Supporting information for

DNA methylation detection with end-to-end nanorod assemblies enhanced surface plasmon resonance

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Experimental section

Apparatus. The surface plasmon resonance responses were measured on a SPR Navi 200 Kretschmann-type spectrometer (BioNavis Ltd., Tampere, Finland) with a light-emitting diode light source (l=670 nm, prism refraction index n=1.61). A gold-coated glass disk mounted on a prism through a thin layer of index-matching oil form the base of a two-channel cuvette. Different samples can be added into the two independent channels. In a kinetic measurement mode, molecular adsorption on gold-coated glass disks is followed by monitoring SPR angle (θ) or angle shifts ($\Delta\theta$) over time. All electrochemical measurements were conducted in a standard electrochemical cell using an electrochemical analyzer (CHI660E, CH Instruments, USA). Non-denaturing polyacrylamide gel electrophoresis (PAGE) was performed on DYCZ-28C electrophoresis power supply equipped with WD-9413A gel documentation & analysis systems from Beijing Liuyi Instrument Factory (Beijing, China). UV/Vis absorption spectra were obtained with a Cary 50 Series Spectrophotometer (Varian, Australia). The sizes of the Au nanorods (AuNRs) were verified by transmission electron micrograph (TEM) using a JEOL JEM-2100EX microscope (Japan).

Chemicals. All oligonucletides used in the present study were purchased from Takara Biotechnology Co., Ltd (Dalian, China), and their sequences were provided in Supplementary Table S1. Dam MTase, Dpn I endonuclease，S-adenosyl-L-methiolnine (SAM), Klenow DNA polymerase, Nb.BbvCI nicking endonuclease were purchased from New England Biolabs Inc. Hexadecyltrimethylammonium bromide (CTAB) were purchased from Sigma. Tris(2-carboxyethyl)phosphine (TCEP, 98%) was purchased from Alfa Aesar (MA, USA).

Tris(hydroxymethyl)aminomethane (Tris), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) were purchased from Sigma-Aldrich. Other chemicals employed were all of analytical grade and double distilled water was used throughout the experiments.

Table S1 DNA sequences used in the present experimental.

Name	Sequences
S_1	5'-GTA GAT GA ACTG GA TCC AGTT-3'
S_2	3'-CAT CTA CTT GAC CT CGACTCC GCT CCG ATG GCT CCT TAG CTC ATC CCA CTG -5'
S_3	3'-CCT TAG CTC ATC CCA CTG-$(CH_2)_6$-SH-5'
S_4	3'-SH-$(CH_2)_6$-ACTCC GCT CCG ATG GCT-5'
S_5	5'-TGAGG CGA GGC TAC CGA-$(CH_2)_6$-SH-3'
b	5'-TGAGG CGA GGC TAC CGA GGA ATC GAG TAG GGT GAC-3'

Preparation of AuNRs. Au NRs with an aspect ratio of approximately 2.6 were prepared by a slightly modified seed-mediated growth procedure.[1] Synthesis of Au seeds: 0.5 mM $HAuCl_4$ solution (1 mL) was added to 0.20 M CTAB solution (1 mL). Then, freshly prepared 0.01 M $NaBH_4$ solution (0.12 mL) was added in one portion. After stirring rapidly for 2 min, the solution was left to reaction at 25 °C for 2 h. Growth of Au NRs: 1 mM $HAuCl_4$ solution (5 mL) was mixed with 0.2 M CTAB solution (5 mL). Then, 70 μL of 0.079 M ascorbic acid solution and 0.25 mL of 0.004 M $AgNO_3$ solution were added to the reaction mixture followed by mixing for about 2 min, which resulted in a colorless solution. Finally, 12 μL of the seed solution was added with gentle mixing for about 20 s and left at 30 °C aging for 4 h.

Synthesis of Single-Strand DNA-Modified Au NRs. AuNRs were centrifuged at 10,000 rpm for 10 min and were resuspended in 200 μL of 5 mM CTAB solution (three times concentrated). The redispersed Au NRs were used in all of the following experiments. The Au NRs modification of $\mathbf{S_4}$ or $\mathbf{S_5}$ was carried out at 25 °C for 12 h with a reaction ratio of 80:1 between the Au NRs and the DNA sequences, which were preactivated with TCEP for 1 h. Then unreacted DNA sequences were removed by centrifuging (7,000 rpm, 10min) and redispersed in 200 μL of 5 mM CTAB solution.

Since CTAB is preferentially bound along the {100} facets on the side of NRs as opposed to {111} facets at the ends of the NRs,[2] the ends of the NRs are more reactive in respect to thiolated DNA, therefore, the $\mathbf{S_4}$ and $\mathbf{S_5}$ were attached to the ends of the AuNRs.

Self-Assembly of Au NRs line. 40 μL of $\mathbf{S_4}$-modified AuNR and 40 μL of $\mathbf{S_5}$-modified AuNR were mixture in 20 μL of 10 mM Tris-HCl buffer (pH 7.5, 20 mM $MgCl_2$, 0.01%SDS), and incubated at 37 ºC with gentle shaking for 12 h. The end-to-end AuNR assemblies were characterized with TEM and UV-Vis spectra.

DNA Methylation and Cleavage of Dpn I Endonuclease. 40 μL of palindromic **S1** (0.1 μM) was added into 60 μL of Tris-HCl buffer and incubated at 37 ºC with gentle shaking for 1 h to obtain double-strand DNA (dsDNA) hybrids. The methylation experiment was carried out in 50 μL of methylase buffer (10 mM Tris-HCl, 50 mM NaCl, 10 mM $MgCl_2$,1mM DTT, pH 7.5) containing 0.02 μM dsDNA probe, 80 μM SAM, a varying amount of Dam MTase, and 4 U Dpn I at 37 °C for 2 h.

Polymerization and nicking reaction. 10 units Nb.BbvCI nicking endonuclease, 5 units Klenow DNA polymerase, 5 μL $\mathbf{S_2}$ (1 μM) and 1mM dNTPs mixed in 50 μL of NEBuffer (1×) were added

to the above solution, and the reaction was initiated by incubating the mixture at 37 °C for 1 h.

SPR detection. Au films coated onto BK7 glass slides were purchased from Biosensing Instrument Inc. and annealed in a hydrogen flame to eliminate surface contaminants. The gold chip was dipped in piranha solution (H_2SO_4/H_2O_2, 3:1 V/V) for 5 min to make the gold hydrophilic. Then the cleaned chip was wash with double distilled water and dried by nitrogen gas prior to use. 20 μL of 1.0 μM capture DNA $\mathbf{S_3}$, preactivated with TCEP for 1 h, was added to the gold chip to self-assemble on the surface for 16 h in wet environment. Subsequently, the 6-mercapto-1-hexanol (MCH) was added to block the nonspecific binding sites for 2 h. Finally, the gold chips were successively rinsed with D.I. water and PBS solution to remove free and excess DNA. The modified gold chip was washed by flowing PBS buffer in SPR. After the baseline became stable, injecting of the above reaction solution and the end-to-end AuNR assemblies into the fluidic channels led to a hybridized surface at 5 μL/min for 1 h. The surface plasmon resonance responses were measured on a SPR Navi 200 Kretschmann-type spectrometer with a light-emitting diode light source (l=670 nm, prism refraction index n=1.61).

Cell culture. HeLa cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 100 IU/mL penicillin Streptomycin. The cell density was determined by using a hemocytometer prior to any experiments. Approximately one million cells dispersed in RPMI 1640 cell media buffer were centrifuged at 3000 rpm for 5 min and rinsed with 5 mL of dye-free cell media three times and were then redispersed in cell media buffer (1 mL). The cells were disrupted by sonication for 20 min at 0 °C and the lysate was centrifuged at 18 000 rpm for 20 min at 4 °C to remove the homogenate of cell debris. Different concentrations of Dam MTase and Dpn I were spiked into the cytosol.

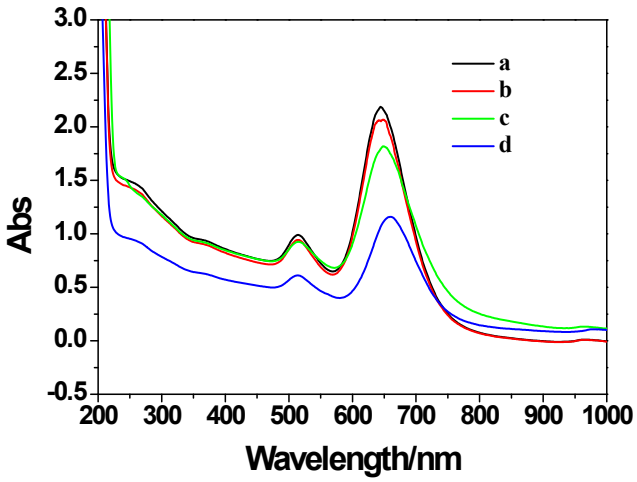

Fig. S1 UV-vis spectra of free AuNRs (a), $\mathbf{S_4}$-AuNRs (b), $\mathbf{S_5}$-AuNRs (c), and ETE AuNR assemblies (d).

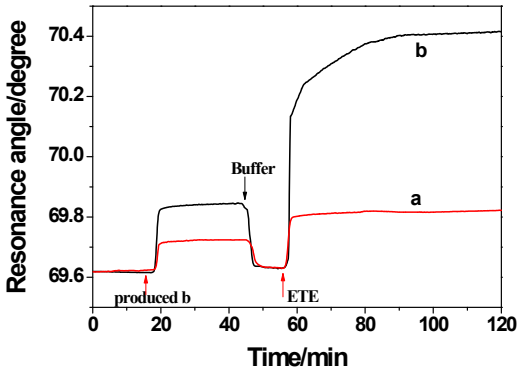

Fig. S2 SPR sensorgrams corresponding to injections of ETE probe at 10 μL/min into fluidic channels wherein the substrate had been exposed to (a) $\mathbf{S_3}$ modified Au chip, (b) $\mathbf{S_3}$ modified Au chip and hybridized with produced **b**. The arrows indicate the beginnings of injections.

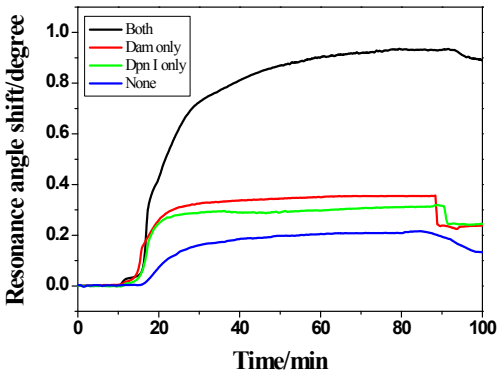

Fig. S3 SPR sensorgrams in the absence (blue line) or presence of Dam MTase (red line), Dpn I (green line), and Dam MTase and Dpn I (black line), respectively. The amount of Dam MTase and Dpn I were 120 U/mL and 8 units, respectively.

Optimization of the concentration of the reporter probes S_4 and S_5. The low concentration of reporter probes could not bind to each other sufficiently, however, high concentration might induced accumulation of AuNRs. The concentrations of reporter sequences **S_4** and **S_5** were investigated. Different aliquots of **S_4** and **S_5** were added into the prepared AuNRs solution, respectively. Then **S_4**-modified AuNRs and **S_5**-modified AuNRs were mixed and incubated at 37 ºC to get the ETE AuNR assemblies. Fig. S4 showed the variance of SPR signal with different concentrations of reporter probe. When the ratio of **S_4** or **S_5** with AuNRs was 80:1, the SPR signal reached a maximum value.

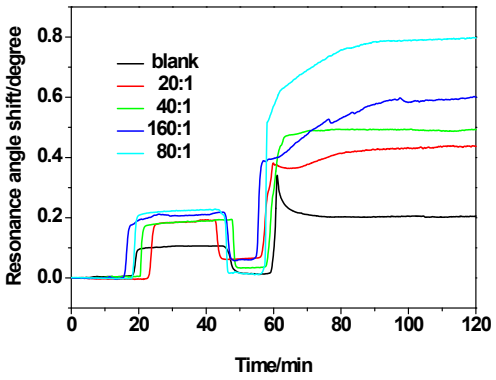

Fig. S4 SPR sensorgrams of different concentrations of reporter sequences. The concentration of Dam MTase was 90 U/mL.

Optimization of the amount of enzymes and SAM. The methylation process was affected by many molecular species including enzymes and SAM. To get a rapid methylation reaction and high SPR angle shift, the concentrations of Dpn I, SAM, Nb.BbvCI nicking endonuclease and Klenow DNA polymerase were optimized. As shown in Fig. S5, SPR signal increased with the Dpn I concentration from 0 to 8 units. Then, the saturation was reached by persistently increasing Dpn I. Afterward, the effect of different concentrations of SAM was studied. The SPR signal

increased with the increase of SAM and reached a stabilized platform with 80 μM. The amounts of Nb.BbvCI nicking endonuclease and Klenow DNA polymerase were optimized as 10 units and 5 units, respectively.

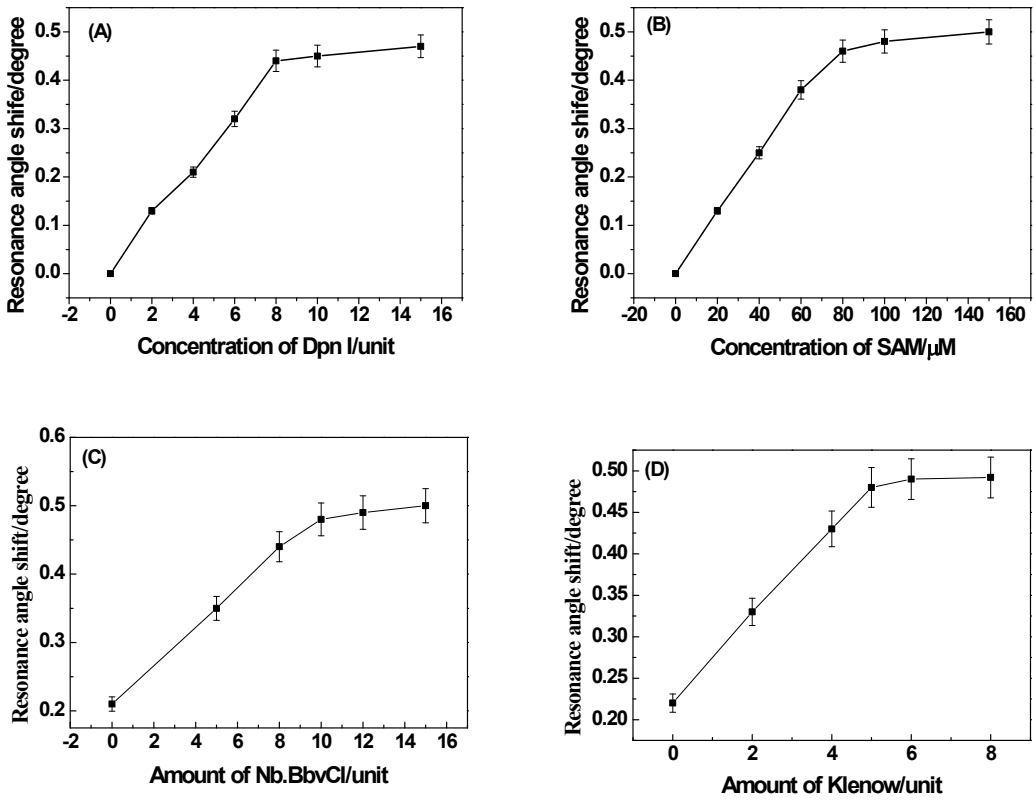

Fig. S5 Effect of different concentrations of Dpn I (A), SAM (B), Nb.BbvCI nicking endonuclease (C) and Klenow DNA polymerase (D) on the SPR signal. The concentration of Dam MTase was 60 U/mL for both. Error bars show the standard deviation of three experiments.

Control experimental with monomer AuNR as probe. To prove the amplification effect by ETE AuNR assembly, the control experimental with monomer AuNR as probe was carried out. The corresponding real-time resonance angle responses were shown in Fig. S6. Comparing Fig. 3A with Fig. S6A, the SPR responses were different with each other. For both cases, the ETE AuNR assembly or monomer AuNR probes were added at 10 min, respectively. The SPR signal increased slowly after the introduction of monomer AuNR probes, until 50 min the signal increased rapidly (Fig. S6A), while the SPR signal for ETE AuNR assembly increased quickly after the addition of the probes (Fig 3A). This might be due to the high SPR effect of the ETE AuNR assembly amplification. Using monomer AuNRs as probes, the detection limit for Dam MTase is 1.0 U/mL.

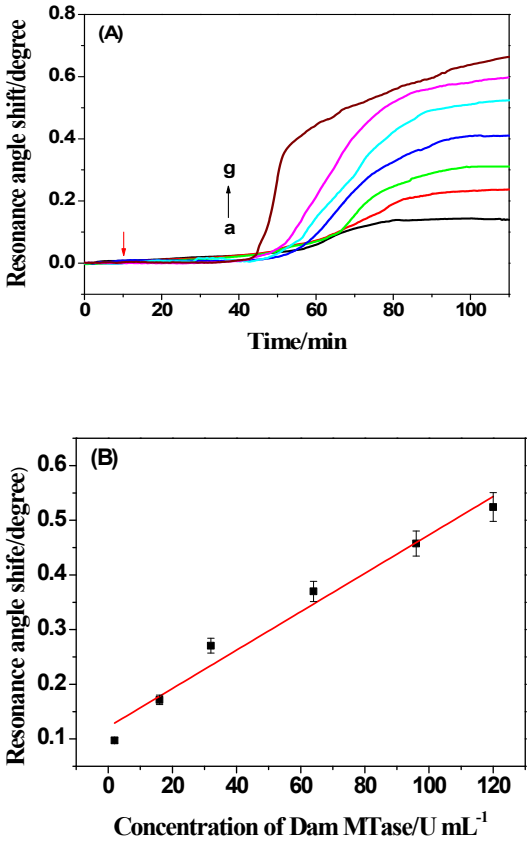

Fig. S6 (A) Real-time resonance angle responses of biosensor for Dam MTase detection with monomer AuNR probes. The concentration of Dam MTase from a to g: 0, 2.0, 15, 30, 60, 90, 120 U mL^{-1}. (B) Calibration curve of the determination of Dam MTase with monomer AuNR probes. Red arrow indicates the introduction of the AuNR probes.

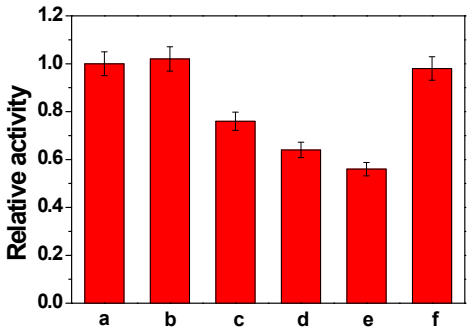

Fig. S7 Influence of different drugs on the activity of Dam MTase: (a) no drug, (b) amoxicillin, (c) benzylpenicillin sodium, (d) gentamycin sulfate, (e) fluorouracil, (f) mitomycin. [dsDNA probe] = 0.02 μM, [Dam] = 60 U/mL, [SAM] = 80 μM, Dpn I = 8 unit. The error bar was calculated from three detection points.

Table S2 Determination of Dam MTase in cell lysate.

samples	Spiked (U/mL)	Detection (U/mL)	RSD (%, n=5)	Recovery (%, n=5)
1	1.0	0.96	6.8	96.0
2	10	10.8	5.4	108.0
3	20	22.4	7.9	112.0
4	50	46.7	8.2	93.4
5	100	97.6	6.3	97.6

References

1 B. Nikoobakht and M. A. El-Sayed, *Chem. Mater.,* 2003, **15**, 1957–1962.

2 L. Wang, Y. Zhu, L. Xu, W. Chen, H. Kuang, L. Liu, A. Agarwal, C. Xu and N. A. Kotov, *Angew. Chem. Int. Ed.*, 2010, **49**, 5472–5475.