

## Electronic Supplementary Information (ESI)

### **A Sensitive Electrochemical Biosensor for DNA Methyltransferase Activity by Combining DNA Methylation-Sensitive Cleavage and Terminal Transferase-Mediated Extension**

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#### **Experimental**

##### **Materials and Reagents**

The hairpin probe 5'-GAGGGCCTGCAGGATCATTGGCTTTTGCCAATGATCCTGCAGGCCCTCTTTTTTTT-(SH)-3' (S1), control sequence 5'-GAGGGCCTGCAGAGTCATTGGCTTTTGCCAATGACTCTGCAGGCCCTCTTTTTTTT-(SH)-3' (S2) were synthesized by Sangon Biotch Co. Ltd (Shanghai, China). Dam methyltransferase (Dam MTase), methylation sensitive restrictive endonuclease Dpn I (Dpn I), terminal transferase (TdTase), S-adenosylmethionine (SAM) were obtained from New England Biolabs. Ltd (Beijing, China). The dUTP-biotin and inhibitor 5-fluorouracil (5-FU) were purchased from Sangon Biotch Co. Ltd (Shanghai). 1-Naphthyl phosphate (1-NP) was purchased from Sigma-Aldrich (Shanghai). Bovine serum albumin (BSA) and streptavidin-alkaline phosphatase (SA-ALP) were from

Beijing Dingguo Biotechnology Company (Beijing). All of the other chemicals were of analytical reagent grade and used without further purification. The ultrapure water with an electrical resistance larger than 18.2 M $\Omega$  was used throughout this work.

The phosphate buffer solution (PBS) was a 10mM KH<sub>2</sub>PO<sub>4</sub>-Na<sub>2</sub>HPO<sub>4</sub> solution (pH 7.5). The tween-20 containing PBS (PBST) consisted of 0.05% tween-20 and a PBS. The PBS containing 5 mM K<sub>3</sub>[Fe(CN)<sub>6</sub>], 5 mM K<sub>4</sub>[Fe(CN)<sub>6</sub>], and 100 mM KCl was used in electrochemical impedance spectroscopic (EIS) measurements. The measuring buffer for the differential pulse voltammetric (DPV) experiments consisted of 100 mM tris-HCl (pH 9.8), 1 mM Mg<sup>2+</sup> and 4 mM 1-NP.

## **Apparatus**

DPV and EIS experiments were carried out on a CHI 760B electrochemical workstation (Shanghai Chenhua Apparatus, Shanghai, China). A conventional three-electrode system contained a gold electrode (2.0 mm diameter) as the working electrode, a platinum foil as the auxiliary electrode, and a saturated calomel electrode (SCE) as the reference. All experiments were performed in a 10 mL voltammetric cell at ambient temperature (25°C).

## **Hairpin probe self-assembled on gold electrodes**

Before self-assembly of hairpin probe, the gold electrode was polished sequentially with 0.3  $\mu$ m and 0.05  $\mu$ m alumina powder, followed by ultrasonic cleaning in ethanol and water. Subsequently, the gold electrode was dipped in freshly prepared piranha

solution ( $\text{H}_2\text{SO}_4/\text{H}_2\text{O}_2$ , 7:3) for 20 min, and then rinsed thoroughly with water. Then, the electrochemical pretreatments were performed by cycling the potential between -0.3 to +1.5 V (vs. SCE) in 0.5 M  $\text{H}_2\text{SO}_4$  solution for 10 min. Finally, the electrode was washed with water and dried under a nitrogen stream.

The gold working electrode was coated with 10  $\mu\text{L}$  of 1  $\mu\text{M}$  hairpin probe solution (PBS contains 1 M NaCl) and kept overnight at 4°C. After the incubation step, the electrode was rinsed with PBST. Then the unmodified region of the electrode was blocked by immersing the electrode into 1 mM 6-mercaptohexanol (MCH) solution for 10 min and then rinsing the surface with PBST.

### **Electrochemical detection of Dam MTase**

An aliquot of the reaction mixtures consisting 10 mM tris-HCl (pH 7.5), 10 mM  $\text{MgCl}_2$ , 50 mM NaCl, 80  $\mu\text{M}$  SAM, 80 U/mL of Dpn I, and various amounts of Dam MTase was dropped onto the surface of hairpin probe self-assembled gold electrode and incubated at 37°C for 40min. After cleaning with PBST, the electrode was immersed in extension solution (20 mM tris-HCl, 50 mM KCl, 10 mM  $\text{Mg}(\text{Ac})_2$ , 0.25 mM  $\text{CoCl}_2$ , pH 7.9, 200 U/mL TdTase, 10  $\mu\text{M}$  dUTP-biotin) and incubated at 37°C for 60 min. A BSA solution (20  $\mu\text{L}$  of 1% BSA) was dropped onto the electrode and incubated at 37°C for 30 min to block the nonspecific site. Then, 20  $\mu\text{L}$  SA-ALP (1:100 dilution using 1% BSA solution) was added onto the electrode surface and incubated at 37°C for 40 min.

After all those steps, the electrode was immersed into 3 mL of measuring buffer for

3 min. DPV voltammogram was recorded at a potential range from 0 to 0.6 V (vs. SCE) with a 100 mV s<sup>-1</sup> scan rate.

### **Influence of inhibitor on Dam MTase activity**

In this section, 5-FU as a inhibitor for Dam MTase was studied. Firstly, the methylation reaction solution (10 mM tris-HCl (pH 7.5), 10 mM MgCl<sub>2</sub>, 50 mM NaCl, 80 μM SAM, 80 U/mL Dam MTase, different concentration of 5-FU) was dropped onto the hairpin probe assembled electrode and incubated at 37°C for 40 min. After cleaning, the methylated probe was cleaved by Dpn I (10 mM tris-HCl pH 7.5, 10 mM MgCl<sub>2</sub>, 50 mM NaCl, 80 U/mL Dpn I at 37°C for 40 min). The other processes were the same as the Dam MTase activity detection.

It was reported that 5-FU had inhibitory effect on the activity of Dpn I endonuclease, thus interfering with the assay of Dam MTase.<sup>1,2</sup> Thus we also investigated the influence of 5-FU. As shown in Fig. S5, a strong DPV peak is obtained in our separate reaction protocol in which Dpn I endonuclease was added after washing the methylatin reaction mixture containing Dam MTase and 5-FU (100 μM). In contrast, the current peak becomes much smaller (~41% of the peak current for the separate reaction protocol) when the methylatin and cleavage reactions proceed with Dam MTase, 5-FU (100 μM) and Dpn I endonuclease in a one-pot process (curve b). This finding reveals that high concentration of 5-FU can have substantial inhibitory effect on the activity of Dpn I, and our separate reaction protocol can eliminate this interference in our assay.

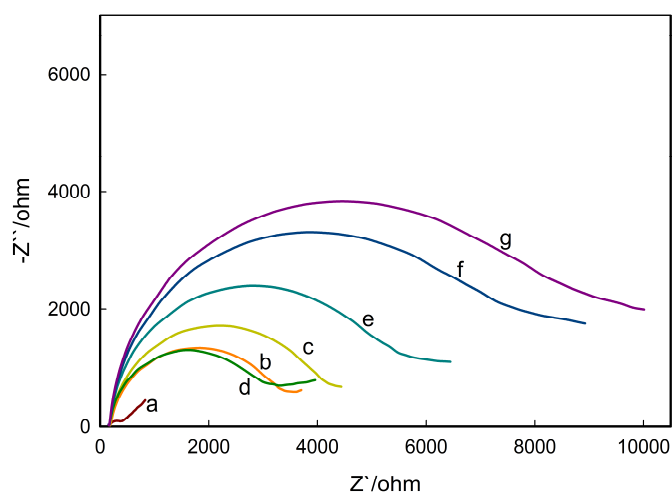
## Characterization of modified electrode using electrochemical impedance spectroscopy

The feature of surface-modified electrode was studied by the electrochemical impedance spectroscopy. As shown in Fig. S1, starting from the bare gold electrode (curve a), the electrochemical impedance changed sequentially after each step of association. When hairpin probe was assembled on the electrode, the impedance increased (curve b). MCH blocked the unmodified region of the electrode resulting in the impedance increase (curve c). With introduction of Dam MTase and Dpn I, the hairpin probe was methylated and cleaved, and the impedance decreased (curve d). After TdTase-mediated dUTP-biotin extension, the impedance increased (curve e). BSA blocking the nonspecific site (curve f) caused the impedance also increased. After the SA-ALP binding specifically to the biotin, the impedance further increased (curve g). This study indicated that a sensing interface was effectively constructed and successfully applied in the analysis of the activity of Dam MTase and confirmed the feasibility of the biosensor.

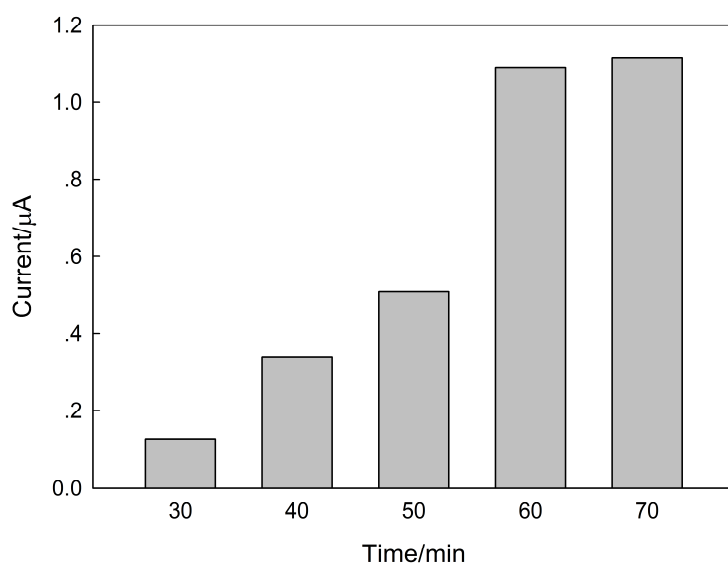
### References

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- 2 W. Li, Z.L. Liu, H. Lin, Z. Nie, J.H. Chen, X.H. Xu, S.Z. Yao, *Anal. Chem.*, 2010, **82**, 1935;

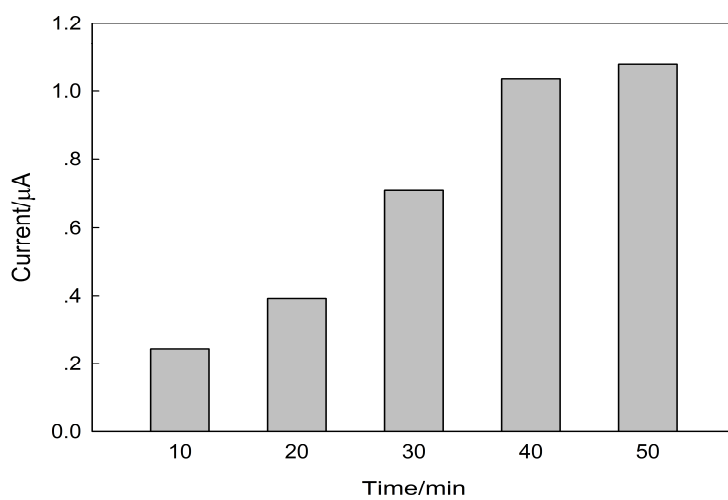
## Supporting Figures



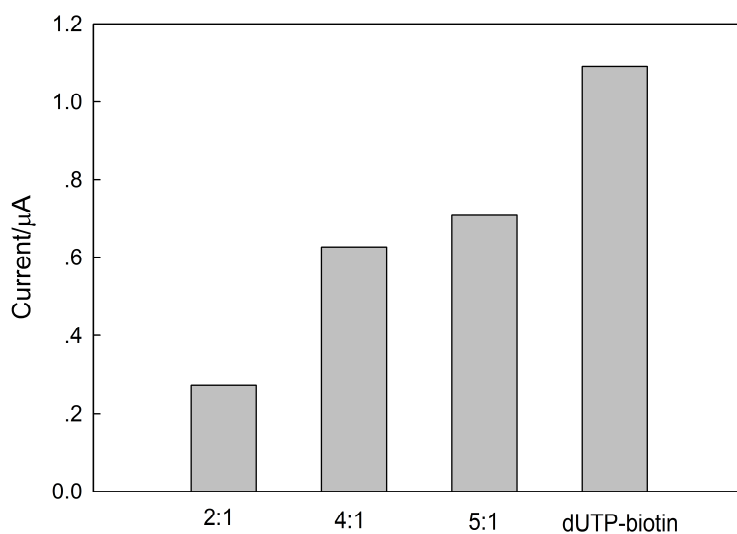
**Fig. S1** Electrochemical impedance spectra(EIS, in the frequency range of 0.1-10 KHZ) after different steps of modification. (a) bared gold electrode, (b) DNA probe modified electrode, (c) DNA probe/MCH modified electrode, (d) Dam MTase and Dpn I catalyzed DNA probe methylation and cleavage, (e) TdTase-mediated dUTP-biotin extension, (f) BSA blocked the nonspecific site, (g) SA-ALP bound with biotin. All the measurements were performed in 5 mM  $\text{K}_3\text{Fe}(\text{CN})_6 / \text{K}_4\text{Fe}(\text{CN})_6$  solution containing 0.1 M KCl.



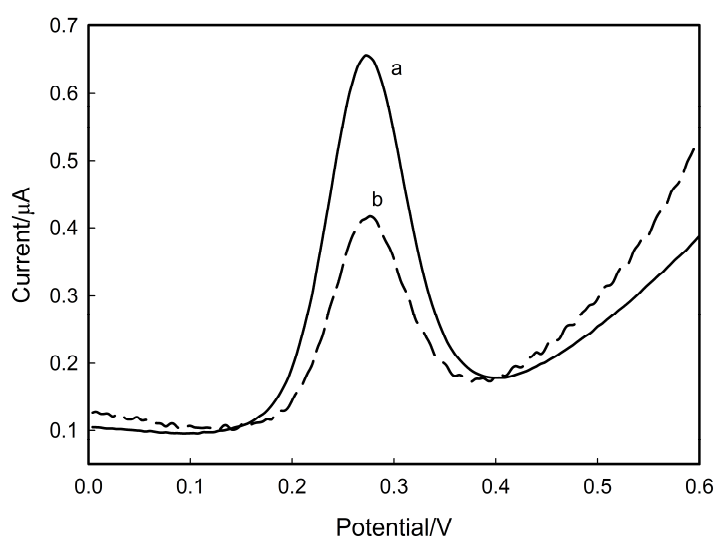
**Fig. S2** Different TdT-mediated dUTP-biotin extension reaction time at the condition: 80 U/mL Dam MTase, 40 min; 10 μM dUTP-biotin, 200 U/mL TdT. (at 37°C)



**Fig. S3** Different methylation time at the condition: 80 U/mL Dam MTase; 10 μM dUTP-biotin, 200 U/mL TdTase, 60 min. (at 37°C)



**Fig. S4** Different ratio of dUTP-biotin:dATP. The amount of dUTP-biotin and dATP was 10 μM.



**Fig. S5** Differential pulse voltammograms between 0 and 0.6 V versus SCE using separate reaction protocol (a) and one-pot process (b).