Supplementary Material (ESI) for Chemical Communications

Supporting Information

A label-free supersandwich electrogenerated chemiluminescence method for the detection of DNA methylation and assay of the methyltransferase activity

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Reagents and Materials

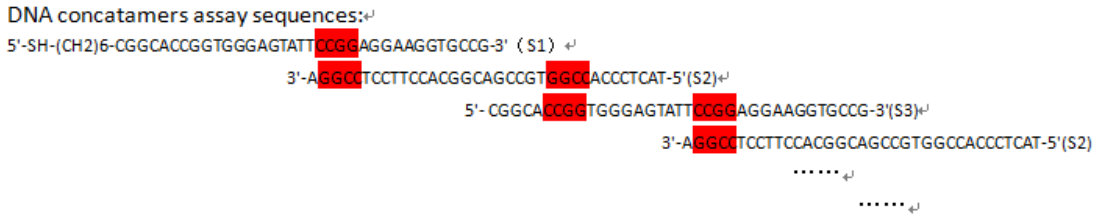
Tris(hydroxymethyl) aminomethane (Tris), tris(2-carboxyethyl) phosphine hydrochloride (TCEP, 98%), Tripropylamine (TPrA), dithiothreitol (DTT),dichlorotris(1, 10-phenanthroline) ruthenium hydrate ($Ru(phen)_3Cl_2 \cdot H_2O$), mercaptohexanol (MCH), S-adenosylmethionine (SAM), 5-azacytidine (5-Aza), and 5-aza-2'-deoxycytidine (5-Aza-dC) were purchased from Sigma-Aldrich and used without further purification. *E. coli* CpG methyltransferase M.SssI and *E. coli* restriction endonuclease *Hpa*II were supplied by New England BioLabs (Ipswich, MA). Milli-Q water (18 MΩ, Millipore System Inc.) was used throughout the experiment. The synthetic DNA oligonucleotides using for the detection were provided by Sangon Bioengineering Co. Ltd. (Shanghai, China), with the following sequences: S1: 5'-SH-$(CH_2)_6$-CGG CAC CGG TGG GAG TAT TCCG GAG GAA

GGT GCCG-3' (capture probe DNA)

S2: 3'-**AGGC CTC CTT CCA CGGC** A *GCC GTG GCC ACC CTC AT*-5' (the bold fragment at the 3' end is complementary with the underlined fragment of the capture probe DNAS1)

S3:5'-**CGG CAC CGG TGG GAG TA** T TCCG GAG GAA GGT GCCG-3' (the bold fragment at the 5' end is complementary with the italic fragment of S2, meanwhile, dotted line labeled fragment is complementary with the bold fragment of S2)

S4: 5'-TA CTC CCA C***T***G GTG CCG A CGGC ACC TTC CTC ***T***GG A-3' (Excepting two mismatched bases ***T***, S4 has the same sequence as S2)



The red sections of the DNA concatamers were specifically recognized by CpG methyltransferase M.SssI

Apparatus

The ECL emission was detected by 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 reference electrode and a modified gold electrode or bare gold electrode as working electrode.

Assembly of DNA Sensors

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 H_2SO_4 from −0.2 V to 1.6 V at 100 mV/s until a reproducible cyclic voltammogram (CV) was achieved. After being rinsed with water and dried with N_2, the clean electrode was modified with S1 by immersed in 1 μM S1/10 mM TCEP in 10 mM Tris-HCl buffer containing 0.1 M NaCl (pH 7.4) for 2 hours at room temperature (RT). The electrode was then washed with water and passivated with 10 μL MCH (1 mM MCH in 10 mM Tris-HCl, pH 7.40) for 40 min to block the nonspecific site and optimize the orientation of S1 to make later hybridization easier. After that, the prepared electrodes were washed with water, dried with N_2 and incubated with 5 μL partially hybridized S2&S3 (1 μM S2&S3 in 10 mM Tris-HCl, 0.1 M NaCl, pH 7.40) for 2 h at RT. In order to intercalate $Ru(phen)_3^{2+}$ molecules, a droplet of 5μL 2 mM $Ru(phen)_3^{2+}$ was dropped onto the modified electrode and incubated for 5 h. When finishing assembly, the modified electrode was thoroughly rinsed and dried. This electrode was used as an ECL biosensing electrode.

ECL measurements

The M. SssI assay solution contained buffer (10 mM Tris-HCl pH 7.9, 1mM dithiothreitol (DTT), 10 mM $MgCl_2$, 50 mM NaCl), 160 μM SAM and different activity (concentration) of M. SssI. For the detection of M. SssI, an ECL biosensing electrode was immersed in 500μL various concentration of M. SssI assay solution and incubated at 37 °C for 2 h to form the methylation S1/S2&S3 superhybrid. Subsequently, the methylation S1/S2&S3 superhybrid modified electrode was immersed in 500μL of 10 mM Tris-HCl buffer (pH 7.40) containing 20 U/mL *Hpa*II, 50 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 200 μg/mL BSA, and 50% glycerol for 2 h at 37 ºC to digest the unmethylated cytosines, thereafter rinsed with 10 mM Tris-HCl buffer (pH 7.40). Finally, the cleaved electrode was transferred into 2.0 mL of 0.10 M

PBS (pH 7.40) containing 0.05 M TPA to detect the ECL response. The CV was conducted from 0 to 1.20 V (vs. Ag/AgCl) with a scan rate of 100 mV/s and ECL signal was recorded.

For analysis of the inhibition effects of two typical anticancer drugs, 5-Aza and 5-Aza-dC, on the M. SssI activity, the hybridized electrode prepared above was incubated in 500 μL of MTase assay solution containing 250 U/mL M. SssI and different concentration of the inhibitors at 37 °C for 2 h. The ECL measurement was the same as for the detection of M. SssI.

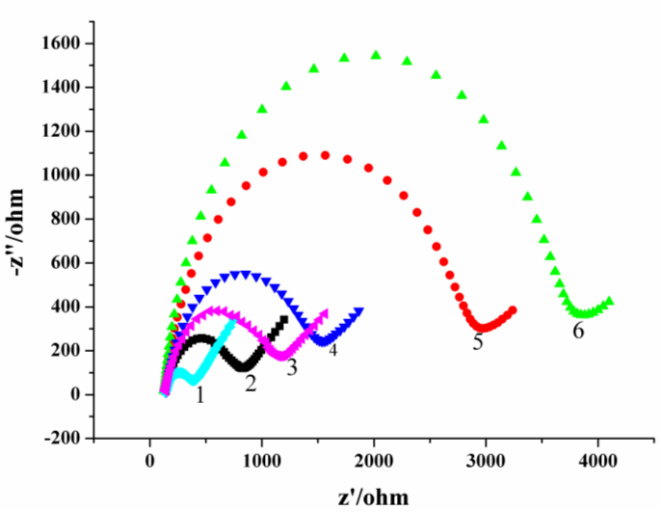
Supporting Figures

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

Detection technique	Liner range (U/mL)	Detection limit (U/mL)	reference
Colorimetry	-	0.25	1
Fluorescence	-	0.4	2
Electrochemistry	0.5-355	0.1±0.02	3
Electrochemistry	0.1-1.0	0.04	4
Electrochemistry	0.1- 20	0.04	5
Electrochemistry	0.1-450	0.05±0.02	6
ECL	0.05-100	0.02	7
ECL	1.0×10^{-5}-1.0	3×10^{-6}	Present method

Fig. S1 (A) Electrochemical impedance spectra corresponding to different electrode. (1) bare gold electrode, (2) capture DNA probe (S1) modified electrode, (3) capture DNA probe/MCH modified electrode,(4) S1/S2&S3/Ru(phen)$_3^{2+}$ modified electrode methylated by 0.01 U/mL M. SssI and then cleaved with 20 U/mL *Hpa*II. (5) S1/S2&S3/Ru(phen)$_3^{2+}$ modified electrode methylated by 10 U/mL M. SssI and then cleaved with 20 U/mL *Hpa*II. (6) S1/S2&S3/ Ru(phen)$_3^{2+}$ modified electrode, All the measurements were implemented in 5 mM $K_3Fe(CN)_6$/ $K_4Fe(CN)_6$ solution containing 0.1 M KCl. The biased potential was 0.235 V. The frequency was between 1 Hz and 100 kHz and the amplitude was 5.0 mV. (B) Demonstrated the corresponding CV profiles of the differently modified electrodes.

(A)



(B)

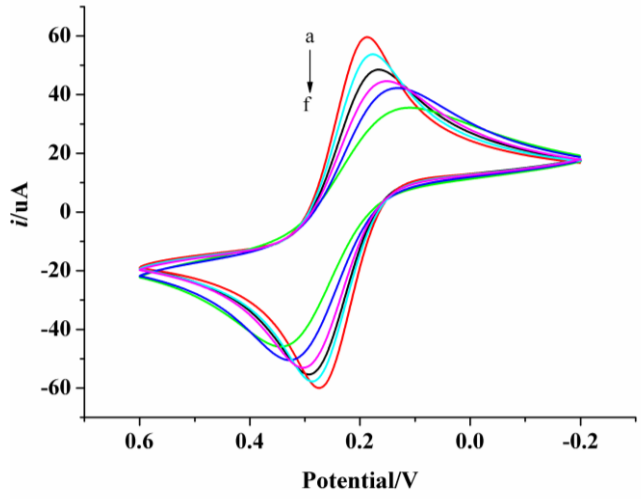


Fig. S2 Dependence of the ECL intensity on the methylation time of the S1/S2&S3 superhybrid with 1 U/mL M. SssI.

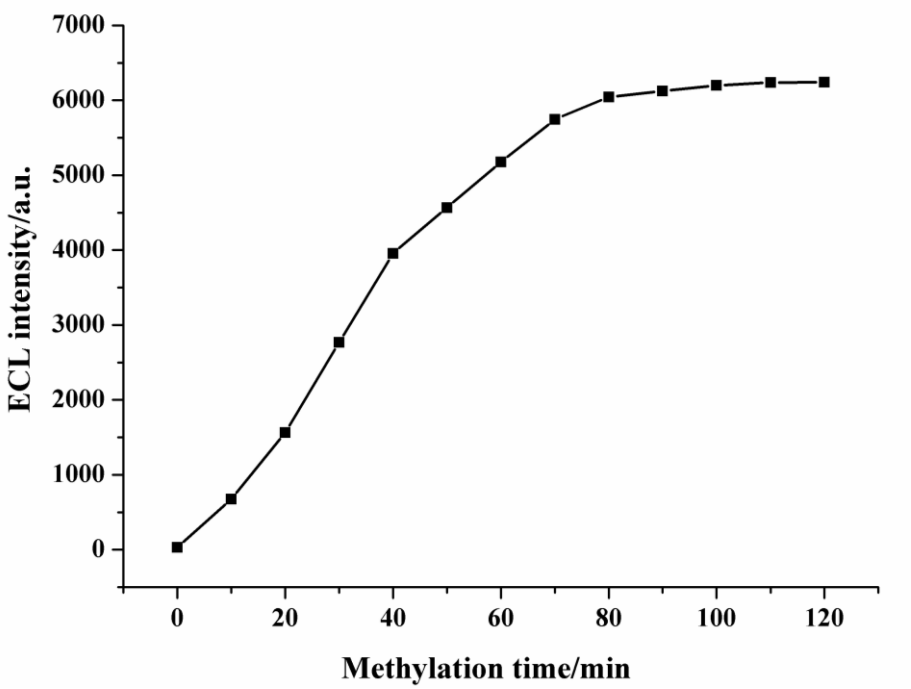


Fig. S3 ECL response of different two kinds of methods. Line a represents the ECL intensityof the sensor based on the supersandwich DNA structure, while line b represents the ECL intensity of the traditional sandwich DNA sensor. The concentration of M. SssI is 1 U/mL.

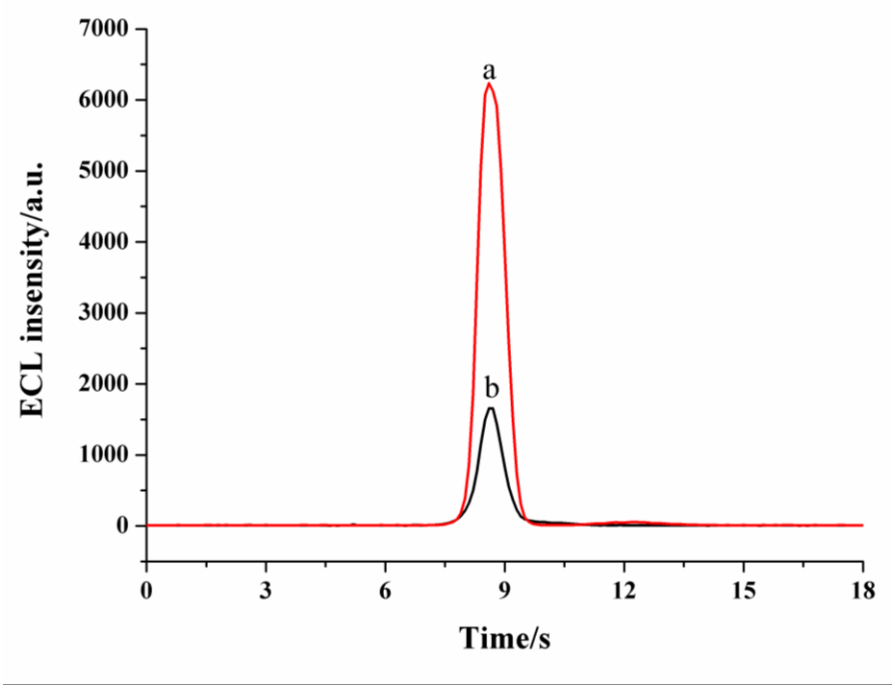
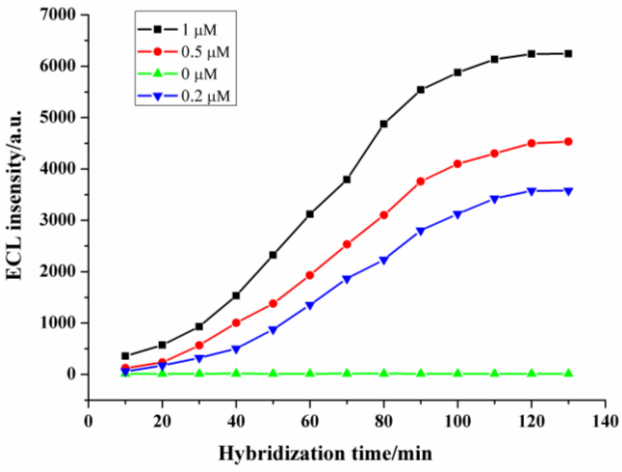
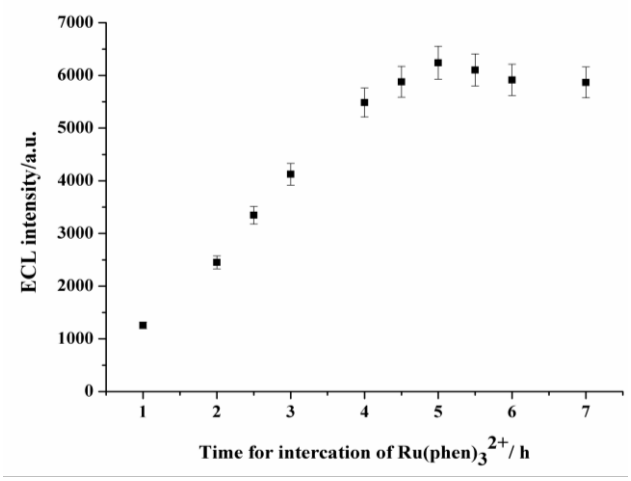


Fig. S4 Optimization of experimental conditions: (A) Hybridization time of S1 with different concentration of partially hybridized S2&S3: 5~130min. (B) Effects of the incubation time for intercalation of $Ru(phen)_3^{2+}$ on the ECL intensity.

(A)

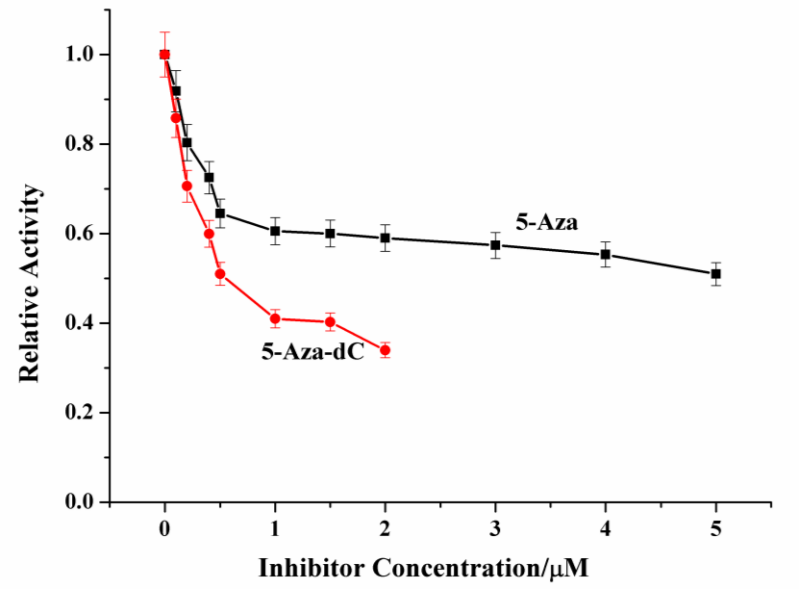


(B)



Herein, the hybridization time of S1 with different concentration of partly hybridized S2&S3 and the incubation time for intercalation of $Ru(phen)_3^{2+}$ were optimized. Maintaining longer time, the ECL intensity increases slightly (Fig. S4A). The ECL signal reached a plateau after 120 min. The optimum response of the incubation time for intercalation of $Ru(phen)_3^{2+}$ was obtained after 5 h (Fig. S4B). Even with longer time, there was no conspicuous change in the ECL intensity. Consequently, 2 h of the hybridization time and 5 h of the incubation time for intercalation of $Ru(phen)_3^{2+}$ were used in the subsequent experiments.

Fig. S5 The feasibility of the present method for inhibiting M. SssI was assessed by using 5-Aza and 5-Aza-dC as model inhibitors. The relative activity of the M. SssI was regarded as the ratio of the ECL signal of the S1/S2&S3/Ru(phen)$_3^{2+}$ superhybrid, which were methylated by M. SssI with various concentrations of inhibitors to that without inhibitors. Before the ECL was recorded, the S1/S2&S3/Ru(phen)$_3^{2+}$ superhybrid were methylated for 2 h by M. SssI (250 U/mL) at different concentrations of 5-Aza or 5-Aza-dC and then cleaved by *Hpa*II (20 U/mL) for 2 h. Every point is an average value of five measurements.



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