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Electronic Supplementary Information

A Label-free Electrochemical Strategy for Highly Sensitive Methyltransferase Activity Assay

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1. Reagents and materials

All oligonucleotides were purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). The sequences of the oligonucleotides were as follows: S1, 5′ - SH - $(CH_2)_6$- CAG T**CC GG**A GGT GAA CCT TAG ATA GAC CAA TTA - 3′; S2, 5′-CAC CT**C CGG** ACT G - 3′.

M.SssI CpG methyltransferase (M.SssI MTase) supplied with 10 × NEBuffer 2, restriction endonuclease HpaII supplied with 10 × CutSmart buffer, HaeIII methyltransferase (HaeIII MTase), AluI methyltransferase (AluI MTase), and *S*-adenosyl-$_L$-methiolnine (SAM) were purchased from New England Biolabs LTD.

Graphene prepared by an oxidation–reduction method was bought from XFNANO Materials Tech Co., Ltd. (Nanjing, China) and used as received. 5-Azacytidine (5-Aza), 5-aza-2′-deoxycytidine (5-Aza-dC), 6-mercapto-1-hexanol (MCH), and tris(2-carboxyethyl)phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich. Tris(hydroxymethyl) aminomethane (Tris) was purchased from Solarbio (Beijing, China). Ascorbic acid (AA) was purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All other chemicals were of analytical grade and used without further purification.

Aqueous solutions used throughout were prepared with ultra pure water (>18 MΩ cm) obtained from a Millipore system. 0.5 mg mL^{-1} graphene solution was prepared by dispersing a certain amount of graphene in ultra pure water under ultrasonication for 1h.

2. Apparatus

All electrochemical measurements including cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and differential pulse voltammetry (DPV) were performed on a CHI 660D Electrochemical Workstation (Chenhua Instrument Company of Shanghai, China). A conventional three-electrode cell was used with a planar gold (Au) electrode (2 mm in diameter) as the working electrode, a platinum wire as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode. The morphology of graphene was characterized by atomic force microscopy (AFM, MultiMode, Veeco Instruments Inc., USA) in the tapping-mode at room temperature and transmission electron microscopy (TEM, JEM-2100F, Jeol, Japan).

3. Preparation of the MCH/ds-DNA/Au electrode

Before the immobilization of the S1/S2 hybrid on the Au electrode, the Au electrode was polished to a mirror-like surface with 0.5 and 0.05 μm alumina slurries, followed by ultrasonication in ultrapure water and anhydrous ethanol. The electrode was then pretreated electrochemically in 0.5M H_2SO_4 aqueous solution by potential cycling in the potential range of −0.3 to 1.6 V at a scan rate of 100 mVs^{-1} until the cyclic voltammogram characteristic of a clean Au electrode was obtained. Then, the Au electrode was washed thoroughly with copious amount of ultrapure water and dried under nitrogen gas.

The S1/S2 hybrid was prepared as follows: the mixture of the two DNA strands (S1 and S2, 1.0 μM each) and 1.0 mM TCEP was prepared in a hybridization buffer (10 mM Tris, 1.0 mM EDTA, 1.0 M NaCl, pH 7.4). The solution was then annealed by heating to 90 ^{o}C for 5 min and followed by slowly cooling to room temperature. The obtained S1/S2 hybrid stock solution (1.0 μM) was kept at 4 °C when not in use.

For the preparation of the MCH/ds-DNA/Au electrode, 10 μL of 1.0 μM S1/S2 hybrid solution was dropped onto the cleaned Au electrode surface for 16 h at room temperature to obtain the ds-DNA/Au electrode. Then, the ds-DNA/Au electrode was further immersed in 1 mM MCH + 0.2 M PBS (20 mM phosphate, 0.15 M NaCl, pH 7.4) for 1 h to block the uncovered Au electrode surface as well as to make the array of ds-DNA on the electrode more regularly, and the obtained electrode was labeled as MCH/ds-DNA/Au electrode. To monitor each immobilization step, the EIS and CV measurements were performed in 0.1 M KCl aqueous solution with 5.0 mM $[Fe(CN)_6]^{3-/4-}$ as the probe. The related electrochemical impedance spectra were recorded within the range of 100 kHz-10 mHz at the formal potential of $[Fe(CN)_6]^{3-/4-}$ and the amplitude of the alternate voltage was 5.0 mV. And the CV investigation was carried out at a scan rate of 100 mV s^{-1} in the potential range of −0.2 to 0.5 V.

4. DNA MTase activity and inhibitor assay

The MCH/ds-DNA/Au electrode was first incubated with 5.0 μL 1× NEBuffer 2 containing different concentrations (from 0 to 400 U mL^{-1}) of M.SssI MTase and 160 μM SAM at 37 ˚C for 2 h to achieve the methylation at the CpG site of the

immobilized ds-DNA. Then the electrode was washed with NEBuffer 2 and dried, incubated with 5.0 μL 1×CutSmart buffer containing 50 U mL^{-1} HpaII restriction endonuclease at 37 °C for 2 h to cleave unmethylated duplex symmetrical sequence 5′-CCGG-3′. After another round of washing with CutSmart buffer and drying, the electrode was incubated in 0.5 mg mL^{-1} graphene aqueous solution for 30 min. After rinsed with PBS thoroughly, the obtained electrode was immersed in the PBS (20 mM phosphate, 0.15 M NaCl, pH 7.4) containing 5.0 mM AA and the electrooxidation of AA was investigated by CV and DPV methods. The CV investigation was carried out at a scan rate of 100 mV s^{-1} in the potential range of −0.2 to 0.6 V. The conditions for DPV investigation was as follows: amplitude, 50 mV; pulse width, 0.2 s; sampling width, 0.0167 s; potential range, −0.2 - 0.6 V.

To further study the inhibition effect of anticancer drug, such as 5-Aza and 5-Aza-dC, on the M.SssI MTase activity, the MCH/ds-DNA/Au electrode was incubated with 5.0 μL 1×NEBuffer 2 consisting of 200 U mL^{-1} M.SssI MTase and 160 μM SAM with different concentrations of inhibitors. After the HpaII digestion and graphene adsorption steps as described above, the electrocatalytic oxidation current of AA was recorded on the inhibitor-treated electrode. The inhibition effect of anticancer drug on the M.SssI MTase activity was expressed as follows:

$$\text{Relative Activity} = \frac{I_{R2} - I_{R0}}{I_{R1} - I_{R0}}$$

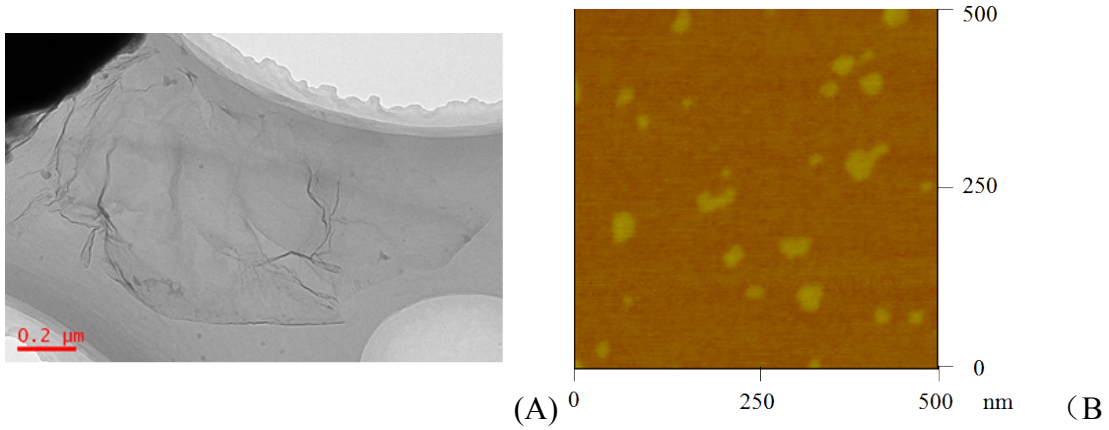
I_{R0} was the AA oxidation peak current obtained on the MCH/ds-DNA/Au electrode treated with 50 U mL^{-1} HpaII and 0.5 mg mL^{-1} graphene. I_{R1} was the AA oxidation peak current obtained on the MCH/ds-DNA/Au electrode treated with 200 U mL^{-1}

M.SssI MTase, 50 U mL^{-1} HpaII and 0.5 mg mL^{-1} graphene. I_{R2} was the AA oxidation peak current obtained on the MCH/ds-DNA/Au electrode treated with the mixture consisting of 200 U mL^{-1} M.SssI MTase and different concentrations of the 5-Aza or 5-Aza-dC, 50 U mL^{-1} HpaII and 0.5 mg mL^{-1} graphene. The solution for AA oxidation was PBS (20 mM phosphate, 0.15 M NaCl, pH 7.4) containing 5.0 mM AA.

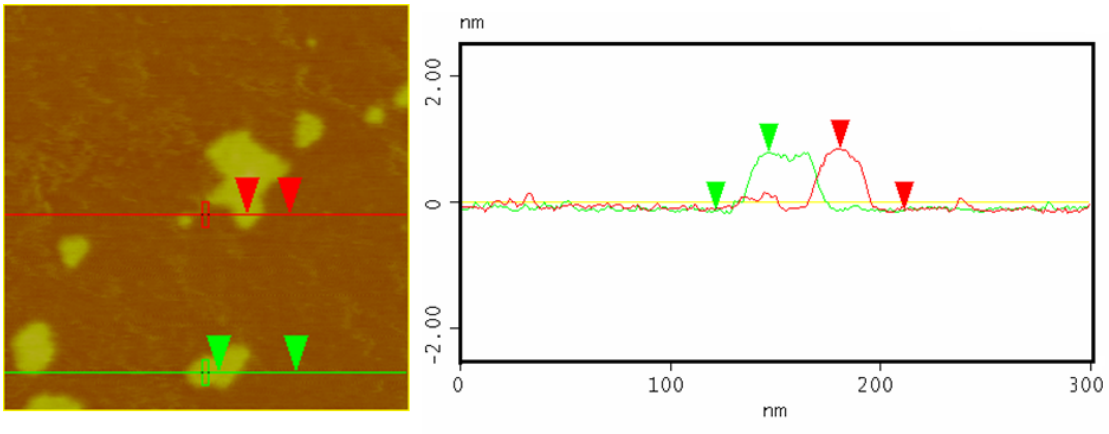
Each electrochemical measurement in this paper was repeated at least three times on the different electrodes prepared in the same procedure and conditions.

5. Characterization of graphene

The morphology of graphene was characterized by TEM and AFM. As shown in Fig.S1 A, the graphene sample looks like transparent film, implying that the obtained graphene should be very thin. From Figs. S1B and S1C, it is noted that the size of graphene is range from 20 - 70 nm and the average thickness of graphene is about 0.95 nm.



(A) (B)



(C)

Fig. S1 . SEM (A) and AFM (B, C) images of graphene.