

Supplementary Material (ESI) for Chemical Communications

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Supporting Information

The determination of DNA methyltransferase activity by quenching of tris(2, 2'-bipyridine)ruthenium electrogenerated chemiluminescence with ferrocene

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Reagents and Materials

Tris(hydroxymethyl) aminomethane (Tris), tris(2-carboxyethyl) phosphine hydrochloride (TCEP, 98%), Tripropylamine (TPrA), dithiothreitol (DTT), 6-mercaptohexanol (MCH), H₂AuCl₄·4H₂O (99% w/w), tris(2,2'-bipyridine)ruthenium(II) (Ru(bpy)₃²⁺), cysteamine (SH-(CH₂)₂-NH₂), ferrocene carboxylic acid, 1-ethyl-3-[(3-dimethylamino)propyl]carbodiimide (EDC), and N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich and used without further purification. S-adenosylmethionine (SAM), *E. coli* DNA adenine methyltransferase (Dam), *E. coli* restriction endonuclease Dpn I and the corresponding buffer solution were provided by New England Biolabs In (Beijing,

China). Milli-Q water (18 MΩ, Millipore System Inc.) was used throughout the experiment. The synthetic DNA oligonucleotides used for the detection were provided by Sangon Bioengineering Co. Ltd. (Shanghai, China), with the following sequences:

S1: 5'-SH-(CH₂)₆-ACAAT GATCA CTATT-3' (probe DNA)

S2: 5'-NH₂-AAAAAAAAAAAAAAAAAAAAA-**AATAG TGATC ATTGT**-3' (the bold fragment at the 3' end is complementary with the underlined fragment of the probe DNA S1, the red font section at the 5' end is the polyA overhang component, which was designed to restrict the access of nonspecific molecules to the surface of AuNP and optimize the orientation of S2 to make later quenching of ECL of Ru(bpy)₃²⁺ by ferrocene acetic acid more facile)

S3: 5'-NH₂-AAAAAAAAAAAAAAAAAAAAA-**AATAG TG***TTC* ATTGT-3' (With the exception of one mismatched base *T*, S3 has the same sequence as S2)

The ferrocene acetic acid- labeled oligonucleotide probes (S2 or S3) were synthesized according to methods in the reported literature.¹

Apparatus

The ECL emission was detected using a model MPI-A electrochemiluminescence analyzer that is produced by Xi'an Remex Electronics (Xi'an, China), and the voltage of the PMT was set at -900 V throughout the process. The conventional three-electrode cell was used, which included a platinum wire as a counter electrode, an Ag/AgCl (3.0 M KCl) as a reference electrode and a modified gold electrode or bare gold electrode as a working electrode. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) measurements were performed with a CHI 660a electrochemical workstation (Shanghai Chenhua Instrument Corporation, China). The scanning electron microscopy (SEM) was carried out using a JSM-6700 (JEOL, Japan).

Preparation of AuNPs and Ru(bpy)₃²⁺-AuNPs Composite

AuNPs with a diameter of ~19 nm were prepared by citrate reduction of HAuCl₄ in aqueous solution according to ref 2. In brief, 100 mL of 0.01% HAuCl₄ was brought to reflux, and then 2.5 mL of 1% sodium citrate was introduced while stirring. The AuNPs suspension was then kept boiling for another 30 min and left to cool to room temperature. The product was stored in a dark glass bottle at 4 °C for further use.

The Ru(bpy)₃²⁺-AuNPs composite was prepared according to the literature.³ 150 μL of 50 mM Ru(bpy)₃²⁺ aqueous solution was added into 20 mL of the AuNPs solution under vigorous stirring at room temperature. After several minutes, a large amount of black precipitate had formed. The resulting Ru(bpy)₃²⁺-AuNPs composite was collected by centrifugation, washed several times with water, and then suspended in water.

Fabrication of the biosensing electrode

Prior to use, the gold electrode (2 mm in diameter) was polished carefully with alumina powder of 0.3 and 0.05 μm, followed by sonication in water, ethanol and water for 2 min in each. The electrode was then scanned in 0.1 M H₂SO₄ from -0.2 V to 1.6 V at 100 mV/s until a reproducible cyclic voltammogram (CV) was achieved. The cleaned electrode was thoroughly rinsed with Milli-Q water and immersed in 0.10 M cysteamine aqueous solution for 2 h at room temperature to form the cysteamine monolayer. Then the electrode was thoroughly rinsed with Milli-Q water to remove physically adsorbed cysteamine. A 2 μL aliquot of the suspension of Ru(bpy)₃²⁺-AuNPs composite was then placed on the cysteamine-derivated gold electrode surface. The prepared electrode was air-dried at room temperature and rinsed thoroughly with Milli-Q water. Finally, the ECL signal composite of Ru(bpy)₃²⁺-AuNPs was immobilized on the gold electrode surface.

For the assembly of S1 on the surface of the Ru(bpy)₃²⁺-AuNPs modified gold electrode, a mixture of 10 μL of S1 solution (10 μM) with 10 μL of TCEP solution (10 mM) was first incubated for 1 h to reduce the disulfide bond at the 5'-terminus of S1 and generate a free thiol group for surface immobilization, followed by diluting the mixture to 100 μL with 10 mM Tris-HCl buffer (pH 7.4). The S1 was immobilized onto the surface of the Ru(bpy)₃²⁺-AuNPs modified gold electrode by incubating the electrode in the diluted S1 solution for 4 h. Then the electrode was rinsed with Tris-HCl buffer and water in turn to remove the physically adsorbed S1 probe. After that, the S1 modified electrode was blocked with 1 mM MCH solution for 30 min. Finally, the S1 modified electrode was immersed into 200 μL of 10 μM S2-Fc for 2 h to form the sensing interface and washed with water. This hybridized electrode was used as an ECL biosensing electrode.

ECL measurements

The Dam assay solution contained buffer (10 mM Tris-HCl (pH 7.5), 10 mmol/L EDTA, 10 mM MgCl₂, 50 mM NaCl), 160 μM SAM and different activities (concentration) of Dam. For the detection of Dam, an ECL biosensing electrode was immersed in 500 μL various concentration of Dam assay solution and incubated at 37 °C for 2 h to form the methylated S1&S2 hybrid. Subsequently, the methylated S1&S2 hybrid modified electrode was immersed in 500 μL of buffer solution containing 1× NEB buffer 4 (50 mM NaCl, 20 mM Tris-Ac, 10 mM Mg(Ac)₂, 1 mM DTT (pH 7.9)) and 50 U/mL Dpn I for 2 h at 37 °C to cleave the methylated adenines and washed with the 10 mM Tris-HCl buffer (pH 7.5). Finally, the cleaved electrode was transferred into 2.0 mL of 0.10 M PBS (pH 7.40) containing 0.02 M TPA to record the ECL response. A constant potential of +0.9 V (vs. Ag/AgCl) was applied and the ECL signal was recorded. The activity of Dam was quantified by the ECL

intensity.

Inhibition of drugs on Dam activity

To investigate the potential application of this sensor in inhibition assay, we used gentamycin (broad-spectrum antibiotic) and 5-fluorouracil (anti-cancer drugs), as two model inhibitors to evaluate the effect of the drugs on Dam activity. The hybridized ECL biosensing electrode prepared above was used in 50 mM Tris-HCl (pH 7.5) containing 160 mM SAM, 50 mM NaCl, 10 mM EDTA, 10 mM MgCl₂, 40 U/mL Dam and various concentration of the inhibitors at 37 °C for 2 h. The ECL measurement was the same as for the detection of Dam.

Supporting Figures

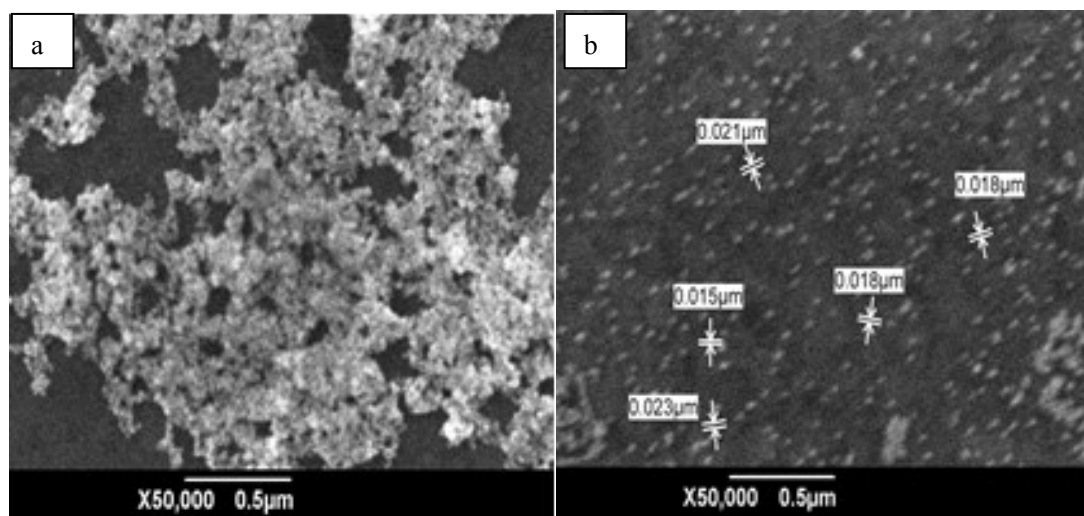
Table S1. Comparison of different methods for assay of the Dam activity

Detection technique	Liner range (U/mL)	Detection limit (U/mL)	reference
Colorimetry	-	0.25	4
Fluorescence	0.8-40	0.8	5
Fluorescence	0.4-20	0.4	6
Fluorescence	0.5-100	0.1	7
Fluorescence	0.08-26	0.05	8
Fluorescence	0.01-10	0.01	9
Fluorescence	1-100	0.16	10
Fluorescence	1-100	1	11
Bioluminescence	0.2-100	0.08	12
Chemiluminescence	0.025-2.5	0.000129	13
Electrochemistry	0.2-10	0.12	14
Electrochemistry	0.1-20	0.04	15
Electrochemistry	0.1-1.0	0.07	16
Electrochemistry	0.1-1.0	0.04	17
ECL	0.1-20	0.03	18
ECL	0.1-100	0.03	Present method

Fig. S1 (A) SEM images of (a) $\text{Ru}(\text{bpy})_3^{2+}$ -AuNPs on Au surface and (b) the AuNPs

/Au surface. (B) EDS of $\text{Ru}(\text{bpy})_3^{2+}$ -AuNPs.

(A)



(B)

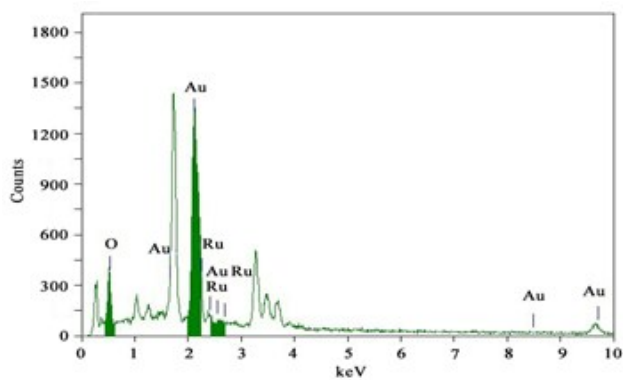


Fig. S2 (A) Electrochemical impedance spectra corresponding to different electrode.

(a) Ru(bpy)₃²⁺-AuNPs/cysteamine modified gold electrode, (b) bare gold electrode, (c) cysteamine modified gold electrode, (d) S1/MCH/Ru(bpy)₃²⁺-AuNPs/cysteamine modified gold electrode, (e) S2&S1/MCH/Ru(bpy)₃²⁺-AuNPs/cysteamine modified gold electrode, (f) S2&S1/MCH/ Ru(bpy)₃²⁺-AuNPs/cysteamine modified gold electrode methylated by 100 U/mL Dam and then cleaved with 50 U/mL Dpn I. All the measurements were performed in 5 mM K₃Fe(CN)₆/ K₄Fe(CN)₆ solution containing 0.1 M KCl. The biased potential was 0.227 V. The frequency was between 1 Hz and 100 kHz and the amplitude was 5.0 mV. (B) Demonstration of the corresponding CV profiles of the differently modified electrodes.

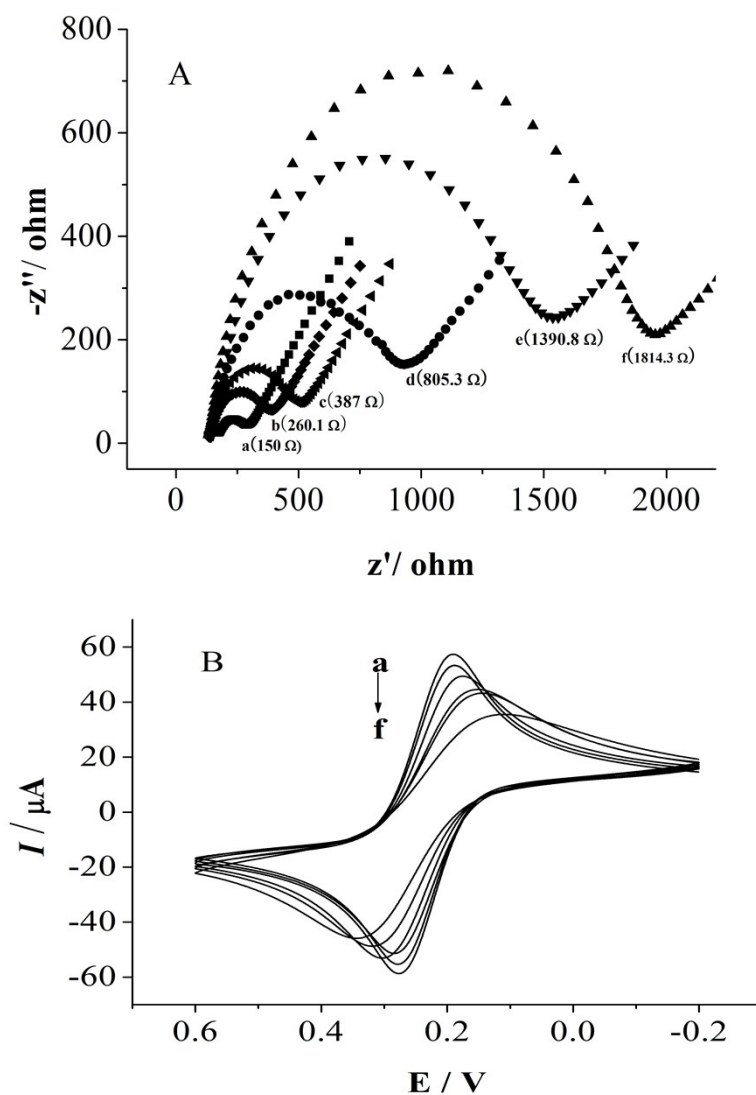


Fig. S3

(A)

Dependence of the ECL intensity on the methylation time of the S1&S2 hybrid with

100 U/mL Dam. (B) The different concentrations of SAM.

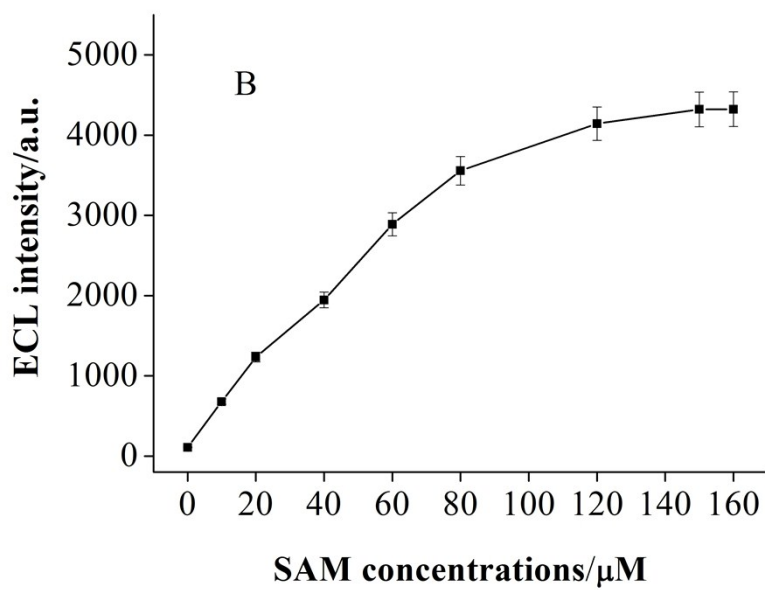
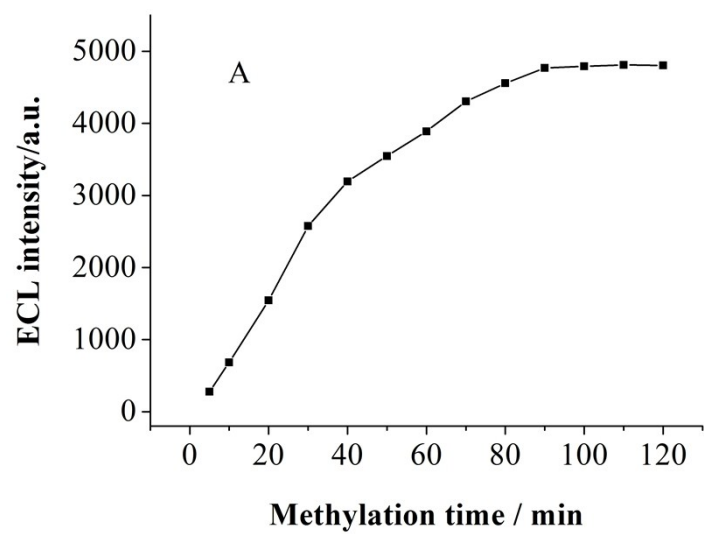
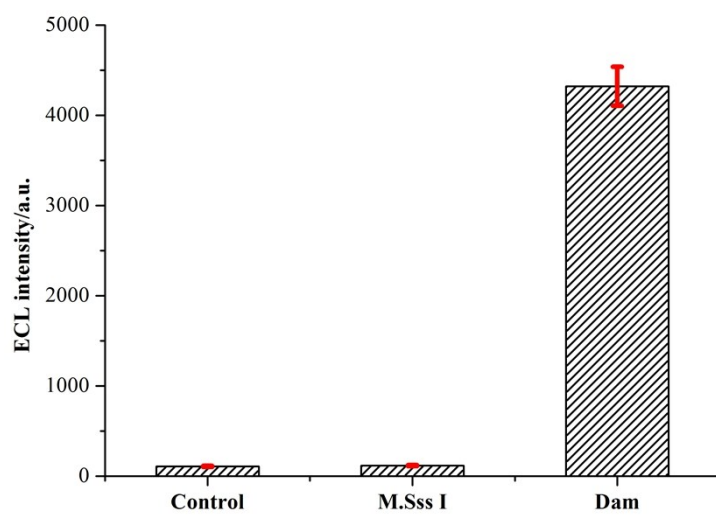


Fig.

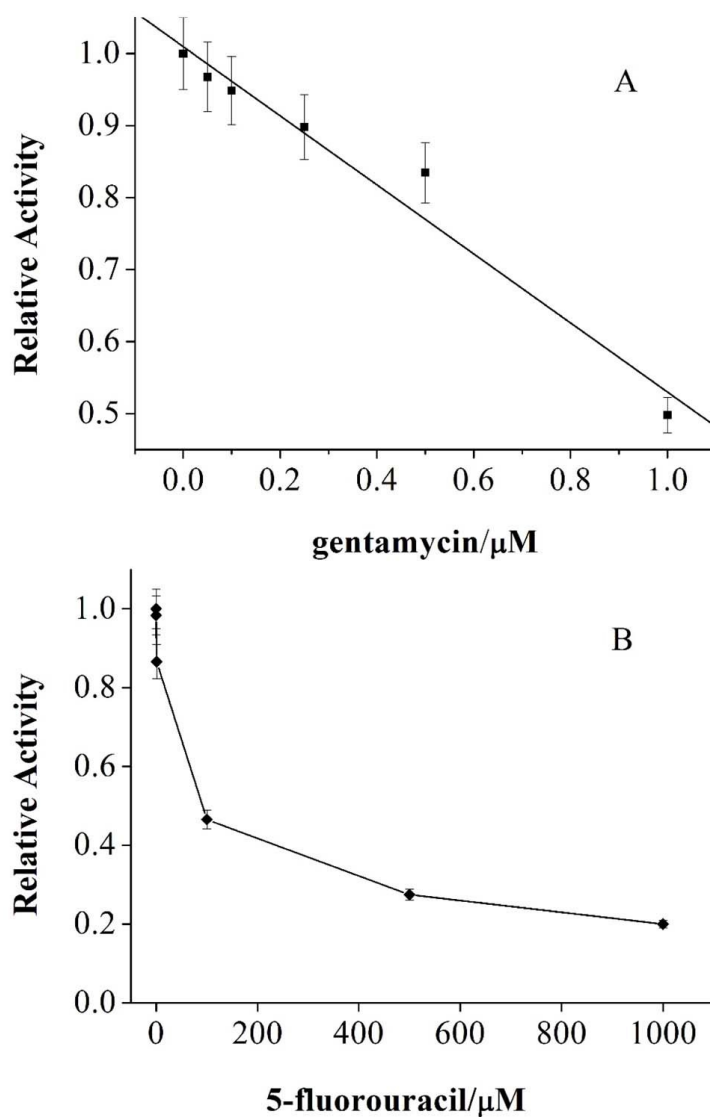
S4



Selectivity

of the proposed method. The concentration of Dam is 50 U/mL, and the concentration of M.SssI methyltransferase is 50 U/mL. Error bars show the standard deviation of three experiments.

Fig. S5 The feasibility of the present method for inhibiting Dam was assessed by using gentamycin and 5-fluorouracil as model inhibitors. The relative activity of the Dam was regarded as the ratio of the ECL signal of the S2&S1 hybrids, which were methylated by Dam with various concentrations of inhibitors, to that without inhibitors. Before the ECL was recorded, the S1&S2 hybrids were methylated for 2 h by Dam (100 U/mL) at different concentrations of gentamycin or 5-fluorouracil and then cleaved by Dpn I (50 U/mL) for 2 h. Every point is an average value of five measurements.



References

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