

# **DNA-mediated supercharged fluorescent protein/graphene oxide interaction for label-free fluorescent assay of base excision repair enzyme activity †**

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## 1. Experimental Section

### 1.1. Materials and Measurements.

Graphene oxide (GO) was purchased from XFNano Material InC., China, and the detailed characteristics was showing in Fig. S1. NaCl, KCl, MgCl<sub>2</sub>, and Tris (hydroxymethy-1) aminomethane (Tris) were obtained from Sinopharm Chemical Reagent InC., Ltd, China. All the chemicals used were analytical grade. Uracil DNA glycosylase (UDG), uracil DNA glycosylase inhibitor (UGI), endonuclease IV (Endo IV), human alkyladenine glycosylase (hAAG), DpnI, and dam methyltransferase (Dam MTase) were purchased from New England Biolabs, Inc. (Ipswich, MA, USA). All oligonucleotides used in this work were synthesized and purified by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China), and their sequences were listed in Table S1. Ultrapure water (18.25 MF • cm) was obtained from a Millipore filtration system and used throughout. UV-Vis absorption spectra were obtained on a BECKMAN DU-800 spectrophotometer. Fluorescence polarization was measured on a Hitachi F-4500 fluorescence spectrophotometer. Fluorescence measurements were carried out on a Synergy™ Mx multi-mode microplate reader (BioTek). Dynamic light scattering (DLS) and Zeta potential were carried on a Nano-z90 Zetasizer (Malvern Instruments Ltd, UK).

### 1.2. Expression and purification of ScGFP and eGFP

Genes of ScGFP and eGFP were cloned by our group and expressed in *E. coli* BL21 according to the reports<sup>1</sup>. Briefly, the construct pET 28a-*scgfp* was electroporated into *E. coli* BL21 and the resulting strain was cultured on LB media with kanamycin. Pick a single colony and culture it in 3 mL LB media overnight at 37 °C. Then 1 ml seed culture was inoculated into 100 ml LB media, and cultured at 37 °C for 3 h. After that, 10 µg/ml IPTG was added and the cells were continued to be cultured at 30 °C for

6 h. Harvest the culture by centrifugation at 6000 rpm for 2 min. The cells can be stored at -20 °C or lysed by sonication. With His-tag at the N-terminal, both ScGFP and eGFP were purified from the cell lysate through nickel-affinity chromatography column, and changed the buffer into 10 mM Tris-HCl, 100 mM NaCl, pH 7.4 through hydrophobic column (AKTA purifier, Box-900) according to the manual. Then the purified proteins were stored at -20 °C. ScGFP was quantified by absorbance with an extinction coefficient of  $8.33 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$  at 488 nm.

### **1.3. Analysis of the ScGFP/GO interaction and DNA-mediated ScGFP/GO interaction**

To confirm the ratio of GO to ScGFP, different concentrations of GO from 0.5, 1, 2, to 5 µg/mL was respectively added into 100 µL reaction system (20 mM Tris-HCl, 100 mM NaCl, pH 7.5, 25 °C) containing with 50 nM ScGFP. After mixing for 1 min, 100 µL solution was taken to record the fluorescence intensity in the microplate reader. All samples were illuminated at an excitation wavelength of 480 nm, and the fluorescence emission was scanned from 500 to 600 nm for the fluorescent intensity at 510 nm.

To ensure the binding time of GO and ScGFP, 1 µg/mL GO and 50 nM ScGFP were mixed for 0 to 2 min in the 100 µL reaction system (20 mM Tris-HCl, 100 mM NaCl, pH 7.5, 25 °C). Then, the fluorescent intensity was recorded as the same procedure above.

To illustrate the function of His-tag on adsorption of eGFP and ScGFP on GO, different concentrations of thrombin were introduced to the 100 µL buffer (20 mM Tris-HCl, 100 mM NaCl, pH 7.5, 25 °C) containing 50 nM eGFP or ScGFP for 30 min, then 1 µg/mL GO was introduced for 1 min, and the fluorescence intensity was recorded by the microplate reader.

The influence of DNA length on its ability of combining with ScGFP was analyzed

by performing the experiment in the 100  $\mu$ L buffer (20 mM Tris–HCl, 100 mM NaCl, pH 7.5, 25  $^{\circ}$ C) containing 50 nM ScGFP, 1  $\mu$ g/mL GO, and DNA with different length. In the reaction, different concentrations of DNA from 100 nM to 500 nM was firstly mixed with ScGFP in the buffer, then GO was added, and the fluorescence intensities was recorded in the microplate reader. Based on the result, 200 nM DNA-10 or DNA-10U was used for the following experiment.

#### **1.4. Assay of UDG and UGI**

The UDG enzymatic reactions were performed in 80  $\mu$ L 1X UDG reaction buffer (20 mM Tris–HCl, 100 mM NaCl, 1 mM EDTA, 1 mM DTT, pH 7.5) containing 250 nM DNA-10U, 50 U/mL Endo IV, and a varying amount of UDG, and incubated at 37  $^{\circ}$ C for 30 min. After the reaction, 10  $\mu$ L ScGFP (500 nM) and 10  $\mu$ L GO (10  $\mu$ g/mL) were added sequentially with the homogeneously mixing respectively. Then, the 100  $\mu$ L solution was taken out to record the fluorescent intensity in the microplate reader at 25  $^{\circ}$ C. The parallel tests were performed three times for each sample (n=3).

For optimization of the UDG assay, 1X UDG reaction buffer with pH regulated from 6.5 to 9.5 by NaOH or with the concentration of NaCl from 50 mM to 400 mM was compared. Then, pH 7.5 and 100 mM NaCl were selected for the reaction system. Similarly, the influence of Endo IV solo on the proposed sensor was analyzed. Then, 50 U/mL Endo IV was obtained as the optimal concentration in this work.

The UDG inhibition and the selectivity experiments were operated similarly to UDG detection, except that, instead of UDG, the same amount of UDG and UGI, or 10 U/mL of other DNA-related enzymes (DpnI, Dam MTase, and hAAG) were incubated with 250 nM DNA-10U and 50 U/mL Endo IV at 37  $^{\circ}$ C for 30 min. Similarly, the reliability experiment of the assay was carried out by mixing the proposed sensor with no UDG as the background, 1.0 U/mL UDG, or 1.0 U/mL UDG and 1.0 U/mL UGI, respectively.

## 1.5. High Throughput Screening Calculation

$Z'$  factor was calculated according to the equation:

$$Z' = \left( \mu_{pos} - \frac{3\sigma_{pos}}{\sqrt{n}} \right) - \left( \mu_{neg} + \frac{3\sigma_{neg}}{\sqrt{n}} \right) / (\mu_{pos} - \mu_{neg})$$

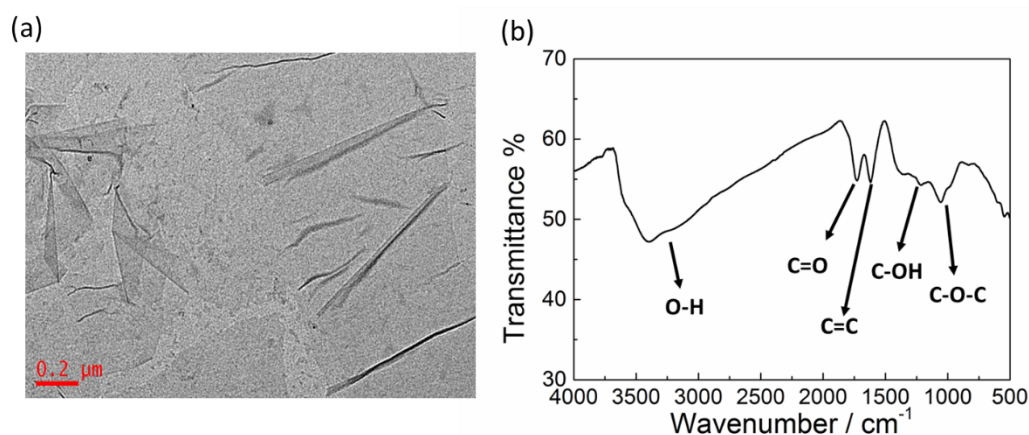
Where  $n$  is the number of replicates,  $\mu_{pos}$  and  $\mu_{neg}$  are the average value of the positive and negative controls, respectively, and  $\sigma_{pos}$  and  $\sigma_{neg}$  are the standard deviation of the positive and negative controls, respectively. For the  $Z'$  factor of UDG, the  $\mu_{pos}$  and  $\mu_{neg}$  are the average quenching ratio responding to UDG and the background, respectively, and the  $\sigma_{pos}$  and  $\sigma_{neg}$  are the standard deviation corresponding to the  $\mu_{pos}$  and  $\mu_{neg}$ , respectively. For the  $Z'$  factor of UGI, the  $\mu_{pos}$  is the average quenching ratio in the presence of UDG and UGI and  $\mu_{neg}$  is the average quenching ratio with UDG only, and the  $\sigma_{pos}$  and  $\sigma_{neg}$  are the standard deviation corresponding to the  $\mu_{pos}$  and  $\mu_{neg}$ , respectively.

## 2. Supplementary Table

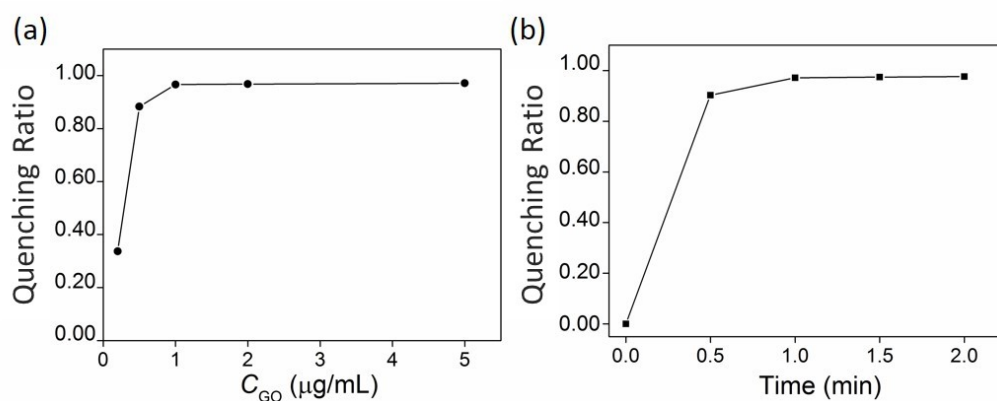
**Table S1** Sequences of the oligonucleotides used in the experiments

DNA name	Base number (nt)	DNA sequence
DNA-5	5	5'-GGTAT-3'
DNA-7	7	5'-GGTATGG-3'
DNA-9	9	5'-GGTATGGTA-3'
DNA-10	10	5'-GGTATGGTAT-3'
DNA-10U	10	5'-GGTAUGGTAT-3'

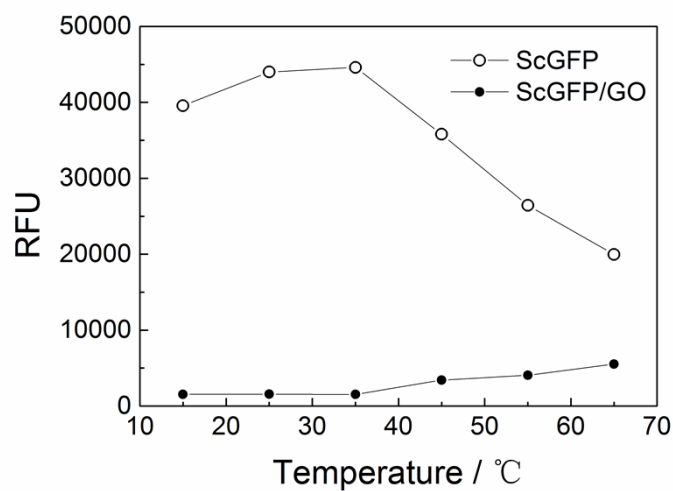
### 3. Supplementary Figures



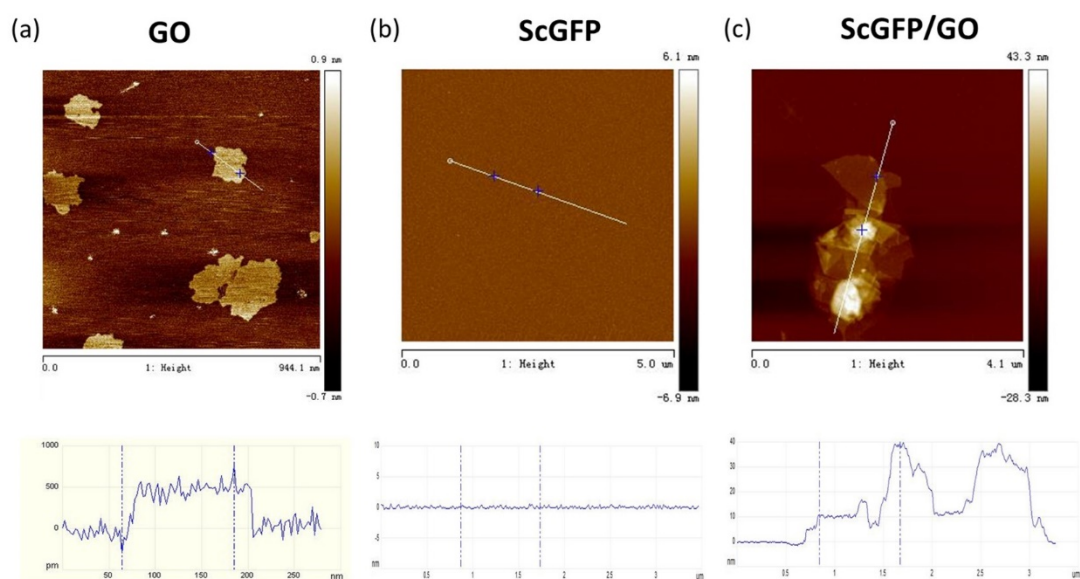
**Fig. S1** The size of GO (1 μg/mL) characterized by TEM (a) and the functional groups on the surface of GO reflected by FT-IR spectrum (b).



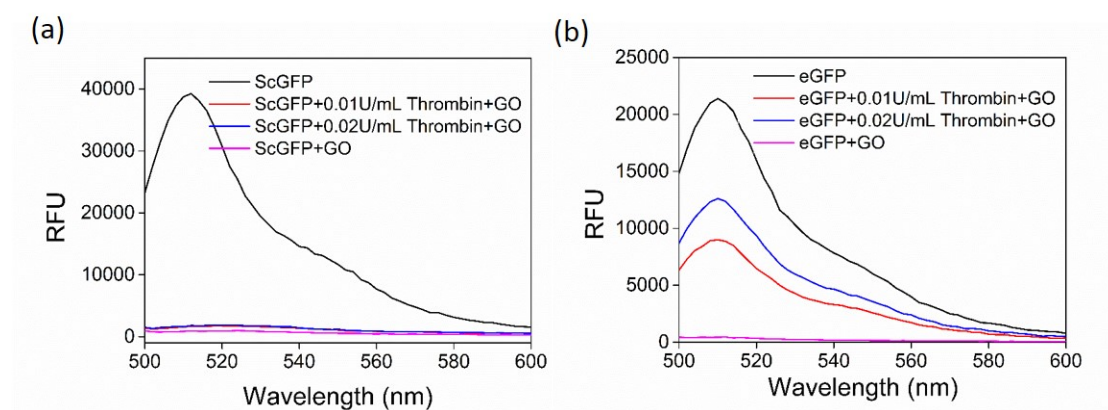
**Fig. S2** Quenching ratios of ScGFP (50 nM) by different concentrations of GO (a) and by GO (1 μg/mL) with different mixing time.



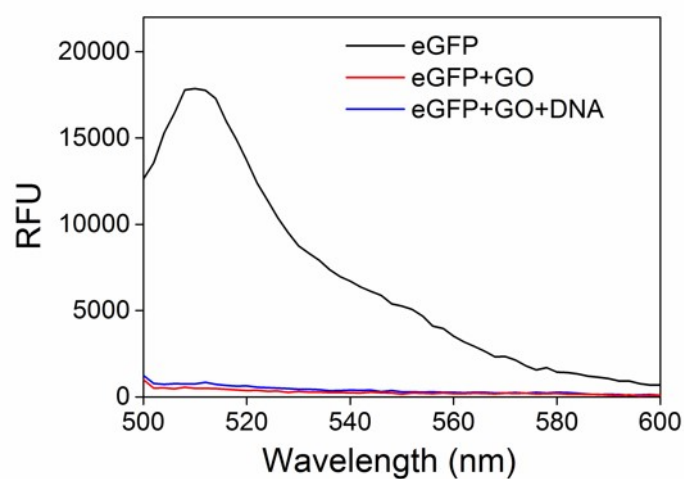
**Fig. S3** The influence of temperature on the fluorescence intensity of ScGFP (50 nM) at 510 nm in the presence (solid dots) or absence (soft points) of GO (1 μg/mL).



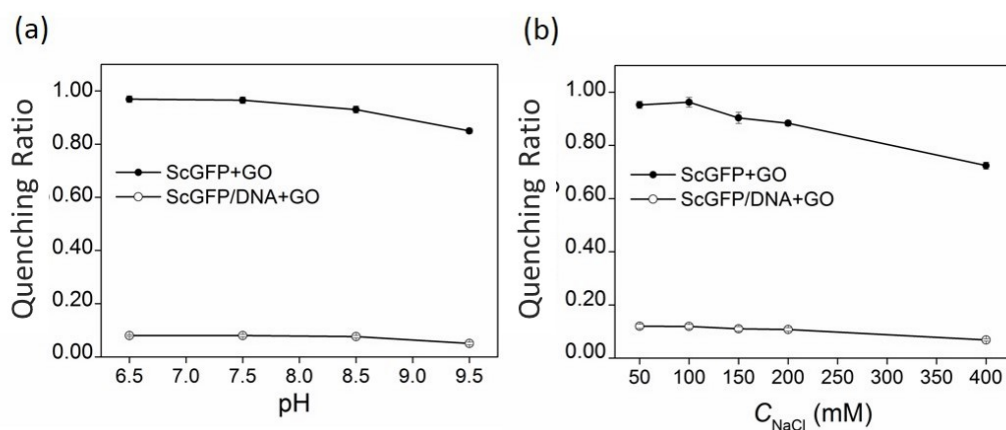
**Fig. S4** AFM image and height profiles of GO (1  $\mu\text{g/mL}$ ) (a), ScGFP (50 nM) (b), and ScGFP/GO (c).



**Fig. S5** Fluorescence spectra of ScGFP (50 nM) (a) and eGFP (50 nM) (b) coexisting with GO (1  $\mu\text{g/mL}$ ), before and after cleavage by different concentrations of thrombin.

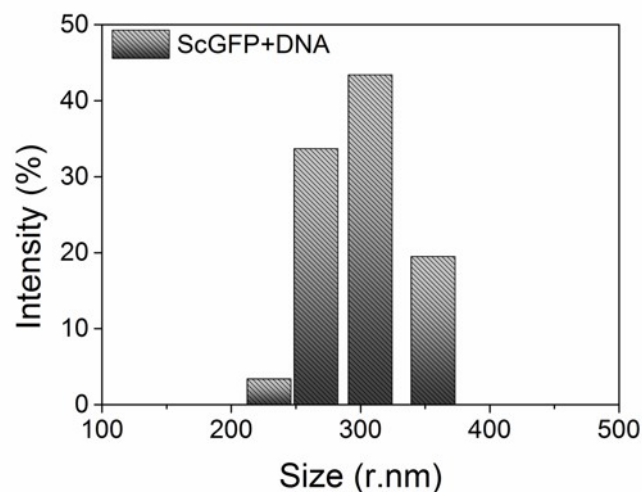


**Fig. S6** Fluorescence spectra of eGFP (50 nM), eGFP mixing with GO (1  $\mu\text{g/mL}$ ), and eGFP coexisting with DNA-10 (200 nM) and GO (1  $\mu\text{g/mL}$ ), respectively.

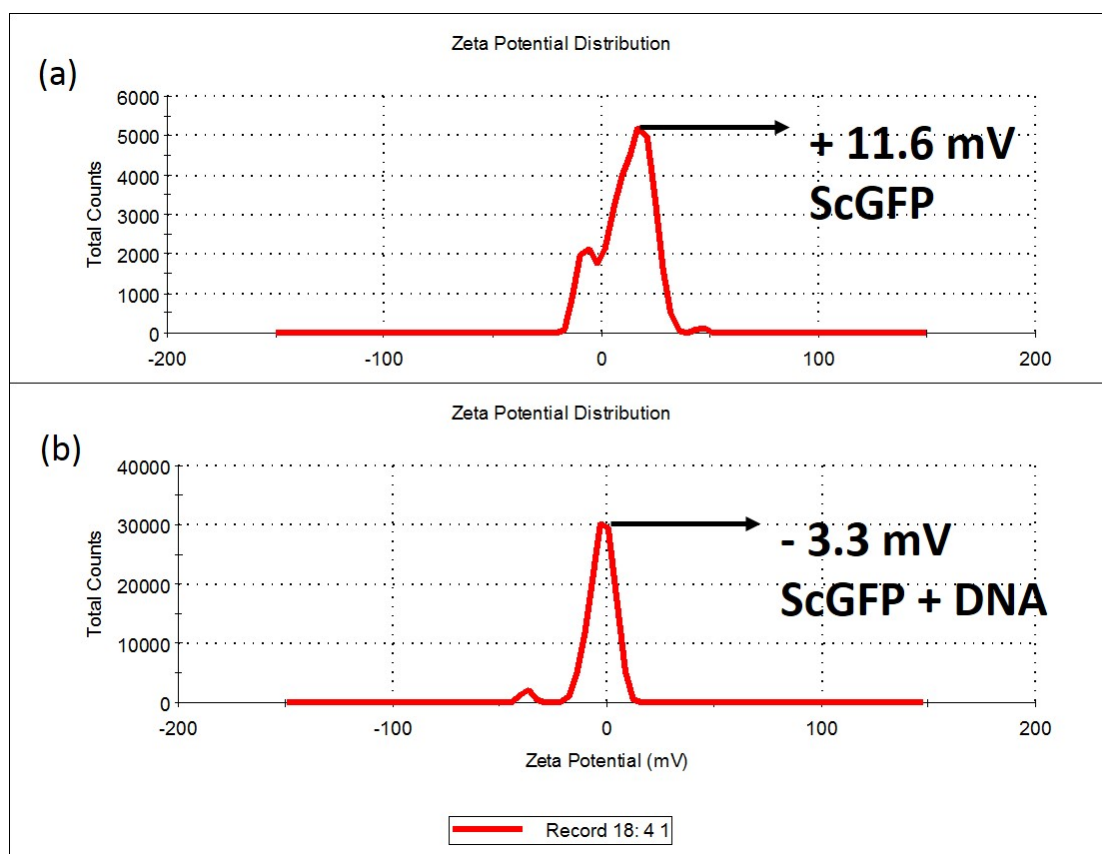


**Fig. S7** The influence of pH (a) and NaCl concentrations (b) on the quenching ratios of ScGFP/GO system when ScGFP was pre-mixed with DNA (soft points) or not (solid dots).

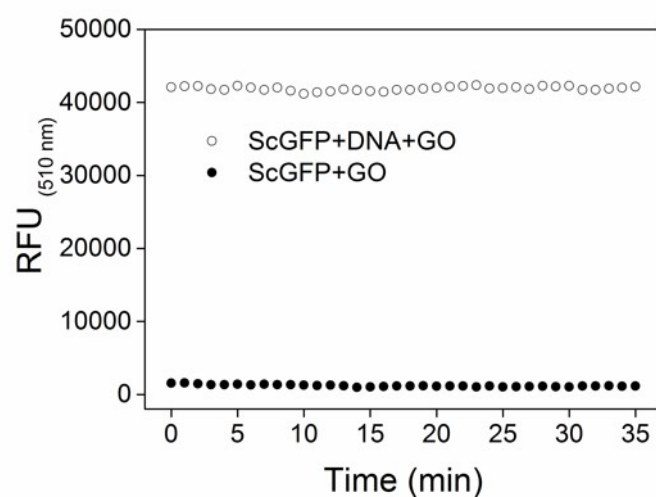




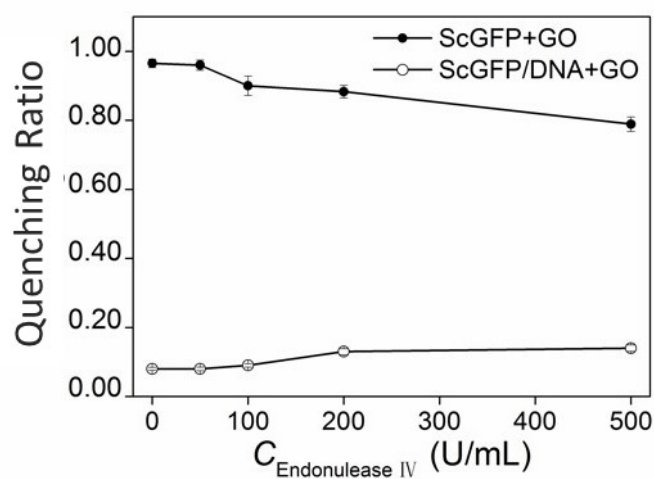
**Fig. S8** DLS of ScGFP (50 nM) mixed with DNA-10 (200 nM) to be ScGFP/DNA nano-complex, and DLS of ScGFP cannot be detected.



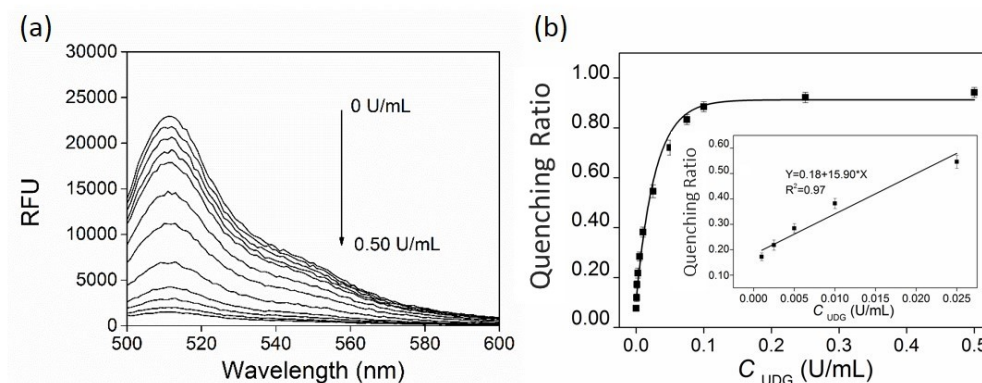
**Fig. S9** Zeta potential of ScGFP (50 nM) (a) and ScGFP mixed with DNA-10 (200 nM) (b) to be ScGFP/DNA nano-complexes



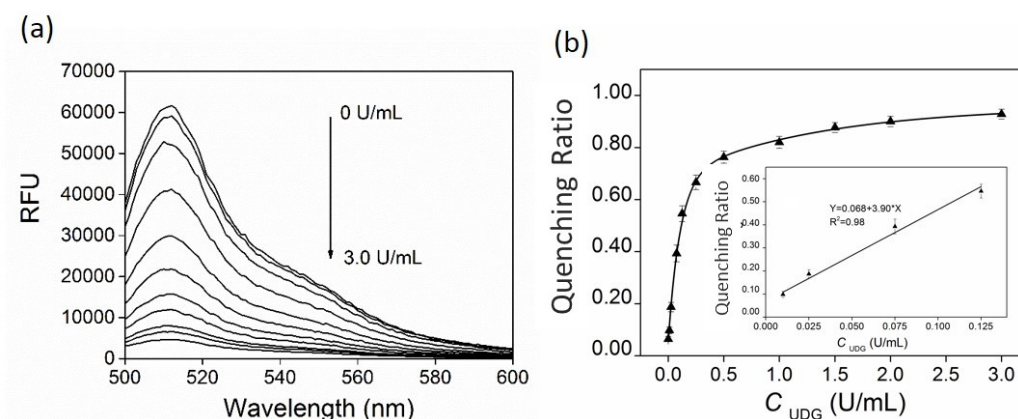
**Fig. S10** The fluorescent intensity of ScGFP/GO system varying with time when ScGFP (50 nM) was pre-mixed with DNA-10 (200 nM) (soft points) or not (solid dots).



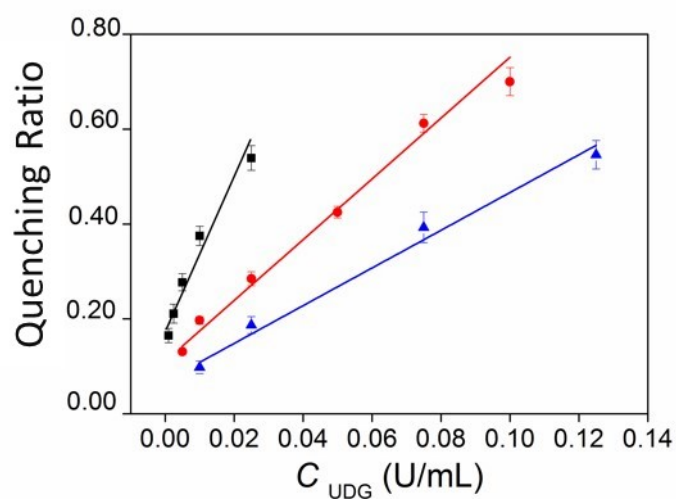
**Fig. S11** The influence of Endo IV concentrations on the quenching ratios of ScGFP/GO system when ScGFP was pre-mixed with DNA (soft points) or not (solid dots).



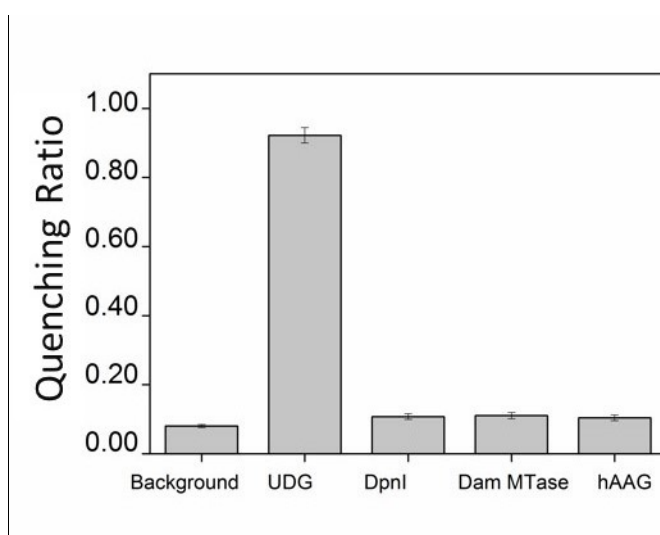
**Fig. S12** The fluorescence spectra of the proposed sensor (25 nM ScGFP, 100 nM DNA-10U) corresponding to the concentration of UDG from 0 - 0.50 U/mL (a) and the calibration curve of quenching ratio as a function of the UDG concentration (b). Inset in (b) shows the linear relationship between the quenching ratio and the UDG concentration from 0.0010 U/mL to 0.013 U/mL.



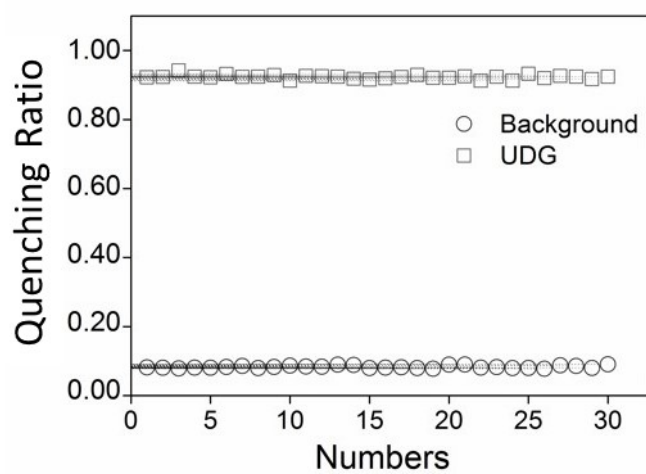
**Fig. S13** The fluorescence spectra of the proposed sensor (100 nM ScGFP, 400 nM DNA-10U) corresponding to the concentration of UDG from 0 – 3.0 U/mL (a) and the calibration curve of quenching ratio as a function of the UDG concentration (b). Inset in (b) shows the linear relationship between quenching ratio and the UDG concentration from 0.010 U/mL to 0.13 U/mL.



**Fig. S14** The linear relationship between quenching ratio and the UDG concentration at different conditions of the proposed sensor (black line 25 nM ScGFP and 100 nM DNA-10U, red line 50 nM ScGFP and 200 nM DNA-10U, blue line 100 nM ScGFP and 400 nM DNA-10U). The lowest LOD was 0.0003 U/mL based on  $3\sigma$  which was obtained in the condition of 25 nM ScGFP and 100 nM DNA-10U .



**Fig. S15** The selectivity of the UDG assay with the addition of UDG (1.0 U/mL) or other nonspecific proteins (10 U/mL DpnI, Dam MTase, hAAG, respectively).



**Fig. S16** Quenching ratio of the proposed sensor in 30 times parallel tests with UDG (1.0 U/mL) or not (background).

#### 4. Reference

1. D. B. Thompson, J. J. Cronican and D. R. Liu, *Methods in enzymology*, 2012, **503**, 293-319.