

Electronic Supplementary Information (ESI)

A highly sensitive strategy for base excision repair enzyme activity detection based on graphene oxide mediated fluorescence quenching and hybridization chain reaction

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Experimental Section

Reagents and Apparatus. The DNA oligonucleotides used in this study (sequences shown in Table S1) were synthesized and purified through HPLC by Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China), 5× TBE buffer (225 mM Tris-Boric Acid, 50 mM EDTA, pH 8.0) and Low MW DNA Marker (25-500 bp) were obtained from Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China). Uracil-DNA glycosylase, bovine serum albumin (BSA), 8-oxoguanine DNA glycosylase (hOGG1), and uracil glycosylase inhibitor (UGI) were obtained from New England Biolabs (Ipswich, MA). Graphene Oxide was purchased from Nanjing XFNano Material Tech Co., Ltd. (Nanjing, China). Other chemicals used in this work were of analytical grade and directly used without further purification. The solutions used in all experiments were prepared using ultrapure water which was obtained through a Millipore Milli-Q water purification system (Billerica, MA) and had an electric resistance >18.2 MΩ.

The fluorescence spectra were recorded at room temperature in a quartz cuvette on an F-7000 fluorescence spectrophotometer (Hitachi, Japan). The excitation wavelength was 495 nm with a recording emission range from 505 to 600 nm and the excitation and emission slits were set at 5 nm.

UDG Activity Assay. The typical UDG activity was performed in a 30 μL reaction mixture containing 1× UDG reaction buffer (20 mM Tris-HCl, 1 mM EDTA, 1 mM DTT, pH 8.0), 50 nM HP and different concentrations of UDG at 37 °C for 60 min. Subsequently, 10 μL hybridization buffer (25 mM Tris-HCl, pH 8.0, 250 mM MgCl₂), 2 μL H1 (2.5 μM), 2 μL H2 (2.5 μM) and 6 μL H₂O were added, the mixture was

incubated at 37 °C for 60 min. Then, 20 µL 0.1 mg/mL GO nanosheets and ultrapure water were added into the system with final reaction volume of 100 µL. The mixture was incubated at room temperature for 10 min before fluorescence detection.

Gel electrophoresis analysis. Gel electrophoresis analysis was carried out on 3% (w/w) agarose gels containing 0.5 µg/mL GoldView and 0.5 µg/mL ethidium bromide running in 0.5× TBE buffer at room temperature. The electrophoresis was performed at a constant potential of 101 V for 2 h after loading 10 µL of each sample into the lanes. After electrophoresis, the gel was visualized via a Tanon 4200SF gel imaging system (Tanon Science & Technology Co., Ltd., China).

Cell culture and sample preparation. HeLa (cervical cancer cell lines) was cultured in RPMI 1640 medium (Thermo Scientific Hyclone) supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin at 37 °C in a humidified atmosphere containing 5% CO₂. Cells (1×10^6) were dispensed in an RNase-free 1.5 mL centrifuge tube, washed twice with PBS (137 mM NaCl, 2.7 mM KCl, 10 mM phosphate, pH 7.4), centrifuged at 2000 rpm for 3min, and then suspended in 100 µL lysis buffer (10 mM Tris-HCl with pH 8.0, 150 mM NaCl, 1% (w/v) NP-40, 0.25 mM sodium deoxycholate, 1% glycerol and 0.1 mM 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride). The lysates were incubated for 30 min on ice, and then centrifuged at 12000 rpm for 20 min at 4 °C. The extract was used immediately for UDG assay or stored at -80 °C.

Table S1. Sequences of DNA probes used in this work^a

Name	Sequence (5'-3')
HP	GUUGAGUUAACCAGTCTAGGATTCGGCGTGGGTAACT CAAC
H1	<i>TTAACCCACGCCGAATCCTAGACTCAAAGTAGTCTAGG ATTCGGCGTG</i>
H2	AGTCTAGGATTCGGCGTGGGTAAACACGCCGAATCCT AGACTACTTTG-FAM

^a Boldface type indicates stem sequences of hairpin DNA probes. Italic type in H1 and H2 shows sticky ends. U in HP probe denote the uracil deoxyribonucleotide modification. FAM at the 3' terminus of H2 probe is 6-carboxyfluorescein modification.

Fig. S1. Agarose gel electrophoresis images of HCR products. Lane M: DNA marker (25-500 bp); lane 1: 1 μ M HP; lane 2: 1 μ M H1; lane 3: 1 μ M H2; lane 4: 500 nM H1 + 500 nM H2; lane 5: 100 nM probe + 500 nM H1 + 500 nM H2; lane 6: 100 U/mL UDG + 100 nM probe + 500 nM H1 + 500 nM H2.

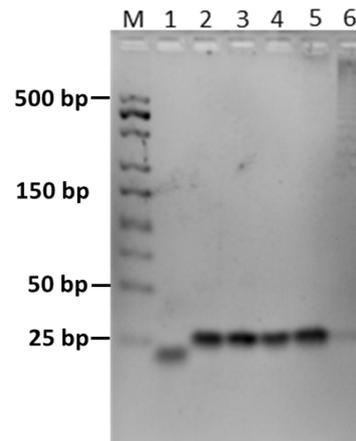


Fig. S2. The fluorescence signal ratio of the assay at different reaction time. F_0 and F are the fluorescence signals in the absence and the presence of UDG, respectively. Error bars are standard deviations of three repetitive experiments.

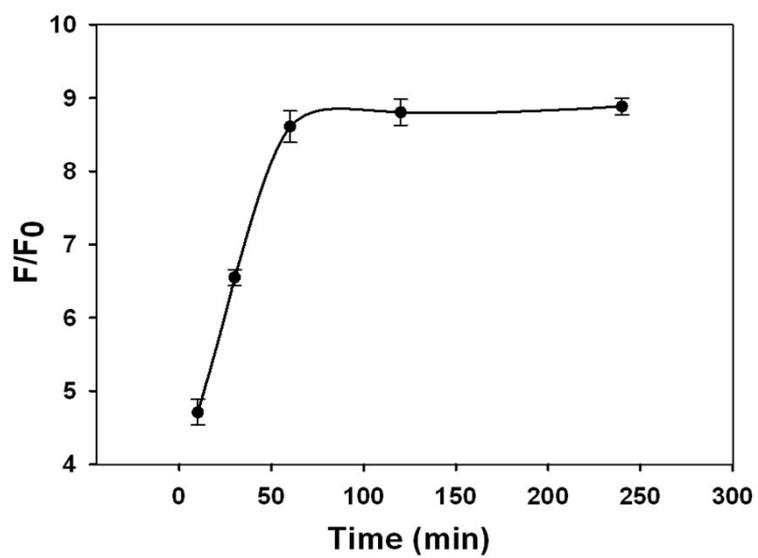


Fig. S3. The variation in fluorescence signal ratio with different concentration of HP.

F_0 and F are the fluorescence signals in the absence and the presence of UDG, respectively. Error bars are standard deviations of three repetitive experiments.

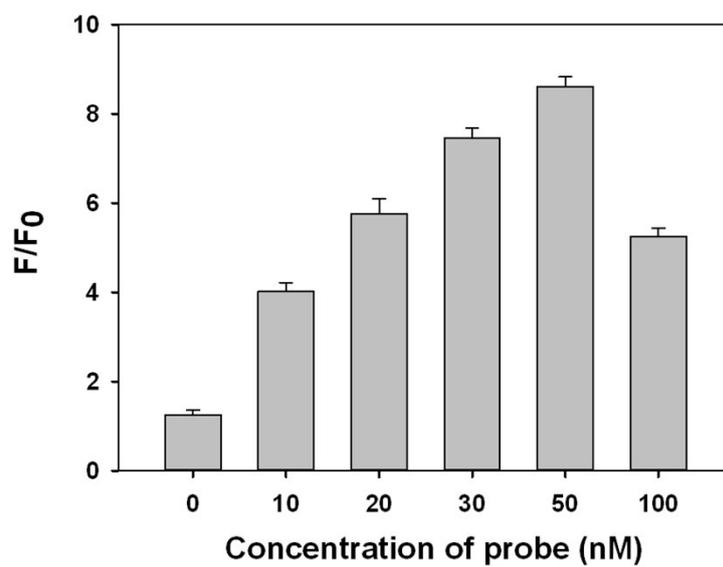


Fig. S4. Optimizing the concentration of GO nanosheets. F_0 and F are the fluorescence signals in the absence and the presence of UDG, respectively. Error bars are standard deviations of three repetitive experiments.

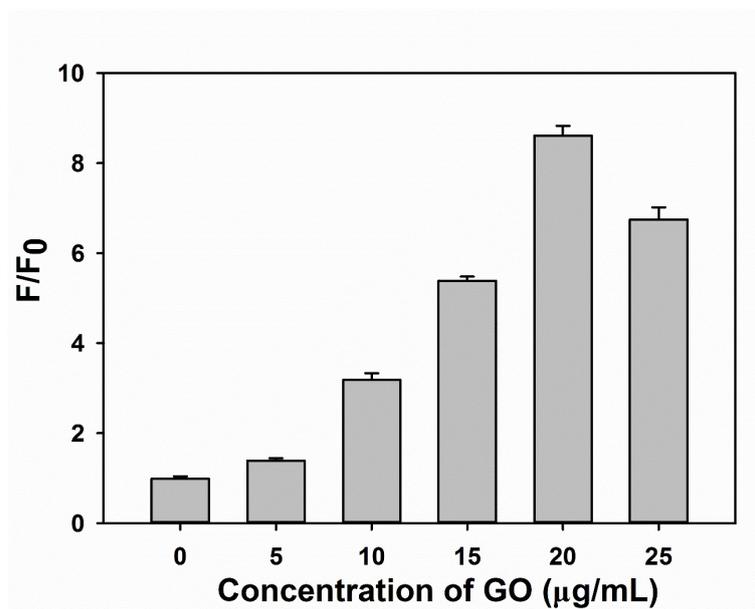


Fig. S5. Specificity of UDG assay. Bars represent the fluorescence intensity of the assay in the presence of varying proteins: 10 U/mL hOGG1, 10 μ g/mL BSA, 10 U/mL UDG. Error bars are standard deviations of three repetitive experiments.

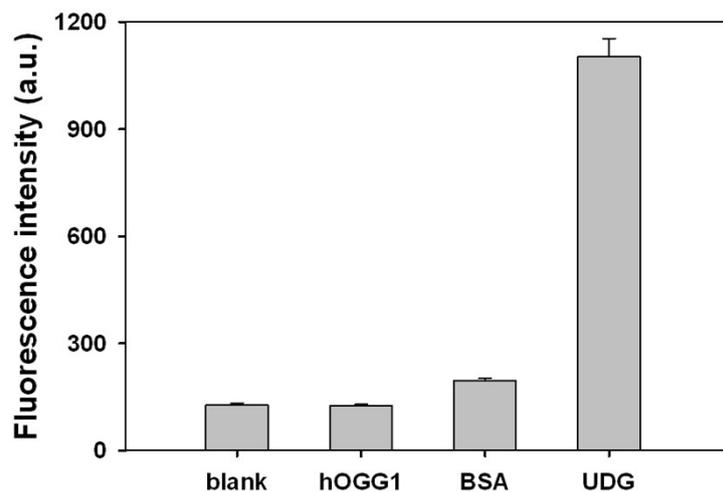


Fig. S6. (A) Fluorescence spectra for UDG activity in cell lysates. (B) Fluorescence intensity of cell lysates of different HeLa cell numbers. Error bars are standard deviations of three repetitive experiments.

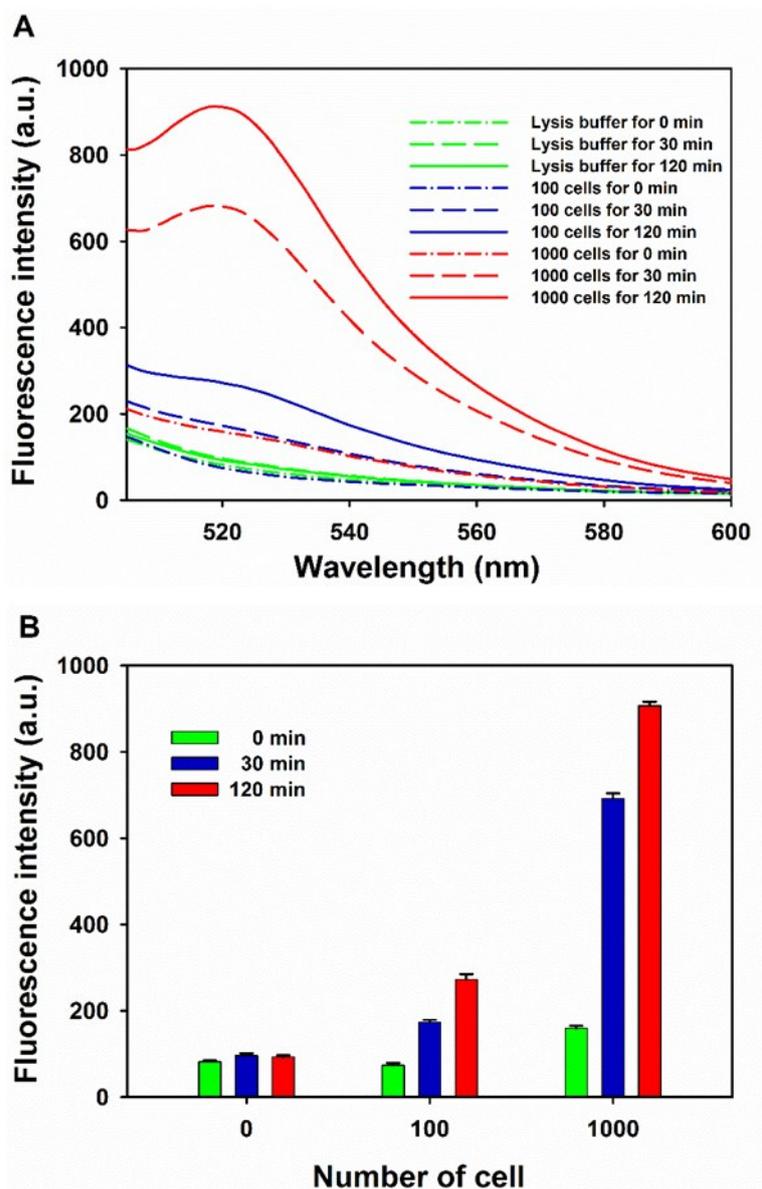


Fig. S7. (A) Fluorescence spectra for UDG activity in cell lysates incubate with UGI.

(B) Fluorescence intensity of cell lysates incubate with UGI. Error bars are standard deviations of three repetitive experiments.

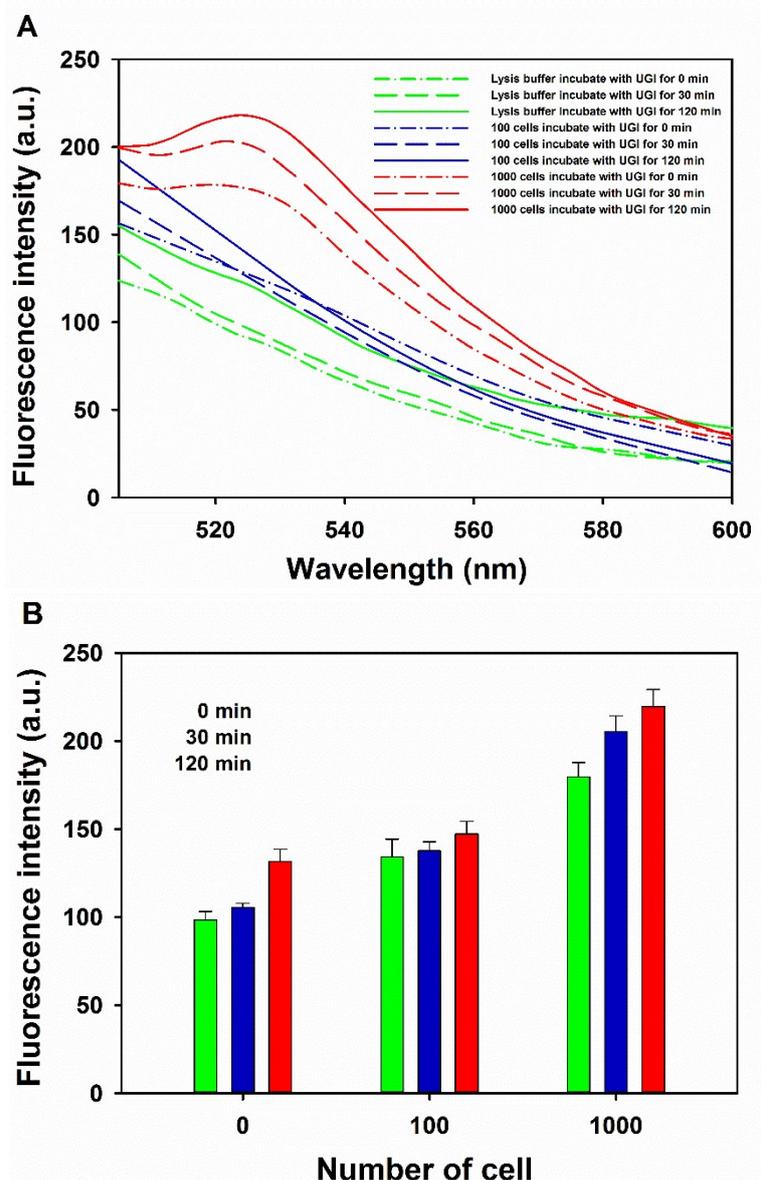


Fig. S8. TEM image of GO nanosheets.

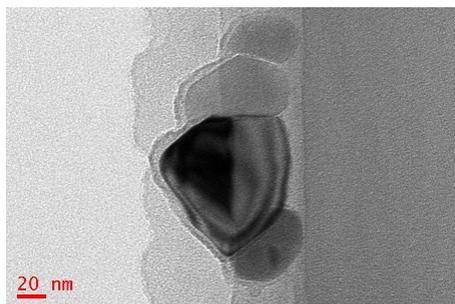


Fig. S9. AFM image and height profile of GO nanosheets on mica substrate.

