

## Electronic Supplementary Information

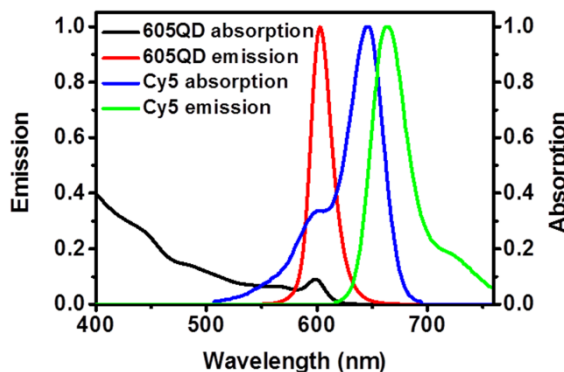
### Construction of single quantum dot nanosensor with the capability of sensing methylcytosine sites for sensitive quantification of methyltransferase

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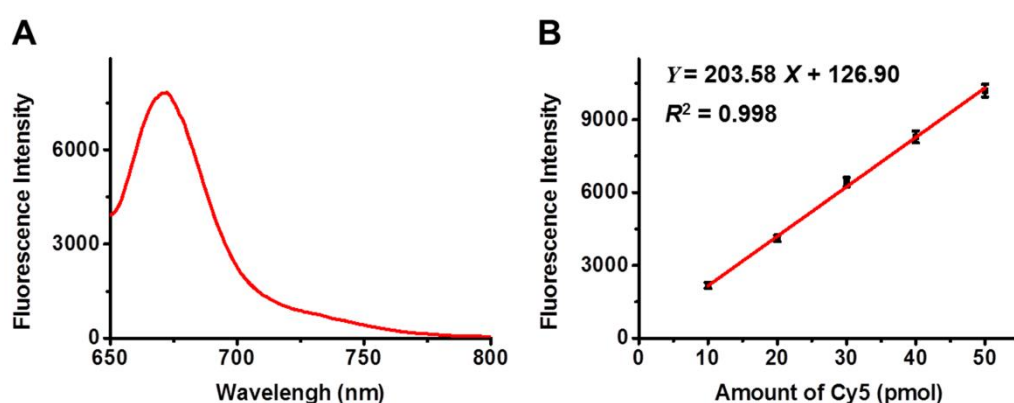


**Fig. S1** Normalized absorption and emission spectra of the 605QD and Cy5. Black line, absorption spectrum of the 605QD; red line, emission spectrum of the 605QD; blue line, absorption spectrum of Cy5; green line, emission spectrum of Cy5.

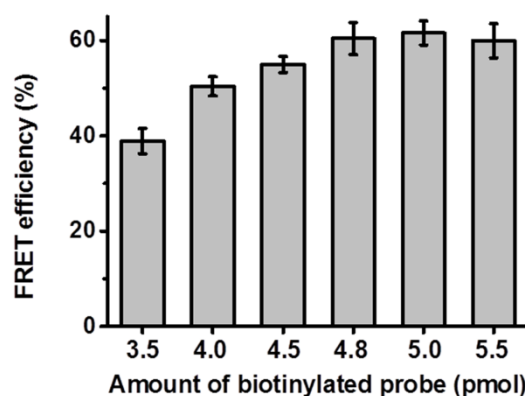
#### Calculation of the number of Cy5 molecules at the end of dsDNA fragment

The number of Cy5 molecules at the end of dsDNA fragment is calculated by using fluorescence emission spectra. After the methylation of M.SssI MTase, Glal endonuclease-mediated cleavage and TdT-assisted polymerization reaction were performed in the presence of biotinylated probes (4.8 pmol), the obtained biotin-/multiple

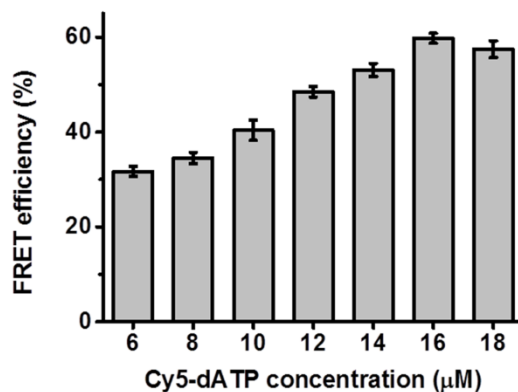
Cy5-labeled dsDNAs were captured by the streptavidin-coated magnetic beads (0.05 mg, Invitrogen Corporation). The free Cy5-dATPs were removed by a magnetic field. Then the Cy5 molecules were released from the magnetic bead-coupled biotin-/multiple Cy5-labeled dsDNAs after the treatment with 20 U of exonuclease III and 10 U of exonuclease I at 37 °C for 1 h. Subsequently, the supernatant solution containing the released Cy5 molecules was added into a cuvette for the measurement of emission spectra at an excitation wavelength of 630 nm (Fig. S2A). The number of Cy5 molecules is estimated to be 37.83 pmol according to the calibration curve in Fig. S2B. The average number of Cy5 molecules at the end of dsDNA fragment is calculated to be 8 according to  $37.83 \text{ pmol} / 4.8 \text{ pmol} = 8$ .



**Fig. S2** (A) Fluorescence emission spectra of the released Cy5 molecules from the streptavidin-coated magnetic beads. (D) Variance of Cy5 fluorescence intensity as a function of the amount of Cy5 molecules (Cy5-dATP).



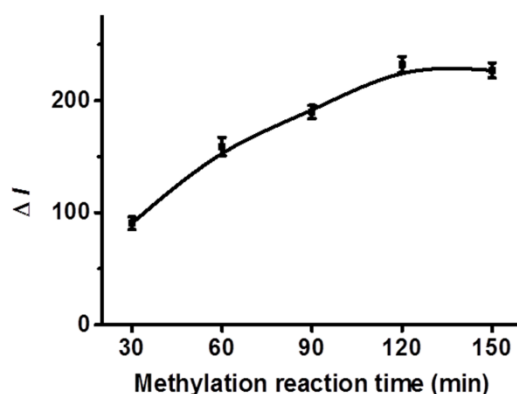
**Fig. S3** Variance of FRET efficiency with the increasing amount of biotinylated probe. Error bars represent the standard deviation of three independent experiments.



**Fig. S4** Variance of FRET efficiency with the increasing concentration of Cy5-dATP. Error bars represent the standard deviation of three independent experiments.

#### Optimization of the methylation reaction time of M.SssI MTase

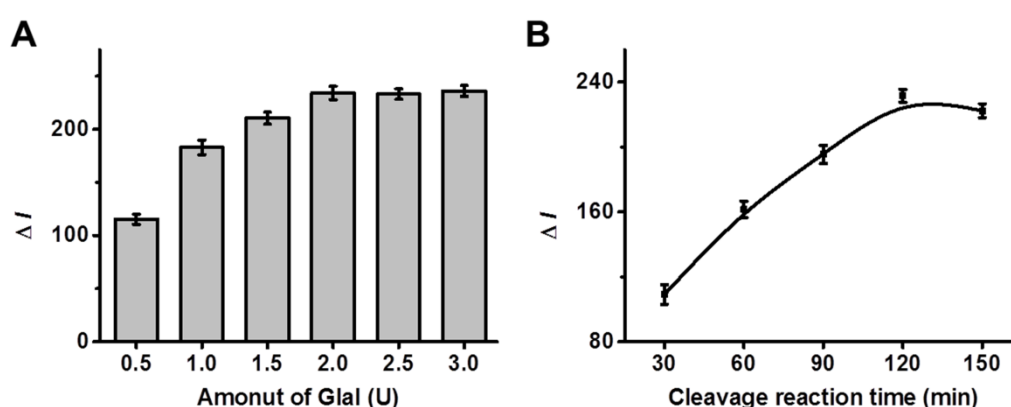
To determine the optimized methylation reaction time of M.SssI MTase, we measured the  $\Delta I$  value with the incubation time from 30 to 150 min (Fig. S5). The  $\Delta I$  value ( $\Delta I = I - I_0$ , where  $I$  and  $I_0$  are the Cy5 fluorescence intensity in the presence and absence of M.SssI MTase, respectively) enhances with the increasing reaction time from 30 to 120 min and reaches a plateau beyond 120 min. Therefore, methylation reaction time of 120 min is used in the subsequent research.



**Fig. S5** Variance of the  $\Delta I$  value with the increasing methylation reaction time of M.SssI MTase.  $\Delta I = I - I_0$ , where  $I$  and  $I_0$  are the Cy5 fluorescence intensity in the presence and absence of M.SssI MTase, respectively. Error bars represent the standard deviation of three independent experiments.

### Optimization of amount of Glal endonuclease and the incubation time of Glal endonuclease-mediated cleavage reaction.

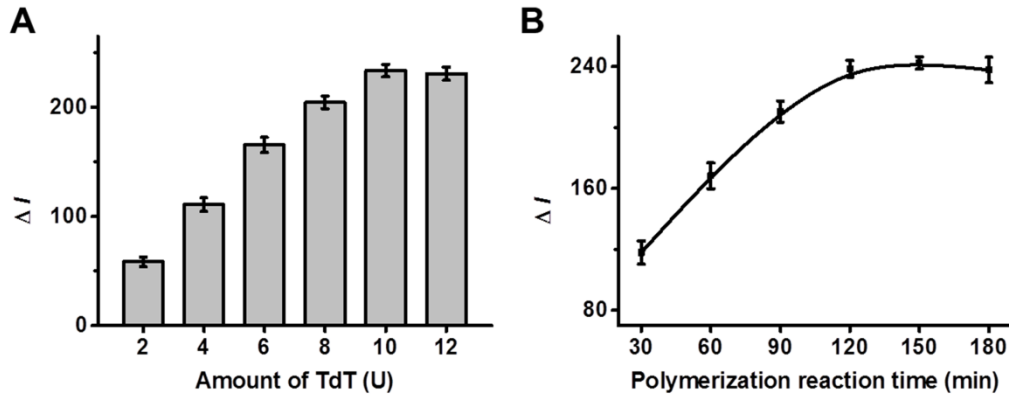
We optimized the enzyme amount of Glal endonuclease. As shown in Fig. S6A, the  $\Delta I$  value enhances with the increasing amount of Glal endonuclease from 0.5 to 2 U, and reaches a plateau at the amount of 2 U. Therefore, 2 U of Glal endonuclease is used in the subsequent research. We further optimized the incubation time of Glal endonuclease-mediated cleavage reaction. The  $\Delta I$  value enhances with the increasing reaction time from 30 to 120 min, and reaches the highest value at 120 min (Fig. S6B). Therefore, the optimal incubation time of Glal endonuclease-mediated cleavage reaction is 120 min.



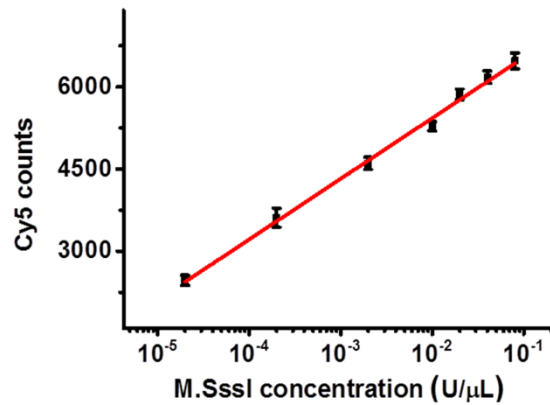
**Fig. S6** (A) Variance of the  $\Delta I$  value with the increasing amount of Glal endonuclease. (B) Variance of the  $\Delta I$  value with the incubation time of Glal endonuclease-mediated cleavage reaction.  $\Delta I = I - I_0$ , where  $I$  and  $I_0$  are the Cy5 fluorescence intensity in the presence and absence of M.SssI MTase, respectively. Error bars represent the standard deviation of three independent experiments.

### Optimization of the TdT-assisted polymerization reaction

We optimized the enzyme amount of TdT. As shown in Fig. S7A, the  $\Delta I$  value enhances with the increasing amount of TdT from 2 to 10 U, and reaches a plateau at the amount of 10 U. Therefore, 10 U of TdT is used in the subsequent research. In addition, we optimized the incubation time of TdT-assisted polymerization reaction. The  $\Delta I$  value enhances with the reaction time from 30 to 120 min, and reaches the highest value at 120 min (Fig. S7B). Thus, polymerization reaction time of 120 min is used in the subsequent research.



**Fig. S7** (A) Variance of the  $\Delta I$  value with the increasing amount of TdT. (B) Variance of the  $\Delta I$  value with the incubation time of TdT-assisted polymerization reaction.  $\Delta I = I - I_0$ , where  $I$  and  $I_0$  are the Cy5 fluorescence intensity in the presence and absence of M.SssI MTase, respectively. Error bars represent the standard deviation of three independent experiments.

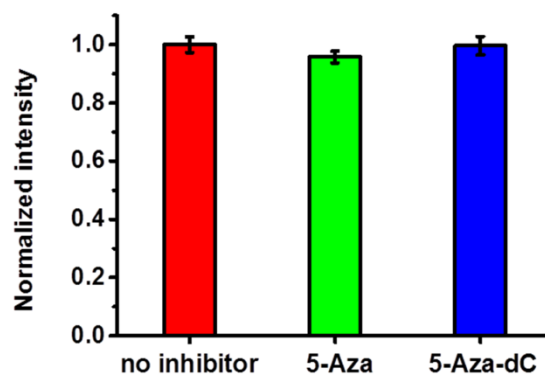


**Fig. S8** Variance of Cy5 counts with different-concentration M.SssI MTase in 10% serum. Error bars represent the standard deviation of three independent experiments.

#### Effect of 5-Aza and 5-Aza-dC upon Glal endonuclease and TdT enzyme

We investigated the effect of 5-Aza and 5-Aza-dC upon Glal endonuclease and TdT enzyme. The 1  $\mu$ M dsDNA containing a 5'-G-mC-G-mC-3'/3'-mC-G-mC-G-5' site was incubated with 0.2 U/ $\mu$ L Glal endonuclease, and 2  $\mu$ M 5-Aza (or 2  $\mu$ M 5-Aza-dC) in 10  $\mu$ L of 1 $\times$  SE-Buffer Y for 2 h at 30  $^{\circ}$ C, followed by inactivation at 80  $^{\circ}$ C for 20 min. Then 10  $\mu$ L of reaction products, 10 U of TdT, 133  $\mu$ M dNTP, and 1 $\times$  TDT reaction buffer were added the reaction solution with a final volume of 30  $\mu$ L at 37  $^{\circ}$ C for 2 h, followed by incubation at 80  $^{\circ}$ C for 10 min. We measured the fluorescence emission spectra of TdT-assisted polymerization products with SYBR Gold as the

fluorescent indicator. As shown in Fig. S9, the fluorescence signal in the presence of 5-Aza (or 5-Aza-dC) shows no significant change compared with that in the absence of any inhibitors. These results demonstrate that neither 5-Aza nor 5-Aza-dC affects the activities of Glal endonuclease and TdT enzyme.



**Fig. S9** Effect of 5-Aza and 5-Aza-dC on Glal endonuclease and TdT enzyme. Red column, in the absence inhibitor; green column, in presence of 2  $\mu$ M 5-Aza; blue column, in presence of 2  $\mu$ M 5-Aza-dC. The concentration of Glal endonuclease is 0.2 U/ $\mu$ L. The amount of TdT enzyme is 10 U.

**Table S1.** Comparison of the proposed method with the reported methods for M.SssI MTase assay

strategy	mechanism for the evaluation of M.SssI MTase	C- or mC-positive assay	linear range	limit of detection	ref.
HRP-IgG-based electrochemical immunosensing	sandwich-type immunoassay	mC-positive	0.5–50 U/mL	0.1 U/mL	1
Ag NPs/carbon nanocubes -based electrochemical immune assay	sandwich-type immunoassay	mC-positive	0.05–120 U/mL	0.03 U/mL	2
strand displacement-based fluorescence assay	enzyme-blocked digestion	C-positive	0.5–80 U/mL	0.019 U/mL	3
enhanced CdS QDs electrochemiluminescence assay	enzyme-blocked digestion	C-positive	1.0–120 U/mL	0.05 U/mL	4
surface plasmon resonance assay	enzyme-blocked digestion	C-positive	0.5–50 U/mL	0.09 U/mL	5
circular dichroism assay based on the assembled AuNP dimers	enzyme-blocked digestion	C-positive	0.5–150 U/mL	0.27 U/mL	6
single QD-based nanosensor	methylcytosine sites cleavage using GluI endonuclease	mC-positive	$2 \times 10^{-3}$ – $2 \times 10^2$ U/mL	$2.1 \times 10^{-4}$ U/mL	this work

## References

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