Magnetic Nanoparticles-Cooperated Fluorescence Sensor for Sensitive and Accurate Detection of DNA Methyltransferase activity Coupled with Exonuclease III-Assisted Target Recycling

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Optimization of assay conditions

Effect of DpnI and SAM concentration

To obtain the best analytical performance, the effects of the concentrations of DpnI and SAM were investigated, respectively. Dpn I endonuclease can only cut the sequence of 5'-G-Am-T-C-3' when the internal adenine is methylated. Therefore, it is important to investigate the effect of Dpn I concentration on the assay. As can be seen from(**Fig. S1A**), with the increasing concentrations of DpnI, the fluorescence intensity increased and tended to a maximum at 10 unit. Thus, 10 unit of DpnI was chosen for the following experiments.

(A)   (B)
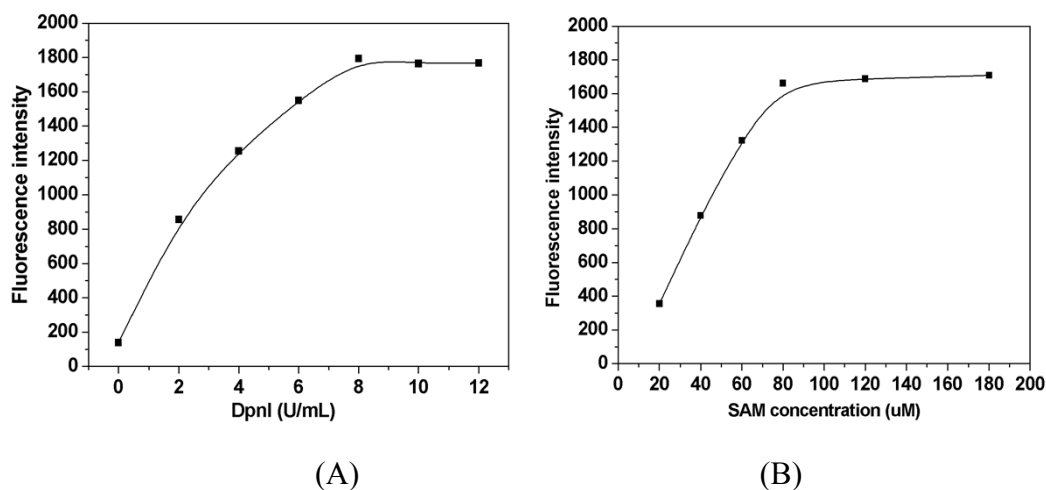
Fig. S1 The effect of (A) DpnI concentration and (B) SAM concentration on the fluorescence response of the sensing system.

As the donor of methyl group, SAM plays an important role in DNA methylation process catalyzed by Dam MTase. The concentration of SAM therefore was optimized and the result is shown in **Fig. S1B**. It could be seen that the fluorescence signal increased gradually as the SAM concentration increased, and then reached an equilibrium value at the concentration of 80 mM. However, considering that SAM is unstable in vitro experiments, a higher concentration of 160 mM was employed for the sensing system.

Effect of molar ratio of oligo 1 to MB, the concentration of oligo 1 and the amounts of Exo III

In the sensing system, the assembly density of duplex DNA probe on the MNPs surface would have an important effect on the performance of fluorescence magneto-nanobiosensor for DNA methyltransferase activity. The duplex DNA hybridization has a significant influence on the assembly density of duplex DNA probe on the MNPs surface. The full hybridization equilibrium between oligo 1 and MB is a key factor for ensuring efficient assembly density of duplex DNA probe on the MNPs surface. Therefore, the effect of molar ratio of oligo 1 and MB was evaluated using a fixed concentration of oligo 1, namely 2 μM. As shown in **Fig. S2A(see supporting information in ESI†)**, the fluorescence intensity increased with the increase in molar ratio. When the ratio value reached 1 : 1.5, the maximum net signal (F-F0) was achieved, where F and F0 are the fluorescence intensities of the biosensing platform in the presence and absence of Dam MTase, respectively. Thereafter, the fluorescence response exhibited a gradual decrease with a further increase in the molar ratio. This was probably because a large excess of MB disturbed their hybridization with oligo 1 in a head-to-tail fashion and the subsequent hybridization efficiency of trigger DNA (tDNA1) from the methylation and cleavage reation of hairpin probe to the duplex DNA probe owing to the steric hindrance effect. As a result, the molar ratio of 1 : 1.5 was selected for further investigation. The hybridization of oligo 1 with MB is a prerequisite for the efficient assembly of duplex DNA probe on the MNPs surface in the fluorescence magneto-nanobiosensor. In addition, oligo 1 also serves as a signal probe for the formation of the fluorescent ZnPPIX/G-quadruplex supramolecular structure after the assembly of duplex DNA probe on the MNPs surface. **Fig. S2B(see supporting information in ESI†)** shows the variance of fluorescence intensity with the concentration of oligo 1. As a result, 2 μM was selected as the optimal concentration due to its maximum net signal (F-F0).

In addition, Exo III-aided DNA recycling amplification was a crucial step. To achieve the best sensing performance of the biosensing system, the amount of Exo III was optimized in the presence of 50 U/mL Dam methyltransferase. As shown in **Fig. S2C (see supporting information in ESI†)**, the fluorescence response to Dam MTase increased quickly with increasing Exo III concentration (the black line).

However, in the absence of Dam MTase, the control group also showed a slight increase in background fluorescence intensity (the red one). As a result, the signal to background ratio (S/N) reached the maximum value at the Exo III amount of 60 U. Thus, 60 U of Exo III was chosen as the optimal experimental conditions, and used in the subsequent experiments.


（A）

（B）

（C）
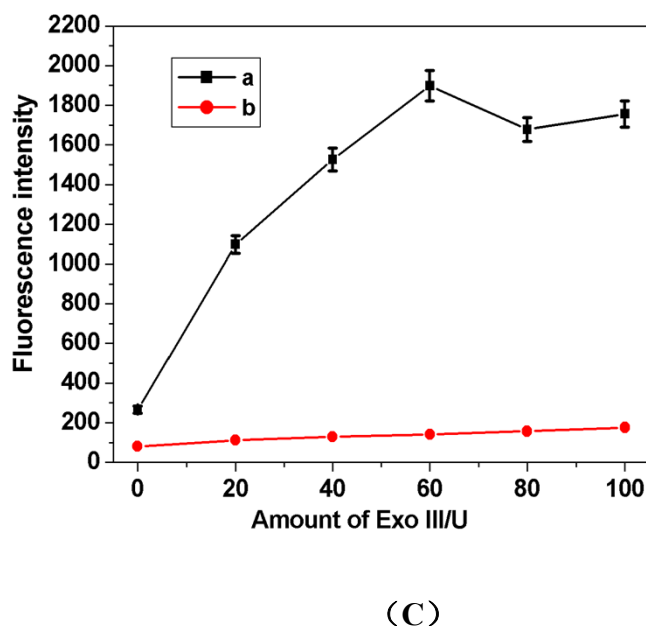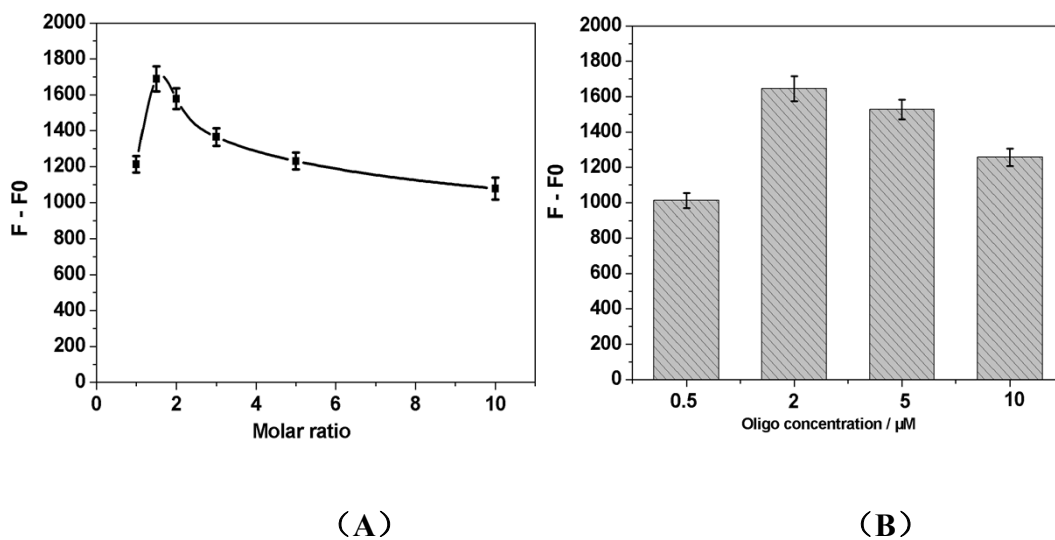
Fig. S2 The effect of (A) molar ratio of oligo 1 to MB, (B) the concentration of oligo 1 and (C) the amounts of Exo III on the fluorescence response of the sensing system.

Effect of the time for DNA methylation and the reaction time of Exo III

The time for DNA methylation is an important factor for the sensing systems. The signal primer for Exo III-assisted recycling amplification reaction derived from the methylation and cleavage reaction. Therefore, the fluorescence intensity of different reaction time were evaluated. It is clear from **Fig. S3A(see supporting information in ESI†)** that the response increases substantially when reaction time changes from 0 to 60min. No significant increase in the sensor response occurred from 60 to 100 min. As a result, the optimal reaction time for the methylation was selected as 60 min in subsequent studies. Finally, 60 min was selected as the optimal reaction time for the methylation due to its maximum net signal (F-F0), where F and F0 are the fluorescence intensities of the biosensing platform in the presence and absence of Dam MTase, respectively.

We further investigated the influence of Eox III reaction time upon the fluorescence signal. As shown in **Fig. S3B (see supporting information in ESI†)**, in the presence of 60 U of Exo III, the net signal (F-F0) reached maximum at the reaction time of 120 min. Therefore, the Eox III reaction time of 120 min is used in the subsequent research.

In the fluorescence magneto-nanobiosensor, the fluorescence signal generated by ZnPPIX/G-quadruplex is dependent on the amount of the ZnPPIX molecule bound to G-quadruplex. To achieve the best sensing performance, the concentration of ZnPPIX was also optimized. As shown in **Fig. S3C (see supporting information in ESI†)**. The experimental results indicated that a concentration of 20 μM ZnPPIX could provide maximum S/N ratio for the sensing system.

(A) **(B)**

(C)
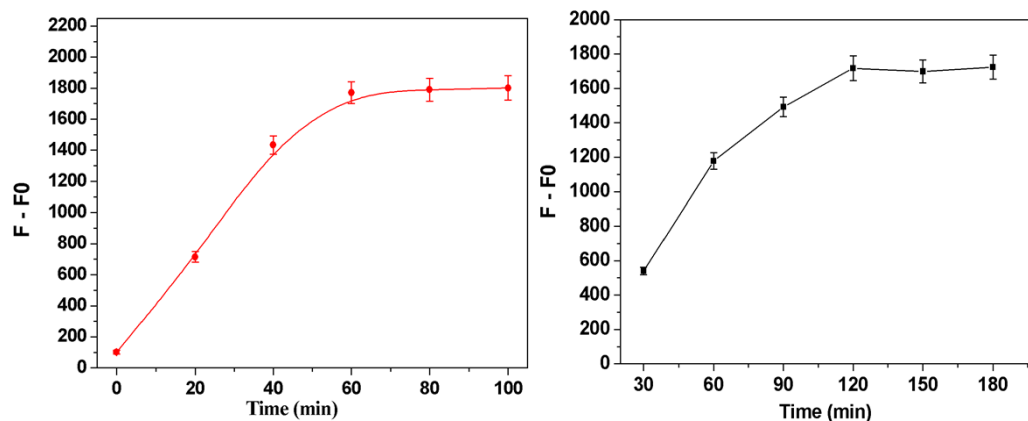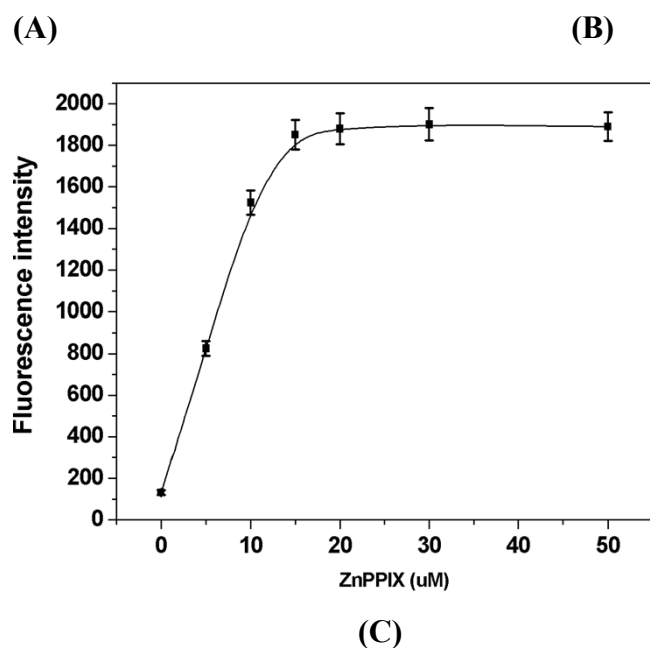
Fig. S3 (A) Variance of the fluorescence intensity with different time for DNA methylation with 50 U / mL Dam MTase, (B) The influence of Eox III reaction time upon the fluorescence signal, (C) Variance of the fluorescence intensity with the concentration of ZnPPIX.
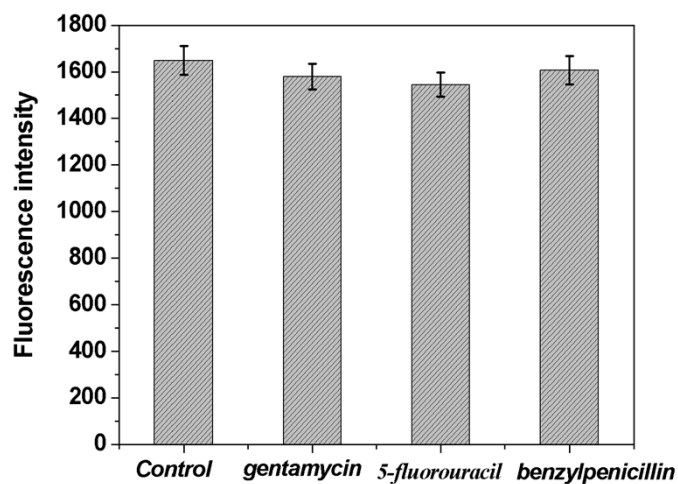
Fig. S4 Influence of different drugs on the activity of both DpnI and Eox III. The concentrations of all the drugs are 1μM.

Table S1. Comparison of LOD of Different Signal Amplification-Based Dam MTase Assays

Method	analyte	LOD	reference
HCR-BRCA[1]	MTase activity	0.52 U/mL	chemiluminescence
PG-RCA[2]	MTase activity	1.29×10^{-4} U/mL	chemiluminescence
SWCNTs signal amplification[3]	MTase activity	0.04 U/mL	electrochemical
AuNPs signal amplification [4]	MTase activity	0.12 U/mL	electrochemical
Nicking enzyme-assisted signal amplification[5]	MTase activity	0.06 U/mL	fluorescence
Carbon nanotube signal amplification[6]	MTase activity	1.0×10^{-4} U/mL	fluorescence polarization
Graphene Oxide signal amplification[7]	MTase activity	0.05 ± 0.02 U/mL	electrochemical
SDA-assisted signal amplification[8]	MTase activity	0.25 U/mL	colorimetric
Exo III assisted signal amplification[9, 10]	MTase activity	0.01-0.04 U/mL	Fluorescence/ electrochemical
This work	MTase activity	2.0×10^{-4} U/mL	fluorescence

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