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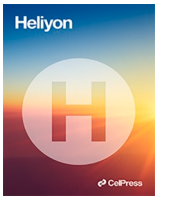

Fluorescence turn-off strategy for sensitive detection of DNA methyltransferase activity based on DNA-templated gold nanoclusters

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ABSTRACT

DNA methylation results in a variety of human diseases and the DNA methylation process is mediated by DNA methyltransferases, which have therefore become potential targets for disease treatment. In this study, a turn-off nanogold biological probe system was successfully created for determining the activity of DNA methyltransferases (M.SssI MTase). A dumbbell-shaped DNA probe with a site-recognizable region of M. SssI MTase and a fluorescent signal probe based on a DNA-templated gold nanocluster (DNA-AuNC) probe combined for the quantitative detection of M. SssI MTase. This dumbbell-shaped DNA probe was methylated by M. SssI MTase, and the dumbbell-shaped DNA probe with a methyl group was recognized by an endonuclease (*Gla*I) and cleaved into hairpin DNA. The dGTP was added to the 3′-OH terminus of hairpin DNA fragments in the presence of terminal deoxynucleotidyl transferase (TdT), and the hairpin DNA was extended with a G-rich sequence that can be used as an inactivation probe. When the inactivation probe was combined with the signal probe, the fluorescent signal disappeared due to the photoinduced electron transfer effect. Methyltransferase activity was then detected based on the turn-off principle of the fluorescence signal from the DNA-AuNCs. The bioprobe enabled sensitive detection of M. SssI MTase with a detection limit of 0.178 U mL^{-1} and good specificity. The bioprobe demonstrated good detection efficiency in both human serum and cell lysates, and its unique fluorescence turn-off mechanism provided good resistance to interference, thus increasing its potential application in complex biological samples. Moreover, it is suitable for screening and assessing the inhibitory activity of M. SssI MTase inhibitors, and therefore has significant potential for disease diagnosis and drug discovery.

1. Introduction

DNA methylation is a common enzymatic reaction in cells that is associated with the regulation of numerous important physiological functions in living organisms [1,2]. Methylation is the process in which a methyl group is transferred from *S*-adenosylmethionine (SAM) to adenine or cytosine remnants through the action of DNA methyltransferases [3,4]. Abnormal DNA methylation can

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cause abnormalities in nucleic acid conformation and thus affect gene expression [2,5], leading to serious human diseases, such as cancer [6–9]. Abnormal activity of DNA methyltransferases directly leads to incorrect DNA methylation. Thus, unusual DNA methyltransferase activity can be used as an indicator for detection of disease at an early stage [10,11]. It has been widely documented that DNA methyltransferases can be used as markers for early clinical diagnosis and are important targets for clinical treatment [12,13]. Screening for inhibitors of DNA methyltransferases is also part of the development of novel cancer drugs. Therefore, it is important to develop an efficient and sensitive bioassay system for determining methyltransferase activity.

In recent years, the detection of methyltransferases has been roughly divided into two categories. The first method depends on the amplification processes of rolling circle amplification (RCA) [14,15], hybridization chain reaction (HCR) [16,17], and strand displacement amplification (SDA) [18–21], which aim to amplify the signal, improve the detection efficiency, and achieve lower detection limits. The second method is based on novel materials, such as nanoparticles [22,23] and quantum dots [24,25], which are used as signal outputs for molecular detection. However, this amplification method may cause nonspecific amplification of nucleic acid fragments, which may increase the background signal of the assay and be detrimental to subsequent detection. As new materials, metal nanoclusters are attracting increasing interest because of their stable physical properties, good biocompatibility [26–29], and simple synthesis method [30–32]. Among these, gold nanoclusters are used in bioimaging [33,34], biomolecular detection [35,36], and treatment of diseases [37]. DNA templates that produce metal nanoclusters with fluorescent signals as the ultimate signal output of biosensing systems have been increasingly used in biosensing [38–41]. There is also growing interest in gold nanoclusters templated by DNA.

A closed dumbbell-shaped DNA probe with a methyltransferase (M.SssI MTase) is often used to study the modified genomes of methyltransferases in higher eukaryotes because it can mimic the pattern of the genome [42,43]. It is also used to study the function of cytosine methylation in higher eukaryotic organisms and to compensate for the difficulty in obtaining methyltransferases in humans [44]. Therefore, it was used as a model enzyme in here. In this study, a bioprobe system for turn-off fluorescence was developed based on the properties of DNA-templated gold nanoclusters (DNA-AuNCs). DNA-AuNC probes with stable fluorescence were synthesized as signal probes using a simple heat reduction reaction. A M. SssI MTase recognition site was released from the system by the action of a methyltransferase and an endonuclease with a 3′-hydroxyl group (3′-OH) at the hairpin end. Using terminal deoxynucleotidyl transferase (TdT) [45–50], a G-rich sequence was added to the end of the hairpin DNA as a quenching probe. The DNA-AuNC probe bound to the quenched probe through complementary base pairing, and the fluorescent signal burst because of photoinduced electron transfer due to the proximity of the signal probe to guanine, thus completing the detection of methyltransferase activity [51,52]. In summary, bioprobes, such as M. SssI MTase, provide a sensitive and selective method for identifying methyltransferases. They can also be used for the sensitive detection of more complex biological samples, including human serum and cancer cell lysates. Importantly, it can screen for inhibitors; therefore, the bioprobe assay system has significant potential for biomedical investigations and clinical diagnostics.

2. Experimental methods

2.1. Reagents

All the oligonucleotides used in this study were synthesized and HPLC-purified from Sangon Biotechnology Co., Ltd. (Shanghai, China), and all the sequences were shown in Table S1. T4 DNA ligase、ExoI exonuclease、ExoIII exonuclease and M. SssI MTase were bought from New England Biolabs (Beijing, China). DNA endonuclease (*Gla*I) with 10 × SEBuffer Y (660 mM potassium acetate, 330 mM Tris-acetate, and 100 mM magnesium acetate, 10 mM DTT, pH 7.9) were bought from SibEnzyme Ltd. (Ak, Novosibirsk, Russia). T4 PNK were bought from Takara Bio. (Dalian, China). 5-Azacytidine (5-Aza) was bought from Sigma-Aldrich. The chemical reagents used in this study were analytical grade.

2.2. Materials

Native polyacrylamide gel electrophoresis (PAGE) was shown by a Molecular Imager Pharos FX™ Plus system (BioRad, Hercules, CA, USA). Transmission electron microscopy (TEM) studies were made using a Tecnai G2 Spirit 120 kV. Acquisition of fluorescence spectral data by microplate reader (Tecan Infinite M1000 Pro, Männedorf, Switzerland).

2.3. Preparation of gold nanocluster probes (DNA-AuNCs)

Based on a previously reported method used with slight modifications, gold nanocluster probes were produced using poly(A)-DNA as the template and sodium citrate as the reducing agent [53,54]. An aqueous solution (0.5 mL) containing 20 μL of 1 mM $HAuCl_4$, 20 μL of 10 μM poly-A DNA probe, and 10 mM sodium citrate (SC) (pH 6) was made for the reaction to take place in. Next, the solution was incubated at 90 °C for 30 min.

2.4. Preparation of enclosed the dumbbell-shaped DNA probe

First, 30 μL reaction mixture containing 3 μL 10 × T4 DNA ligase buffer and 10 μM of DNA solution was heated at 95 °C for 5 min. To ensure that a dumbbell-shaped structure could be formed, the solution was slowly cooled to room temperature. Next, 240 U of T4 DNA ligase was added to the mixture and the reaction was incubated overnight at room temperature. Subsequently, 12 U ExoI and 60 U ExoIII were added to the reaction mixture. This solution was incubated at 37 °C for 1 h to ensure complete digestion of the leftover

unreacted substrate. Finally, the mixture was heated at 80 °C for 20 min to terminate the reaction. The solution containing the closed dumbbell-shaped DNA probe can be used directly for subsequent experiments or stored at −20 °C until use.

2.5. Assay for M.SssI MTase activity

In 20 μL of reaction mixture containing 1 μM dumbbell probe, 1 × SEBuffer Y, 10 U *Gla*I, 160 μM SAM, and various concentrations of M. SssI MTase, the methylation and cleavage reactions were performed. The mixture was incubated at 37 °C for 2 h, then the temperature was increased to 80 °C for 20 min to terminate the reaction. Ten microliters of the above reaction solution was removed, and 3 μL of 10 × TdT buffer, 2 μL of dGTP, and 3 μL $CoCl_2$ (2.5 mM) was added and mixed well. Then, 0.75 μL (15U) of TdT was added to obtain a total volume of 30 μL and incubated at 37 °C for 1 h. The mixture solution was then heated at 80 °C for 20 min to inactivate the TdT polymerase. The above solutions were combined with DNA-AuNC probes and incubated at 37 °C for 30 min. Subsequently, the fluorescence of the mixed solutions was measured.

2.6. Selectivity assay for M.SssI MTase activity

To study the selectivity of the bioprobe detection system and whether it is specific and sensitive for the assay of M. SssI MTase, bovine serum albumin (BSA), Taq DNA ligase, T4 polynucleotide kinase (T4 PNK), Dam MTase and M. SssI MTase were added as targets of the assay system. The remainder of the procedure was with the same as that for the methyltransferase assay.

2.7. Inhibition assay of M.SssI MTase activity

5-azacytidine (5-Aza) incubated at various concentrations with dumbbell-shaped DNA probes and 1 × SEBuffer Y, and later with 160 μM SAM, 100 U mL^{-1} M. SssI MTase, and 10 U of *Gla*I were incorporated into the mixed solution and incubated for 2 h at 37 °C. Subsequent experiments were performed as previously described. The relative activity (RA) of M. SssI MTase was quantified using Equation (1):

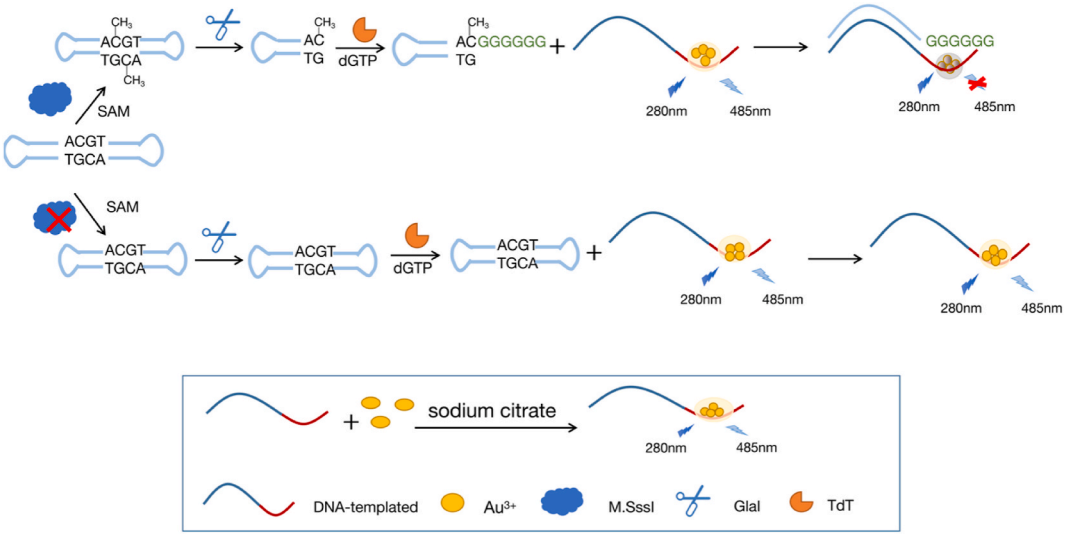
$$RA\% = \frac{(F_0 - F_i)}{(F_0 - F_t)} \times 100\% \quad (1)$$

Where F_0 is the fluorescence intensity in the absence of M. SssI MTase, F_t is the fluorescence intensity with M. SssI MTase included, and F_i is the fluorescence intensity when both M. SssI MTase and the inhibitor are present in the system.

2.8. M.SssI MTase recovery assay from human serum sample and cell lysates

The M. SssI MTase recovery analysis and assay in biological samples were the same as the normal assay for M. SssI MTase activity, but with the addition of 1% human serum sample or 5% cell lysate in the methylation step. The remainder of the procedure was the same as described above. The mean recovery rate was calculated using Equation (2):

$$\text{Recovery } (\%) = \left(C_{\text{mean found}} \ / \ C_{\text{added}}\right) \times 100\% \quad (2)$$



Scheme 1. Schematic showing the bioprobe assay of M. SssI MTase activity.

2.9. Native polyacrylamide gel electrophoresis (PAGE) analysis

The DNA products were analyzed using 12% PAGE. The electrophoresis buffer was 1 × TBE buffer (40 mM Tris, 20 mM acetic acid, and 1 mM ethylenediaminetetraacetic acid [EDTA], pH 8.3). Electrophoresis was maintained at a constant voltage of 110 V for 55 min. The cells were imaged using a Molecular Imager Pharos FX™ Plus system (Bio-Rad, Hercules, CA, USA).

3. Results and discussion

3.1. Experimental principles and feasibility

First, the feasibility of the detection system was analyzed (Scheme 1). We synthesized DNA-AuNC probes with fluorescent signals were synthesized. Therefater, M. SssI MTase was selected as the detection target and designed a dumbbell-shaped DNA with the sequence of 5′-ACGT-3,' which can be recognized by M. SssI MTase and transfer the methyl group from SAM to the dumbbell-shaped DNA probe. After methylation, dumbbell-shaped DNA is recognized by the endonuclease *Gla*I and cleaved into two hairpin structures containing hydroxyl tails. The hairpin structure released by this process is extended by terminal deoxynucleotidyl transferase and dGTP to form a structure with a G-rich sequence. Subsequently, DNA-AuNC probes with fluorescent signals were quenched by hybridization to a G-rich sequence. Based on the principle of photoinduced electron transfer, the fluorescence signals of the DNA-AuNC probe changed when a G-rich sequence was close to the DNA-AuNC probe. Methyltransferase activity can be detected based on the variation in fluorescence emission.

The feasibility of the proposed bioprobe for the determination of methyltransferase activity was verified using 12% PAGE. As shown in the PAGE diagram (Fig. 1A), lane 1 shows a dumbbell-shaped DNA probe formed in the presence of T4DNA ligase, and lane 2 shows a dumbbell-shaped DNA probe recognized and cleaved by *Gla*I after the addition of a methyl group in the presence of M. SssI MTase. This comparison led to the conclusion that with the migration rate of nucleic acid molecules in lane 1, the subsequent DNA in lane 2 migrates at a faster rate than that in lane 1. This demonstrated that the dumbbell-shaped DNA was cleaved into a hairpin structure with a hydroxyl group. The hairpin DNA was extended using the TdT cleavage product. The addition of dGTP at the end of the hairpin extends the G-rich sequence, and as seen in lane 3, DNA band migration is slowed by end lengthening. As a result, it was demonstrated that the dumbbell-shaped DNA probe is eventually cleaved and a segment with a G-rich sequence is added by TdT. These native PAGE experiments demonstrated the viability of the bioprobe.

Fluorescence control experiments were conducted to validate the feasibility of this bioprobe. Control experiments were conducted to determine whether the dumbbell-shaped DNA probe or other enzymes interfered with the fluorescence signal. Within the four sets of control experiments, the fluorescence signal of the bioprobe system decreased when M. SssI MTase, *Gla*I, TdT, or dumbbell-shaped DNA probes were added. According to the fluorescence spectra (Fig. 1B), the fluorescence signal within the system significantly diminished in the presence of the target. In contrast, the fluorescence signal from the gold nanoclusters in the sensing systems was not obviously diminished when M. SssI MTase, *Gla*I, TdT, or dumbbell-shaped DNA probes were absent in the sensing system. This provided a closer validation of the feasibility of this bioprobe for the detection of methyltransferases.

3.2. Characteristic of DNA-AuNCs probe

DNA-AuNC probes with stable fluorescence were synthesized using the above method and were characterized as fluorescent signal probes. As shown in Fig. 2A, DNA-AuNCs had a maximum excitation wavelength of 280 nm and a maximum emission wavelength of 485 nm. The shapes of the prepared DNA-AuNCs were characterized by TEM, which indicated that the DNA-AuNCs were both spherical and well dispersed, with particle sizes averaging approximately 5 nm (Fig. 2B). We also verified that during the formation of gold nanoclusters, gold nanoclusters with a fluorescent signal could only be formed when a DNA sequence containing poly-A was present in the buffer (Fig. S1).

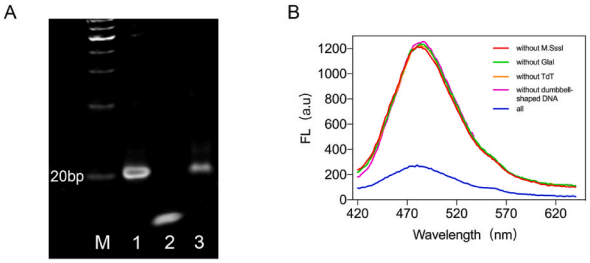


Fig. 1. The feasibility of the bioprobe system was verified by fluorescence and polyacrylamide gel electrophoresis experiments. (A). Demonstrating the feasibility of this strategy by 12% PAGE: Lane M, the DNA ladder marker; Lane1, the dumbbell-shaped DNA probe; Lane2 , the dumbbell-shaped DNA probe + M. SssI + *Gla*I; Lane3, the dumbbell-shaped DNA probe + M. SssI + *Gla*I + TdT. (B). Fluorescence assay for different sensing systems.

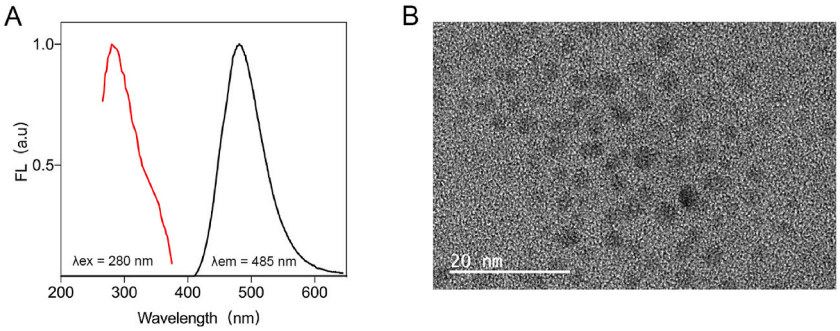


Fig. 2. The characteristic of DNA-AuNCs. (A) Fluorescence excitation spectra data and fluorescence emission spectra data of DNA-AuNCs. (B) TEM images of DNA-AuNCs.

3.3. Optimization of conditions for the proposed bioprobe detection system

After verifying the feasibility of the gold nanocluster fluorescent bioprobe system, the optimal conditions for bioprobe detection were determined. In addition, the amount and time of the enzyme required for the bioprobe assay were optimized. The details are as follows: (a) optimization of the methylation/cleavage reaction time, (b) optimization of the concentration of *Gla*I, (c) Optimization of TdT amount, and (d) optimization of the reaction time of TdT catalysis. (e) Optimization of incubation times for binding of DNA-AuNCs probes to G-rich sequences.

The optimum reaction conditions for this bioprobe detection system were determined experimentally: (a) methylation/cleavage reaction time of 2 h (Fig. 3A); (b) *Gla*I concentration of 10 U (Fig. 3B); (c) TdT amount of 15 U (Fig. 3C); and (d) TdT-catalyzed extension reaction time of 1 h (Fig. 3D). (e) The DNA-AuNCs probe binding to the G-rich sequence incubation time of 30 min (Fig. S2) The response results were evaluated using the ratio of F0–F (fluorescence intensity in the absence of M. SssI MTase in the system–fluorescence intensity in the presence of M. SssI MTase in the system).

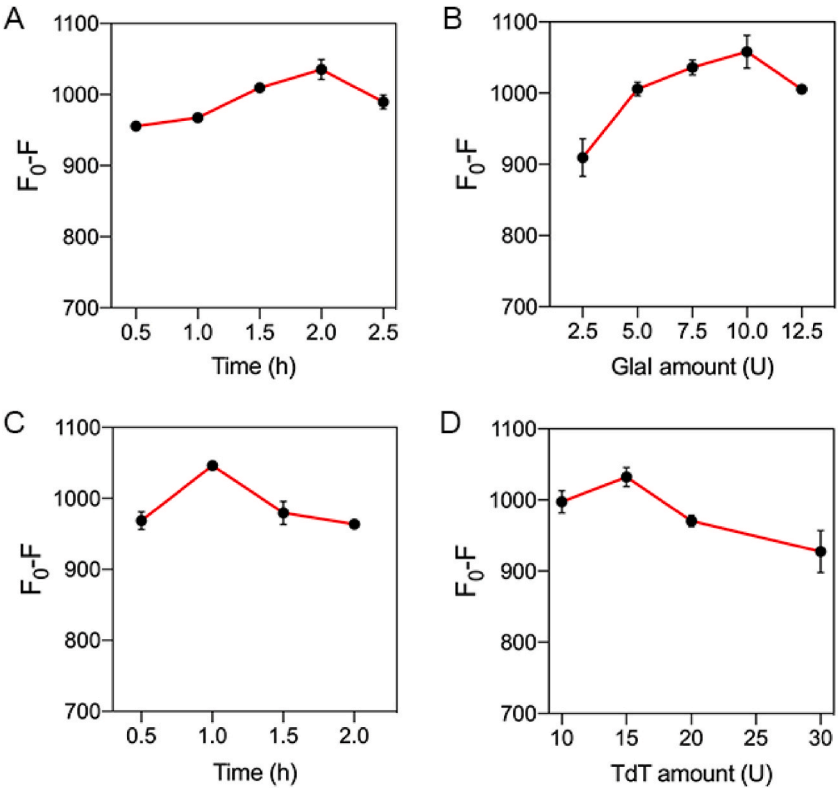


Fig. 3. Optimization of the experimental conditions (A) Optimization of methylation/cleavage reaction time. (B) Optimization of the concentration of *Gla*I. (C) Optimization of TdT-catalyzed extension reaction time. (D) Optimization of TdT amount. The error bars show the standard deviation based on three parallel measurements.

3.4. Sensitivity of bioprobe detection systems for the assay of M.SssI MTase

The experiments described above clearly show that the bioprobe detection strategy can be used to detect M. SssI MTase activity. Based on the best experimental conditions that have been obtained, the sensitivity of the bioprobe detection system was assessed by measuring the change in the fluorescence signal against M. SssI MTase. With increasing concentrations of M. SssI MTase, an increasing number of dumbbell-shaped DNA probes are cleaved by methyltransferases and endonucleases. The hairpin DNA formed by the above process was used for the subsequent extension reaction catalyzed by TdT. As shown in Fig. 4A, the fluorescence intensity of the gold nanoclusters at 485 nm varied with the concentration of M. SssI MTase, and it can be concluded that the fluorescence gradually decreased with increasing concentration of the target. By fitting the equation (Fig. 4B), there was a clear linear relationship between the gold nanocluster fluorescence intensity and M. SssI MTase concentration, especially in the range 0.5–40 U mL^{-1}. The linear regression equation was $F_0–F = 14.23C + 339.50$, as shown by the correlation coefficient (r^2) of 0.9769. Where F_0 and F are the fluorescence intensities at 485 nm in the absence and presence of M. SssI MTase, respectively. And C is the concentration of M. SssI MTase. The limit of detection was calculated as 0.178 U mL^{-1} by assessing the mean response of the blank control with a 3-fold standard deviation. Thus, this method enables the sensitive detection of methyltransferases. The response results were evaluated using the $F_0–F$ (fluorescence intensity in the absence of M. SssI MTase in the system–fluorescence intensity in the presence of M. SssI MTase in the system).

3.5. Detection of M.SssI MTase activity in human serum and cell lysate

To study the application of this detection system to complex biological samples, four different concentrations of M. SssI MTase (10, 20, 40, and 100 U mL^{-1}) were added to the assay system containing 1% human serum. As the enzyme concentration increased, the value of $F_0–F$ also gradually increased. The bioprobe showed almost identical detection efficiency in the complex assay biological samples compared to the buffer (Fig. 5A). This indicated that the proposed approach can be applied to complex biological samples. Thus, M. SssI MTase could be detected properly in a buffer system containing 1% human serum. The fitted standard curve shows that the target concentration of the bioprobe ranged 0.5–40 U mL^{-1} (Fig. 5B). The concentration exhibited a good linear relationship with the fluorescence signal value of the gold nanoclusters. The equation is $F_0–F = 12.47C + 382.70$, $r^2 = 0.9746$, and the detection limit is 0.199 U mL^{-1}. This result was consistent with the detection of the bioprobe in the buffer solution. Therefore, the proposed method can be used for complex systems involving human serum samples. We also tested a bioprobe for recovery of the assay in 1% human serum. Table 1 presents the results of the study. The recovery of this method ranged from 92.58 to 110.58%. Additionally, a bioprobe was used to detect methyltransferase activity in cancer cell lysates (MCF-7 cells). As shown in Fig. S3, compared to the sample in the buffer, the bioprobe maintained virtually the same detection efficiency in the system with the addition of 5% cell lysate. Thus, the detection efficiency of our method was stable in complex biological samples.

3.6. Selectivity of bioprobe detection systems for the assay of M.SssI MTase

With the aim of validating the selectivity of the approach for this bioprobe detection system, the following studies were undertaken to verify the specificity of the proposed strategy. As Fig. 6A shows, using the same assay procedure, four detection systems were added with 0.1 g L^{-1} BSA, 100 U mL^{-1} Taq DNA ligase, 100 U mL^{-1} T^4 PNK, 100 U mL^{-1} Dam MTase and 100 U mL^{-1} M. SssI MTase. The results of the response are evaluated by the $F_0–F$ (fluorescence intensity in the absence of M. SssI MTase in the system - fluorescence intensity in the presence of M. SssI MTase in the system). M. SssI MTase specifically recognizes and labels the dumbbell-shaped DNA probe. The fluorescence was quenched only when M. SssI MTase was present in the system. These results demonstrate the specificity of the bioprobe detection system.

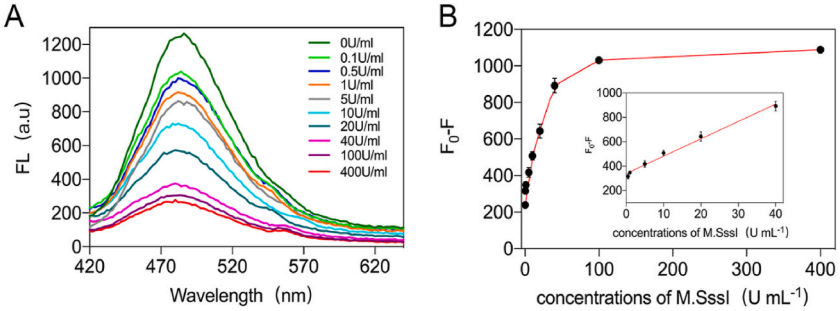


Fig. 4. Sensitivity of the M. SssI MTase bioprobe detection system. (A) The fluorescence spectra data of bioprobe assay systems using different concentrations of M. SssI MTase. (B)Variation of M. SssI MTase concentration versus fluorescent signal values at 485 nm ($F_0–F$). And the inset shows the linear relationship between the fluorescence intensity and the concentration of M. SssI MTase.

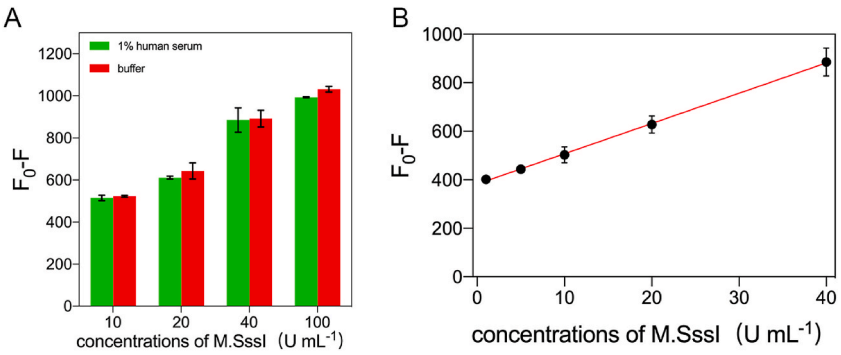


Fig. 5. Application of detection strategies in complex environments. (A) Fluorescence intensities of various concentrations of M. SssI MTase in buffer with 1% human serum samples in the proposed detection strategy. (B) The linear relationship between the fluorescence intensity (F_0–F) and the concentration of M. SssI MTase in 1% human serum.

Table 1
Recovery experimental results in human serum samples.

Add (U mL-1)	Found (U mL-1)	Recovery (%)	RSD (%)
10	11.06	110.58%	1.46
20	22.05	110.25%	0.53
40	37.03	92.58%	1.13

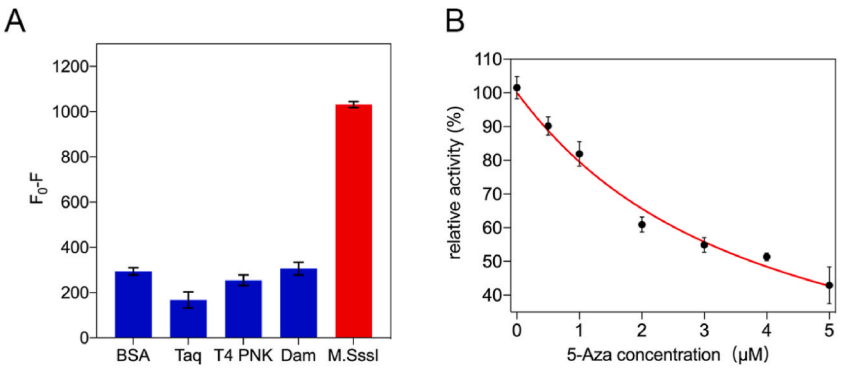


Fig. 6. Selectivity of the M. SssI MTase sensing system and screening of M. SssI MTase inhibitor. (A) Fluorescence intensity of different assay system with 100 U mL^{-1} Taq DNA Ligase, 100 U mL^{-1} T^4 PNK, 0.1 g L^{-1} BSA, 100 U mL^{-1} Dam MTase and 100 U mL^{-1} M. SssI MTase respectively. F_0 and F are the fluorescence intensities at 485 nm in the absence and presence of M. SssI MTase. (B) Inhibition of M. SssI MTase activity by 5-Aza, M. SssI MTase was added at a concentration of 100 U mL^{-1}.

3.7. Analysis of the inhibitory activity of M. SssI MTase inhibitors

DNA methylation is a common DNA modification that significantly affects gene expression. Aberrantly methylated DNA is closely associated with the development and progression of tumors; therefore, the development of useful methyltransferase inhibitors that hinder the activity of this enzyme has become a hot topic. 5-Aza acts as a model inhibitor [55]. The structure of 5-Aza is shown in Fig. S4. M. SssI MTase inhibitors were assessed using the bioprobe described above with the addition of 5-Aza to the assay system. Before conducting the inhibitor activity assays, we excluded the potential effects of the inhibitor on *Gla*I and TdT. First, the effects of these two experiments were excluded. In the first experiment, M. SssI MTase and *Gla*I were treated with a dumbbell-shaped DNA probe, followed by the addition of 5-Aza (Fig. S5A), the fluorescence intensity of this bioprobe system was not significantly different from the original values, indicating that 5-Aza had no obvious effect on TdT activity. Next, 5-Aza and *Gla*I (or *Gla*I alone) were added after treating the dumbbell-shaped DNA probe with M. SssI MTase. The results again indicated that the sensing systems with or without 5-Aza exhibited similar fluorescence outputs (Fig. S5B), indicating that 5-Aza also had no significant effect on *Gla*I activity. The ability of 5-Aza to inhibit M. SssI MTase was tested after eliminating the effects of the inhibitors on other enzymes. As shown in Fig. 6B the value of the IC_{50} (resulting in 50% inhibition of M. SssI MTase activity at 5-Aza concentrations) was calculated to be 3.76 μM. This result is consistent with those reported in the literature [24], indicating that this bioprobe detection system can be used to screen for M. SssI MTase inhibitors and evaluate their inhibitory capacity.

4. Conclusions

In this study, a turn-off bioprobe system based on the properties of Au nanoclusters was designed. The bioprobe was highly sensitive in detecting methyltransferase activity without any amplification process and had a detection limit of 0.178 U mL^{-1}. Its unique principles make this method highly specific, and it does not respond to enzymes or proteins other than M. SssI MTase. Even in complex biological environments (e.g., human serum and cell lysates), bioprobe systems are sensitive to target activity. Importantly, this bioprobe system can be used to screen for methyltransferase inhibitors. In this study, the IC_{50} value of the methyltransferase inhibitor 5-Aza was 3.76 μM. The sensitive, highly adaptable, and uncomplicated bioprobe assay system designed in this study for the detection of methyltransferase activity has a promising future in biomedical detection and drug screening, and presents a new method for the early screening of human diseases.

Author contribution statement

Fangyu Zhou: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Hui Chen, Tingting Fan, Zixia Guo: Contributed reagents, materials, analysis tools or data.

Feng Liu: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

No data was used for the research described in the article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e17724.

References

[1] G.W. Rebeck, L. Samson, Increased spontaneous mutation and alkylation sensitivity of escherichia-coli strains lacking the ogt o6-methylguanine DNA-repair methyltransferase, J. Bacteriol. 173 (6) (1991) 2068–2076, https://doi.org/10.1128/jb.173.6.2068-2076.1991.

[2] K.D. Robertson, E. Uzvolgyi, G.N. Liang, C. Talmadge, J. Sumegi, F.A. Gonzales, P.A. Jones, The human DNA methyltransferases (DNMTs) 1, 3a and 3b: coordinate mRNA expression in normal tissues and overexpression in tumors, Nucleic Acids Res. 27 (11) (1999) 2291–2298, https://doi.org/10.1093/nar/27.11.2291.

[3] Z.D. Smith, A. Meissner, DNA methylation: roles in mammalian development, Nat. Rev. Genet. 14 (3) (2013) 204–220, https://doi.org/10.1038/nrg3354.

[4] X.D. Cheng, R.J. Roberts, AdoMet-dependent methylation, DNA methyltransferases and base flipping, Nucleic Acids Res. 29 (18) (2001) 3784–3795, https://doi.org/10.1093/nar/29.18.3784.

[5] G. Rajendran, K. Shanmuganandam, A. Bendre, D. Mujumdar, A. Goel, A. Shiras, Epigenetic regulation of DNA methyltransferases: DNMT1 and DNMT3B in gliomas, J. Neuro Oncol. 104 (2) (2011) 483–494, https://doi.org/10.1007/s11060-010-0520-2.

[6] V. Shukla, X. Coumoul, T. Lahusen, R.-H. Wang, X. Xu, A. Vassilopoulos, C. Xiao, M.-H. Lee, Y.-G. Man, M. Ouchi, et al., BRCA1 affects global DNA methylation through regulation of DNMT1, Cell Res. 20 (11) (2010) 1201–1215, https://doi.org/10.1038/cr.2010.128.

[7] J.D. Roll, A.G. Rivenbark, W.D. Jones, W.B. Coleman, DNMT3b overexpression contributes to a hypermethylator phenotype in human breast cancer cell lines, Mol. Cancer 7 (2008) 721–732, https://doi.org/10.1186/1476-4598-7-15.

[8] M. Esteller, P.G. Corn, S.B. Baylin, J.G. Herman, A gene hypermethylation profile of human cancer, Cancer Res. 61 (8) (2001) 3225–3229. PMID: 11309270.

[9] D.K. Vanaja, M. Ehrich, D. Van den Boom, J.C. Cheville, R.J. Karnes, D.J. Tindall, C.R. Cantor, C.Y.F. Young, Hypermethylation of genes for diagnosis and risk stratification of prostate cancer, Cancer Invest. 27 (5) (2009) 549–560, https://doi.org/10.1080/07357900802620794.

[10] S.B. Baylin, M. Esteller, M.R. Rountree, K.E. Bachman, K. Schuebel, J.G. Herman, Aberrant patterns of DNA methylation, chromatin formation and gene expression in cancer, Hum. Mol. Genet. 10 (7) (2001) 687–692, https://doi.org/10.1093/hmg/10.7.687.

[11] J.F. Costello, M.C. Fruhwald, D.J. Smiraglia, L.J. Rush, G.P. Robertson, X. Gao, F.A. Wright, J.D. Feramisco, P. Peltomaki, J.C. Lang, et al., Aberrant CpG-island methylation has non-random and tumour-type-specific patterns, Nat. Genet. 24 (2) (2000) 132–138, https://doi.org/10.1038/72785.

[12] F.M. Faraci, S. R. Hyperhomocysteinemia Lentz, Oxidative stress, and cerebral vascular dysfunction, Stroke 35 (2) (2004) 345–347, https://doi.org/10.1161/01.Str.0000115161.10646.67.

[13] X.J. Yan, J. Xu, Z.H. Gu, C.M. Pan, G. Lu, Y. Shen, J.Y. Shi, Y.M. Zhu, L. Tang, X.W. Zhang, et al., Exome sequencing identifies somatic mutations of DNA methyltransferase gene DNMT3A in acute monocytic leukemia, Nat. Genet. 43 (4) (2011) 309–U351, https://doi.org/10.1038/ng.788.

[14] Z.-y. Wang, P. Li, L. Cui, Q. Xu, C.-y. Zhang, Construction of a universal and label-free chemiluminescent sensor for accurate quantification of both bacteria and human methyltransferases, Anal. Chem. 92 (19) (2020) 13573–13580, https://doi.org/10.1021/acs.analchem.0c03303.

[15] Y. Wang, Y. Han, F. Zhou, T. Fan, F. Liu, Simple detection of DNA methyltransferase with an integrated padlock probe, Biosensors 12 (8) (2022) 569, https://doi.org/10.3390/bios12080569.

[16] X.L. Peng, J.L. Zhu, W. Wen, T. Bao, X.H. Zhang, H.P. He, S.F. Wang, Silver nanoclusters-assisted triple-amplified biosensor for ultrasensitive methyltransferase activity detection based on AuNPs/ERGO hybrids and hybridization chain reaction, Biosens. Bioelectron. 118 (2018) 174–180, https://doi.org/10.1016/j.bios.2018.07.048.

[17] S. Zhang, W. Shi, K.-B. Li, D.-M. Han, J.-J. Xu, Ultrasensitive and label-free detection of multiple DNA methyltransferases by asymmetric nanopore biosensor, Anal. Chem. 94 (10) (2022) 4407–4416, https://doi.org/10.1021/acs.analchem.1c05332.

[18] L.J. Wang, X. Han, C.C. Li, C.Y. Zhang, Single-ribonucleotide repair-mediated ligation-dependent cycling signal amplification for sensitive and specific detection of DNA methyltransferase, Chem. Sci. 9 (28) (2018) 6053–6061, https://doi.org/10.1039/c8sc02215a.

[19] H.-C. Hu, S.-H. Wu, L.-X. Jin, J.-J. Sun, Plasmonic Au nanocube enhanced SERS biosensor based on heated electrode and strand displacement amplification for highly sensitive detection of Dam methyltransferase activity, Biosens. Bioelectron. 210 (2022), 114283, https://doi.org/10.1016/j.bios.2022.114283.

[20] Q. Wen, D. Li, H. Xi, G. Huang, W. Zhu, Methylation-blocked cascade strand displacement amplification for rapid and sensitive fluorescence detection of DNA methyltransferase activity, J. Pharm. Biomed. Anal. 219 (2022), 114935, https://doi.org/10.1016/j.jpba.2022.114935.

[21] Q. Wen, D. Li, G. Huang, H. Xi, H. Pan, L. Zhang, Z. Li, X. Xiao, W. Zhu, Ultrasensitive detection of DNA methyltransferase activity: a novel dual-amplification fluorescence technique, Analyst 147 (22) (2022) 4980–4985, https://doi.org/10.1039/d2an01302a.

[22] L.-J. Wang, X. Han, J.-G. Qiu, B. Jiang, C.-Y. Zhang, Cytosine-5 methylation-directed construction of a Au nanoparticle-based nanosensor for simultaneous detection of multiple DNA methyltransferases at the single-molecule level, Chem. Sci. 11 (35) (2020) 9675–9684, https://doi.org/10.1039/d0sc03240a.

[23] M. Wu, M. Zhang, Z. Fan, X. Qin, X. Zhu, H. Ji, Y. Qin, Q. Wang, L. Wu, Ultrasensitive DNA methyltransferase activity sensing and inhibitor evaluation with highly photostable upconversion nanoparticle transducer, Microchim. Acta 188 (5) (2021) 169, https://doi.org/10.1007/s00604-021-04831-z.

[24] J. Hu, Y. Liu, C.-y. Zhang, Construction of a single quantum dot nanosensor with the capability of sensing methylcytosine sites for sensitive quantification of methyltransferase, Nanoscale 12 (7) (2020) 4519–4526, https://doi.org/10.1039/c9nr10376g.

[25] L. Meng, K. Xiao, X. Zhang, C. Du, J. Chen, A novel signal-off photoelectrochemical biosensor for M.SssI MTase activity assay based on GQDs@ZIF-8 polyhedra as signal quencher, Biosens. Bioelectron. 150 (2020), 111861, https://doi.org/10.1016/j.bios.2019.111861.

[26] Y.L. Zhang, Y. Huang, J.H. Jiang, G.L. Shen, R.Q. Yu, Electrochemical aptasensor based on proximity-dependent surface hybridization assay for single-step, reusable, sensitive protein detection, J. Am. Chem. Soc. 129 (50) (2007), 15448, https://doi.org/10.1021/ja0773047.

[27] Y.L. Zhang, Y. Wang, H.B. Wang, J.H. Jiang, G.L. Shen, R.Q. Yu, J.H. Li, Electrochemical DNA biosensor based on the proximity-dependent surface hybridization assay, Anal. Chem. 81 (5) (2009) 1982–1987, https://doi.org/10.1021/ac802512d.

[28] R. Hu, T. Fu, X.B. Zhang, R.M. Kong, L.P. Qiu, Y.R. Liu, X.T. Liang, W.H. Tan, G.L. Shen, R.Q. Yu, A proximity-dependent surface hybridization strategy for constructing an efficient signal-on electrochemical DNAzyme sensing system, Chem. Commun. 48 (76) (2012) 9507–9509, https://doi.org/10.1039/c2cc34848a.

[29] Z.M. Ying, H.Y. Xiao, H. Tang, R.Q. Yu, J.H. Jiang, Light-up RNA aptamer enabled label-free protein detection via a proximity induced transcription assay, Chem. Commun. 54 (64) (2018) 8877–8880, https://doi.org/10.1039/c8cc04498h.

[30] L.L. Zhang, J.J. Zhao, H. Zhang, J.H. Jiang, R.Q. Yu, Double strand DNA-templated copper nanoparticle as a novel fluorescence indicator for label-free detection of polynucleotide kinase activity, Biosens. Bioelectron. 44 (2013) 6–9, https://doi.org/10.1016/j.bios.2012.12.037.

[31] Y. Tao, M.Q. Li, J.S. Ren, X.G. Qu, Metal nanoclusters: novel probes for diagnostic and therapeutic applications, Chem. Soc. Rev. 44 (23) (2015) 8636–8663, https://doi.org/10.1039/c5cs00607d.

[32] H.B. Wang, H.D. Zhang, Y. Chen, Y.M. Liu, A fluorescent biosensor for protein detection based on poly(thymine)-templated copper nanoparticles and terminal protection of small molecule-linked DNA, Biosens. Bioelectron. 74 (2015) 581–586, https://doi.org/10.1016/j.bios.2015.07.021.

[33] C. Ding, Y. Tian, Gold nanocluster-based fluorescence biosensor for targeted imaging in cancer cells and ratiometric determination of intracellular pH, Biosens. Bioelectron. 65 (2015) 183–190, https://doi.org/10.1016/j.bios.2014.10.034.

[34] C. Zhao, L. Lai, F.U. Rehman, C. Qian, G. Teng, H. Jiang, X. Wang, In vivo target bio-imaging of cerebral ischemic stroke by real-time labeling of zinc, RSC Adv. 6 (112) (2016) 110525–110534, https://doi.org/10.1039/c6ra23507g.

[35] L. Qin, K. Zhang, B. Feng, P. Zhang, T. Qing, J. Fei, Proximity sequence-dependent spectral conversion of silver nanoclusters and construction of ratiometric nanoprobe, Chem. Eng. J. 441 (2022), 136001, https://doi.org/10.1016/j.cej.2022.136001.

[36] D. Qin, S. Meng, Y. Wu, G. Mo, B. Deng, Aggregation-induced electrochemiluminescence resonance energy transfer with dual quenchers for the sensitive detection of prostate-specific antigen, Sensor. Actuator. B Chem. 367 (2022), 132176, https://doi.org/10.1016/j.snb.2022.132176.

[37] Y. Wang, C. Xu, J. Zhai, F. Gao, R. Liu, L. Gao, Y. Zhao, Z. Chai, X. Gao, Label-free Au cluster used for in vivo 2D and 3D computed tomography of murine kidneys, Anal. Chem. 87 (1) (2015) 343–345, https://doi.org/10.1021/ac503887c.

[38] J. Yin, F. Liu, T. Fan, Y. Ren, Y. Jiang, Rapid detection of methyltransferases utilizing dumbbell DNA-templated copper nanoparticles, Sensor. Actuator. B Chem. 276 (2018) 499–506, https://doi.org/10.1016/j.snb.2018.08.140.

[39] W. Liu, H. Lai, R. Huang, C. Zhao, Y. Wang, X. Weng, X. Zhou, DNA methyltransferase activity detection based on fluorescent silver nanocluster hairpin-shaped DNA probe with 5'-C-rich/G-rich-3' tails, Biosens. Bioelectron. 68 (2015) 736–740, https://doi.org/10.1016/j.bios.2015.02.005.

[40] S. Zhang, W. Shi, K.-B. Li, F. Chen, J. Su, D.-M. Han, Graphene oxide/silver nanoclusters based logic devices and their application to multiplexed analysis of miRNA, Sensor. Actuator. B Chem. 273 (2018) 408–417, https://doi.org/10.1016/j.snb.2018.06.027. Article.

[41] S. Zhang, K. Wang, K.-B. Li, W. Shi, W.-P. Jia, X. Chen, T. Sun, D.-M. Han, A DNA-stabilized silver nanoclusters/graphene oxide-based platform for the sensitive detection of DNA through hybridization chain reaction, Biosens. Bioelectron. 91 (2017) 374–379, https://doi.org/10.1016/j.bios.2016.12.060. Article.

[42] M. Takiguchi, W.E. Achanzar, W. Qu, G.Y. Li, M.P. Waalkes, Effects of cadmium on DNA-(Cytosine-5) methyltransferase activity and DNA methylation status during cadmium-induced cellular transformation, Exp. Cell Res. 286 (2) (2003) 355–365, https://doi.org/10.1016/s0014-4827(03)00062-4.

[43] W.J. Lee, B.T. Zhu, Inhibition of DNA methylation by caffeic acid and chlorogenic acid, two common catechol-containing coffee polyphenols, Carcinogenesis 27 (2) (2006) 269–277, https://doi.org/10.1093/carcin/bgi206.

[44] M. Fatemi, M.M. Pao, S. Jeong, E.N. Gal-Yam, G. Egger, D.J. Weisenberger, P.A. Jones, Footprinting of mammalian promoters: use of a CpG DNA methyltransferase revealing nucleosome positions at a single molecule level, Nucleic Acids Res. 33 (20) (2005) e176, https://doi.org/10.1093/nar/gni180.

[45] Y. Zhou, W. Fang, K. Lai, Y. Zhu, X. Bian, J. Shen, Q. Li, L. Wang, W. Zhang, J. Yan, Terminal deoxynucleotidyl transferase (TdT)-catalyzed homo-nucleotides-constituted ssDNA: inducing tunable-size nanogap for core-shell plasmonic metal nanostructure and acting as Raman reporters for detection of Escherichia coli O157:H7, Biosens. Bioelectron. 141 (2019), 111419, https://doi.org/10.1016/j.bios.2019.111419.

[46] S. Deng, J. Yan, F. Wang, Y. Su, X. Zhang, Q. Li, G. Liu, C. Fan, H. Pei, Y. Wan, In situ terminus-regulated DNA hydrogelation for ultrasensitive on-chip microRNA assay, Biosens. Bioelectron. 137 (2019) 263–270, https://doi.org/10.1016/j.bios.2019.04.053.

[47] Y.-C. Du, Y.-J. Zhu, X.-Y. Li, D.-M. Kong, Amplified detection of genome-containing biological targets using terminal deoxynucleotidyl transferase-assisted rolling circle amplification, Chem. Commun. 54 (6) (2018) 682–685, https://doi.org/10.1039/c7cc09337c.

[48] Y. Yuan, W. Li, Z. Liu, Z. Nie, Y. Huang, S. Yao, A versatile biosensing system for DNA-related enzyme activity assay via the synthesis of silver nanoclusters using enzymatically-generated DNA as template, Biosens. Bioelectron. 61 (2014) 321–327, https://doi.org/10.1016/j.bios.2014.05.038.

[49] Z. Liu, W. Li, Z. Nie, F. Peng, Y. Huang, S. Yao, Randomly arrayed G-quadruplexes for label-free and real-time assay of enzyme activity, Chem. Commun. 50 (52) (2014) 6875–6878, https://doi.org/10.1039/c4cc03103b.

[50] J. Faber, H. Kantarjian, W.M. Roberts, M. Keating, E. Freireich, M. Albitar, Terminal deoxynucleotidyl transferase-negative acute lymphoblastic leukemia, Arch. Pathol. Lab Med. 124 (1) (2000) 92–97, https://doi.org/10.5858/2000-124-0092-TDTNAL.

[51] M. Torimura, S. Kurata, K. Yamada, T. Yokomaku, Y. Kamagata, T. Kanagawa, R. Kurane, Fluorescence-quenching phenomenon by photoinduced electron transfer between a fluorescent dye and a nucleotide base, Anal. Sci. 17 (1) (2001) 155–160, https://doi.org/10.2116/analsci.17.155.

[52] L.B. Zhang, J.B. Zhu, S.J. Guo, T. Li, J. Li, E.K. Wang, Photoinduced electron transfer of DNA/Ag nanoclusters modulated by G-quadruplex/hemin complex for the construction of versatile biosensors, J. Am. Chem. Soc. 135 (7) (2013) 2403–2406, https://doi.org/10.1021/ja3089857.

[53] H.B. Wang, Y. Li, H.Y. Bai, Y.M. Liu, DNA-templated Au nanoclusters and MnO2 sheets: a label-free and universal fluorescence biosensing platform, Sensor. Actuator. B Chem. 259 (2018) 204–210, https://doi.org/10.1016/j.snb.2017.12.048.
[54] H.B. Wang, Y. Li, H.Y. Bai, Z.P. Zhang, Y.H. Li, Y.M. Liu, Development of rapid and label-free fluorescence sensing of tetracyclines in milk based on poly (adenine) DNA-templated Au nanoclusters, Food Anal. Methods 11 (11) (2018) 3095–3102, https://doi.org/10.1007/s12161-018-1289-8.
[55] J.K. Christman, 5-Azacytidine and 5-aza-2 ′-deoxycytidine as inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy, Oncogene 21 (35) (2002) 5483–5495, https://doi.org/10.1038/sj.onc.1205699.