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# DNA methyltransferase activity detection based on graphene quantum dots using fluorescence and fluorescence anisotropy

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ABSTRACT

DNA methyltransferase enzyme has a crucial role in many biological processes. Abnormal expression level of this enzyme has been regarded as a predictive cancer biomarker. Herein a novel method based on DNA modified GQDs was developed for fluorescence based assay and anisotropic investigation of M.SssI activity. An amine modified ds-DNA was designed including a recognition site for both methyltransferase M.SssI and endonuclease HpaII. By conjugation of ds-DNA to GQDs, fluorescence was decreased to 45%. When M.SssI was introduced, ds-DNA was methylated at palindromic sequence 5′. . .CCGG. . .3′ and become resistant to cleavage by HpaII and no change was observed in fluorescence intensity. Contrarily without sufficient M.SssI enzyme, HpaII cleaved DNA strands and induced enhancement of fluorescence. The proposed method exhibited a low detection limit of 0.7 U/ml. Additional fluorescence anisotropy method was performed to confirm the DNA modification and release of DNA strands from the surface of GQDs in the presence of M.SssI and HpaII respectively. The application of the assay in the serum sample was successfully investigated. This simple method can be a useful tool to apply in diagnosis and biomedical research.



## 1. Introduction

DNA methylation, a prevalent epigenetic modification, has close relationship to gene activation, gene imprinting, chromatin stability, X-chromosome inactivation in females and various diseases such as cancers [1]. It has been confirmed that DNA methylation is catalyzed with DNA methyltransferase by transferring methyl group to DNA using the cofactor S-adenosyl-L-methionine (SAM) [2]. Many studies have indicated that abnormal activity of DNA methyltransferase in CpG islands can result in hypermethylation which can develop cancers by silencing tumor suppressor genes in mammals and virulence in bacteria [3–6]. In particular DNA methyltransferase enzyme (till now abbreviated DNA MTase) have become a significant biomarker of diseases especially cancers [7–10]. Therefore, the detection of DNA methyltransfrase activity could be a critical strategy in life science research. To date, some traditional methods utilize for DNA methylation detection and assay of DNA MTase activity including radioactive labeling of [mrthul-$^3$H]-SAM [11], Polymerase Chain Reaction (PCR) [12,13], High Performance Liquid Chromatography (HPLC) [14], western blotting [15], bisulfite treatment [16] and immune reaction [17]. Although the above methods have their own advantages but they have some challenges such as time consuming, DNA consuming, using expensive, sophisticated and large scale instruments. Recently new strategies such as electrochemical biosensors [18,19], colorimetric assay [20], fluorescence, chemiluminescence and biochemiluminescence [21–23] and photoelectrochemistry methods [24] have been established. These methods are more advanced but it is necessary to develop more sensitive, rapid, effective and facile strategies for the detection of methylation and DNA MTase activity.

Carbon based nanodots are classified into three categories: (1) Carbon nanodots (CNDs): amorphous quasi-special nanodots which lacks in quantum confinement. (2) Carbon quantum dots (CQDs): spherical nanodots present quantum confinement and have crystalline structure. (3) Graphene quantum dots (GQDs):

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π-conjugated single sheet or disks of graphene in the size range of 2–20 nm [25]. Graphene quantum dots have shown unique electro-optical properties and outstanding performance in photovoltaic devices photocatalysis, biosensing and biological imaging [26]. Compared with conventional dye molecules including organic or inorganic fluorophores they exhibited good photostability, low toxicity, high quantum yield, easy modulation, low costly, resistant to photobleaching and excellent biocompatibility [27]. Due to this excellent fluorescence performance and biocompatibility, GQDs are expected to show promising features in bioimaging and biosensing applications [28–35]. DNA modified graphene quantum dots [28,32,33,35–37] and other nanomaterials with quantum confinement [38,39] are used as fluorescent sensing platforms. In this work highly sensitive fluorescence approach for DNA methylation detection and DNA MTase activity assay based on biocompatible GQDs was established. According to this method, DNA CpG methyltransferase (M.SssI) and HpaII are chosen as DNA MTase and methylation resistant endunoclease model enzyme respectively.

As depicted in Scheme 1 the double stranded DNA (ds DNA) used in this study has CCGG palindromic site that is recognized by both M.SssI and HpaII. The DNA probe is connected to GQDs from the end which contains CCGG sequence. The fluorescence intensity is suppressed due to conjugation of DNA strands to GQDs. With sufficient catalytic activity of M.SssI, these sites are methylated and become resistant to cleavage by HpaII. By increasing the amount of M.SssI, more DNA would be methylated and remain connected to GQDs leading to decrease in fluorescence intensity. The fluorescence intensity of ds-GQD has a linear relationship with the M.SssI MTase activity in the range of 2–30 U/ml, with the detection limit of 0.7 U/ml without any amplification which is comparable with previous reports as seen in Table 1. Additionally, this method can be performed in human serum with satisfying recovery. We have also used the current probe for the detection of fluorescence anisotropy (FA) to assay M.SssI activity in the same concentration range of enzyme (0–30 U/ml). The change in GQDs volume or mass via conjugation of DNA strands will alter their FA [40–42] (Scheme 2).

## 2. Experimental

### 2.1. Reagents and materials

Deionized water was used throughout the experimental processes. CpG methyltransferase (M.SssI), S-adenosyl methionine (SAM), restriction endonuclease HpaII were purchased from Thermoscientific Company. According to the supplier, M.SssI is stored at −20 °C in a buffer containing 10 mM potassium phosphate(pH 7.0), 400 mM KCl, 1 mM DTT (Dithiothreiol), 1 mM EDTA, 0.2 mg/ml BSA (Bovine Serum Albumine) and 50%(v/v) glycerol. HpaII was stored at −20 °C in Tango buffer.

The DNA sequence oligonucleotides are as follows:

DNA S1 sequence: 5′-$NH_2$-$(CH_2)_6$-TGCCGGTGGGAGGGAGAGAGTAATAA-3′

DNA S2 sequence: 5′-TTATTACTCTCCCTCCCACCTCCGGCA-3′

The double stranded DNA was selected from Adenomatous polyposis coli (APC) tumor suppressor gene sequence. Above DNA sequences were synthesized and purified with PAGE by Shanghai Generay Biotech Co. The oligonucleotides were prepared with TE buffer (containing 1 M tris–HCl and 0.5 ml EDTA, pH 7.5) and kept in −20 °C. Citric acid monohydrate,1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC.HCl) and N-hydroxylsuccinimide sodium salt (NHS) were purchased from Merck. Phosphate buffer solution was prepared by mixing stock solutions of $NaH_2PO_4$ and $Na_2HPO_4$ at pH 7.4. All chemicals were of analytical grade and used without further purification.

### 2.2. Apparatus

Fluorescence measurements were performed using a Perkin Elmer LS-45 fluorescence spectrometer (Bu Kingham Shire, UK). Transmission Electron Microscope (TEM) image was taken using Zeiss, EM10C, 80 KV, Germany. UV–vis absorption was measured using Specord 250 spectrophotometer (analytic jena, Germany). Zeta potential was detected using the zetasizer (Marvalen, Model of nano-zs). Fourier transform infrared spectroscopy (FTIR) was carried out with a bruker Tensor 27 Spectrometer.

### 2.3. Synthesis of graphene quantum dots (GQDs)

The GQDs were synthesized using the method described by Dong et al. [43] Briefly 2 g citric acid was put into a 5 ml beaker and heated to 200 °C by a heating mantle for 30 min, until the citric acid of white solid changed to an orange liquid. Then, a certain amount of 10 mg/ml NaOH solution was added into the orange liquid drop by drop, with continuously vigorous stirring, until the pH value of the solution was adjusted to 7 to obtain GQDs solution. The obtained GQDs solution was stored at 4 °C in the refrigerator.

### 2.4. Preparation of dsDNA-GQDs

The GQDs were first diluted with PBS solution (10 mM, PH7.4). After that EDC (60 mg) and NHS (60 mg) were added to the above solution to activate the surface carboxylic group with stirring for 30 min at room temperature. Then amine modified ssDNA (10 μl, 100 μM) added to resulting solution through condensation reaction for 24 h at room temperature with continuous stirring. To remove the free nonconjugated DNA, the ssDNA-GQD were separated by centrifugation at 16300 × *g* for 20 min and washed with PBS buffer three times. Afterward, the complementary DNA strands were added into the resulted solution and were incubated for 1 h in 37 °C to form $S_1$-$S_2$ DNA- GQD. To confirm the immobilization of DNA on GQDs the zeta potential value of the GQDs before and after DNA modification was measured. Zeta potential value changed to −9.5 from −4.6 after modification. This negative shift is due to negative charge of functionalized DNA. In addition, Circular dichroism (CD) spectra of DNA and DNA-GQD indicating a change in the conformation when DNA binds to GQD [36] (Fig. 5S).

### 2.5. Detection of M.SssI activity

In a typical experiment M.SssI methylation was performed by incubating the 50 μl dsDNA-GQDs solution and a desire amount of M.SssI, SAM and 1× M.SssI buffer (10 mM Tris–HCl, 100 mM KCl, 1 mM $MgCl_2$, 0.1 mg/ml BSA, pH 7.5) at 37 °C for 30 min. Subsequently the above solution was incubated with 40 U/ml HpaII and 1 × Tango buffer (33 mM Tris-acetate, 10 mM Magnesium acetate, 66 mM potassium acetate, 0.1 mg/ml BSA) per each reaction at 37 °C for 20 min. The fluorescence spectra were recorded by fluorescence spectrometer.

### 2.6. Measurement of fluorescence anisotropy

For the measurement of the fluorescence anisotropy, the FAs of samples were measured using a Perkin Elmer LS-45 fluorescence spectrometer (Bu Kingham Shire, UK). During the assay the similar procedure were performed with mentioned above method. The fluorescence anisotropy of the standard sample was set to 0.206 and blank correction protocol was used for FA of samples.