Electronic Supplementary Material (ESI) for Analyst

Supporting information for

A general fluorescent sensor design strategy for "turn-on" activity detection of exonucleases and restriction endonucleases based on graphene oxide

1. Characterization of GO

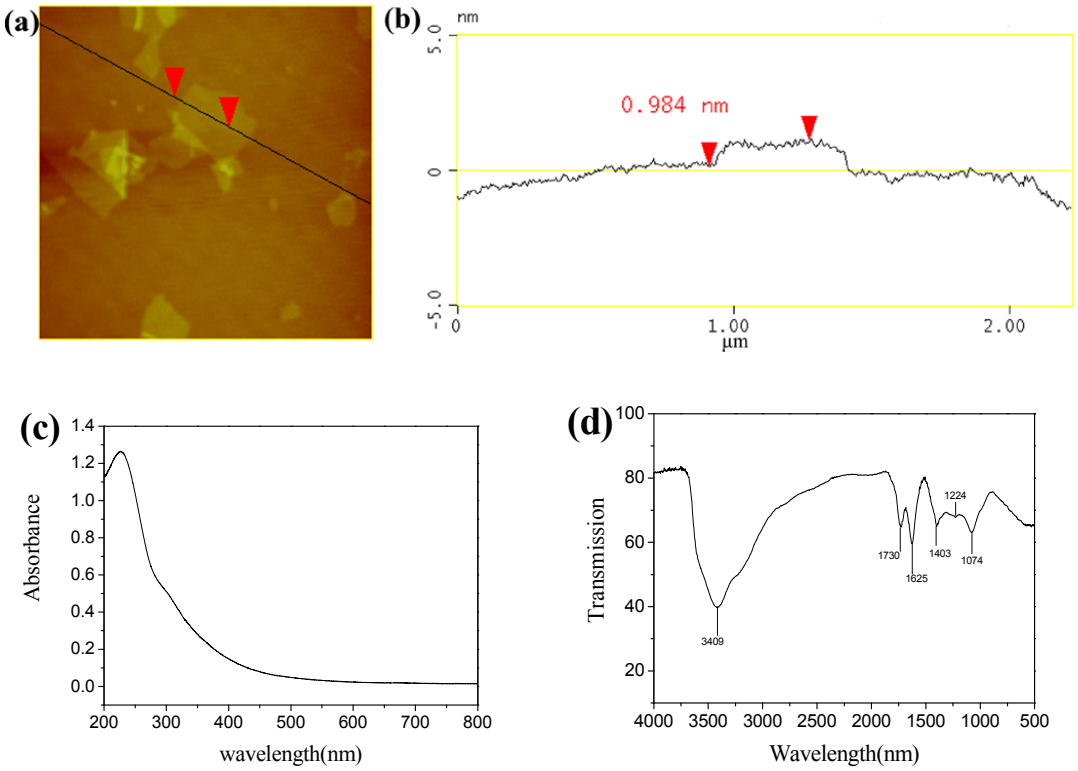

Fig. S1 Characterization of GO. (a) AFM image and (b) height profile of GO show the dimensions of as-prepared GO as 0.1-0.3 μm in width and 0.98 nm in height. (c) UV spectra and (d) FT-IR spectra of GO indicate characteristic peaks of oxygen functional group.

2. Raman characterization of two GOs with different oxidation degrees

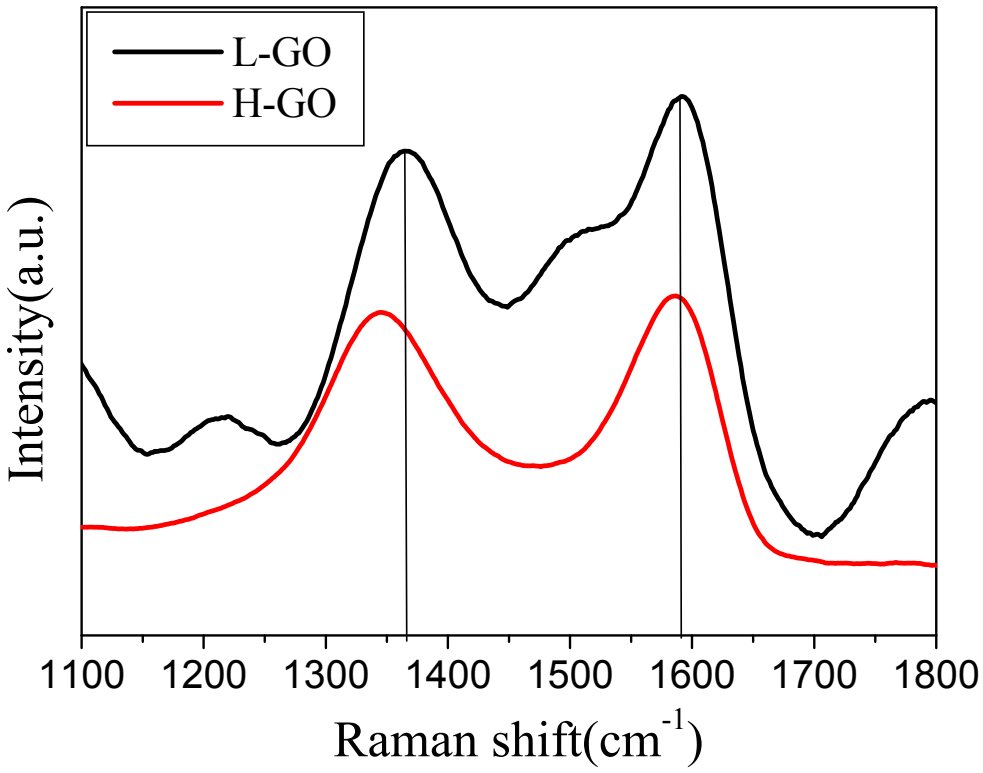

Fig. S2 Raman spectra of two GOs with different oxidation degrees.

3. X-ray photoelectron spectroscopy (XPS) characterization of two GOs with different oxidation degrees

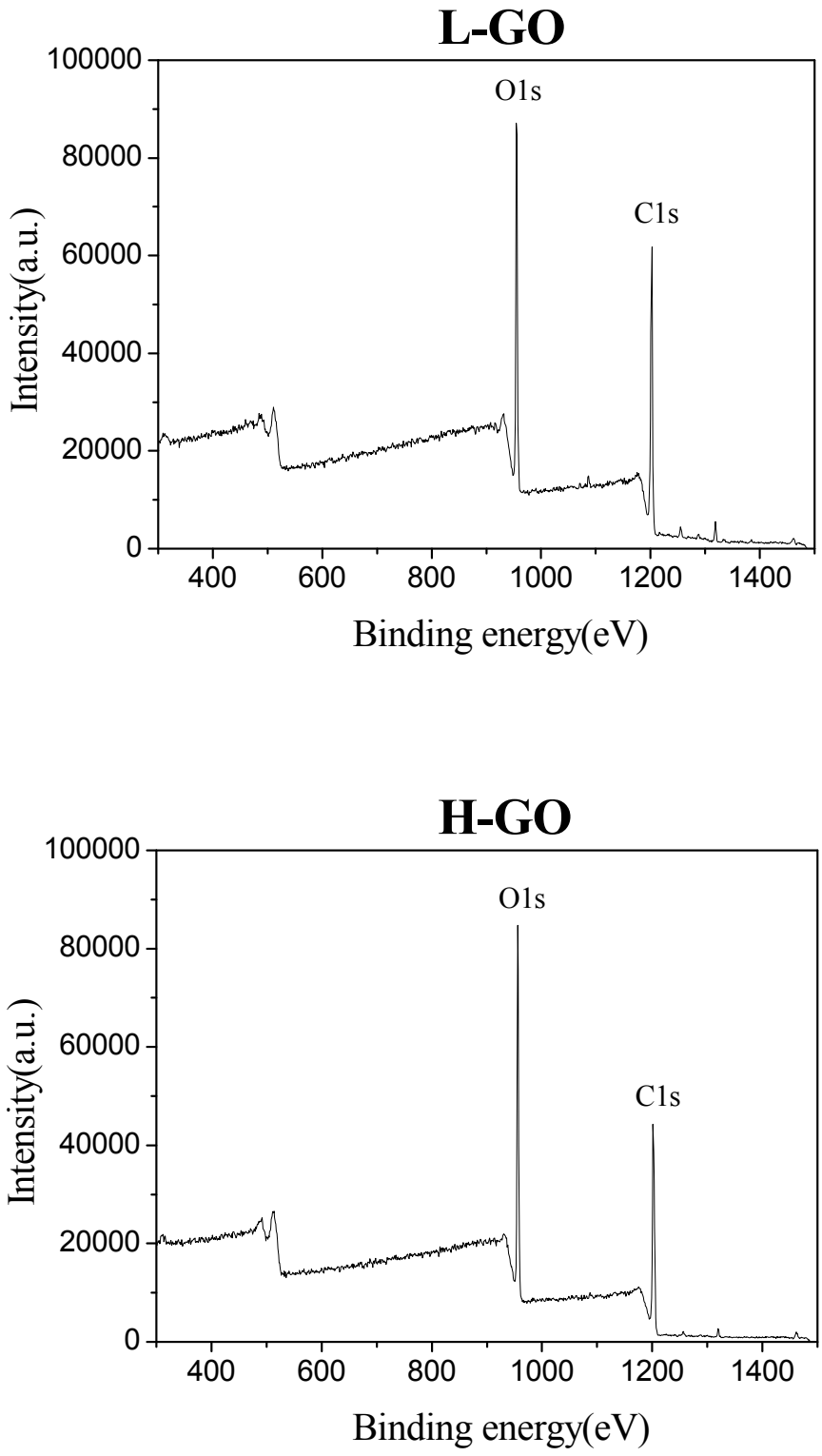

Fig. S3 XPS wide spectra of L-GO(top) and H-GO (bottom).

4. Fluorescence quenching of fluorescent labeled ss-DNAs by the two GOs with different oxidation degrees

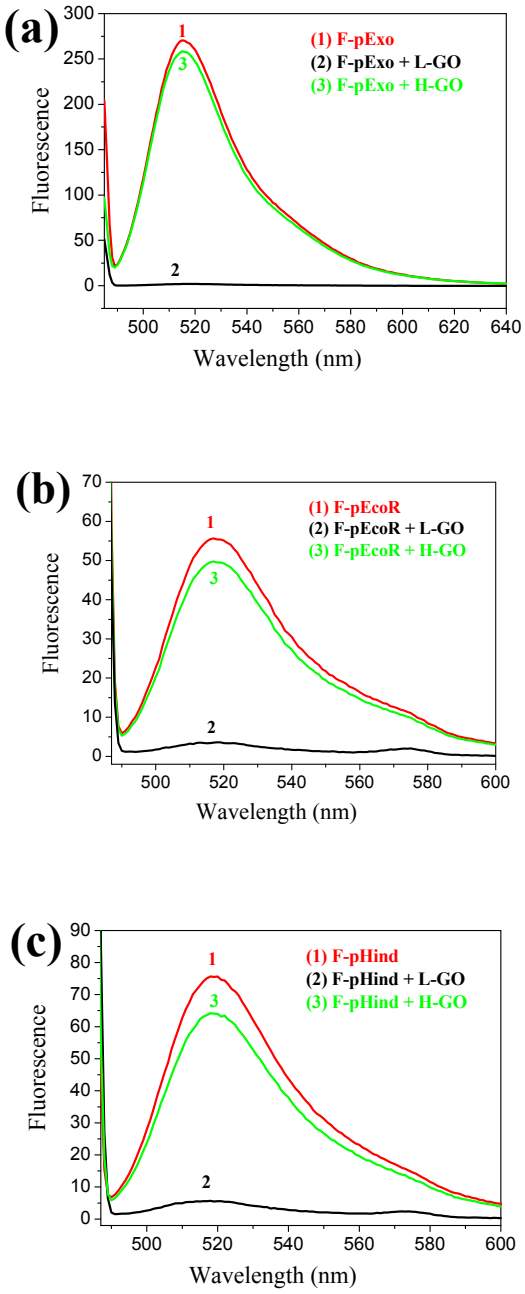

Fig. S4 Fluorescence emission spectra of fluorescent labeled ss-DNAs under different conditions. (a) The fluorescent labeled F-pExo was used. [F-pExo] = 200 nM, [L-GO] = 16 μg/100 μL, [H-GO] = 50 μg/100 μL; (b) The fluorescent labeled F-pEcoR was used. [F-pEcoR] = 100 nM, [L-GO] = 8 μg/100 μL, [H-GO] = 20 μg/100 μL; (c) The fluorescent labeled F-pHind was used. [F-pHind] = 100 nM; [L-GO] = 10 μg/100 μL; [H-GO] = 25 μg/100 μL.

5. Thickness change of L-GO before and after Exo 1 treatment

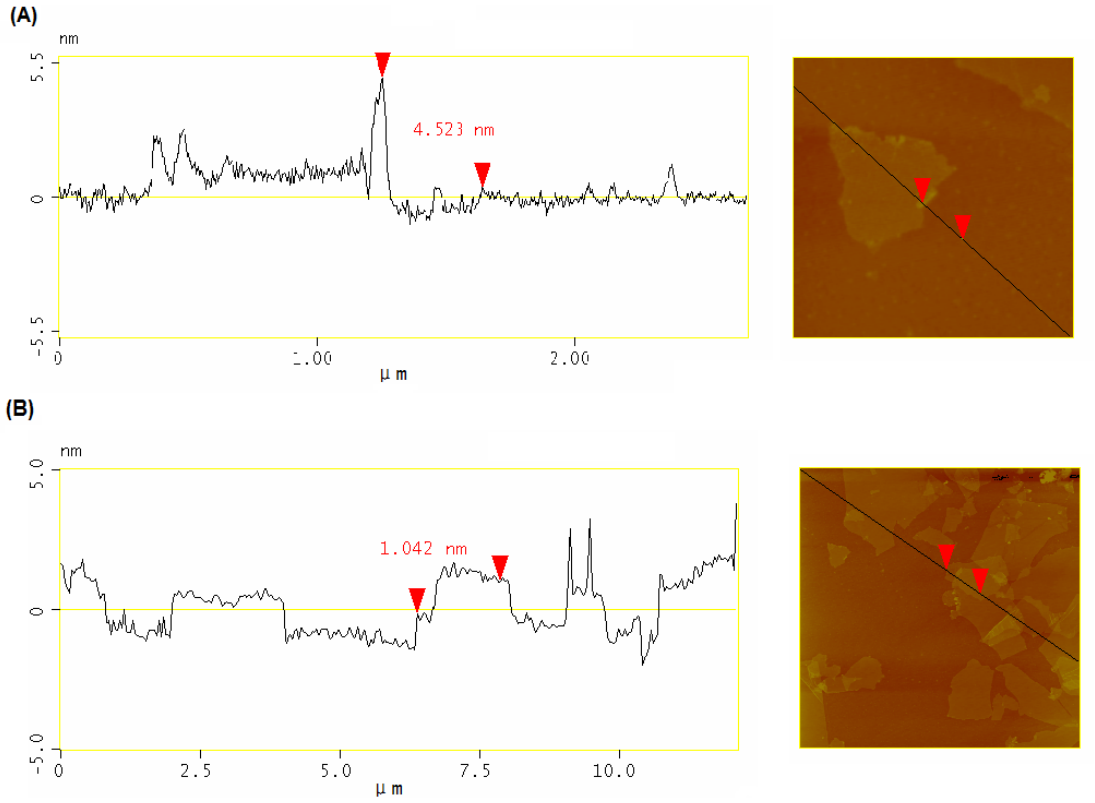

Fig. S5 AFM images of the L-GO/F-pExo mixture before (A) and after (B) treatment with Exo 1.

6. The morphology of GO in different conditions

The morphology of GO in different conditions was characterized by high-resolution transmission electron microscopy (HRTEM). Before ultrasound treatment, only large black spots could be observed, indicating that the synthesized GOs aggregated together (Fig. S6A). However, after an ultrasound treatment, wrinkled and silk–like sheets were observed on the top of the grid, which is the typical HRTEM image of single-layered GO (Fig. S6B). The presence of fluorescent labeled ss-DNA (F-pExo) and Exo 1 nearly had no effect on the morphology of the L-GO. The large sheet still wrinkled but not folded (Fig. S6C,D).

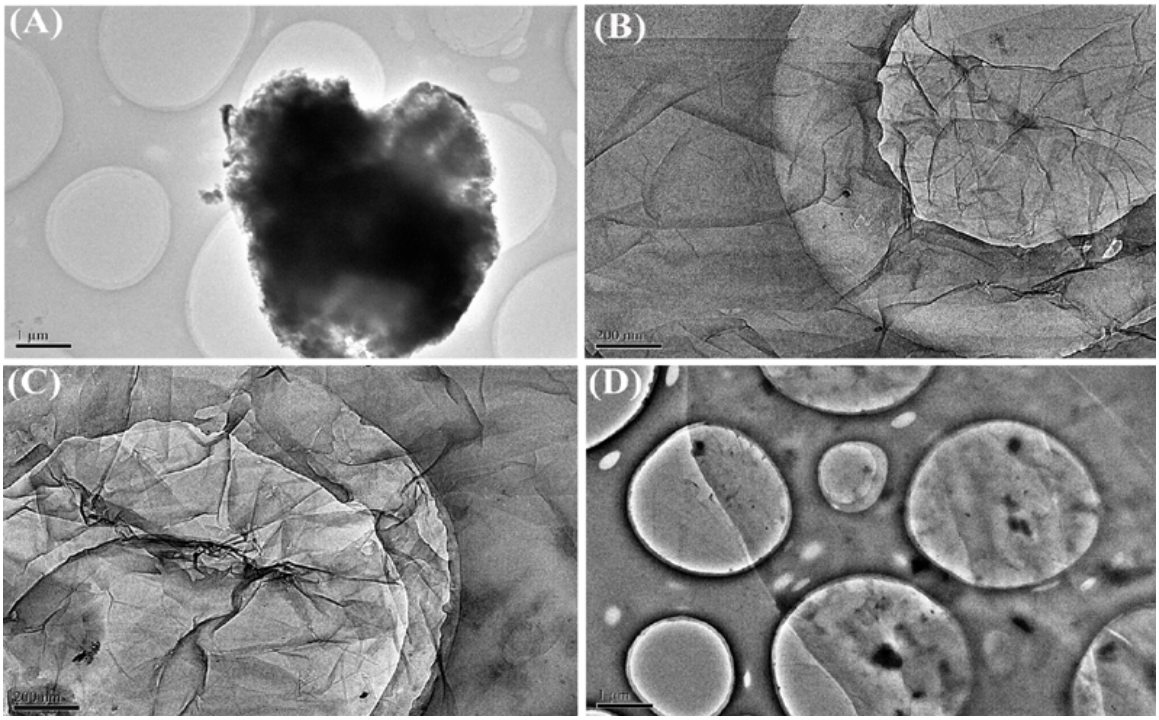

Fig. S6 HRTEM images of GO in different conditions. (A) GO before ultrasound treatment; (A) GO after ultrasound treatment; (C) The mixture of L-GO and F-pExo; (D) The mixture of L-GO and F-pExo after treatment with Exo 1.

7. Optimization of digestion reaction time for Exo 1 detection

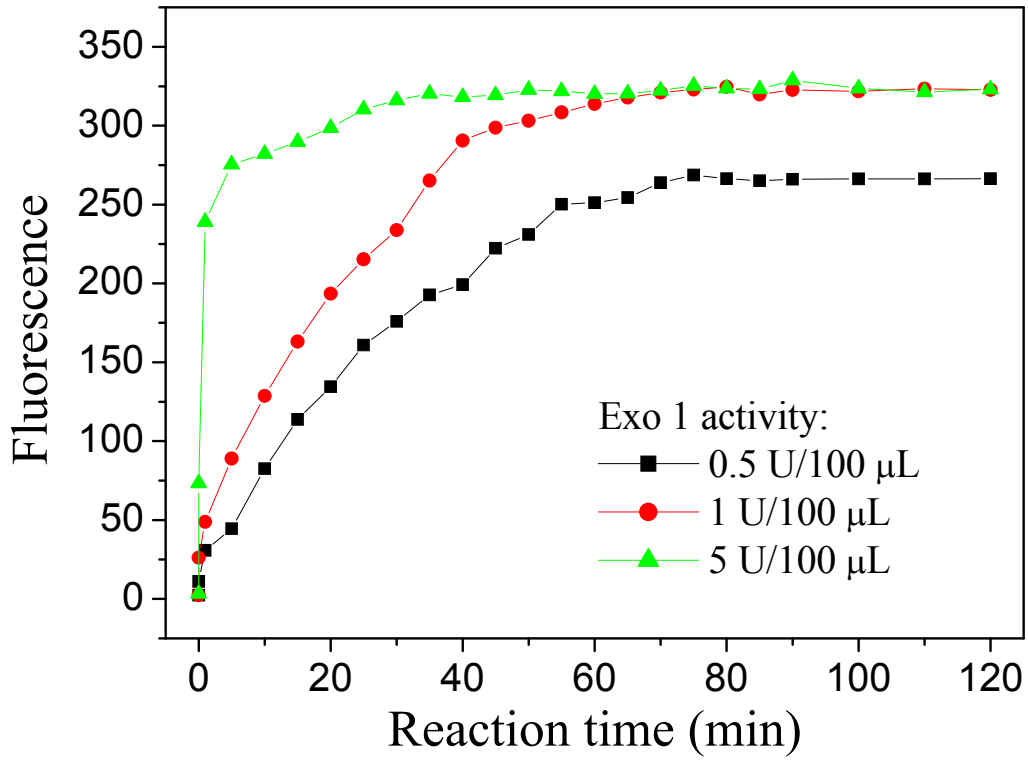

Fig. S7 Digestion time-dependent fluorescence change of the sensing system. [F-pExo] = 200 nM. Reaction temperature was 37°C.

8. Examination of EcoR I-triggered cleavage of F-pEcoR by polyacrylamide gel electrophoresis (PAGE)

5 μL of 100 μM F-pEcoR solution was dissolved in 35 μL Tris-HCl buffer (pH = 7.5) containing 50 mM Tris-HCl, 100 mM NaCl, 10 mM $MgCl_2$ and 1 mM DTT. The mixture was heated at 95 ^{o}C for 5 min, then cooled slowly to room temperature (25 ^{o}C) and incubated at 25 ^{o}C for 30 min.. Different concentrations of EcoR I were added. The final volume of the mixture was 50 μL. After incubation at 37 ^{o}C for 2 h, 5 μL loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol, 10 mM EDTA) was added. 30 μL of samples were loaded on 21% polyacrylamide gel. Electrophoresis was carried out in 1 × TBE buffer at 10 V/cm for 3h at room temperature. The gel was photographed using a Gel Documentation system (Huifuxingye, Beijing, China).

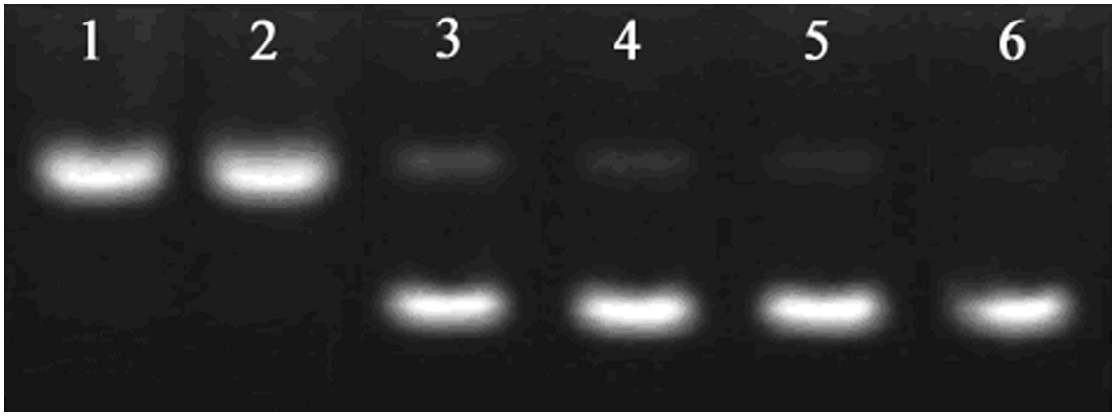

Fig. S8 PAGE analysis of EcoR I-dependent cleavage of F-pEcoR. Lane 1: F-pEcoR only; Lane 2: F-pEcoR is treated with 40 U/100 μL inactive EcoR I; Lane 3-6: F-pEcoR is treated with 10, 20, 40 or 100 U/100 μL EcoR I, respectively. [F-pEcoR] = 10 μM.

9. Feasibility of the proposed EcoR I sensor

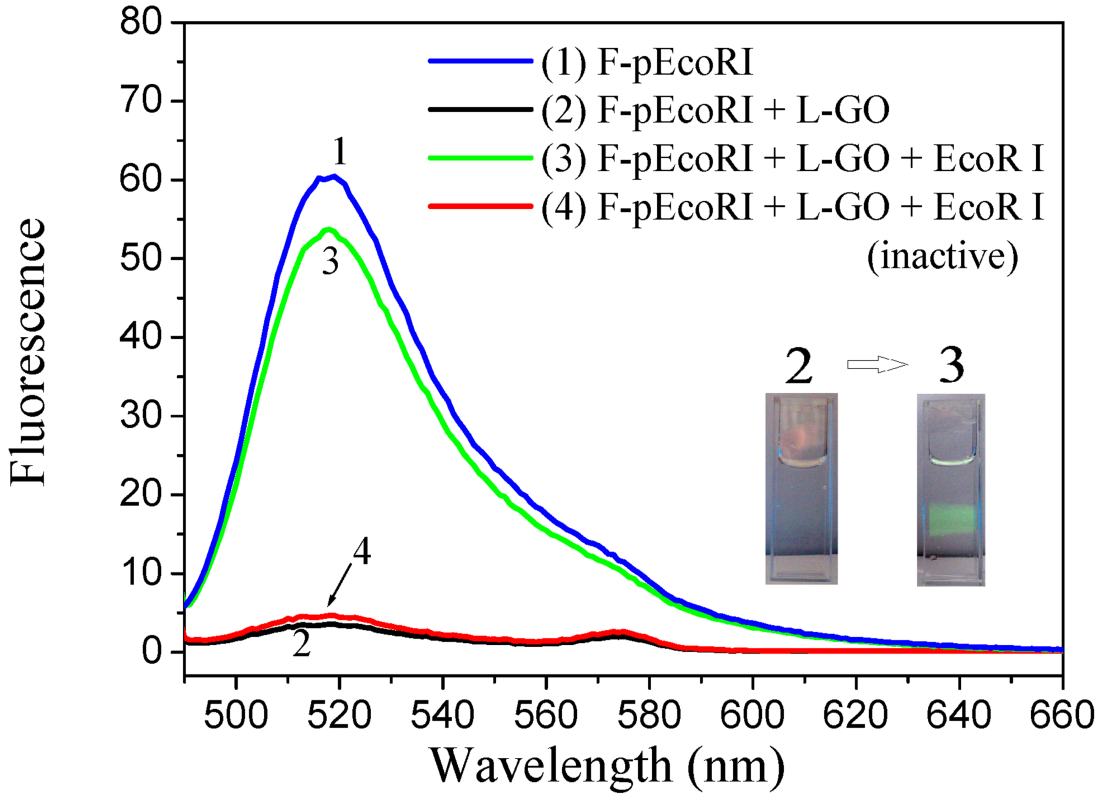

Fig. S9 Fluorescence emission spectra under different conditions. The insert shows the photographed images of the detection system in the absence or presence of EcoR I when they are irradiated with light of 480 nm. [F-pEcoR] = 100 nM; [L-GO] = 8 μg/100 μL; [EcoR I] = 0.15 U/100 μL. The inactive EcoR I was prepared by heating the enzyme solution at 95 ^{o}C for 20 min.

10. Optimization of L-GO concentration for EcoR I detection

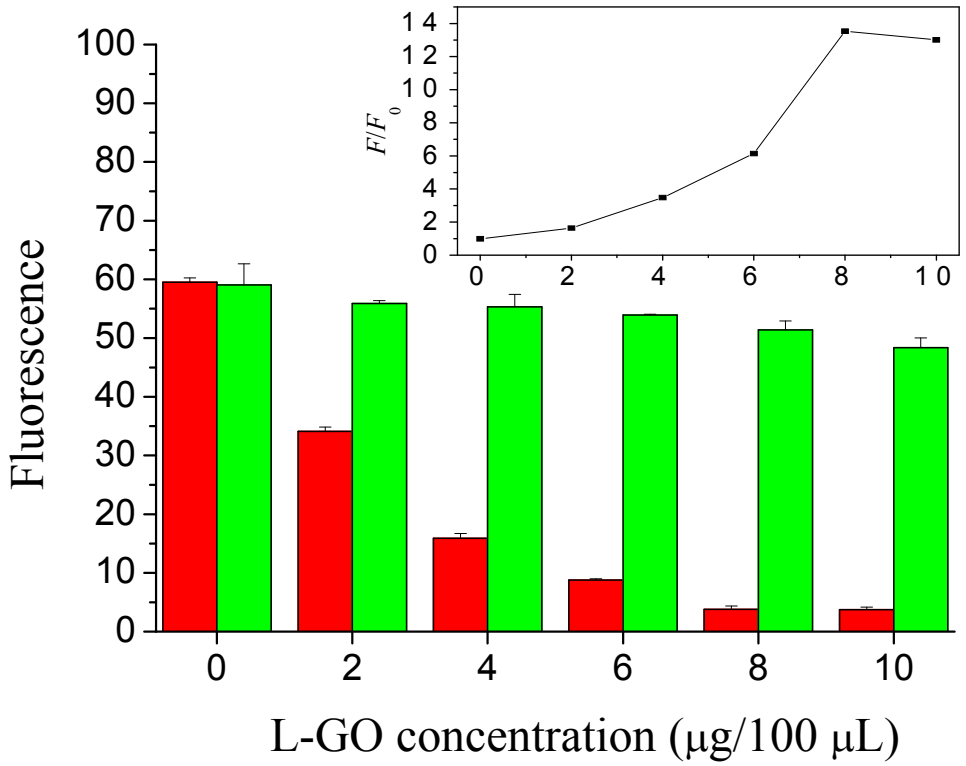

Fig. S10 Fluorescence signals of the sensing system containing different L-GO concentrations in the absence or presence of 0.15 U/100 μL EcoR I. F/F_0 is defined as the signal-to-background ratio, F and F_0 represent the fluorescence intensities in the presence and absence of EcoR I, respectively. [F-pEcoR] = 100 nM. λ_{ex} = 480 nm, λ_{em} = 519 nm.

11. Optimization of digestion reaction time for EcoR I detection

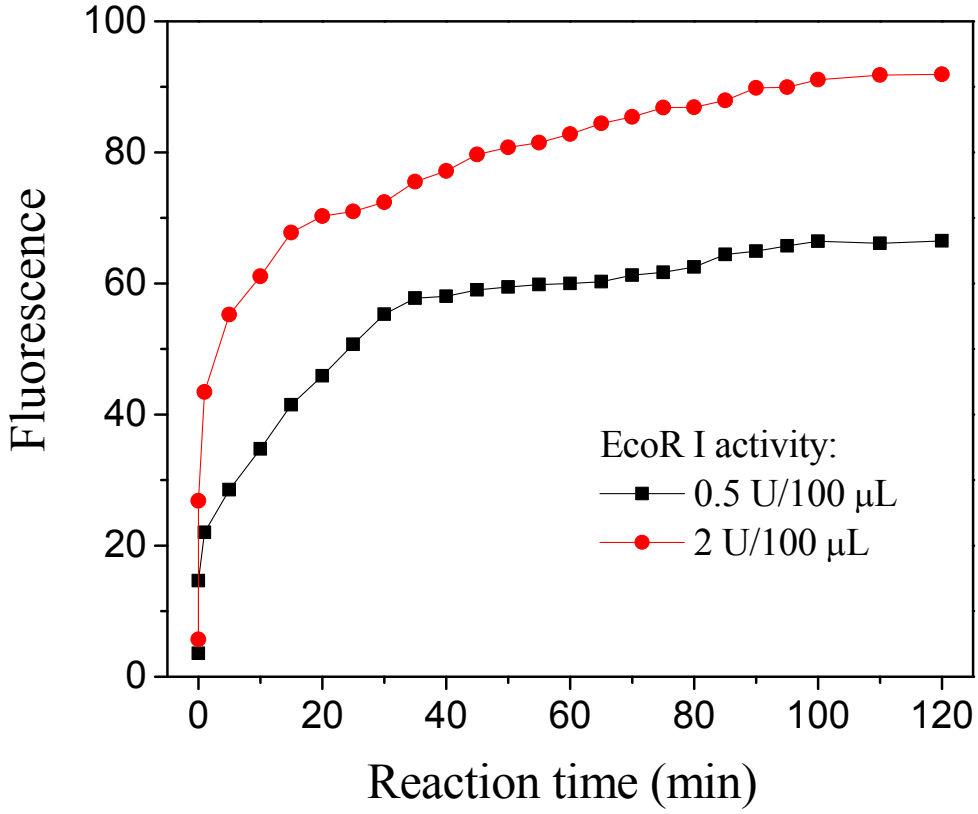

Fig. S11 Digestion time-dependent fluorescence change of the sensing system. [F-pEcoR] = 100 nM. Reaction temperature was 37 °C.

12. Comparison of our EcoR I activity detection method with other reported methods

Table S1 Comparison of several EcoR I activity detection methods

Method	Linear range (U/mL)	Detection limit (U/mL)	Reference
gold nanorods	1.0-100	0.65	17
Enzyme-responsive vanoparticle	25-150	Not given	11
Cationic conjugated polymer/DNA complexes	Not given	0.15	34
G-quadruplex DNAzyme-based fluorescent probe	0.1-5	0.1	35
SYBR Green I method	40 -300	8	36
This method	0.1-2 U/100 μL	0.06	

13. Optimization of L-GO concentration for Hind III detection

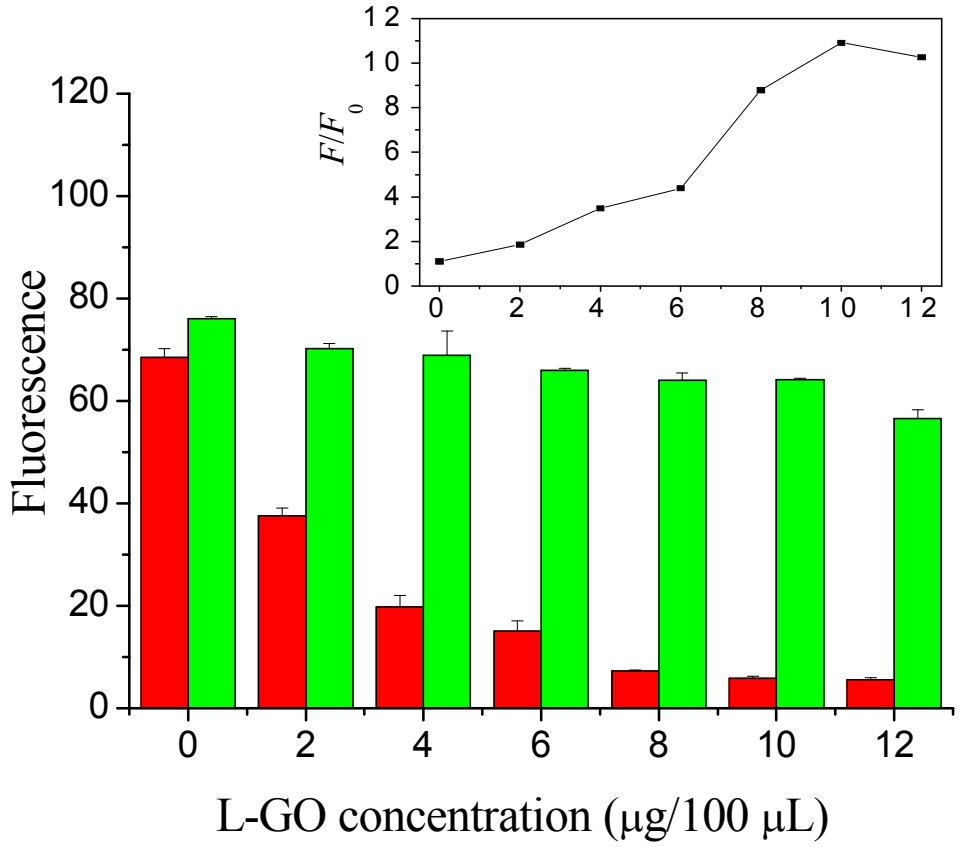

Fig. S12 Fluorescence signals of the sensing system containing different L-GO concentrations in the absence or presence of 0.19 U/100 μL Hind III. F/F_0 is defined as the signal-to-background ratio, F and F_0 represent the fluorescence intensities in the presence and absence of Hind III, respectively. λ_{ex} = 480 nm, λ_{em} = 519 nm. According to the result, 10 μg/100 μL was selected as the optimal L-GO concentration.

14. Hind III activity detection using the proposed Hind III sensor.

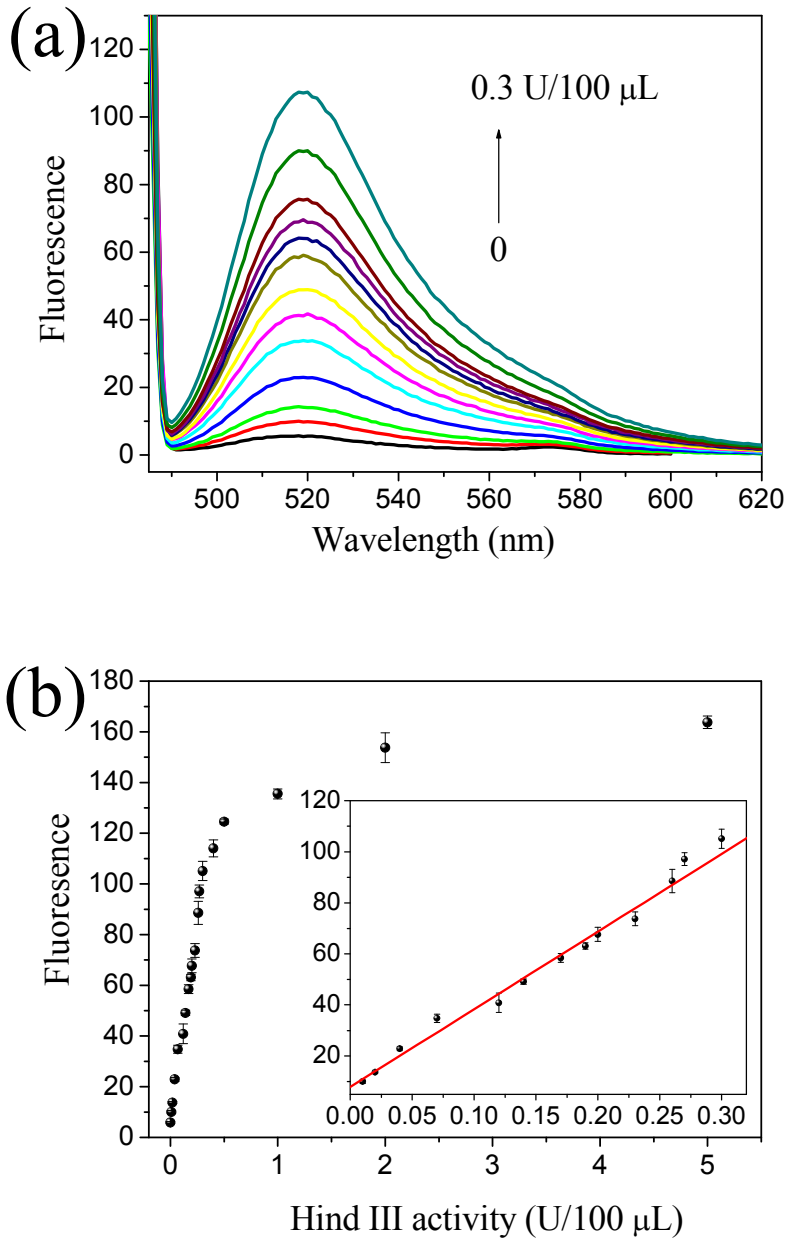

Fig. S13 Sensitivity of GO-based Hind III sensor. (A) Hind III activity-dependent change in fluorescence spectra of the sensing system. The activities of Exo1 are (arrow direction): 0, 0.01, 0.02, 0.04, 0.07, 0.12, 0.14, 0.17, 0.19, 0.20, 0.23, 0.26, 0.30 U/100 μL. (B) Hind III activity-dependent change in the fluorescence signal at 519 nm. The insert shows the absorption signal change in the Hind III activity range of 0.01-0.30 U/100 μL. The solid line represents a linear fit to the data. All experiments were performed in triplicate.