**Electronic Supporting Information**

# Label-free electrochemical detection of DNA methyltransferase activity *via* DNA tetrahedron-structured probe

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## 1. Experimental

### Chemicals and materials

All oligonucleotides synthesized and modified by Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. (Shanghai, China) and the sequences are shown in Table S1. Ethylene diamine tetraacetic acid (EDTA), tris(2-carboxyethyl)phosphine hydrochloride (TCEP), 6-Mercapto-1-hexanol (MCH), aniline (99.5%), 5-Aza-z′-deoxycytidine (5-Aza), and procaine were obtained from Sigma-Aldrich Co. Ltd. (St. Louis, MO). SAM, E. coli CpG methyltransferase (M.SssI), E. coli restriction endonuclease (HpaII), and Dam MTase were obtained from New England BioLabs (Ipswich, MA). Hemin was purchased from Porphyrin Products (Logan, UT, USA) and used without further purification. Hemin stock solution was prepared in dimethyl sulfoxide (DMSO) and stored in the dark at -20 °C. All other reagents of certified analytical grade were supplied from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Milli-Q water (18.2 MΩ at 25 °C, Barnstead, Thermo Scientific, USA) was used throughout the experiments.

The buffer solutions employed in this study were as follows: 1×PBS (pH 7.2 ~ 7.4, 136.89 mM NaCl, 2.67 mM KCl, 8.24 mM $Na_2HPO_4$, 1.76 mM $NaH_2PO_4$); TE buffer (10 mM Tris, pH 8.0, 1 mM EDTA); TM buffer (20 mM Tris, pH 8.0, 50 mM $MgCl_2$); MTase work buffer (10 mM Tris-HCl, pH 7.9, 50 mM NaCl, 10 mM $MgCl_2$, 160 μM SAM, 1 mM DTT); Cleavage buffer (33 mM Tris-acetic acid (Tris-Ac), pH

7.9, 10 mM $Mg(Ac)_2$, 66 mM KAc, 0.1 mg/mL BSA); TK buffer (20 mM Tris-HCl, pH 8.0, 50 mM KCl, 2 μM hemin); Deposition buffer (100 mM HAc-NaAc, pH 4.3, 120 mM aniline, 100 mM $H_2O_2$, 50 mM KCl, prepared daily); Electrolyte (100 mM HAc-NaAc buffer, pH 4.3); Electrochemical impedance spectroscopy (EIS) buffer (1×PBS, 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1), 100 mM KCl).

**Self-assembly of DNA probes on gold electrode surfaces**

Gold electrodes (2 mm diameter) were cleaned following the reported protocol.[1,2] DNA tetrahedron-structured probes (DTP) were formed as reported previously.[3-6] In brief, tetra-A and three thiol-modified single strands (tetra-B, tetra-C, and tetra-D) were dissolved in TE buffer, yielding a final concentration of 50 μM. A quantity of 1 μL of each strand was mixed with 1 μL of TCEP (500 mM) and 45 μL of TM buffer. The resulting mixture was heated to 95 °C for 10 min, then cooled to 4 °C for 30 min using a thermal cycler S1000TM (MJ Research Inc., SA). The resulting product was analyzed by using 12.5% polyacryamide gel electrophoresis (Fig. S1). Then, 3 μL of DTP (1 μM) were added to the cleaned gold electrode for 12 h at room temperature for immobilization. The process of ssP without the tetrahedral-structure immobilized on the gold electrode as following: 3 μL of 1 μM ssP in TM buffer was injected on the surface of the gold electrode for 12 h at room temperature for immobilization. Subsequently, the resulting electrode was incubated in 1 mM MCH for 1 h to eliminate nonspecific binding. The resulting DTP and ssP electrodes were rinsed with 1×PBS and dried lightly with nitrogen before hybridization.

**Hybridization of target DNA with DTP or ssP**

The DTP or ssP modified gold electrode was immersed into TM buffer with 1 μM target DNA for 2 h at 37 °C. After hybridization, the gold electrode was thoroughly rinsed with 1×PBS.

**M.SssI MTase activity assay**

The methylation of hybrids were performed at 37 °C for 3 h in the MTase working buffer with various concentration of M.SssI ranging from 0 to 20 U/mL. HpaII digestion was performed at 37 °C in the cleavage buffer containing 50 U/mL HpaII for 3 h. After each modification, the gold electrode was thoroughly rinsed with 1×PBS.

**Inhibition of M.SssI MTase activity**

To study the inhibition effects of 5-Aza and procaine on the M.SssI activity, the hybrids were incubated with MTase working buffer containing 2.5 U/mL M.SssI, and inhibitors of various concentrations at 37 °C.

**Formation of HRP-mimicking DNAzyme and deposition of PANI**

The above modified electrodes were immersed in TK buffer for 60 min at room temperature. For the deposition of PANI, the modified electrodes were incubated in deposition buffer for 90 min at room temperature.

**Characterization and measurements**

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were performed with a CHI 660D electrochemical analyzer (Chen Hua Instruments, Shanghai, China). EIS was performed on a VersaSTAT 3 workstation (Princeton Applied Research, USA). A three-electrode system was employed with platinum wire as the auxiliary electrode, saturated calomel electrode (SCE) as the reference electrode, and gold electrode or modified gold electrode as the working electrode, respectively. The electrochemical signal was measured with CV and DPV by scanning from -0.1 V to 0.5 V in electrolyte, which was degassed with nitrogen for 15 min. EIS was recorded with a frequency range of 0.01 to $10^5$ Hz. Surface plasmon resonance (SPR, DyneChem HiTech Ltd. China) was used to investigate the binding performance of DNA and PANI deposition on SPR chip. The morphology of PANI was analyzed using atomic force microscopy (AFM) (Nanoscopy IIIa, USA) and scanning electron microscopy (SEM) system (JEM-2100, JEOL, Japan).

All experiments were performed in compliance with the relevant laws and institutional guidelines issued by the ethical committee of the second hospital of Nanjing. The ethical committee had approved the experiments and informed consent was obtained for any experimentation with human subjects.

**Table S1** Sequences of oligonucleotides employed in this work.[a]

| name | Sequence and modifications (from 5′-3′) |
|---|---|
| tetra-A | ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAG AGCCGCCATAGTATTTTTGTAGAT**CCGG**TTCATA |
| tetra-B | SH-$(CH_2)_6$-TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAA TAGATGCGAGGGTCCAATAC |
| tetra-C | SH-$(CH_2)_6$- TCAACTGCCTGGTGATAAAACGACACTACGTGGGA ATCTACTATGGCGGCTCTTC |
| tetra-D | SH-$(CH_2)_6$- TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGT TTGTATTGGACCCTCGCAT |
| ssP | SH-$(CH_2)_6$-GTAGAT**CCGG**TTCATA |

Target DNA ***GGGTAGGGCGGGTTGGG***ATGAA**CCGG**ATCTAC

[a]The bold font of CCGG was the recognized bases by M.*Sss*I MTase and restriction endonuclease *Hpa*II. The bold/italic font of GGGTAGGGCGGGTTGGG was the G-rich segments in target DNA sequence.

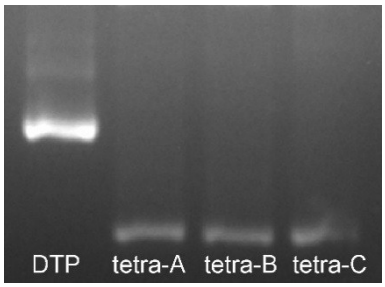


**Fig. S1** The gel results indicated that the successful formation of the DNA tetrahedron. The DNA tetrahedron-structured probe (DTP) shifted slower than the single stranded DNA (tetra-A, B, C, and D).

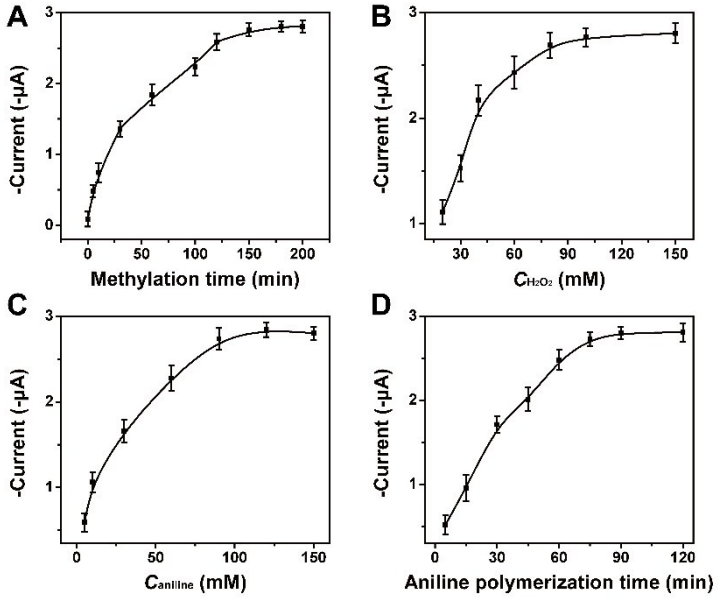


**Fig. S2** The influence of (A) different methylation time: 0, 5, 10, 30, 60, 100,120, 150, 180 and 200 min; (B) different $H_2O_2$ concentrations: 20, 30, 40, 60, 80, 100, and 150 mM; (C) different aniline concentrations: 5, 10, 30, 60, 90, 120, and 150 mM; (D) different aniline polymerization time: 5, 15, 30, 45, 60, 75, 90, and 120 min on the response of the biosensor. Error bars show the standard deviation of three experiments.

## 2. References

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