Supporting Information

Ultrasensitive electrochemical detection and inhibition evaluation of DNA methyltransferase based on cascade strand displacement amplification

Ruizhi Liu,[a] Yuge Wang,[a] Hua Chai,[bd] Peng Miao[*bc]

[a.] Center of Reproductive Medicine and Center of Prenatal Diagnosis, the First Hospital of Jilin University, Changchun 130021, China

[b.] Suzhou Institute of Biomedical Engineering and Technology, Chinese Academy of Sciences, Suzhou 215163, China

[c.]Shandong Laboratory of Advanced Biomaterials and Medical Devices in Weihai, Weihai 264200, China

[d] Jinan Guoke Medical Technology Development Co., Ltd., Jinan 250103, China

E-mail: miaopeng@sibet.ac.cn (P. Miao).

Experimental

Materials and Instruments. Mercaptoethanol (MCH), gentamycin, sodium chloride (NaCl), magnesium chloride ($MgCl_2$), and ethylenediaminetetraacetic-acid (EDTA) were obtained from Sigma-Aldrich (USA). Dam MTase, M.SssI MTase, BSA, DpnI endonuclease, Klenow fragment polymerase, dNTPs, and Nb.BbvCI NEase were purchased from New England Biolabs (Beijing, China). DNA strands were synthesized and modified by Sangon Biotech Co., Ltd. (Shanghai, China). Human serum samples were provided by First Hospital of Jilin University. Related studies were approved by the ethics committee of the hospital in accordance with the Helsinki Declaration. Double-distilled water used in this work was purified with a Millipore system (18 MΩ cm resistivity). Electrochemical experiments were performed using an electrochemical workstation (CHI 660E). Polyacrylamide gel (PAGE) images were taken with the GelDoc XR^+ System (Bio-Rad, USA). Mass spectra data were acquired by a the MALDI mass spectrometry (MassARRAY®CPM 96/384, Agena Bioscience, USA) equipped with a 337 nm nitrogen laser in a linear negative-ion mode. The software of Typer Analyzer was used for the data analysis.

Pretreatment of Electrode and DNA Modification. The gold electrode was treated with piranha solution for 5 min (*Caution: highly dangerous!*). Then, it was polished to mirror-smooth using P5000 sand paper and alumina slurry of different sizes. Subsequently, ultrasonication treatment was carried out using ethanol and water, respectively. Afterward, the electrode was electrochemically cleaned via cyclic voltammetry scanning in H_2SO_4 (500 mM). The electrode was then immersed in Probe

D with the concentration of 0.5 μM overnight, followed by the treatment with MCH (100 mM) for 0.5 h.

Cascade Strand Displacement Reactions Assisted by Enzymes. Dam MTase was diluted to form standard solutions. Then, Dam MTase was mixed with Probe A (80 nM) and DpnI (5 U mL^{-1}) with 1×buffer conditions. The solution up to 50 μL was heated to 37 °C for 2 h. Next, it was transferred to cascade strand displacement reaction system (100 μL). The system also included 250 μM dNTPs, Klenow fragment polymerase (60 U mL^{-1}) and Nb.BbvCI NEase (100 U mL^{-1}). Probe D modified electrode was then immersed in the solution, which was incubated at 30 °C (60 min) followed by a procedure of 85 °C incubation (20 min). It was then carefully washed before the following measurements.

Electrochemical Characterizations. A traditional three-electrode system was applied. Electrochemical impedance spectroscopy (EIS) involved the use of the electrolyte of $[Fe(CN)_6]^{3-/4-}$ (5 mM) and KCl (1 M). Square wave voltammetry (SWV) involved the use of the electrolyte of Tris-HCl (20 mM, pH 7.4) containing NaCl (140 mM) and $MgCl_2$ (5 mM). For EIS, the bias potential of 0.22 V, the amplitude of 5 mV, and the frequency range from 0.1 to 100000 Hz were set. For SWV, modulation amplitude of 25 mV, step potential of 4 mV, and frequency of 70 Hz were set.

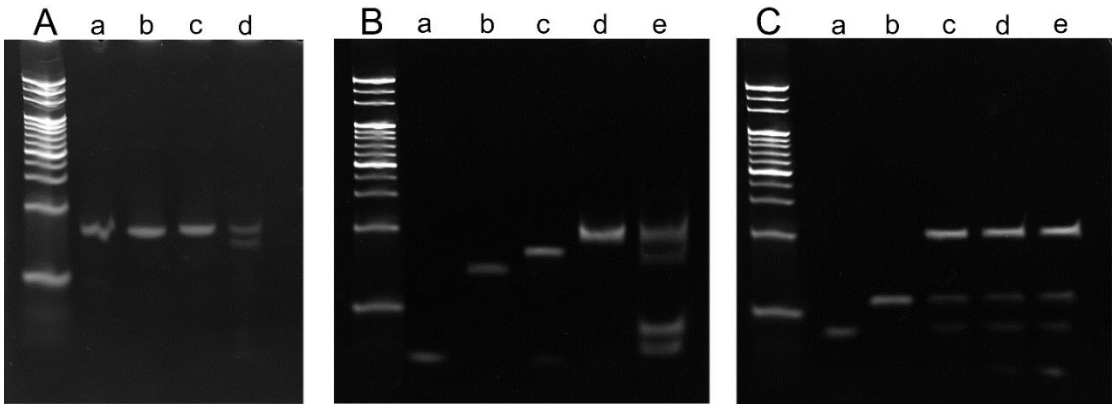

Figure S1. Polyacrylamide gel electrophoresis analysis. (A) Enzyme catalyzed reactions (Dam and DpnI): Probe A, Probe A and Dam, Probe A and DpnI, Probe A, Dam and DpnI (from left to right). (B) SDP cycles in the solution: Probe B, Probe A*, Probe B and A*, after Klenow catalyzed SDP, after Klenow and Nb.BbvCI catalyzed cycles (from left to right). (C) SDP cycles at the electrode surface: Probe C, Probe D, Probe C and D, Probe C, D and E, after Klenow catalyzed SDP (from left to right). The marker was 20 bp DNA Ladder.

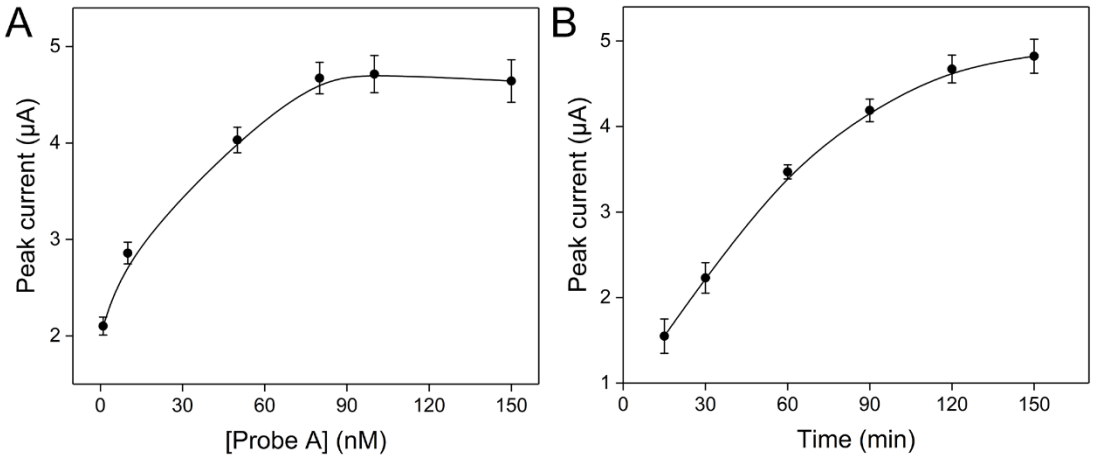

Figure S2. Optimizations of (A) the concentration of Probe A, (B) reaction time of Dam MTase and DpnI.

Table S1. DNA and RNA sequences used in this work.

Name	Sequence (from 5’ to 3’)
Probe A	CGACCGATCAATAAGACTTCAACCTCAGCCTCACTCTTATT GATCGGTCG
Probe A*	TCAATAAGACTTCAACCTCAGCCTCACTCTTATTGA
Probe B	TCAATAAGAGTGAGGC
Probe C	TGAGGTTGAAGTCTTATTGA
Probe D	TCAATAAGACTTCAACCTCATTGAAGTCTTATTTTTTTT-$(CH_2)_6$-SH
Probe E	Fc-TTTTAAGACTTC
Probe F	CCGATCAACTCTCTTGATCGG

Table S2. Comparison of the analytical performances of recent MTase assays.

Technique	Strategy	Detection range ($U\ mL^{-1}$)	LOD ($U\ mL^{-1}$)	Ref
fluorescence	highly photostable upconversion nanoparticle transducer	8×10^{-2} to 24	5.7×10^{-2}	1
nanopore	nanopore technique coupled with enzyme-linkage reactions	0 to 50	3×10^{-2}	2
FCS	single molecule FCS and polystyrene polymer dots	2.5×10^{-2} to 3	2.5×10^{-2}	3
PEC	RGO-CdS: Mn nanoparticles and CdTe@DNA network	10^{-2} to 80	7.1×10^{-3}	4
fluorescence	strand displacement coupled with RCA	2×10^{-2} to 4	6.7×10^{-3}	5
fluorescence	DNA walkers and hyperbranched RCA	10^{-3} to 2×10^{-1}	10^{-3}	6
SERS	Au@SiO_2 array substrate and RCA	5×10^{-3} to 50	2.51×10^{-4}	7
SWV	Y-motif-mediated primer-free cyclic signal amplification	5×10^{-4} to 10	2.5×10^{-4}	8
SERS	Au nanocubes and strand displacement	10^{-4} to 5×10^{-1}	8.65×10^{-5}	9
fluorescence	dual-color light-up RNA nanosensor	10^{-4} to 10^{2}	7.21×10^{-5}	10
SWV	cascade strand displacement reaction	10^{-6} to 10^{-1}	10^{-6}	this work

FCS, fluorescence correlation spectroscopy; PEC, photoelectrochemistry; RCA, rolling circle amplification; SERS, surface-enhanced Raman scatting.

Table S3. Evaluation of Dam MTase in human serum samples.

Sample	Spiked (U mL^{-1})	Detected (U mL^{-1})	RSD (%)	Recovery (%)
1	0.1	0.103	2.3	103.0
2	0.2	0.214	3.9	107.0
3	0.3	0.279	4.3	93.0
4	0.5	0.486	4.6	97.2
5	1.0	1.05	3.7	105.0

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