Supporting Information

Highly sensitive detection of DNA methyltransferase activity and its inhibitor screening by coupling fluorescence correlation spectroscopy with polystyrene polymer dots

Yuyang Huang, Liyun Deng, Di Su, Xiangyi Huang*, Jicun Ren*

School of Chemistry & Chemical Engineering, State Key Laboratory of Metal Matrix Composites, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai, 200240, P. R. China.

Email: huangxy@sjtu.edu.cn, jicunren@sjtu.edu.cn

Conjugation of PS Pdots and streptavidin.

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) was used as an activation reagent for carboxyl groups in amide synthesis. In the conjugation reaction, 20 μL of PEG (Mw = 2000, 5% w/v), 20 μL of 1.0 M HEPES (pH = 6.5) and 6 μL 10 mg mL^{-1} freshly prepared EDC solutions were added to 1 mL PS Pdots solution (50 μg/mL), then mixed thoroughly on a vortex mixer and placed for 5 minutes. After that 60 μL 1 mg/mL streptavidin solution was added to the mixed solution and incubated for 4 h at room temperature. Finally, the streptavidin conjugated PS Pdots (SA-PS Pdots) were separated from free streptavidin molecules through a centrifugal filtration device (Amicon Ultra-4, MWCO 100 KDa) at 3200 rpm for 4 min.

TEM and DLS measurements

For the TEM measurements, 30 μL of the nanoparticle dispersion was placed on a cleaved carbon support film. Repeat the process five times after evaporation of the water. After air drying overnight, the physical size was imaged with JEM-2100HR TEM. For the DLS measurements, 1 mL of the nanoparticle dispersion was used for hydrodynamic size with sizer Nano ZS92."

FCS detection and data analysis.

The FCS setup is shown in Figure S1. The samples of conjugation, DNA methylation, and enzyme digestion products were measured by FCS. The 488 nm laser power is about 75 μW. Every single detection time was 30 s and repeated five times.

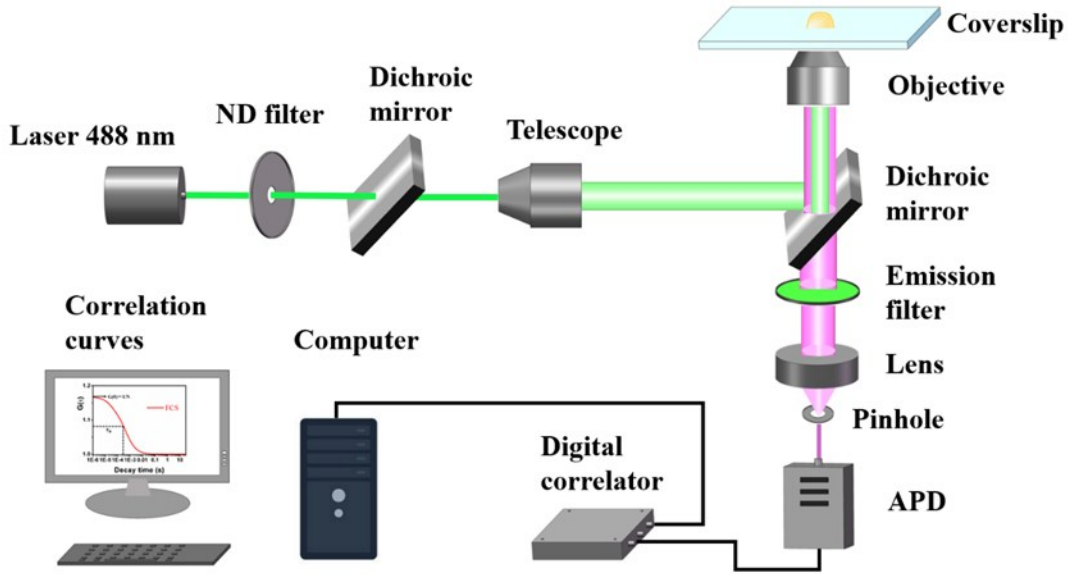

Fig. S1 FCS system combined with the detection sample on the coverslip.

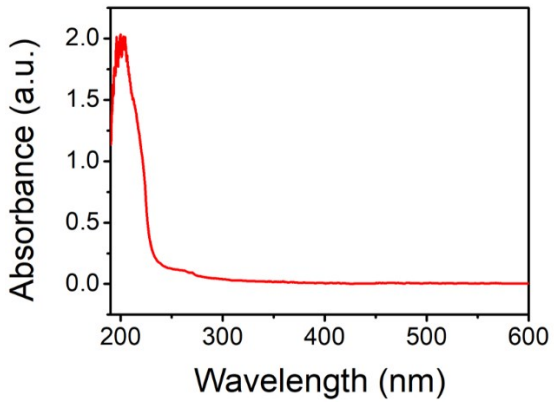

Fig. S2 UV-vis spectra of PS Pdots.

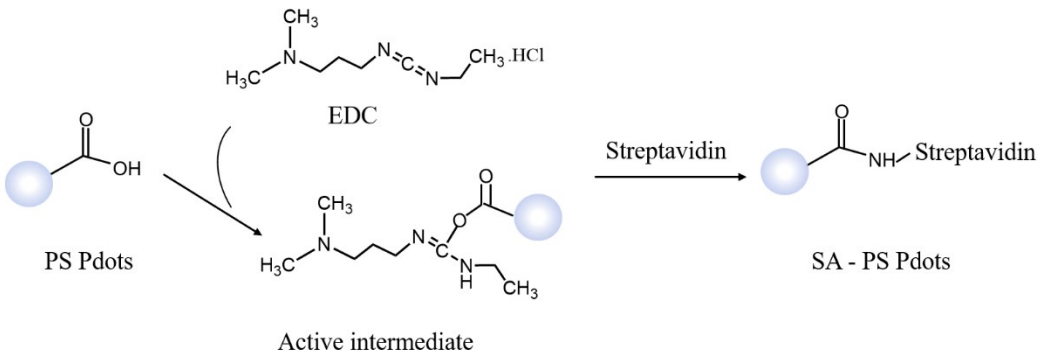

Fig. S3 Schematic of the conjugation of PS Pdots and streptavidin

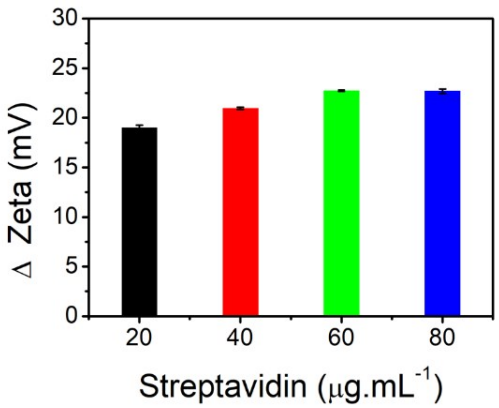

Fig. S4 Δ Zeta of PS Pdots and streptavidin-PS Pdot conjugates when used different concentration of streptavidin. (Δ Zeta = $Zeta_{conjugates}$ - $Zeta_{PS\ Pdots}$)

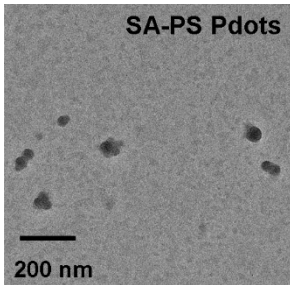

Fig. S5 TEM image of streptavidin-PS Pdots.

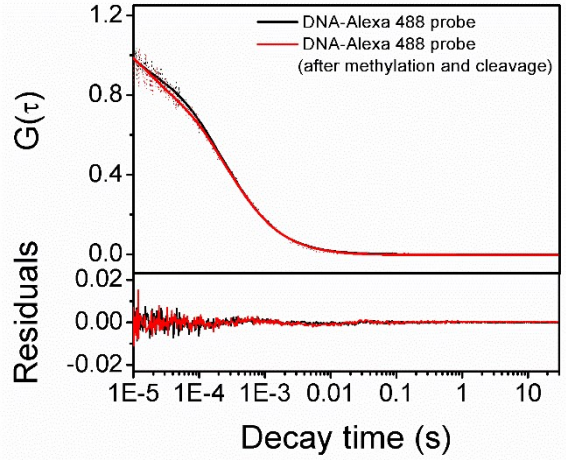

Fig. S6 Normalized FCS curves and fitting residuals of DNA-Alexa 488 probe (black) and DNA-Alexa 488 probe after methylated and cleaved (red), respectively.

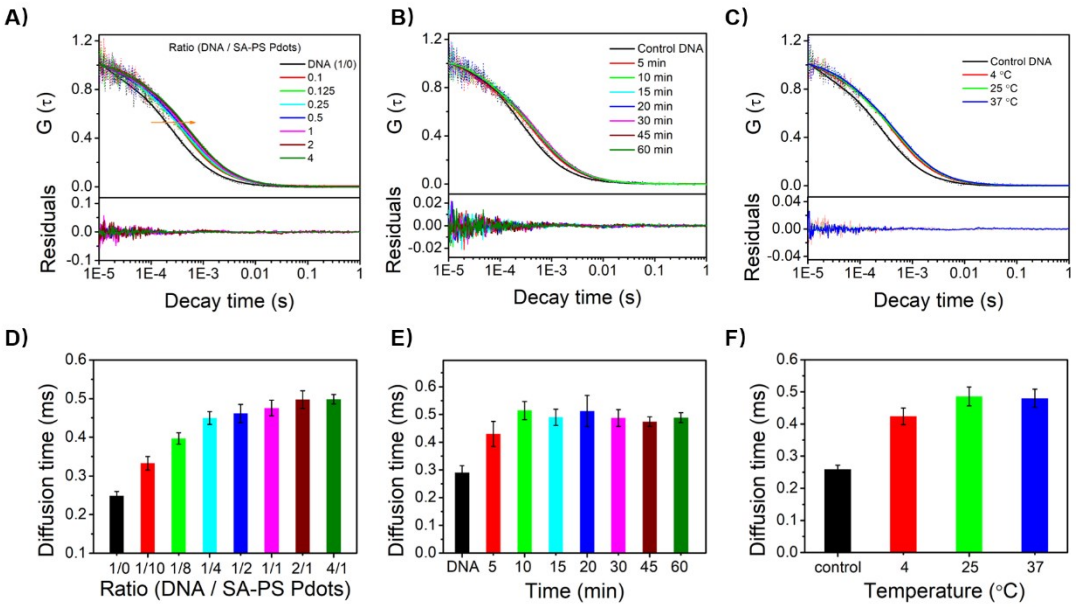

Fig. S7 Optimization of the reaction conditions between biotin-DNA and SA-PS Pdots. (A) Normalized FCS curves and fitting residuals of probes under different reaction ratios of biotin-DNA and SA-PS Pdots. (B) Normalized FCS curves and fitting residuals of probes under different binding time. (C) Normalized FCS curves and fitting residuals of probes under different reaction temperature. (D) Comparison of the characteristic diffusion times (τ_D) of probes under different reaction ratios of biotin-DNA and SA-PS Pdots. (E) Comparison of the characteristic diffusion times (τ_D) of probes under different binding time. (F) Comparison of the characteristic diffusion times (τ_D) of probes under different reaction temperature. The concentration of biotin-DNA was 5 nM. The FCS measurement time was 30 s. Error bars stand for the standard deviation of three experiments.

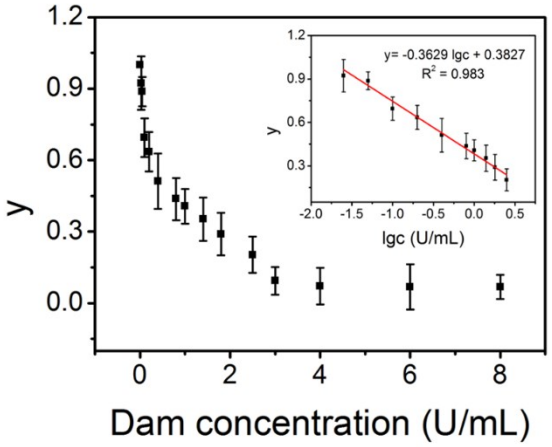

Fig. S8 The relationship of y and the Dam MTase concentrations. y is the relative concentration fraction of the unmethylated PS Pdots-streptavidin-biotin-DNA-Alexa 488 complex to the initial total DNA-Alexa 488 probe concentration.