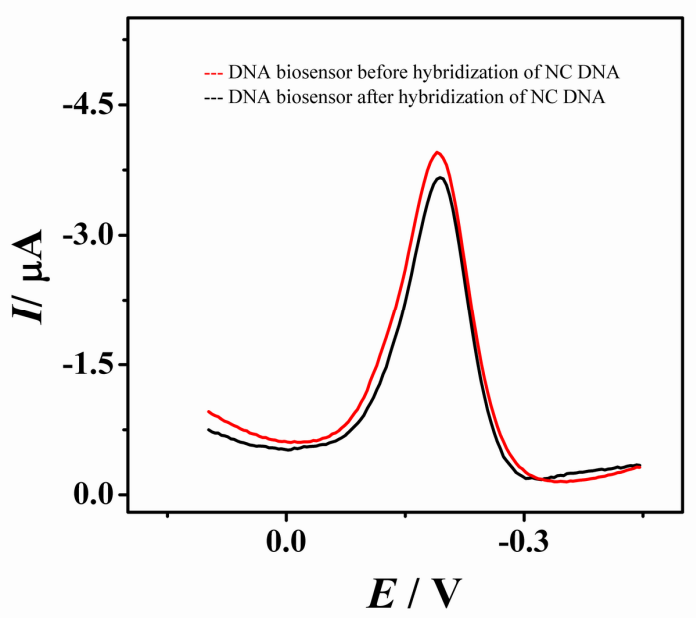

Fig. S6 Square wave voltammegrams of DNA biosensor in 15 µM MB in BR buffer (pH 6.09) containing 25 mM NaCl before (black line) and after (red line) hybridization with 10 µM non-complementary DNA of 5'-ATC TCT AG**T** CCG GTA TAA TG-3'. The increase potential, amplitude and frequency of SWV are 0.004 V, 0.025 V and 30 Hz, respectively.

Table S1 Analysis of DNA samples with 40% mthylation degree at different concentration

Sample concentration (μM)	Methylation degree (%)	Tested (%)	RE (%)
0.05	40.0	37.8	-5.5
0.5	40.0	38.4	-4.0
5.0	40.0	39.2	-2.0
10.0	40.0	40.8	2.0

Table S2 Sensitivity of gene-specific methylation assay

Methods	Detection limit	Sensitivity	Ref
MS-PCR	—	0.1%	[2]
COBRA	—	1.6%	[3]
MS-DBA	¤ < 2%	—	[4]
MS-MLPA	>10%	—	[5]
Our method	9.8%	0.8%	

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