## Electronic Supplementary Information:

Highly sensitive methyltransferase activity assay and inhibitor screening based on fluorescence quenching of graphene oxide integrating with the site-specific cleavage of restriction endonuclease

Lijuan Ji, Zhewei Cai,<sup>+</sup> Yingdan Qian, Ping Wu, Hui Zhang\* and Chenxin Cai\*

Jiangsu Key Laboratory of New Power Batteries, Jiangsu Collaborative Innovation Center of Biomedical Functional Materials, College of Chemistry and Materials Science, Nanjing Normal University, Nanjing 210097, P.R. China.

<sup>+</sup> Undergraduate student of Department of Chemical and Environmental Engineering, University of Arizona. He is currently involved in a project of innovation study in Nanjing Normal University during his summer vacation.

\* Corresponding author, E-mail: <u>zhangh@njnu.edu.cn</u> (H. Zhang), <u>cxcai@njnu.edu.cn</u> (C. Cai)

## 1. Experiemntal details

**Chemicals.** All chemicals and solvents were of reagent grade or better. Graphite powder (99.998%, 325 mesh) was from Alfa Aesar; 1,4-dithiothreitol (DTT), tris(hydroxymethyl)-aminomethane (Tris), 5-azacytidine (5-Aza), and disodium ethylenediaminetetraacetic acid (EDTA) were from Sigma-Aldrich and used as received. 5-aza-2'-deoxycytidine (5-Aza-dC) and 2',3'-dideoxy-3'-thiacytidine (Lamivudine, 3-TC) was from TCI. *S*-adenosyl-L-methionine (SAM), M.SssI, HaeIII, and AluI MTase, and HpaII endonuclease were purchased from New England BioLabs (Ipswich, MA).

The oligonucleotides were purchased from BioSune Biological Engineering Technology Co. (Shanghai, China). They are FAM-labeled single-stranded 96-mer probe DNA (P1, 5'-FAM-<u>TTC TCT</u> <u>TCC TCT GTG CGC CGG TCT CTC CCA GG</u>A CTA TGT GCC GAA TAT CAA GGA CAG TTG TAG CTA TGT GCC GAA TCG TAC CGT GAG TAA TGC GAC-3'. The underlined bases (32-mer) are responsible for hybridizing with target; the italic and bolded sequence (CCGG) is the HpaII recognition sequence), 32-mer target DNA from the promoter region (exon 8) of the *Homo sapiens p53* gene (T1, 5'-CC TGG GAG AGA CCG GCG CAC AGA GGA AGA GAA-3'), and 32-mer modified *p53* gene containing one mismatch base (T2, 5'-CC TGG GAG AGA CCT GCG CAC AGA GGA AGA GAA-3'. The mismatched base (T) is italicized and bolded).

**Preparation of graphene oxide (GO) sheets.** GO was prepared by a modified Hummers method, starting from graphite powder. The detailed procedures have been reported in our previous work.<sup>1</sup>

**MTase activity assay.** The assay was performed in buffer solution (10 mM Tris-HCl, 50 mM NaCl, 10 mM EDTA, and 1 mM DTT) at 37 °C and pH 7.9. First, the recovered fluorescence of FAM in absence of M.SssI was measured. 10  $\mu$ L of GO dispersion (200  $\mu$ g/mL) and 1  $\mu$ L of P1 (1  $\mu$ M) were mixed together and diluted to 100  $\mu$ L using buffer solution (step (a), Figure 1). After a stable background fluorescence of the solution was attained, 1  $\mu$ L of T1 (1  $\mu$ M) were added to the solution and allowed to hybridize with P1 for 1 h (step (b), Figure 1), which is long enough to ensure the complete hybridization. Then, HpaII with final concentration of 20 U/mL was added into the solution to allow the cleavage for 2 h (step (c), Figure 1). The fluorescence signal at each step was monitored.

Then, the recovered fluorescence of FAM in presence of M.SssI was measured. SAM with final concentration of 160 µM and different concentrations of M.SssI were added to the system to achieve the methylation at the CpG dinucleotide site of the P1/T1 duplex before the addition of HpaII (step (d), Figure 1). After the mixtures were incubated at 37 °C for 2 h, HpaII with final concentration of 20 U/mL was added into the solution to allow the cleavage reaction to take place for 2 h (step (e), Figure 1). The fluorescence signal was recorded. Fluorescence emission spectra were collected with a Fluorosens fluorescence spectrophotometer (Gilden Photonics) under excitation of 492 nm.

**Selectivity of M.SssI activity assay.** To evaluate the selectivity of the proposed M.SssI activity assay, two other cytosine methyltransferases, namely, HaeIII and AluI, were selected as the potential interfering MTase. The measurement was conducted with 10 U/mL HaeIII or AluI in the same way as the M.SssI activity assay, except for that M.SssI was replaced by HaeIII or AluI in the methylation step.

**Evaluating inhibition of anticancer drugs to M.SssI activity.** The inhibition effects of three representative anticancer drugs, 5-Aza, 5-Aza-dC, and 3-TC, on the M.SssI activity were evaluated. The methylation of P1/T1 duplex was performed at 37 °C in buffer containing 160  $\mu$ M SAM, 100 U/mL M.SssI, and various concentration of the inhibitors. After the HpaII digestion, the fluorescence signal was recorded. The relative activity of M.SssI is estimated using the equation:

relative activity = 
$$\frac{F_0 - F_2}{F_0 - F_1}$$

where  $F_0$ ,  $F_1$ , and  $F_2$  are the recovered fluorescence intensity of the P1/T1 duplex untreated with M.SssI (0 U/mL M.SssI), treated with 100 U/mL M. SssI, and treated with 100 U/mL M. SssI in the presence of various dose of inhibitors, respectively, in the methylation step.

## References

1 Y. Hu, P. Wu, Y. Yin, H. Zhang and C. Cai, Appl. Catal., B, 2012, 111-112, 208.



**Fig. S1** Fluorescence spectrum of P1 (10 nM) recorded immediately (curve a) and 10 h (curve b) after preparation.



**Fig. S2** (A) Fluorescence spectra of FAM-labeled P1 (10 nM) at GO surface with GO concentrations of (a-l) 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 22 µg/mL. The inset shows the fluorescence spectra of GO alone at a concentration of (from bottom to top) 2, 5, 10, 15, and 25 µg/mL. (B) Dependence of the fluorescence intensity of P1 at 520 nm on GO concentration. The inset shows the plot of  $F_0/(F_0-F)$  against the concentration of GO. (C) Time-dependent fluorescence signal P1 (10 nM) quenched by GO (20 µg/mL). The inset shows the fluorescence spectra of FAM-labeled P1 (10 nM) after it was immobilized on the surface of GO sheet for (a-i) 0, 1, 3, 5, 7, 9, 11, 15, and 20 min. Error bars were based on five measurements.

In the absence of GO in the solution, P1 (10 nM) exhibits a strong characteristic fluorescence signal of FAM at ~520 nm (a, Fig. S2A). Addition of GO into solution causes the rapidly quenching of the fluorescence of FAM (b-l, Fig. 1A, and Fig. S2B). The fluorescence intensity decreased with the increase of the GO concentration (the concentration of P1 was kept at 10 nM) and was almost completely quenched at GO concentration of 20  $\mu$ g/mL (Fig. S2B), indicating good fluorescence quenching ability of our prepared GO. We estimated the quenching constant (*K*) of GO for P1 to be ~31 mL/mg using the modified Sterm-Volmer equation:

$$\frac{F_0}{F_0 - F} = \frac{1}{fK} [\text{GO}] + \frac{1}{f}$$

where  $F_0$  and F are the fluorescence intensities of P1 at 520 nm in the absence and presence of GO, respectively. [GO] is the concentration of GO in the system. f is the fractional maximum fluorescence intensity of P1. K is the quenching constant. The related linear plot is depicted in the inset of Fig. S2B. The high quenching constant implies that GO has high quenching efficiency for P1. Note that the fluorescence signals of GO do not affect the fluorescence features of FAM because the fluorescence signals of GO alone are very low under our experimental conditions (the inset, Fig. S2A). Kinetic studies showed that the fluorescence quenching of GO is fairly fast because the fluorescence intensity decreased rapidly to ~32% of the initial intensity in ~1 min and was completely quenched within 3 min (Fig. S2C and the inset).



Fig. S3 Fluorescence spectra of P1/T1 duplex at GO surface for (a) 0, (b) 1, (c) 2, and (d) 4 h.



**Fig. S4** (A) Dependence of the fluorescence intensity of P1/T1 duplex on the cleavage time of HpaII (20 U/mL). (B) Dependence of the fluorescence intensity of P1/T1 duplex on the concentration of HpaII (2 h cleavage). (C) Dependence of the methylated P1/T1 duplex on the methylation time. The concentration of M.SssI MTase is 10 U/mL and the concentration of HpaII and the cleavage time are 20 U/mL and 2 h, respectively.



**Fig. S5** Fluorescence spectra of P1/T1 duplex at GO surface before (b) and after cleaved by heat-treated HpaII (a). Curve (c) is the fluorescence spectra of the P1/T2 duplex after cleavage with HpaII. Curves (d) and (e) are P1-functionalized GO before (e) and after (d) cleavage of HpaII.



**Fig. S6** The fluorescence spectra of the methylated P1/T1 duplex after 2-h cleavage with the HpaII (20 U/mL). The methylation time is 2 h and the concentration of M.SssI is (a-j) 0, 0.1, 0.5, 1, 2, 5, 10, 20, 50, and 100 U/mL, respectively.



Fig. S7 Dependence of the fluorescence signal ( $\Delta F = F_0 - F_1$ ) on the kinds of the MTases. Error bars were based on five measurements. The inset shows the fluorescence spectra of P1/T1 duplex on GO surface after 2-h treatment with 10 U/mL M.SssI (b), AluI (c), and HaeIII MTase (d) in methylation step followed by 2-h cleavage of HpaII (20 U/mL). (a) and (e) are the fluorescence spectra of P1/T1 duplex (without methylation) on GO surface before (a) and after (e) cleavage with HpaII.