Electronic Supplementary Information

Single quantum dot-based nanosensor for signal-on detection of DNA methyltransferase

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MATERIALS AND METHODS

Materials. The detection probe 5'-black hole quencher 2 (BHQ2)-ACA GTG ATC ATT GTT TTC AAT GAT CAC TGT-Cy5-TTT TTT-Biotin-3' was purchased from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). Dam Methyltransferase, CpG Methyltransferase (M.SssI), Hhal Methyltransferase, diplococcus pneumoniae G41 restriction enzyme (DpnI), S-adenosylmethionine (SAM), and the corresponding buffer solutions were purchased from New England Biolabs (Ipswich, MA, USA). The Qdot® 605 streptavidin conjugates (605QDs) were obtained from Invitrogen Corporation (California, USA). Gentamycin, bovine serum albumin (BSA) and other chemicals with analytical grade were purchased from Sigma-Aldrich Co (St.

Louis, MO, USA). Ultrapure water obtained from a Millipore filtration system was used throughout in this research.

Detection of DAM MTase and Assembly of 605QD/Probe/Cy5/BHQ2 Nanostructure. The detection of Dam MTase activity involves three consecutive steps. First, all oligonucleotides were diluted with 10× Tris-EDTA buffer to prepare the stock solutions. The hairpin probe substrates were prepared by incubating 1 μM hairpin probe in a buffer containing 50 mM NaCl and 5 mM Tris-HCl (pH 8.0) at 95°C for 4 min, followed by slowly cooling to room temperature. The obtained hairpin probe substrates were stored at 4°C for further use. Second, the methylation and cleavage of hairpin probe were carried out in 50 μL of reaction mixture containing 96 nM hairpin probe, various amounts of Dam MTase, 1× Dam buffer (50 mM Tris-HCl, pH 7.5, 10 mM EDTA, 5 mM 2-mercaptoethanol, 160 μM SAM, 6 U Dpn I and 1 × CutSmart buffer (50 mM KAc, 20 mM Tris-Ac, 10 mM Mg $(Ac)_2$, 100 μg/mL BSA, pH 7.9). The experiment was performed at 37 ℃ for 1 h, followed by inactivation at 80℃ for 20 min. Third, 50 μL of reaction products was incubated with 2 nM 605QDs in 100 μL of incubation buffer (3 mM $MgCl_2$, 100 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, pH 8.0) at room temperature for 10 min to form the 605QD/Probe/Cy5/BHQ2 complexes.

Gel Electrophoresis. The products of methylation and cleavage reaction were analyzed by 12% non-denaturating polyacrylamide gel electrophoresis (PAGE) in 1× TBE buffer (9 mM Tris-HCl, pH 7.9, 9 mM boric acid, 0.2 mM EDTA) at a 110 V constant voltage for 50 min at room temperature.

Single-Molecule Detection and Data Analysis. Before single-molecule detection, the reaction products were diluted 10-fold in imaging buffer (1 mg/mL glucose oxidase, 0.4% (w/v) D-glucose, 0.04% mg/mL catalase, 50 μg/mL BSA, 67 mM glycine-KOH, 1 mg/mL trolox, 2.5 mM $MgCl_2$, pH 9.4). For TIRF imaging, 10 μL of sample was directly pipetted to the coverslip. A sapphire 488 nm laser (50 mW, Coherent, USA) was used to excite the 605QDs. The photons from the 605QD and Cy5 were collected by a 100× objective (Olympus, Japan) and imaged with an exposure time of 500 ns by an Andor Ixon DU897 EMCCD. A region of interest (200 × 400 pixels) of the images was selected for Cy5 molecule counting by using image J software.

Selectivity and Inhibition Assay. The M.SssI MTase, HhaI MTase and BSA were used to investigate the selectivity of the proposed method. The 40 U/mL interference enzyme and 20 nM BSA were used in the experiments with same procedure for Dam activity assay. To study the inhibition effect of gentamycin, gentamycin at various concentrations was incubated in the solution containing 1× Dam buffer (50 mM Tris-HCl, pH 7.5, 10 mM EDTA, 5 mM 2-mercaptoethanol) and 96 nM hairpin probe substrate at 37 °C for 15 min. Then 40 U/mL Dam MTase, 6 U DpnI and 160 μM SAM were added into the solution, followed by incubation at 37 °C for 1 h and inactivation at 80°C for 20 min. The Dam activity was measured using same procedure described above. The relative enzyme activity is quantitatively determined on the basis of eq. 1.

$$\text{Relative activity } (\%) = \frac{N_i - N_0}{N_t - N_0} \times 100\% \qquad (1)$$

where N_0, N_t and N_i represent the Cy5 counts in the absence of Dam, in the presence of Dam, and in the presence of both Dam and gentamycin, respectively.

Detection of DAM MTase in Serum Sample. A total volume of 50 μL of sample mixture containing 5% human serum sample spiked with various-concentration Dam, 96 nM hairpin probe, 160 μM SAM, 6 U DpnI, 1× CutSmart buffer (50 mM KAc, 20 mM Tris-Ac, 10 mM $Mg(Ac)_2$, 100 μg/mL BSA, pH 7.9) were incubated at 37°C for 1 h, followed by inactivation at 80°C for 20 min. The Dam activity was measured using same procedure described above.

SUPPLEMENTARY RESULTS

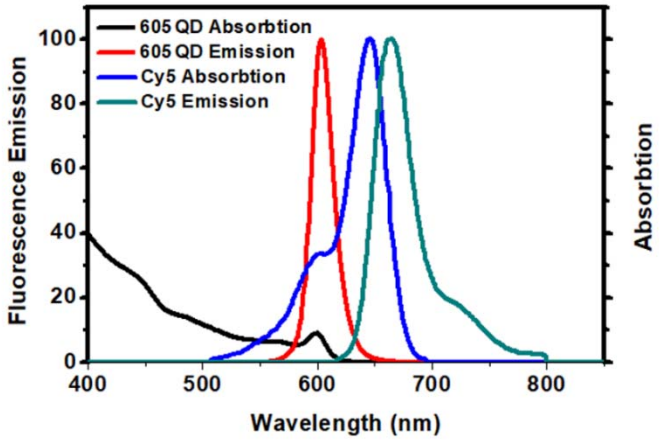

Fig. S1 Normalized absorption and emission spectra of the 605QD and Cy5. Black line, absorption spectrum of the 605QD; red line, emission spectrum of the 605QD; blue line, absorption spectrum of Cy5; green line, emission spectrum of Cy5.

We verified by PAGE analysis with SYBR Gold as the indicator. As shown in Fig. S2, a distinct band is observed in the presence of detection probe (lane 1), and no change in band intensity is observed in the presence of either DpnI (lane 2) or Dam (lane 3), indicating no cleavage reaction occurs. In contrast, the decrease in band intensity of detection probe is observed when both Dam and DpnI are present (lane 4), indicating the cleavage of the detection probes. In

principle, three cleavage fragments should be observed after the reaction, however, only a single cleavage fragment is observed in Fig. S2, which may be ascribed to that the other two cleavage fragments are too short to be stained by SYBR Gold.

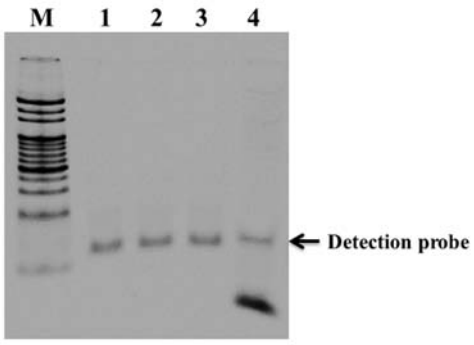

Fig. S2 PAGE analysis of reaction products with SYBR Gold as the indicator. Lane M, marker; Lane 1, 1 μM probe; Lane 2, 1 μM M probe + 6 U DpnI; Lane 3, 1 μM probe + 20 U Dam; 1 μM probe + 6 U DpnI + 20 U Dam.

Fluorescence Spectra Analysis. To further verify the proposed assay, we measured the fluorescence spectra at the excitation wavelength of 488 nm. As shown in Fig. S3, in the presence of either DpnI (blue line) or Dam (purple line), no Cy5 emission (667 nm) is observed, indicating the Cy5 is completely quenched in the 605QD/probe/Cy5/BHQ2 complex. In contrast, in the presence of both Dam and DpnI (red line), significant Cy5 emission is observed, indicating the cleavage of Dam methylation products by DpnI and the recovery of Cy5 emission.

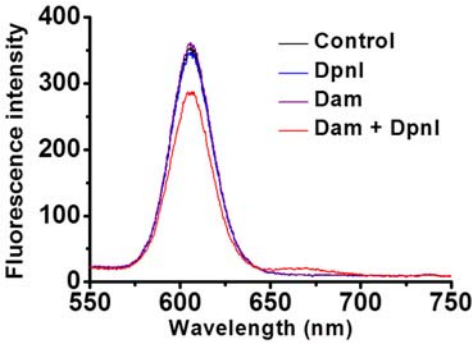

Fig. S3 Fluorescence spectra in response to DpnI, Dam, and the coexistence of DpnI and Dam. The Dam concentration is 20 U/mL, and the amount of DpnI is 6 U. The sample without any enzyme treatment is used as the control.

Optimization of Experimental Conditions. To optimize the reaction time, we measured the Cy5 counts as a function of reaction time in the presence or in the absence of Dam, respectively. As shown in Fig. S4A, no obvious Cy5 signal is detected in the absence of Dam (Fig. S4A, dark line). While in the presence of Dam, the Cy5 counts increase with the reaction time (Fig. S4A, red line), and reach a plateau at 60 min due to either the consumption of all detection probes or the complete loss of Dam activity. To minimize the analysis time, 60 min is used in the subsequent experiments.

We further optimize the DpnI concentration. As shown in Fig. S4B, the Cy5 counts increase with the increasing DpnI amount from 1 to 12 U, and reach a plateau at 6 U due to the complete cleavage of the methylation products by DpnI. To minimize the enzyme consumption, 6 U DpnI is used in the subsequent experiments.

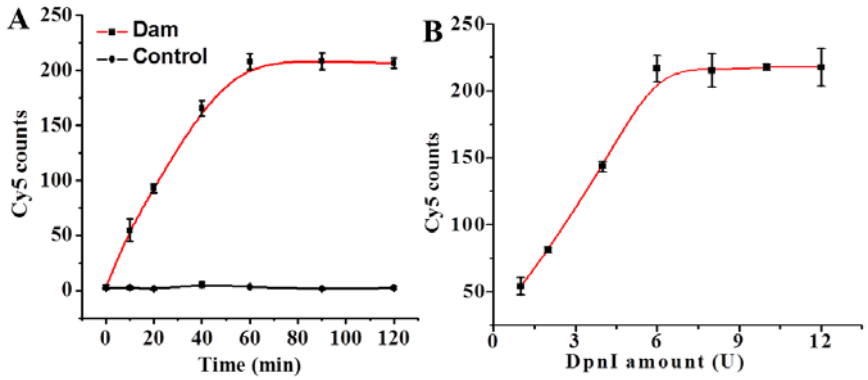

Fig. S4 (A) Variance of Cy5 counts in response to 40 U/mL Dam and the control group without Dam. (B) Variance of Cy5 counts in response to various amounts of DpnI. Error bars show the standard deviations of three experiments.

In QD-based FRET assay, a single QD can accommodate multiple acceptors to improve the FRET efficiency. To achieve the best performance of the proposed assay, the ratio of Cy5-labeled substrate to the 605QD is optimized. Different amount of Cy5-labeled substrates was mixed with a fixed amount of 605QDs, and the fluorescence intensities of both the 605QDs and Cy5 at the excitation wavelength of 488 nm were measured. The FRET efficiency is caudated based on $E = 1 - \frac{F_{DA}}{F_D}$, where F_{DA} is the 605QD fluorescence intensity in the presence of Cy5 acceptors and F_D is the 605QD fluorescence intensity in absence of Cy5 acceptors. The obtained Cy5 fluorescence intensity and FRET efficiency (%) were plotted against the molar ratio of Cy5-labeled substrate to the 605QD. As shown in Fig. S5, the FRET efficiency enhances with the increasing ratio of Cy5-labeled substrate to the 605QD. The maximum Cy5 fluorescence intensity is obtained at the Cy5-labeled substrate-to-605QD ratio of 24:1, followed by the decrease of Cy5 signal beyond the molar ratio of 24:1 due to the inner-filter effect caused by the high local concentration of Cy5.[1] Therefore, the Cy5-labeled Cy5-labeled substrate-to-605QD ratio of 24/1 is used in this research.

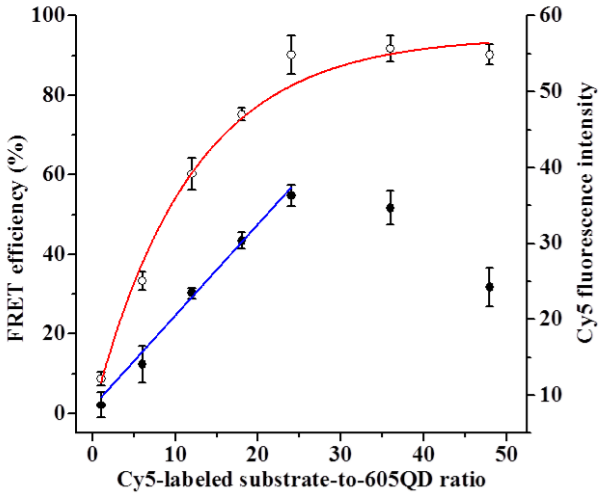

Fig. S5 Variance of FRET efficiency (red color) and Cy5 fluorescence intensity (blue color) as a function of Cy5-labeled substrate-to-605QD ratio. Error bars show the standard deviation of three experiments

Comparison with the QD-free Assay. We investigated the performance of the assay without the QDs. As shown in Fig. S6, more Cy5 molecules (red dots) are observed in the presence of Dam. In contrast, few Cy5 molecules are detected in the absence of Dam. Notably, the Cy5 counts in the control group of QD-free assay are much more than that in the control group of proposed QD-based assay (Fig. 2), suggesting a lower signal-to-noise ratio (SNR) in the QD-free assay. In addition, the SNR is calculated to be 7.59 for the QD-free assay, and 34.17 for the proposed QD-based assay. Thus, the QD-based assay exhibits much higher SNR.

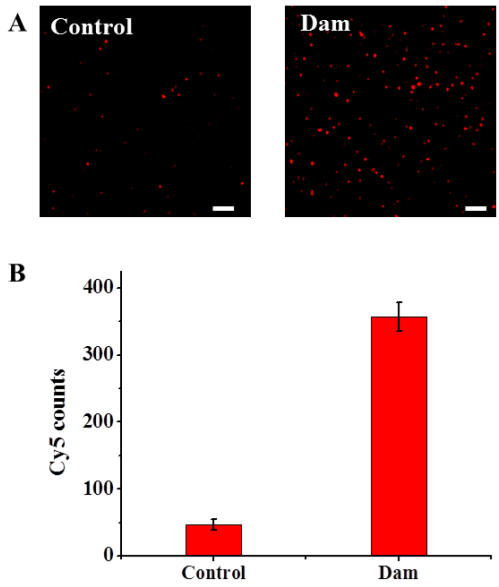

Fig. S6 (A) TIRF images of Cy5 emission in the absence (Control) and in the presence of Dam. The scale bar is 0.5 μm. (B) The Cy5 counts obtained in the absence (Control) and in the presence of Dam. The Dam concentration is 40 U/mL, and the amount of DpnI is 6 U. Error bars show the standard deviations of three experiments.

As shown in Fig. S7, in QD-free assay, the Cy5 counts exhibit a linear correlation with the logarithm of Dam concentration from 0.01 to 2 U/mL. The regression equation is $N = 118.89 + 32.15 \log_{10} C$, where N is the Cy5 counts and C is the Dam concentration (U/mL). The detection limit is calculated to be 0.008 U/mL. Notably, the detection limit of this QD-free assay cannot compare with that of the proposed QD-based assay (0.002 U/mL, Fig. 3).

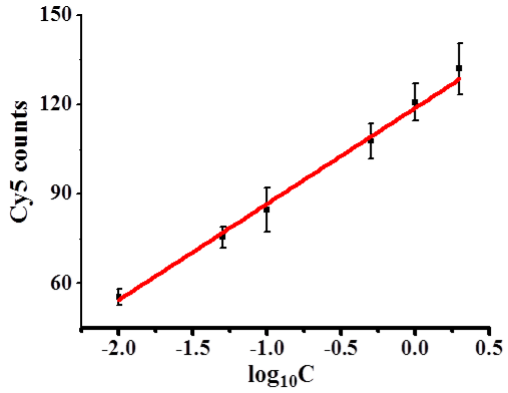

Fig. S7 Linear correlation between Cy5 counts and the logarithm of Dam concentration. The DpnI concentration is 6 U. Error bars show the standard deviations of three experiments.

We also investigated the selectivity of the QD-free assay. As shown in Fig. S8, higher Cy5 signal is overserved in the presence of Dam. In contrast, lower Cy5 signal is detected in the presence of HhaI, M. SssI and BSA. The discrimination ratio is 5.66 between Dam and HhaI, 6.69 between Dam and M.SssI, and 7.51 between Dam and BSA, respectively. In contrast, in the proposed QD-based assay, the discrimination ratio is 29.28 between Dam and HhaI, 22.78 between Dam and M.SssI and 27.69 between Dam and BSA, respectively (Fig. 4), much higher than those of QD-free assay.

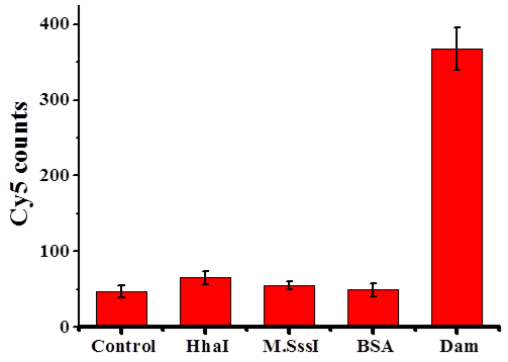

Fig. S8 Measurement of Cy5 counts in the presence of HhaI (40 U/mL), M. SssI (40 U/mL), Dam (40 U/mL), BSA (100 nM). The amount of DpnI is 6 U. The sample without any enzyme treatment is used as the control. Error bars show the standard deviations of three experiments.

In summary, although the proposed assay can be carried out without the QDs, it is accompanied by higher background signal and lower SNR (Fig. S6), leading to lower detection sensitivity (Fig. S7) and poorer selectivity (Fig. S8).

Table S1. Recovery studies in spiked human serum samples.

Added (U/mL)	Measured (U/mL)	Recovery (%)	RSD (%)
80	78.85	98.55	1.36
40	40.59	101.45	1.36
20	19.94	99.72	2.30
10	10.70	102.27	1.04
5	4.79	98.77	2.62

Reference

1. P. A. Porta and H. D. Summers, *J. Biomed. Opt.*, 2005, **10**, 034001-0340018.