Supporting information

A Chemiluminescence Immunoassay for S-adenosylhomocysteine Detection and Its Application in DNA Methyltransferase Activity Evaluation and Inhibitors Screening

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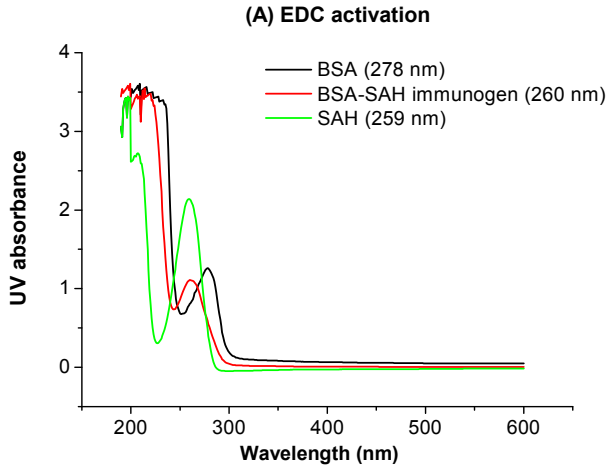
1. Production of Polyclonal Antibodies for SAH

The animal treatments were conducted with the approval of Institutional Authority for Laboratory Animal Care. Two male New Zealand rabbits (2.0 kg) were immunized at multiple sites on the neck and back with immunogens to generate the polyclonal antibodies against SAH.

Before immunization, 1 mL of blood was taken as the negative sample. For the initial immunization, 0.5 mg of immunogen in 0.5 mL of saline were emulsified with 0.5 mL of FA, and subcutaneously injected at multiple sites on the neck and back of the rabbits. For subsequent booster injections, the amount of immunogen was decreased by half, and iFA was used instead of FA. The immunizations were carried out for three times at two-week intervals. At the fifth immunization, 0.25 mg of immunogen was dissolved in 0.5 mL of saline without emulsification and injected. One week later, all rabbits were exsanguinated by heart puncture after general anesthetic. The blood was stored overnight at 4°C, and then centrifuged at 10000 g for 15 min. The supernatant liquor was removed into 1.5 mL of eppendorf tubes, stored at -20°C and served as the antiserum for the next experiments.

2. Characterization of SAH immunogens



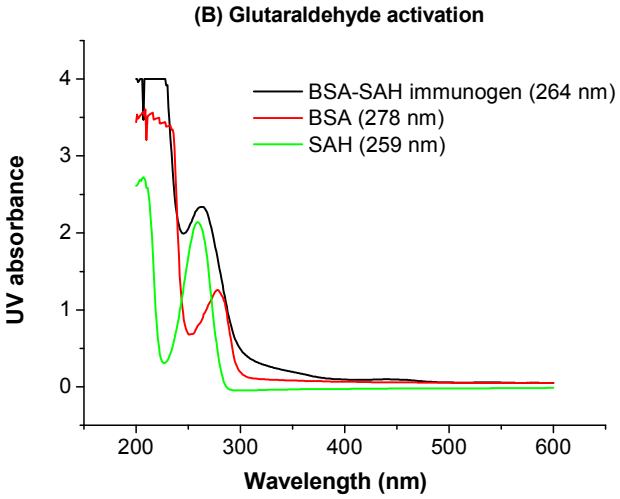
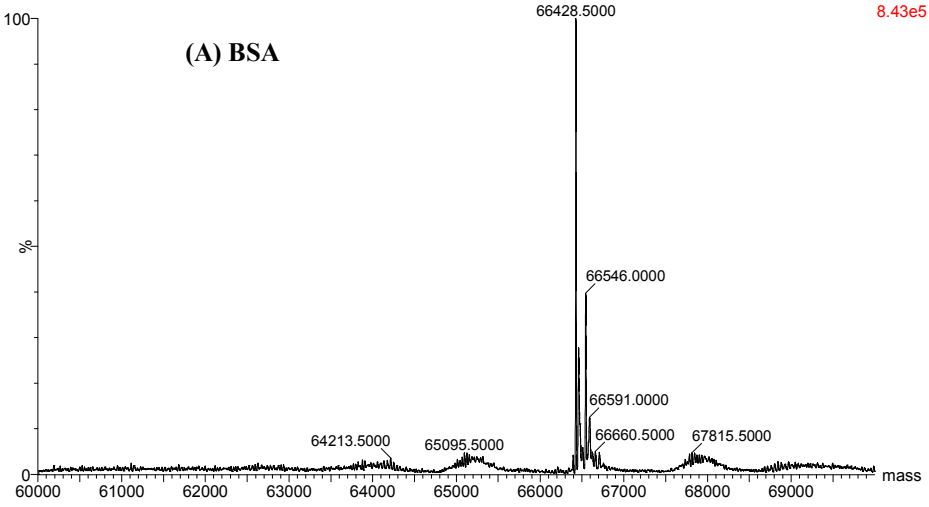
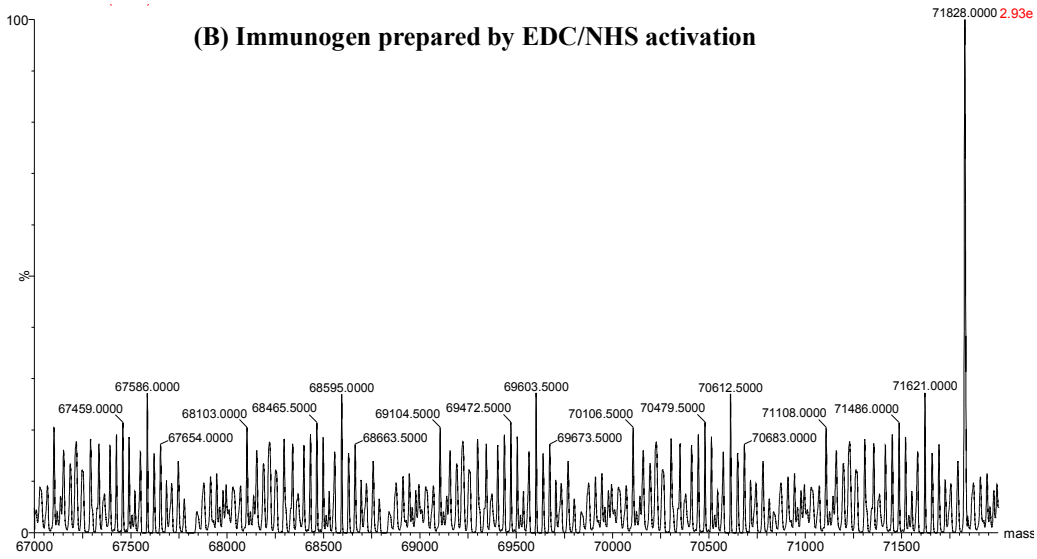


Figure S1. UV spectra of SAH, BSA and immunogens prepared by EDC (A) and glutaraldehyde (B) activation.

The two SAH immunogens were analyzed by UPLC-Q-TOF-MS (Waters, MA, USA) (Figure S2). The immunogen in glutaraldehyde group was composed of five main products with different molecular weight (MW=67779, 68967.5, 70169.5, 71164.5 and 71384.5), however, the immunogen in EDC/NHS group seems to be more unique, for which the coupling ratio of SAH to BSA was calculated to be 14:1.





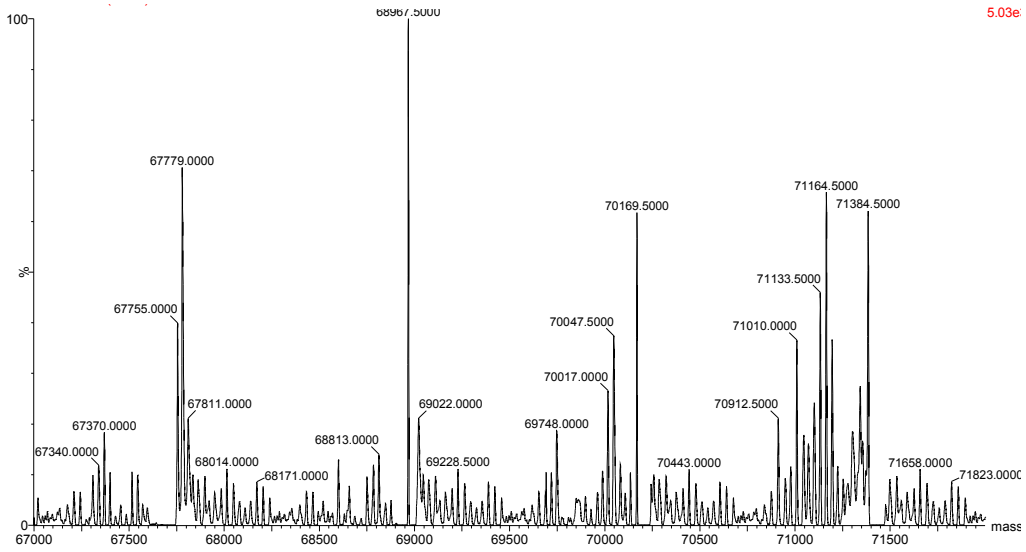


Figure S2. UPLC-MS spectra of SAH immunogens

3. Optimization of concentration for coating antigen and SAH antibodies in the

CLIA test for SAH

When analysis of SAH by the CLIA (Section of Immunoassay Procedure in manuscript), we measured the IC_{50} values of the method under different concentrations of coating antigen (1 μg mL^{-1}, 2 μg mL^{-1} and 5 μg mL^{-1}) and SAH antibody (1:2000 and 1:20000). As shown in Table S1, the method is more sensitive when 1 μg mL^{-1} coating antigen was used. The SAH antibody diluted at 1:20000 led to a little higher sensitivity than 1:2000 group, therefore, they were used in further experiments.

Table S1. Optimization of concentration for coating antigen and SAH antibodies.

Coating antigen	Dilution of SAH antibody	IC_{50} Values (ng mL^{-1})
1 μg mL^{-1}	1:2000	111.7
	1:20000	106.7
2 μg mL^{-1}	1:2000	429.1
	1:20000	162.2
5 μg mL^{-1}	1:2000	320.8
	1:20000	219.6

4. Optimization of methylation time in assay of DNA MTase activity

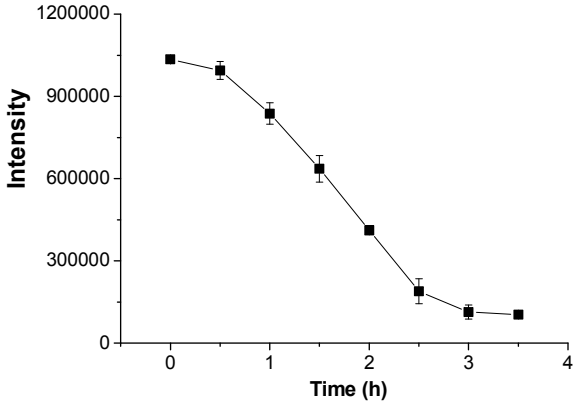


Figure S3. Optimization of DNA MTase methylation time (n=3).

5. Determination of K_m for SAM and DNA in assay of DNA MTase activity

The K_m values for SAM and DNA were measured by a Michaelis-Menten kinetic study.[1] In this procedure, the production of SAH was determined at 1.0 h for different levels of SAM (0.2, 0.4, 1.0, 2.0, 4.0, 12.5, and 18.5 μM) and DNA (0.125, 0.25, 0.5, 1.0, 2.0 and 4.0 μM). The velocity of the methylation reaction was calculated and plotted against substrate concentrations (Figure S4). The V_{max} for each substrate is calculated under saturating substrate concentrations. The K_m is a substrate concentration which leads to a velocity half of the V_{max} determined. As shown in Table S2, the K_m values for both substrates by this assay are consistent with the published values. In addition, the optimal concentration of SAM and DNA in this work could be easily selected for study of enzyme inhibitor activity, since the velocity is very sensitive to changes in substrate concentrations around or below the K_m.[1]

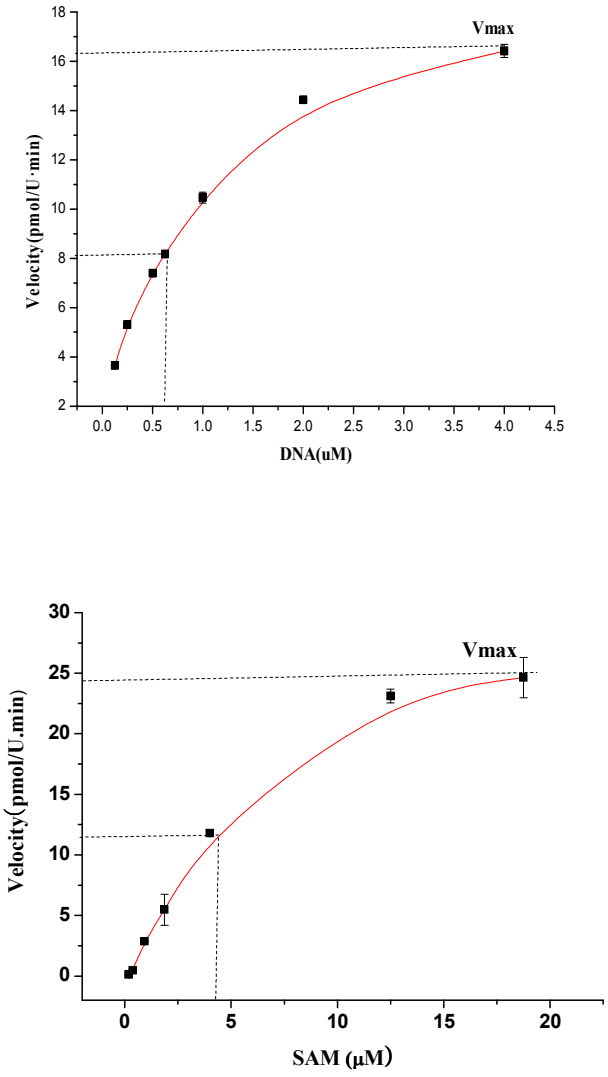


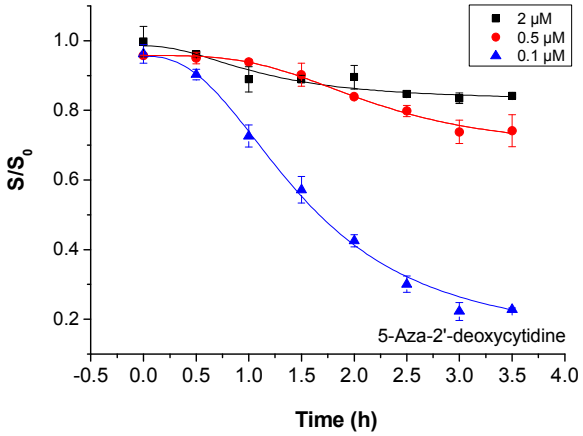
Figure S4. Velocity of methyl transfer reaction as a function of SAM and DNA concentration (n=3).

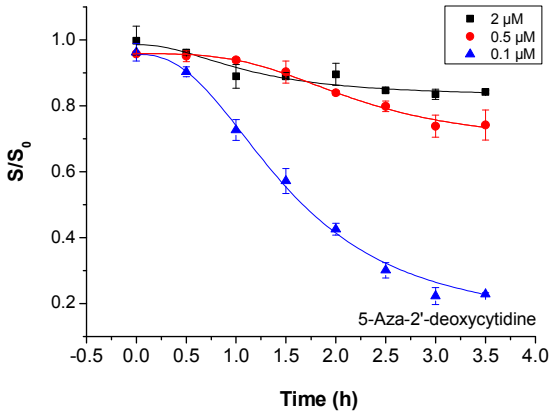
Table S2. The Michaelis constant (K_m) for SAM and DNA detected by the proposed assay.

Substrate	Reported K_m value[2]	Detected K_m	Applied concentration in this assay

SAM	2.6-7.2 μM	4.0 μM	1.4 μM
DNA	0.32-1.32 μM	0.63 μM	0.5 μM

6. Kinetic study of DNA MTase reaction in DNA MTase inhibitors





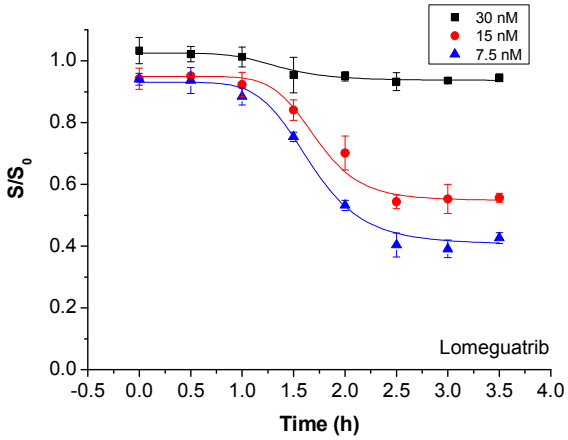


Figure S5. Kinetic study of CL signal (S) decrease for SAH test in presence of DNMT and different inhibitors.

7. The comparison of the proposed method with reported studies

Table S3. Comparison between the proposed DNA MTase assay with other previously reported DNA MTase activity detection methods.

Method	System	Detection range (U/mL)	Detection limit (U/mL)	Time	Reference
Chemiluminescence immunoassay	Chemiluminescence immunoassay of methylation product SAH by preparation of poly antibodies against SAH	0.1-8.0	0.07	4 h for 90 samples	This work
Electrochemical assay	Electrochemical immunosensing system via methlyation resistant cleavage	0.5-50	0.1	6.5 h	3
	Electrochemical assay via methylation resistant cleavage and graphene assisted signal amplification	0-120	Not mentioned	4 h	4
	Electrochemical assay via methylation sensitive cleavage	0.05-200	0.03	4.5 h	5
	Electrochemical immunoassay via AuNPs-IgG-HRP as signal amplification unit	0.05-90	0.02	8 h	6
	Light-activated photoelectrochemical assay via bismuth oxyiodide nanoflake as photoactive electrode materials	0.1-50	0.04	6 h	7
Colorimetric assay	Methylation-blocked cascade amplification strategy	0.8-24	0.4	77-160 min	8
	Methylation-triggered DNAzyme based DNA machine	6-100	0.25	2.5 h	9

Fluorescent assay	Carbon nanoparticles based flurorescent biosensing system	0.5-100	0.1	2 h	10
	Methylation sensitive cleavage coupled with nicking enzyme-assisted signal amplification	0.1-4	0.1	1 h	11
	Label-free fluorescence detection of DNA methylation based on restriction endonuclease and exonuclease	1--10	0.2	5.5 h	12
	Gold nanorods-based fluorescent resonance energy transfer (FRET) assay	0.5-20	0.2	2.5 h	13
Light scattering assay	Methylation sensitive cleavage coupled with light scattering assay	0.2-100	0.1	6 h for 4 samples	14

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