Supporting information:

A carbon nanoparticle-based low-background biosensing platform for sensitive and label-free fluorescent assay of DNA methylation

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Experimental Section

Reagents and Instruments. All oligonucleotides (Table 1) were synthesized by Takara Biotechnology Co. Ltd. (Dalian, China). The Dam MTase (Escherichia coli), Dpn I endonuclease, SAM and the corresponding buffer solution were purchased from New England Biolabs Inc. Other chemicals were of analytical grade and were used without further purification. Deionized water was obtained through Millpore water purification system.

The fluorescence and thermodynamics assays were performed on a PTI QM4 Fluorescence System (Photo Technology International, Birmingham, NJ) with an accessory of temperature controller. Under the excitation wavelength of 498 nm, the fluorescence spectra were recorded from 505-615 nm. Both the excitation and emission slits were set for 5 nm and integration was 0.2 sec. pH was measured on TP310.

Activity Assay of Dam MTase. The methylation experiment was performed in 20 μL of methylase buffer (50 mM Tris-HCl, 50 mM NaCl, 10 mM EDTA, 1 mM DTT, 10 mM $MgCl_2$, pH 7.5) containing 2 μM DNA probe, 80 μM SAM, 4 units DpnI and a varying amount of Dam MTase. The reaction mixture was incubated at 37 °C for 2 h. To protect the activity of

Dam MTase, all of these standard solutions were thawed on ice and stored at -20 °C. After the methylation reaction, 2 μL of SG, 50 μL of 0.1M Tris-HCl and 450 μL of H_2O were added to the sample. Fluorescence spectra were obtained at 498 nm excitation.

Influence of Some Drugs on DNA Methylation. To investigate whether or not drugs have influence on Dpn I, a control experiment was carried out. Firstly, the DNA probe was absolutely methylated by putting DAM MTase, SAM and the DNA probe together for 16 h. Then 1 μM different drugs were added into the solution as well as Dpn I respectively. The influence of drugs on the activity of Dam MTase was similar to that noted above except that drugs were added together with Dam MTase.

Methylation Assay by Gel Electrophoresis. In the gel electrophoresis assay, all the contents in the sample were the same as that aforementioned. After incubation at 37 °C for 6 h, the sample was applied to a polyacrylamide gel (20% acrylamide, 19:1, acrylamide/bisacrylamide) to separate the cleaved products from the substrate. The electrophoresis was carried in tris-borate-EDTA (TBE) (pH 8.0) at 200 V constant voltages for 1 h. After SG staining, the gel was scanned using Tanon 2500R.

Table 1. Oligonucleotides Sequences Used in This Work

Type	Sequences
P1	5′-GTTGGGATCGAGAAG-3′
P2	5′-CTTCT CGATCC CAAC-3′
P3	5′-TCGAGAAG-3′
P4	5′-CTTCTCGA-3′
P5	5′-GTTGGGA-3′
P6	5′-TC CCAAC-3′
P7	5′-GTTGGCTAGGAGAAG-3′
P8	5′-CTTCTCCTAGCCA AC-3′

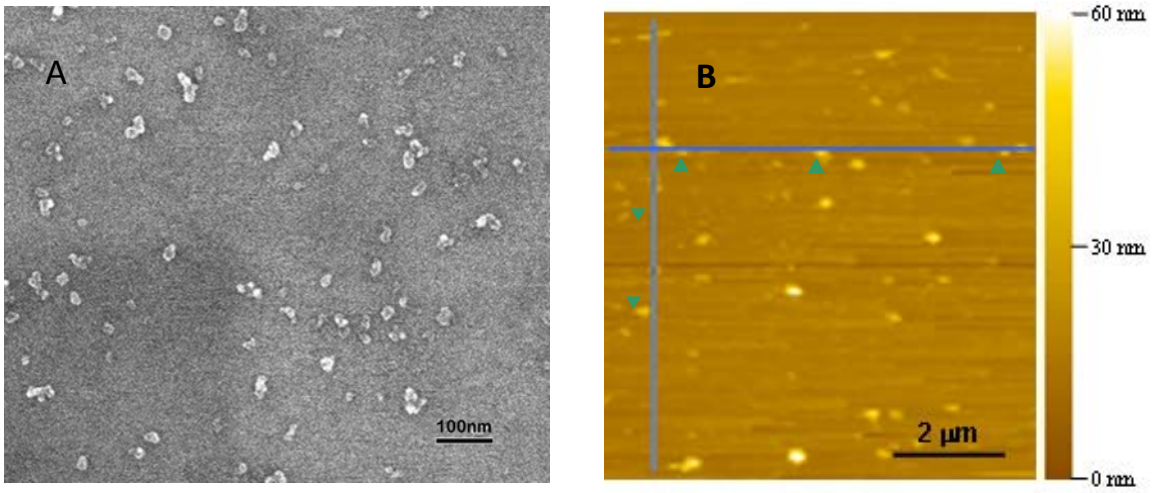

Fig. S1. SEM (A) and AFM (B) image of carbon nanoparticles.

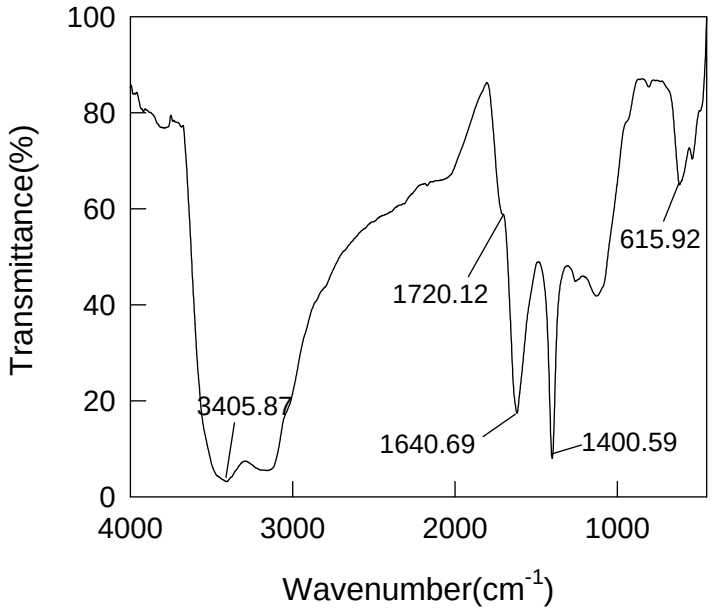

Fig. S2. FT-IR spectroscopy of CNPs. The line at 1720 cm^{-1} is assigned to the C=O stretching mode of the -COOH groups on the cCNPs, and the line at around 3400 cm^{-1} is assigned to the -OH stretching mode of the -COOH groups.

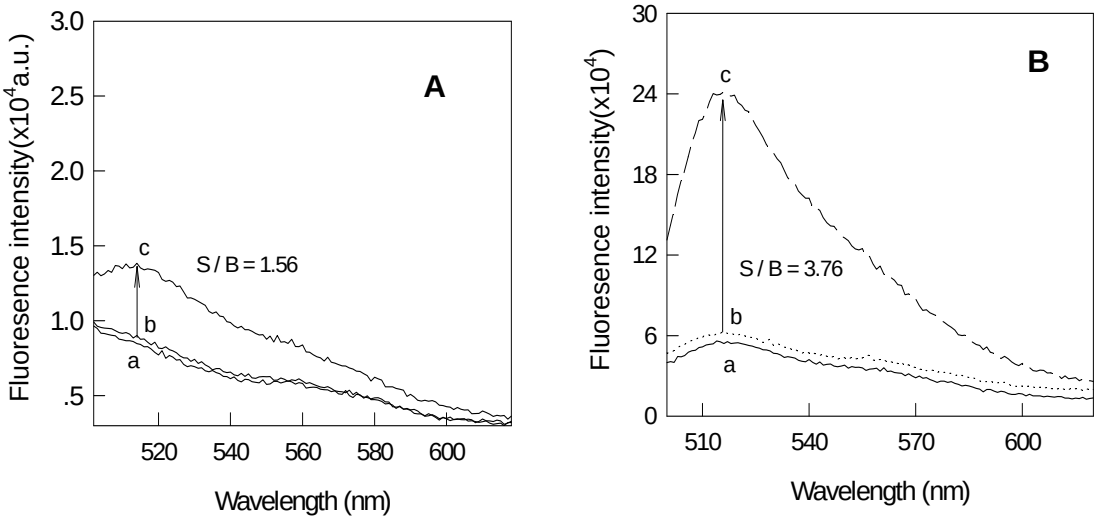

Fig. S3. The fluorescence spectra of SG in the presence of 10 μg/mL graphene (A) and 10 μg/mL SWNTs (B). curve a: SG in Tris-HCl buffer; curve b: a + ssDNA; curve c: a + dsDNA.

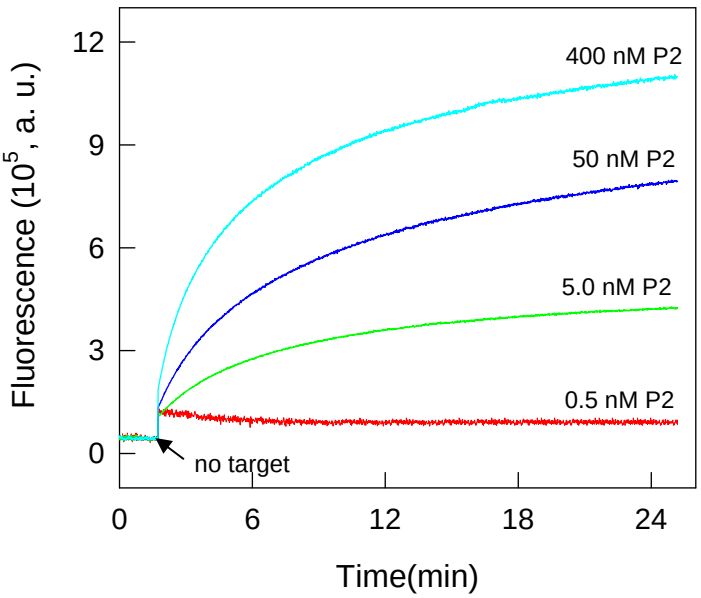

Fig. S4. Kinetics of the interactions of P1/CNPs (25 nM P1+0.02 mg/mL CNPs) with different concentrations of P2 in the Tris-HCl buffer solution at room temperature. The transitions between each regime are marked with an arrow. Fluorescence emission was recorded at 520 nm with an excitation wavelength of 480 nm.

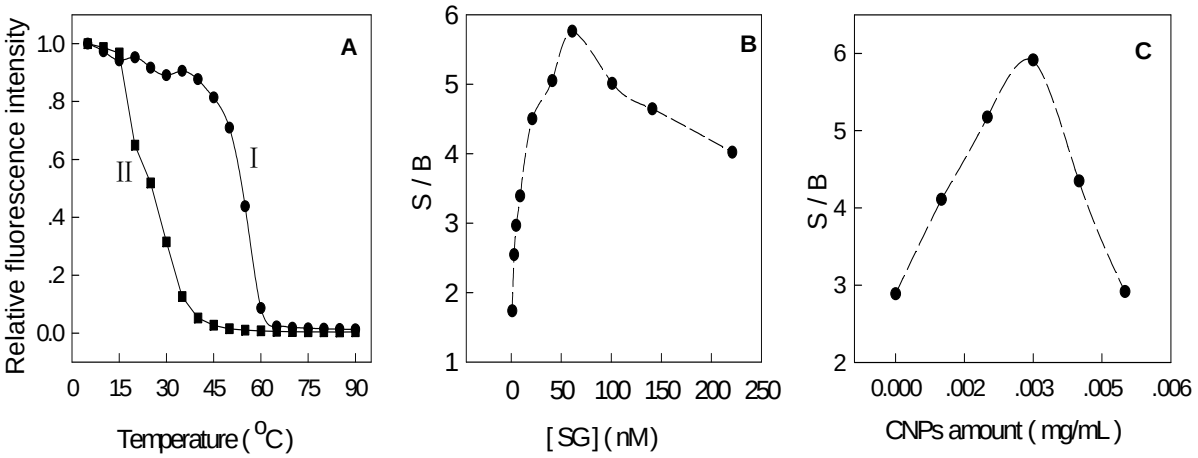

Fig. S5. Optimization of the measuring system. (A) Melting temperature curves of the dsDNA probe (P1+P2, I) and its cleaved products (P3+P4+P5+P6, II); (B) Fluorescence intensity ratio of the dsDNA probe to its cleaved products (S/B) as a function of different concentrations of SG; (C) S/B as a function of different concentrations of CNP.

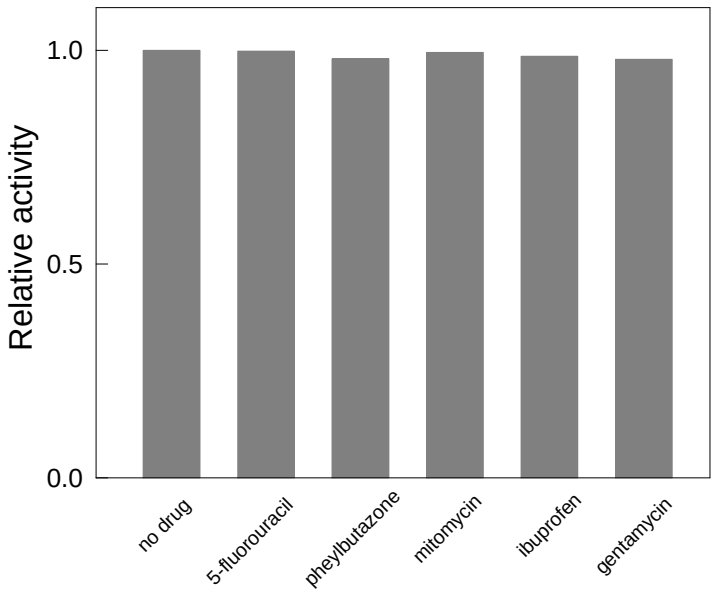

Fig. S6. Influence of different drugs on the activity of Dpn I. The concentration of all the drugs is 1μM.

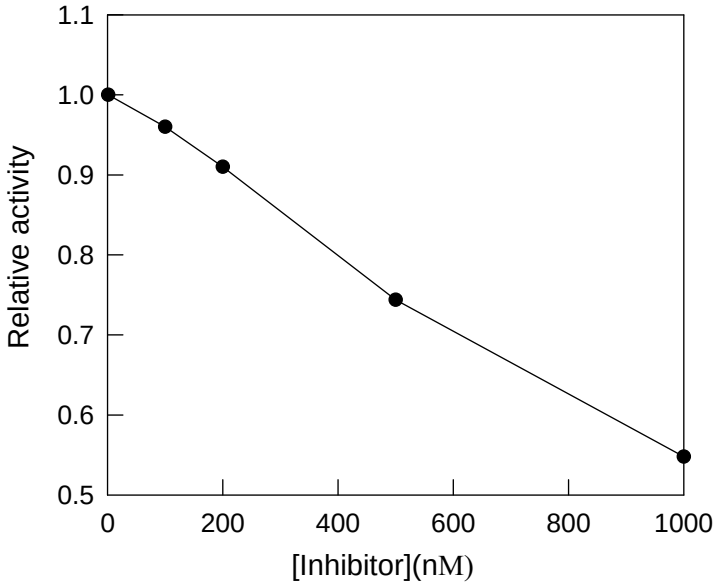

Fig. S7. The inhibition of different concentrations of 5-fluorouracil on the activity of Dam MTase.

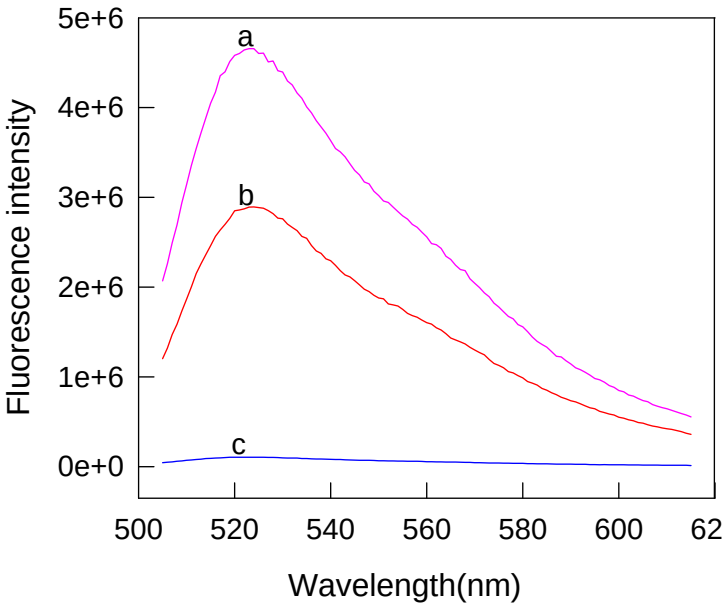

Fig. S8. The fluorescence spectra of SG-dsDNA with (b) and without (a) CNPs, curve c is the SG and CNPs.One can see from the fluorescence spectra that the fluorescence intensity of SG-dsDNA with CNPs is still strong, not significant decrease compared to that of SG-dsDNA. This result clearly indicates that the affinity of SG to dsDNA is stronger than that of SG to the carbon nanomaterials.