Supporting Information

Self-Powered Biosensing Platform Based on “Signal-On”
Enzymatic Biofuel Cell for DNA Methyltransferase Activity
Analysis and Inhibitor Screening

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Materials and Reagents. Thiolated DNA and carboxylated DNA were synthesized and purified by Shanghai Sangon Biotechnology Co. Ltd (Shanghai, China). The sequence of the thiolated-DNA (DNA 1) was 5’-HS-TTA TGA GTC AAT CCG GAG ACT TGT ATG-3’, and the sequence of carboxylated-DNA (DNA 2) was 5’-HOOC-CAT ACA AGT CTC CGG TTA GAC TCA-3’. M.SssI CpG methyltransferase (M.SssI MTase), restriction endonuclease HpaII and S-adenosyl-L-methionine (SAM) were purchased from New England Biolabs (Ipswich, MA). Bilirubin oxidase (BOD, E.C. 1.3.3.5, from Myrothecium verrucaria), glucose dehydrogenase (GDH) from Pseudomonas sp. (NAD) and 6-mercapto-1-hexanol (MCH) were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A). 5-Azacytidine (5-Aza) and epicatechin were purchased from Aladdin (Shanghai, China). Carbon nanotubes (CNT) were obtained from Nanjing Jicang Nano Tech Co., Ltd. (Nanjing, China). Carbon paper (CP) was purchased from Shanghai Hesen Electric Co., Ltd. (Shanghai, China). β-D Glucose was obtained from Tokyo Chemical Industry Co. Ltd. (Japan). Chloroauric acid (HAuCl₄·4H₂O) was obtained from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China). AuNPs were prepared through reducing HAuCl₄ by sodium citrate.¹ A 0.1 M pH 7.4 phosphate buffer (PB) consisting of Na₂HPO₄ and NaH₂PO₄ was employed as the supporting electrolyte. Denatured enzymes were obtained by high temperature treatment. All other reagents were of analytical grade and used without further purification. Ultrapure water (resistivity 418.2 MΩ cm at 25°C) obtained from a Milli-Q water purification system (Millipore Corp., Bedford, MA, USA) was used for all the experiments.

Apparatus and Instrumentation. Transmission electron microscopy (TEM) images were performed and obtained on a HT7700 microscope (Hitachi, Japan), which operated at 100 kV. All electrochemical experiments were carried out on a CHI 660E electrochemical workstation (Shanghai CH Instrument Co., China) at room temperature using a conventional three-electrode system with the constructed cathode or anode as the working electrode, a Ag/AgCl electrode as the reference electrode and a platinum wire as the counter electrode, respectively. The open-circuit voltage (E°_OCV) of EBFC was measured by the connection of the anode with the cathode in the electrolytic cell. Electrochemical impedance spectroscopy (EIS) was conducted on an Autolab PGSTAT 302N electrochemical analyzer (Metrohm Autolab, The Netherlands) within a frequency range of 0.1 Hz to 100 kHz and with 2.5 mM [Fe(CN)₆]³⁻/⁴⁻ used as the probe.
Figure S1. TEM image of AuNPs.
Figure S2. LSV signals of BOD/HpaII/DNA2/MCH/DNA1/AuNPs/CP electrode (a), HpaII/M.SssI/DNA2/MCH/DNA1/AuNPs/CP electrode (b), and denatured BOD/HpaII/M.SssI/DNA2/MCH/DNA1/AuNPs/CP electrode (c) in PB solution (0.1 M, pH 7.4).
Figure S3. EIS of the anode.
Figure S4. CV signals of CNT electrode (a) and the denatured GDH modified CNT electrode (b) in PB solution (0.1 M, pH 7.4) containing 2 mM NAD⁺.
Figure S5. Effect of (a) DNA 1 concentration, (b) DNA 2 concentration, (c) HpaII concentration, (d) BOD concentration, (e) DNA MTase methylation time, (f) HpaII digestion time. All experiments were carried out in the presence of 0.5 U/mL MTase in PB solution (0.1 M, pH 7.4). The error bars are standard deviations of three repetitive measurements.
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<th>Method</th>
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<th>LOD (U/mL)</th>
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<td>ECL&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Multifunctional carbon nitride nanosheets</td>
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<td>0.05 - 80</td>
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<td>ECL&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Dam</td>
<td>0.03</td>
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<td>Dual signal amplification strategy</td>
<td>DNMT1</td>
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<td>1 - 40</td>
<td>5</td>
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<tr>
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<td>Dual-amplification sensing strategy</td>
<td>M.SssI</td>
<td>(2.8 \times 10^{-3})</td>
<td>0.05 - 50</td>
<td>7</td>
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<tr>
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<tr>
<td>Self-Powered Biosensor</td>
<td>Integrated with “signal-on” strategy</td>
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<td>0.005</td>
<td>0.005-100</td>
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</tbody>
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<sup>a</sup>Electrochemiluminescence; <sup>b</sup>Surface-enhanced Raman scattering; <sup>c</sup>Photoelectrochemistry
References