Electronic Supporting information (ESI†)

for

Label-free Colorimetric Assay for DNA Methylation Based on Unmodified Gold Nanorods as a Signal Sensing Probe Coupled with Enzyme-Linkage Reactions

Xiang-Juan Zheng, Jian-Ding Qiu,* Li Zhang, Zhong-Xia Wang, Ru-Ping Liang

Department of Chemistry, Nanchang University, 330031, Nanchang, China

Experimental section

Reagents and Materials.

Dam MTase, Dpn I endonuclease, SAM, NT.ALWI nicking endonuclease, lambda exonuclease, and the corresponding buffer solution were purchased from New England Biolabs. Ltd (Beijing, China). The oligonucleotides (P1-P4) were synthesized and purified by Invitrogen Co. Ltd (Shanghai, China). The methylated oligonucleotides (P5-P6) and DNA polymerase I were purchased from Takara Biotechnoly Co. Ltd (DaLian, China). Tetrachloroauric acid trihydrate ($HAuCl_4 \cdot 3H_2O$), cetyltrimethylammonium bromide (CTAB), L-ascorbic acid and sodium borohydride ($NaBH_4$) were commercially available from Shanghai Chemical Reagent Co (Shanghai, China). All other chemicals were of analytical grade and used without further purification. The ultrapure water with an electrical resistance larger than 18.2 MΩ was used for all the experiments.

The oligonucleotides sequences used in this work were as follows:

Probe 1 (P1): 5′—GTTGG***GATC***GAGAAG—3′

Probe 2 (P2): 5′—CTTCTC***GATC***CCAAC—3′

Probe 3 (P3): 5′—GTTGG***CTAG***GAGAAG—3′

Probe 4 (P4): 5′—CTTCTC***CTAG***CCA AC—3′

Probe 5 (P5): 5′—GTTGG***GAmTC***GAGAAG—3′

Probe 6 (P6): 5′—CTTCTC***GAmTC***CCAAC—3′

Preparation of Au-NRs.

The Au-NRs were synthesized in aqueous solution. Briefly, gold seeds were prepared at first by mixing $HAuCl_4 \cdot 3H_2O$ (2.0×10^{-3} M) with freshly prepared ice-cold $NaBH_4$ (1.0×10^{-2} M) in the presence of CTAB (0.2 M). With vigorous shaking (A QL-901 vortex mixer, Haimen, China) for 30 s, the mixture solution displayed as light brown. After being standing for 2 h at 25 °C, the gold seeds were ready (about 5nm). Then, 2.0×10^{-3} M $HAuCl_4$, 0.2 M CTAB, 0.01 M $AgNO_3$ and 0.1 M freshly prepared ascorbic acid were injected into a clean test tube, the mixture was shaken vigorously for about 2 min. Finally, 110 μL of the above-prepared gold-seed suspension was added. After being shaken vigorously for 30 s, the mixture was placed in water bath for 24 h at 30 °C, Au-NRs was obtained. Scanning electron microscope (SEM) (Quanta 200, FEI, Holland), and plasmon resonance absorption (PRA) spectra (UV-2450, Shimadzu, Japan) were used to characterize the Au-NRs (Fig. S1). ζ potential measurement was used to monitor the surface potential of the Au-NRs with a Zetasizer Nano ZS90 (Malvern Instruments, England). Then, Au-NRs were stored at 4 °C before use. The average aspect ratio of such prepared Au-NRs is about 3 as inferred from SEM images, the concentration of Au-NRs synthesized was estimated by the measured absorbance and corresponding molar extinction coefficient ($4.1 \pm 0.5 \times 10^9$ M^{-1} cm^{-1}) at wavelength maxima of the longitudinal absorption band [1].

Assay of Dam Activity and Extent of DNA Methylation.

A series of standard Dam MTase solutions were prepared from 0.2 to 80.0 U mL^{-1}. To protect the activity of Dam MTase, all the standard solutions were prepared at 4 °C and stored under −20 °C. The methylation experiment was performed in 20 μL of methylase buffer (50mM Tris-HCl pH 7.6, 50 mM NaCl, 10 mM EDTA, 1 mM DTT) containing 1 nM probe dsDNA, 80 μM SAM, 80 U mL^{-1} Dpn I endonuclease, and various amounts of Dam MTase, The reaction mixture was incubated at 37 °C for 5 h. After the methylation by Dam and followed digestion reaction by Dpn I, 80 μL of

as-prepared CTAB-coated Au-NRs suspension was added, the mixture was shaken vigorously on the vortexed about 1 min, the PRA spectrum was monitored using a UV-2450 spectrometer. DNA samples with different extent of DNA methylation were prepared by mixing different concentrations of methylated dsDNA and unmethylated dsDNA. After incubation of the samples with 80 U mL^{-1} Dpn I endonuclease for 5 h at 37 °C, 80 μL of as-prepared CTAB-coated Au-NRs suspension was added, the mixture was shaken vigorously on the vortex about 1 min, the PRA spectrum was monitored using a UV-2450 spectrometer.

Influence of Some Drugs on DNA Methylation.

To study whether or not drugs have influence on Dpn I, a control experiment was carried out. First, an absolutely methylated DNA probe was prepared by putting Dam MTase, SAM, and the DNA probe together for 12 h and subsequently elevated temperatures to 65 °C for 15 min. Then 1 μM drug was added into the solution as well as Dpn I respectively. The influence of drugs on activity of Dam MTase was similar to that described above except that drugs were added together with Dam MTase.

Methylation Assay by Circular Dichroism.

Circular dichroism (CD) spectra of DNA methylation product were recorded with CD spectrophotometer (Mos-450, Bio-logic, France). For measurements in the far-UV region (200–300 nm), a quartz cell with a path length of 1 mm was used for sampling. Three scans with a scan speed of 50 nm/min were performed and averaged. Sample temperature was maintained at 25 °C using a water bath. A spectrum of buffer solution was taken and subtracted from the spectra of DNA methylation product.

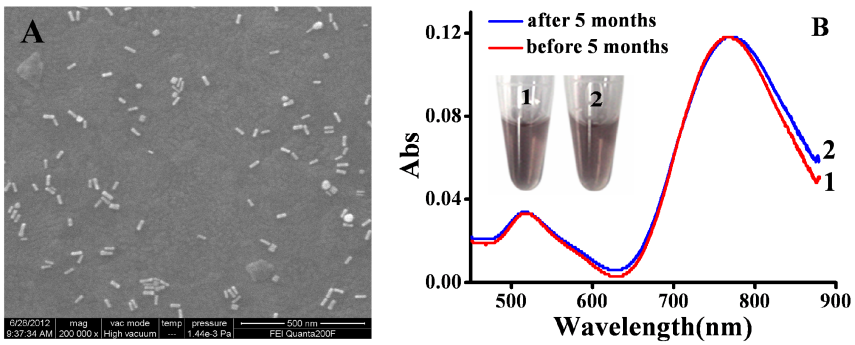
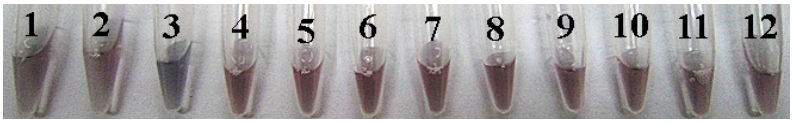


Fig.S1 (A) SEM image of the Au-NRs. (B) PRA spectra of the Au-NRs as-prepared and held on for 5 months.

Table S1 Typical photograph of different external situation to the state of Au-NRs (Nos. 1-12). Various reagents are listed in the column. √ represents the corresponding reagent that was added to each bottle.



	1	2	3	4	5	6	7	8	9	10	11	12
Au-NRs	√	√	√	√	√	√	√	√	√	√	√	√
ssDNA (P1)	√											
ssDNA (P2)		√										
dsDNA(P1+P2)			√									
Tris-HCl,NaCl	√	√	√									
Dam				√		√						
SAM				√		√						
Dam buffer				√		√						
DpnI					√	√						
DpnI buffer					√	√						
DNA polymerase I							√	√				
DNA polymerase I buffer								√				
NT.ALWI									√	√		
NT.ALWI buffer										√		
Lambda Exonuclease											√	
Lambda Exonuclease buffer												√

Measurement of ζ Potential.

To prove that negatively charged ssDNA and dsDNA can bind to CTAB-coated Au-NRs and dsDNA has higher density of surface charge than ssDNA, ζ potential of the Au-NRs solutions mixed with DNA were measured. The charge density of CTAB-coated Au-NRs ζ potential is about +13.21 mV. The Au-NRs ζ potential was slightly reduced upon +11.34 mV when interaction with negative charged ssDNA fragments, but interaction with dsDNA, the Au-NRs ζ potential indeed greatly reduced to +8.17 mV. The larger degree of reduction with dsDNA coating (38.15%) than ssDNA (14.16%) indicates that the dsDNA contributes more negative charges to neutralize the positive charges.

Optimization of Experimental Conditions.

To investigate the effect of the concentration of dsDNA, Dam, SAM, and Dpn I were mixed with different concentrations of probe dsDNA ranging from 0 to 300 nM. The

variation of absorbance intensities (ΔA) with and without Dam was used to assess the sensing performance. It can be seen from Fig. S2A that ΔA gradually increases with increasing concentrations of probe dsDNA ranging from 0 to 1 nM, when the dsDNA concentration increases to 1 nM, ΔA reached the maximum. Meanwhile, it can be observed that the background signal reduced rapidly with increasing the concentration of dsDNA. The results indicated that the optimum concentration of probe dsDNA was 1 nM due to its best signal-to-noise level. To investigate the effect of the concentration of Dpn I, Dam, SAM, and dsDNA were mixed with different concentrations of Dpn I ranging from 0 to 150 U mL^{-1}. It can be seen from Fig. S2B that ΔA increases with increasing concentrations of Dpn I ranging from 0 to 80 U mL^{-1}, implying enhanced cleavage of the methylated dsDNA. Meanwhile, the background signal increased slightly with increasing the concentration of Dpn I. Therefore, for the best signal-to-noised level, 80 U mL^{-1} Dpn I was selected for the cleavage reaction in this work. Furthermore, the highest sensitivity was obtained in 80 μM SAM (Fig. S2C) after incubating for 5 h (Fig. S2D).

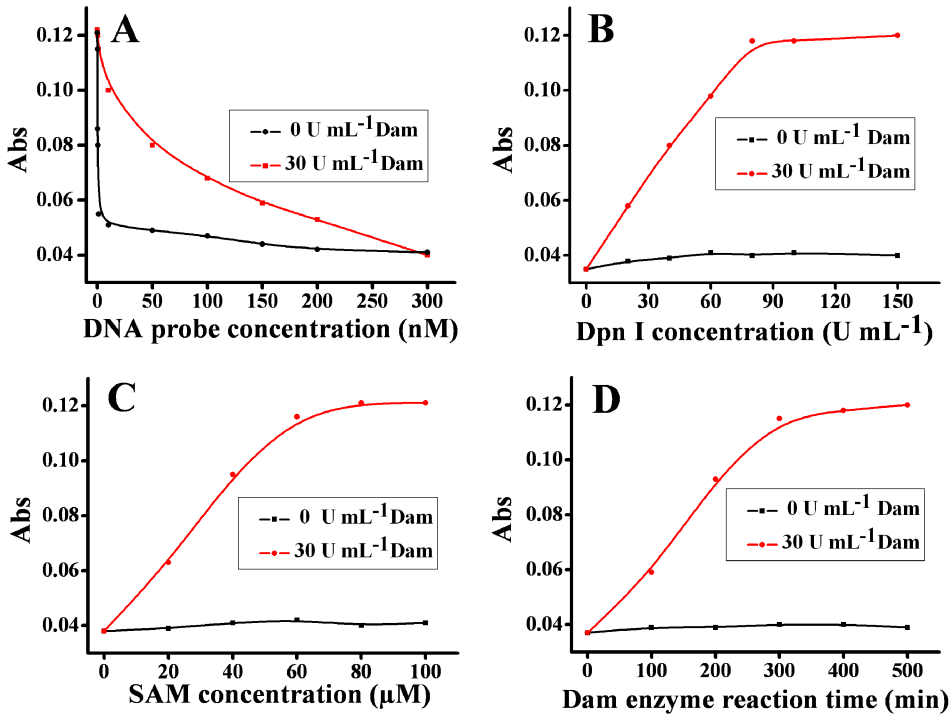


Fig.S2 Optimization of experimental conditions. (A) DNA probe concentration. (B) Effect of Dpn I concentration. (C) Effect of SAM concentration. (D) Effect of enzyme-linked reaction time.

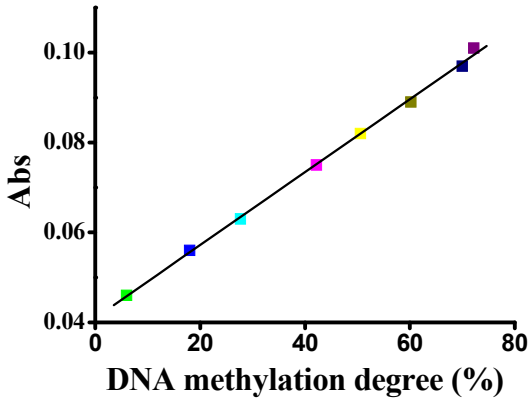


Fig.S3 Dependence of the PRA intensity of Au-NRs on DNA methylation degree.

Table S2 Analysis of DNA samples with different extent of DNA methylation.

DNA concentration (nM)	Extent of methylation (%)	Tested (%)	Relative error (%)
1	20	20.6	3.2
1	30	31.2	4.1
1	40	41.5	3.8
1	50	47.7	-4.6

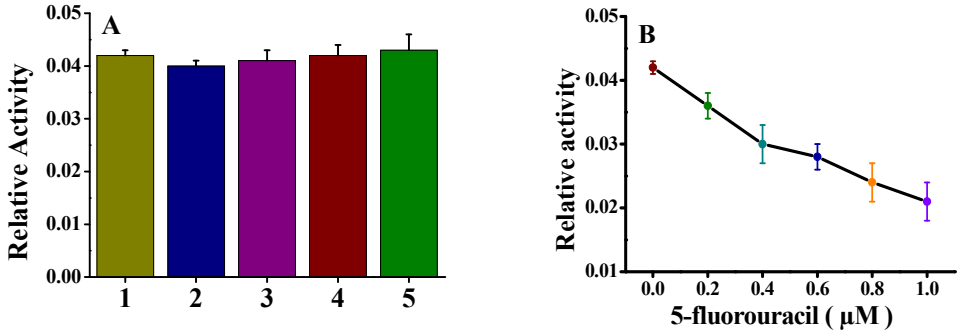


Fig.S 4 (A) Influence of different drugs on the activity of Dpn I. (1) no drug, (2) 5-fluorouracil, (3) platinol, (4) mitomycin, (5) benzylpenicillin. The concentration of all the drugs is 1 μM. (B) The inhibition of different concentrations of 5-fluorouracil on the activity of Dam MTase.

Reference

1 C. J. Orendorff and C. J. Murphy, *J. Phys. Chem. B*, 2006, **110**, 3990.