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A dual-response biosensor for electrochemical and glucometer detection of DNA methyltransferase activity based on functionalized metal-organic framework amplification

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ABSTRACT

DNA methylation is catalyzed by DNA methyltransferase (MTase) and concerned with many biological processes including pathogenesis of various human diseases. The monitoring of MTase activity is thus of great significance in disease diagnosis and drug screening. Herein, we developed a facile way to synthesize biocompatible invertase enzyme modified metal-organic framework (Invertase/MOF) materials, and explored its application in constructing a dual-response Dam MTase sensor for the first time. By using them as signal probes, in which high density of metal sites could be electrochemically detected and invertase could hydrolyze sucrose into glucose for generation of glucometer signal output, dual-response for accurate detection of Dam MTase was realized. In the presence of Dam MTase, the methylation of hairpin probe 1 (HP1) occurred and thus caused the cleavage of HP1 assisted by a restriction endonuclease (*Dpn*I) to produce the binding sequences. The binding sequences then hybridized with the electrode-assembled HP2 to expose their sticky termini which sequentially hybridized with the Invertase/MOFs-tethered capture probes. Finally, the electrodes were incubated with a sucrose solution, followed by the separate electrochemical and glucometer detection. The present assay brought good performance which could detect Dam MTase activity as low as 0.001 U mL^{-1} with wide linear range and good selectivity against other cytosine MTase (M.SssI MTase). Moreover, it also owns ability to be potentially applied for the inhibitors screening by utilization of 5-fluorouracil as an inhibitor model. The results imply that our proposed method provides a convenient platform for early cancer diagnosis and therapeutic applications.

1. Introduction

As an epigenetic modification in genomes, DNA methylation is a process in which a methyl group can be transferred from S-adenosylmethionine (SAM) to adenine or cytosine bases in the specific DNA nucleotides and plays a critical role in gene regulation (Noyer et al., 1993; Okano et al., 1999; Palmer and Marinus, 1994). Recent research also demonstrates that DNA methylation is concerned with pathogenesis of various human diseases (Laird, 2003; Lyko et al., 2005). Since DNA methylation is catalyzed by DNA methyltransferase (MTase) and abnormalities in DNA MTase activity usually occur before other signs of malignancy, DNA MTase has been regarded as a predictive biomarker and therapeutic target in the diagnosis and treatment of various diseases (Robertson, 2005; Mutze et al., 2011). Therefore, sensitive and accurate method for the DNA MTase assay is highly desirable, which may provide an applicable approach for early diagnosis and drug discovery.

Conventional methods for the DNA MTase assay include high-performance liquid chromatography (HPLC), gel electrophoresis, and radioactive labeling strategy, which usually suffer from the disadvantages of laborious operation, radioactive hazard and expensive equipment (Zeng et al., 2013). Such limitations have promoted the development of new methods for determination of DNA MTase activity, such as fluorescent (Ouyang et al., 2012), colorimetric (Y. X. Zhao et al., 2014; Wu et al., 2013), electrochemical (EC) (Su et al., 2012; Zhang et al., 2015), and electrochemiluminescent (Li et al., 2012) methods. Among these methods, EC DNA MTase assay has attracted increasing attention by virtue of its low cost, high sensitivity, fast, and relatively portable devices. The assay is mainly based on a well-designed system, in which DNA MTase can specifically act on the

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corresponding DNA recognition sites for DNA methylation and followed with the methylation-sensitive restriction enzyme cleavage. To improve the sensitivity, various nanomaterials have been adopted in DNA MTase activity assay.

Metal-organic frameworks (MOFs) are a new class of crystalline materials constructed by metal centers/clusters and organic bridging ligands, which have received tremendous attention due to the fascinating structures and intriguing properties (Panella et al., 2006;M. T. Zhao et al., 2014). Their high pore volume, large surface area and tunable physicochemical properties make MOFs promising candidates for application in catalysis, gas storage, separation, drug delivery and analytical sensors (Liu et al., 2007; Decoste et al., 2012; Kumar et al., 2015; Ling et al., 2015). Recently, increasing interests have been focused on the development of functionalized MOFs-based EC biosensors. For instance, incorporation of porphyrin and metalloporphyrin moieties into the emerging porous MOFs materials can produce catalytic current and provide opportunity for the detection of different molecules including DNA, protein and lead ion (Ling et al., 2015; Xie et al., 2015; Cui et al., 2015). Besides, the signal of coordinated metal ions in MOFs can be directly detected in buffer solution (Shen et al., 2015; Liu et al., 2016). This differs from traditional signal tags such as quantum dots (QDs) and metal ions-encapsulated dendrimer which require acid dissolution and preconcentration (Zhang et al., 2011; Gao et al., 2013). Without such laborious operation, the MOFs-based detection steps are thus effectively simplified. Moreover, the easy incorporation of MOFs with metal nanoparticles paves the way to further combine with biomolecules which may be employed for target recognition and signal amplification. So far, the application of MOFs in DNA MTase assay has not been reported to the best of our knowledge.

Another important issue in biosensors is to design a simple and potable detection strategy to meet the requirement of the point-of-care (POC) test applications. According to the recent research, by using invertase enzymes for hydrolyzing sucrose into glucose, the range of personal glucometer has been expanded to monitor other biomolecules (protein and DNA biomarkers) beyond glucose (Xiang et al., 2011; Xiang et al., 2012). We have already fabricated a glucometer-based DNA MTases biosensor labeled with invertase (Chen et al., 2018), which sets the stage for the further development. Herein, a dual-response (EC and glucometer) biosensor for DNA MTase activity detection based on functionalized MOFs was reported for the first time. In this study, gold nanoparticles and copper (Cu^{2+})-MOFs nanocomposites (Au/CuMOFs) acted as not only a well-defined EC redox probe attributing to the coordinated Cu^{2+} but also an ideal loading platform to immobilize invertase for catalyzing sucrose to glucose, which realized the EC and glucometer responses for DNA MTase detection. The high density of metal sites in MOFs and the plentiful invertase on Au/CuMOFs with high efficiency of catalysis realized signal amplifications. The EC and glucometer signal intensities were proportional to the amount of the DNA MTase. The significance of this work lies in the dual-response for accurate detection of DNA MTase, which can reduce false positive result and benefit early diagnosis and therapeutic applications.

2. Experimental section

2.1. Reagents and apparatus

Dam MTase, M.SssI MTase, S-adenosyl-L-methionine (SAM), the restriction endonuclease *Dpn*I and enzyme reaction buffers were provided by New England Biolabs Inc. (Beijing, China). Tris (2-carboxyethyl)phosphine hydrochloride (TCEP), 6-mercaptohexanol (MCH), 1,3,5-benzenetricarboxylic acid (H_3BTC), invertase from baker's yeast and sucrose were purchased from Sigma-Aldrich. Tris (hydroxymethyl) aminomethane (Tris) and ethylenediaminetetraacetic acid (EDTA) were from Solarbio (Beijing, China). All other chemicals are of analytical grade. 5-fluorouracil and all oligonucleotides used in this proposed strategy were ordered from Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). The sequences of the synthetic oligonucleotides are listed below:

Hairpin probe 1 (HP1):5′-ACTTATCAGCTTAAGGATCATAAGACTTCTAAT TGATCCTTAAGCTGATAAGT-3’. (The specific recognition sequence for Dam MTase was underlined.)

Thiolated hairpin probe 2 (HP2):5′-TAAGACTTCTTCAATTAGAAGTCTTATG A−$(CH_2)_6$−SH-3’.

Capture probe (CP): 5′-AGAAGTCTTA−$(CH_2)_6$−NH_2-3’.

Ultrapure water obtained from a Millipore filtration system was used throughout. Phosphate-buffered solution (PBS) (pH 7.0, 0.1 M) containing 10 mM KCl, 2 mM $MgCl_2$ was used as working buffer solution.

A CHI 660 E electrochemical workstation (Chenhua Instrument Company of Shanghai, China) was employed to accomplish all the EC measurements. A three-electrode system was used which consisted of a modified gold working electrode (AuE, 3 mm in diameter), a platinum wire auxiliary electrode and a saturated calomel reference electrode (SCE). The glucometer (Contour™TS) and test strips were obtained from Bayer Healthcare LLC (Mishawaka, IN).

2.2. Preparation of CuMOF

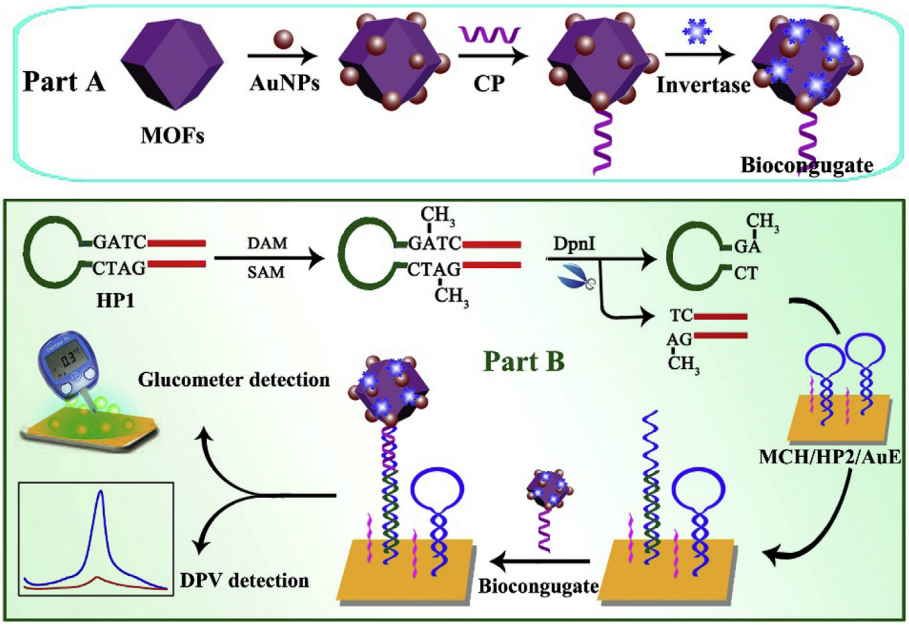
CuMOF was synthesized according to the previous method with slight modification (Chui et al., 1999; Liu et al., 2016). Briefly, 2.101 g H_3BTC was dissolved in 100 mL KOH solution (0.3 mol L^{-1}) under continuous stirring until a clear K_3BTC solution was obtained. Then 9 mL $Cu(NO_3)_2{\cdot}3H_2O$ solution (0.2 mol L^{-1}) was dropped into 9 mL K_3BTC solution with stirring for 2 min to produce blue precipitates. The precipitates were collected via centrifugation and dispersed in ethanol. This was followed by the reaction of the above mixture for 1 h. Finally, the precipitates were washed with water via centrifugation until there was no precipitation appeared when the washing solution was tested with one drop of a mixture composed of HAc and K_4 [$Fe(CN)_6$]. This indicated there was no free Cu^{2+} existing on the precipitates (CuMOF). The obtained CuMOF was collected after vacuum drying and stored for further use.

2.3. Preparation of Au/CuMOF

To obtain the gold nanoparticles (Au NPs), 2 mL of trisodium citrate solution (1%) was added into 50 mL of boiling $HAuCl_4$ solution (0.01%) under reflux for 10 min. The wine-colored solution indicated the formation of the Au NPs. Then the solution was cooled to room temperature under continuous stirring, and stored at 4 °C. Next, 50 mg CuMOF were added to the as prepared Au NPs solution (15 mL) with constant stirring at room temperature. After 48 h reaction of this mixture, the purple precipitates (Au/CuMOF) were achieved, followed by successive washing with ethanol and water. Finally, Au/CuMOF nanocomposites were dispersed in 15 mL PBS (pH 7.0) and stored at 4 °C for further use (referred as Au/CuMOF solution).

2.4. Preparation of the invertase/CP/Au/CuMOF bioconjugates

The stepwise formation of Invertase/CP/Au/CuMOF bioconjugates is shown in the Part A of Scheme 1. Briefly, 40 μL of amido-terminated CP (50 μM, in PBS (pH 7.0)) was pipetted into 2 mL of the above Au/CuMOF solution. This mixture was incubated for 16 h with constant stirring at 4 °C. Then, the sediments were collected via centrifugation at 12 000 rpm for 10 min, and redispersed in 2 mL of PBS (pH 7.0). This was followed by the addition of 1 mg invertase enzyme to the mixture solution for residual active sites blocking on the Au/CuMOF surface. Finally, the Invertase/CP/Au/CuMOF bioconjugates were centrifugated at 12 000 rpm for 20 min, extensively washed with water, resuspended in 2 mL of PBS (pH 7.0), and stored at 4 °C for further use (referred as Invertase/CP/Au/CuMOF solution).



Scheme 1. (A) The stepwise preparation of Invertase/CP/Au/CuMOF bioconjugates (detail in experimental section 2.3 and 2.4). (B) Schematic illustration of the fabrication process of this proposed dual-response biosensor for Dam MTase activity assay (detail in results and discussion section 3.1).

2.5. Methylation and cleavage of HP1

Prior to use, all hairpin structure oligonucleotides were separately annealed by heating to 95 °C for 2 min and then gradually cooled to room temperature for at least 1 h. Next, the methylation of the HP1 was carried out in 20 μL of 1 × Dam buffer (50 mM Tris-HCl, 10 mM NaCl, 10 mM EDTA, 5 mM 2-mercaptoethanol (pH 7.5)) containing various amounts of Dam MTase, 1 μM HP1 and 160 μM SAM at 37 °C for 1 h. Then, 20 μL of the above methylation products and 10 U *Dpn*I enzyme were dispersed in 20 μL of 1 × NEB buffer 4 (50 mM KAc, 20 mM Tris-Ac, 10 mM $Mg(Ac)_2$, 1 mM DTT (pH 7.9)). This mixture was incubated at 37 °C for another 1 h to achieve the *Dpn*I-assisted cleavage reaction and generate binding sequences. Subsequently, the enzymes were inactivated at 80 °C for 20 min.

2.6. Fabrication of sensors

Firstly, all the gold electrodes (AuEs) were pretreated according to the conventional steps (Chen et al., 2018). Each pretreated AuE was incubated with 10 μL of thiolated HP2 (0.5 μM) in the immobilization buffer (IB: 10 mM Tris-HCl, 1 mM EDTA, 1 M NaCl, 1 mM TCEP, pH 7.4) over night at room temperature to obtain the HP2-assembled electrode surface. Then, the surface was washed and blocked with 1 mM MCH for 2 h. This was followed by the incubation of the AuE with 10 μL of the above-mentioned methylation and cleavage reaction (MCR) products for 1 h at 37 °C. This step implemented the capture of the binding sequences in the MCR products by HP2 and unfolding HP2 to further hybrid with CPs tethered on Invertase/CP/Au/CuMOF bioconjugates via incubation for 1 h at 37 °C. After rinsed thoroughly, the resulting modified electrode surface was incubated with 10 μL of sucrose solution (0.5 M) at 37 °C for 45 min. At last, the glucometer analysis of the incubation buffer and the EC measurement of the modified AuE were separately carried out. The differential pulse voltammetric (DPV) measurement was taken in HAc/NaAc buffer solution (pH 4.5) with the following conditions: the potential range was from 0.3 to −0.3 V with pulse amplitude of 25 mV, pulse width was 0.025 s and quiet time was 2 s.

2.7. Native polyacrylamide gel electrophoresis (PAGE)

The freshly prepared 16% native polyacrylamide gel was transferred to the electrophoretic system and gelated for 30 min. After the introduction of different samples, the gel electrophoresis was carried out at 100 V for 90 min in 1 × TBE buffer. Finally, the gel was dyed with GelRed and imaged with a digital camera under a UV light.

2.8. Dam MTase inhibition evaluation

To further extend the potential application of this biosensor in inhibitors screening, 5-fluorouracil was selected to investigate the influence of drugs on Dam MTase activity. First, Dam MTase, SAM, HP1, and different concentration of 5-fluorouracil (0, 0.1, 0.2, 0.3, 0.5, or 1.0 μM) were mixed in 1 × Dam buffer and incubated for 1 h to achieve the absolute methylation. After the addition of *Dpn*I, the mixture was incubated for another 1 h. Then, the enzymes were inactivated. The following procedures were the same as those described above. Finally, the EC and glucometer signals were respectively tested and recorded. The inhibition effect of 5-fluorouracil on the Dam MTase activity was estimated as follows:

$$\text{Relative activity} = (I_2 - I_0)/(I_1 - I_0)$$

In this equation, I_0, I_1 and I_2 are the EC and glucometer signal intensities in the absence of Dam MTase, in the presence of Dam MTase, and in the presence of both Dam MTase and inhibitor, respectively.

3. Results and discussion

3.1. The principle of this dual-response Dam MTase assay

In this study, we developed a facile way to synthesize a biocompatible Au/CuMOF material, which acted as not only a highly efficient signal tag but also an ideal nanocarrier to immobilize numerous biomolecules, and explored its application in constructing a dual-response biosensor for Dam MTase detection. Part B of Scheme 1 illustrates the principle of this Dam MTase activity detection based on Invertase/CP/Au/CuMOF bioconjugate signal probes. There are two hairpin structure DNAs (HP1 and HP2) involving in this sensing

protocol. HP1 consists of two specific regions: one is a recognition sequence for Dam MTase in the stem, another is a binding sequence for H2 initially locked in the loop (the green region in HP1). HP2 are immobilized on the AuE surface. Both hairpin DNAs remain stable in the absence of the Dam MTase. As the result, the attachment of the signal probes is inhibited. However, in the presence of Dam MTase, the adenosine bases of 5′-GATC-3′ sequence of HP1 are methylated to form the *Dpn*I-specific sequence 5′-GmATC-3'. After the cleavage assisted by *Dpn*I, the green binding sequence in HP1 is released to hybridize with the complementary sequence in HP2. Therefore, HP2 is unfolded, exposing a sticky terminus to further hybridize with CP tethered on signal probes. Finally, the electrode is incubated with a sucrose solution to generate glucose catalyzed by the invertase of signal probes, followed by the separate EC and glucometer detection. On the one hand, the corresponding EC current response of Cu^{2+} in MOF can be detected. On the other hand, the above incubation buffer can also be tested by a commercially available glucometer with its digital readings. With the increasing concentration of Dam MTase, more methylation and cleavage toward HP1 will occur to generate numerous binding sequences, which in turns leads to the attachment of plentiful signal probes and amplified both EC and glucometer signal output.

3.2. Electrochemical characterization of the modified AuE

Cyclic voltammetry (CV) was applied to characterize the stepwise fabrication process of AuE with the $[Fe(CN)_6]^{3-/4-}$ redox couple to reveal the EC behaviour of the different modified AuE surface. All the resulting voltammograms were recorded in 0.1 M KCl aqueous solution containing 5 mM $[Fe(CN)_6]^{3-/4-}$ and shown in Fig. 1. A pair of reversible redox peaks of $[Fe(CN)_6]^{3-/4-}$ indicating the good electric conductivity are observed on the bare AuE (curve a). The MCH/HP2 self-assembled AuE (MCH/HP2/AuE) appears a dramatic decrease in peak current (curve b). This is basically attributed to the repellence of the negative-charged DNA phosphate backbones and MCH as well as the steric hindrance of HP2 toward $[Fe(CN)_6]^{3-/4-}$. After the incubation of the modified electrode with the MCR products, a further decrease in peak current is observed (curve c). Such decrease indicates the successful production of the DNA binding sequences and their hybridization with the self-assembled HP2. As expected, the sequential incubation of the above AuE with the CP-tethered Invertase/CP/Au/CuMOF bioconjugates leads to further peak current decrease (curve d), suggesting the poor electrical conductivity bioconjugates are finally attached. All the obtained results demonstrate the accomplishment of our sensing interface.

3.3. The feasibility of the designed sensing assay

To verify the methylation-induced cleavage of HP1, polyacrylamide

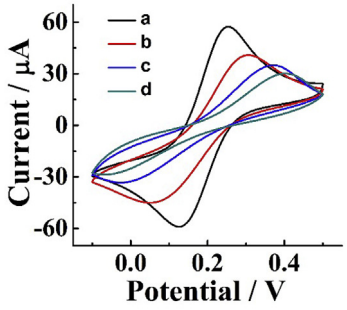


Fig. 1. Cyclic voltammograms of the bare AuE (a), MCH/HP2/AuE (b), (b) treated with MCR products (c), and (c) treated with Invertase/CP/Au/CuMOF bioconjugates (d) in 0.1 M KCl aqueous solution containing 5.0 mM $[Fe(CN)_6]^{3-/4-}$ by scanning the potential from −0.1–0.5 V at a scan rate of 100 mV S^{-1}.

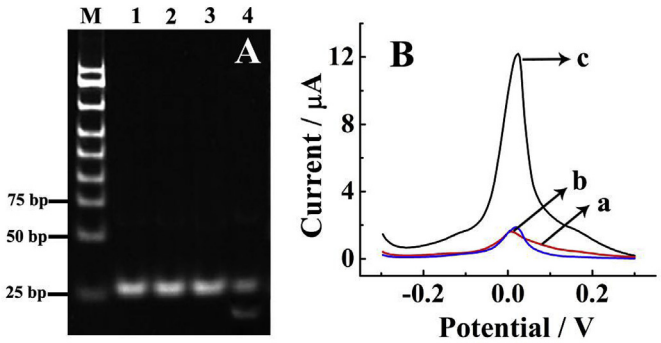


Fig. 2. (A) Native PAGE analysis of different mixtures: (Lane 1) HP1; (Lane 2) HP1 and Dam MTase; (Lane 3) HP1 and *Dpn*I; (Lane 1) HP1, Dam MTase and *Dpn*I. (B) Typical DPV curves of the different modified AuE: (a) MCH/HP2/AuE; (b) MCH/HP2/AuE treated with the mixture of *Dpn*I and HP1 and followed with signal probes incubation; (c) MCH/HP2/AuE treated with the MCR products with the involvement of Dam MTase (0.05 U mL^{-1}) and followed with signal probes incubation.

gel electrophoresis (PAGE) experiments were carried out and the result is demonstrated in Fig. 2A. The distinct band from lane 1 corresponds to HP1. The incubation of either Dam MTase (lane 2) or *Dpn*I (lane 3) alone with HP1 causes insignificant band shift of HP1 (Lane 1), suggesting the failure in cleavage event. For comparison, when both Dam MTase and *Dpn*I enzyme are present with HP1, the original band of HP1 becomes indistinct and a new band with higher electrophoretic mobility can be observed (lane 4). Such result verifies the successful occurrence of the methylation reaction and the methylated DNA is then digested into short segments.

Subsequently, the feasibility of our dual-response biosensor for Dam MTase detection was further investigated by EC and glucometer detection respectively. Fig. 2B shows the DPV curves which were measured after incubation with/without the addition of Dam MTase followed by incubation with bioconjugate signal probes. MCH/HP2/AuE exhibits nearly no current response (curve a). The incubation without the presence of Dam MTase causes neglect changes in current intensity (curve b vs. a), suggesting that the unmethylated HP1 is unable to be cleaved and bind with signal probes. However, when MCH/HP2/AuE is treated with the MCR products with the involvement of Dam MTase and followed with signal probes incubation, a dramatic increase in current intensity (curve c) is observed on the sensing interface. Accordingly, the glucometer readings with/without Dam MTase (0.05 U mL^{-1}) are 10.2 and 2.8 mM. Both consistent comparisons suggest only the presence of Dam MTase along with *Dpn*I leads to signal increase, which can be basically contributed to the methylation-sensitive cleavage of HP1 and the subsequent introduction of numerous bioconjugate signal probes. The preliminary results shown here reveal the great potential of the approach for dual-response detection of Dam MTase.

3.4. Analytical performance of this biosensor

The analytical performance of our proposed biosensor was first monitored by incubating with a series of Dam MTase standard solutions and detecting with CHI660E and glucometer respectively. As can be seen from Fig. 3A, the DPV current intensity of this biosensor increases gradually with an increase in the concentration of Dam MTase. The related standard calibration curve (Fig. 3B) indicates a good linear relationship between DPV responses and the logarithm of Dam MTase concentrations ranging from 0.002 to 1 U mL^{-1}. The corresponding regression equation is $I = 6.388 \lg c + 19.88$ with a detection limit of 0.001 U mL^{-1} according to 3σ rule (σ is the relative standard deviation of a blank solution with different electrodes from the same batch). Furthermore, the calibration curve (inset in Fig. 3C) related to glucometer analysis also shows a linear dependence between the

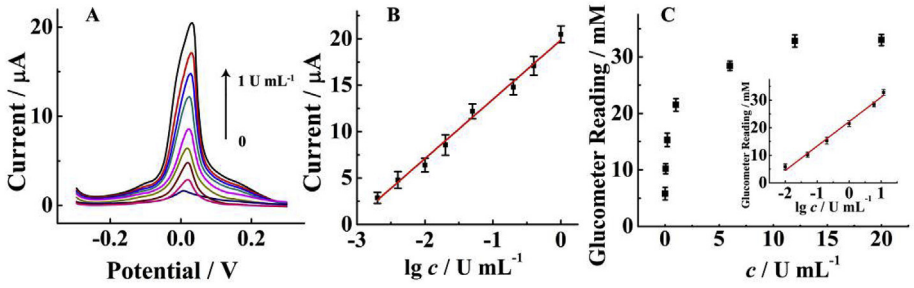


Fig. 3. (A) Typical DPV curves of the sensor in response to Dam MTase at different concentrations: 0, 0.002, 0.004, 0.01, 0.02, 0.05, 0.2, 0.4, 1 U mL^{-1}. (B) The calibration plot of current intensity *vs* the logarithm of Dam MTase concentration ranging from 0.002 to 1 U mL^{-1}. (C) The relationship between the glucometer reading (R) and the corresponding Dam MTase concentration. Inset: The calibration plot of R *vs* the logarithm of the Dam MTase concentration (0.01, 0.05, 0.2, 1, 6, 12 U mL^{-1}). Error bars: SD, n = 3.

glucometer readings and the logarithm of Dam MTase concentration over the range from 0.01 to 12 U mL^{-1}. The corresponding detection limit is determined to be 0.007 U mL^{-1} (3σ). With the mutual calibration of DPV and glucometer curves, a wider linear range from 0.002 to 12 U mL^{-1} is obtained, which is important for practical application. The above results suggest our dual-response biosensor provides an effective way for sensitive and accurate detection of Dam MTase with a wider detection range compared with some previous correlative work (Table S1). The high sensitivity of this method is due to the signal amplification ability of Invertase/CP/Au/CuMOF bioconjugate containing abundant Cu^{2+} ions and invertase enzyme (catalyzing the sucrose to glucose), which in turns produce amplified EC and glucometer signal output.

Selectivity is another vital factor to evaluate the analytical performance of a sensing platform. In this protocol, M.SssI MTase, which can methylate all cytosine residues within the double stranded dinucleotide recognition sequence of 5′—C-G-3′, was selected as an interference enzyme for selectivity investigation. Due to the specific site recognition of Dam MTase toward its substrate (Geier and Modrich, 1979), this method was expected to easily discriminate target Dam MTase from M.SssI MTase. As shown in Fig. 4A, the presence of even 10-fold higher concentrations of M.SssI MTase causes no obvious changes in DPV current intensity in contrast to the blank test. However, significant current enhancement can be observed in the presence of target Dam MTase (0.05 U mL^{-1}). Selectivity was also investigated in a mixture of Dam and M.SssI MTase. The DPV responses from the mixture was almost the same as the response obtained from Dam MTase only. For corresponding glucometer detection, only the presence of Dam MTase leads to a dramatic increase in glucometer signal output (Fig. 4B). Both DPV and glucometer detection results are consistent to validate the good selectivity of this proposed method due to the inherent advantage of the high specific recognition of Dam MTase and the methylation-sensitive *Dpn*I enzymes.

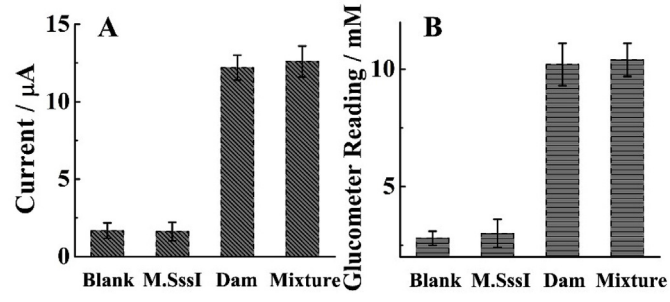


Fig. 4. (A) Selectivity evaluation of this biosensor for the DPV detection of blank detection buffer, M.SssI MTase (0.5 U mL^{-1}), Dam MTase (0.05 U mL^{-1}) and the mixture (0.05 U mL^{-1} Dam MTase and 0.5 U mL^{-1} M.SssI MTase). (B) Selectivity evaluation of this biosensor for the glucometer detection of blank detection buffer, M.SssI MTase (0.5 U mL^{-1}), Dam MTase (0.05 U mL^{-1}) and the mixture (0.05 U mL^{-1} Dam MTase and 0.5 U mL^{-1} M.SssI MTase). Error bars: SD, n = 3.

3.5. Dam MTase activity inhibition investigation

Since Dam MTase has been served as a potential biomarker for early clinical diagnosis and a therapeutic target, pharmacological inhibition of Dam MTase can be applied for antibiotics and anticancer therapeutics. An anticancer drug 5-fluorouracil was selected as a model inhibitor to investigate the feasibility of this method for inhibitor screening, which might be helpful for drugs discovery. Previous study reported that 5-fluorouracil had no inhibition effect on the *Dpn*I activity with the concentration below 10 μM (Li et al., 2010). Therefore, lower concentration of 5-fluorouracil (less than 10 μM) was used to exclude the effect of inhibitors towards *Dpn*I which was involved in the whole process. The DPV and glucometer signals in response to different concentrations of 5-fluorouracil were recorded respectively. As shown in Fig. 5, the corresponding relative activity (see the experimental section) of Dam MTase decreases with the increase of 5-fluorouracil concentration. The IC_{50} value is the inhibitor concentration required for 50% decrease in enzyme activity and usually employed to assess the inhibition efficiency of the inhibitors. The IC_{50} values of 5-fluorouracil based on DPV and glucometer detection are calculated to be 0.39 ± 0.007 μM. The above results exhibit this proposed method has the potential to be applied for the inhibitors screening and antibiotics and anticancer drugs discovery.

4. Conclusion

In summary, we developed a facile way to synthesize a biocompatible Au/CuMOF material and explored its application in constructing a dual-response Dam MTase sensor. The present assay brought good performances with detection limit of 0.001 U mL^{-1} and wide linear range from 0.002 to 12 U mL^{-1}. Such sensitivity was ascribed to the excellent loading capacity of Au/CuMOF providing abundant Cu^{2+} and invertases as well as the high catalytic efficiency of invertase toward substrates. Moreover, the developed method showed good selectivity and could be potentially applied for inhibitors screening. With these merits, this dual-response, accurate and sensitive assay shows promise for applications in early clinical diagnosis and therapeutic applications.

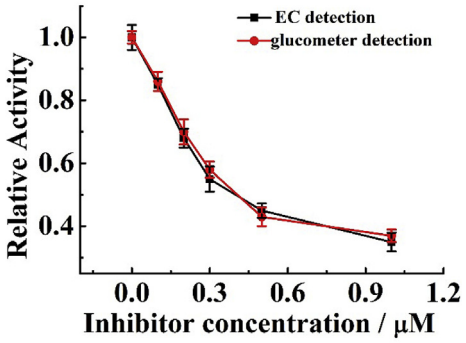


Fig. 5. The inhibitory effects of different concentrations of 5-fluorouracil on Dam MTase activity.

CRediT authorship contribution statement

Ying Chen: Funding acquisition, Methodology, Conceptualization, Investigation, Software, Writing - original draft. **Xian-Zhu Meng:** Investigation, Data curation, Formal analysis. **Hui-Wen Gu:** Investigation, Validation. **Hong-Chao Yi:** Supervision. **Wei-Yin Sun:** Writing - review & editing, Supervision.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2019.03.051.

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