Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Inducible Graphene Oxide Probe for High-Specific Tumor Diagnosis

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1 Materials and animals

N-(2-Aminoethyl) maleimide, 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), *N*,*N*-Dimethylformamide (DMF, anhydrous, 99.8%), and sodium chloroacetate were purchased from Sigma-Aldrich. Propargyl-GPLGVRGC was synthesized by GL Biochem Ltd. (Shanghai, China). The fluorescent dye azide cyanine-5 (N_3-Cy5), were offered by Fanbo Biochemical Co.(Beijing, China). Cell-Counting Kit-8 (CCK8) kit was from the Dojindo Laboratories.

Murine Lewis lung carcinoma (LLC) cells, human fibrosarcoma (HT-1080) cells, and human proximal tubular epithelial (HKC) cells were supplied by ATCC (American Type Culture Collection). Cells were cultured with DMEM cell culture medium added with penicillin (100 U mL^{-1}), streptomycin (100 U mL^{-1}), and 10% fetal bovine serum (FBS) in a humidified incubator at 37 °C and 5% CO_2. DMEM and FBS were purchased from Invitrogen, with culture flasks and dishes from Corning.

Male C57BL/6 mice, 6-8 weeks of age, were ordered from Charles River Laboratories (USA). For tumor-bearing mouse model, a subcutaneous syngeneic transplantable model of lung cancer was established by injecting 1 × 10^6 LLC cells in 100 μL PBS (pH 7.2) subcutaneously at the axillary fossa of male C57BL/6 mice. All animal experiments were performed in compliance with the institutional ethics committee regulations and guidelines on animal welfare (Animal Care and Use Program Guidelines of Peking University).

2 Methods

2.1 GO preparation

Preparation of nano-sized GO sheets was started from the primary GO that made by a modified Hummers method. After sufficient sonication and washing, the GO sheets were separated by making use of specific sedimentation rates of graphene in different size. The centrifugal forces was selected as 10,000-30,000 g.

In virtue of a typical absorption peak at 230 nm, GO concentrations were determined using an Ultrospec 2100 pro UV/Visible spectrophotometer. GO characterization was performed on a BioScope Catalyst AFM (Veeco), operating in tapping mode in air at room temperature.

2.2 Modification of GO with chloroacetic acid

Preparation a 50 mL solution consisting of 2 M sodium chloroacetate in 4 M NaOH. Immediately add GO (50 mL, 100 μg/mL) to above solution, following with stirring. After reacting for 70 min at room temperature, stop the reaction and adjusting the pH to neutral with 6 N HCl. Remove excess reactants by centrifugation.

2.3 Assay of carboxyl groups on GO surface

To detect the carboxyl groups of different GO, we employed a convenient conductometric titration method. In brief, a given quality of GO or GO-COOH was tittered by a conductometric meter. The surface carboxyl intensity was calculated by the following equation. Carboxyl groups intensity (μmol/g) = $10^6 \times M \times (V_2 - V_1)/1000/W$, where M (mol/L) is the concentration of sodium hydroxide (NaOH), $(V_2 - V_1)$ (mL) is the linear fitting volume of NaOH, and W (g) is the GO quality.

2.4 Fabrication of probe

Cy5-GPLGVRGC (p-Cy5) was obtained by conjugating propargyl-GPLGVRGC and N_3-Cy5 through click chemistry, which was confirmed by mass spectrometry (Mw = 1398.54). The freeze-dried GO-COOH was transferred to DMF solvent and mixed with *N*-(2-Aminoethyl) maleimide. The activator EDC was then added following with stirring for 4-6 h. The product was separated and washed with DMF by centrifugation. Then, p-Cy5 was added, and reacts for 2 h. Finally, the probe with grafting ratio of 86.5% was fabricated.

2.5 Cytotoxicity test of GO sheets

A CCK8 assay was applied to examine the effect of GO and probe on cell viability. Typically, 5000 cells were cultured in each well (100 μL) of 96-well plate and allowed to adhere for 12 h, and then 10 μL serial dilutions of GO (ranging from 0 to 20 μg/mL) were added to the culture medium. After 48 h coincubation, cell samples were treated with 10 μL CCK8 for 1 h at 37 ℃. To avoid interference from the residual GO, 80 mL supernatants of all tested samples were transferred to a new 96-well plate before the final absorbance measurement. In addition, we prepared wells for background absorbance measurement that containing all material exceptcells. The water-soluble formazan product, generated by cellular dehydrogenase activity, was determined on an Infinite M200 microplate spectrophotometer (Tecan) at 450 nm. The absorbance was normalized to comparison with the GO-untreated

control.

2.6 *In vivo* NIR fluorescence imaging

The tumor-bearing mice were injected with probe (100 μL, 10 μg/mL). At different time intervals, the mice were anesthetized and scanned using an in-vivo imaging system (FX Pro, Carestream) with an excitation band pass filter at 650 nm and an emission filter at 700 nm. X-ray imaging was acquired for contouring the skeleton.

3 Figures

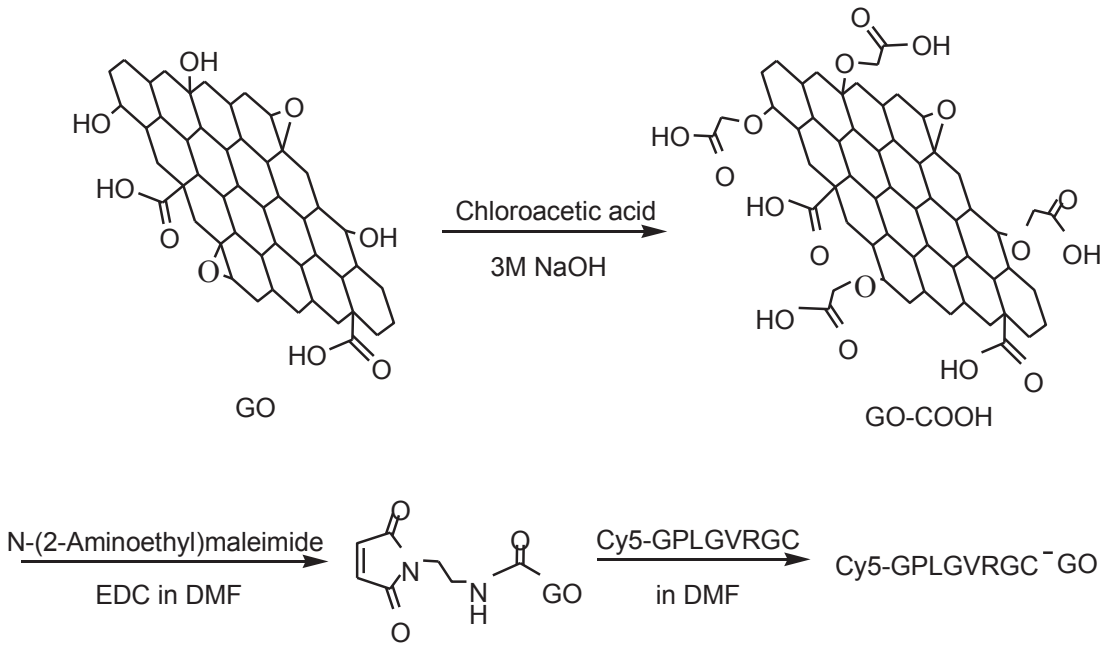

Figure S1. Synthetic scheme of probe.

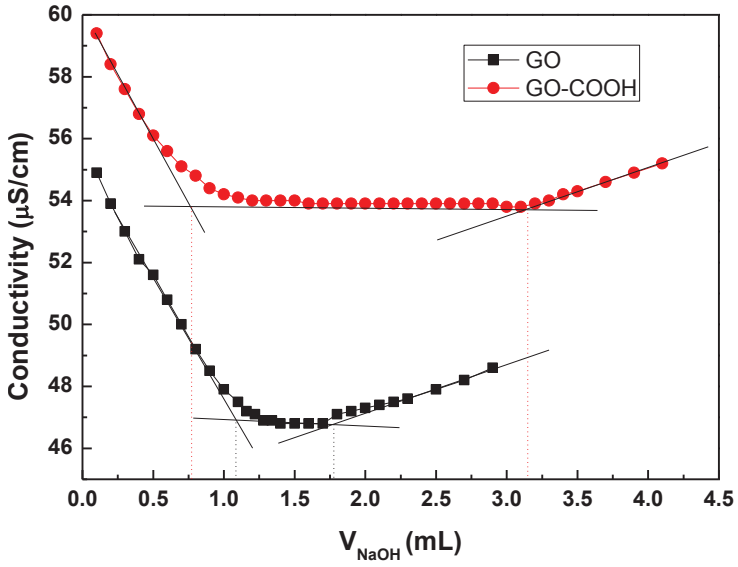

Figure S2. Conductance curve of GO and carboxylated GO indicating the concentration of carboxyl groups.

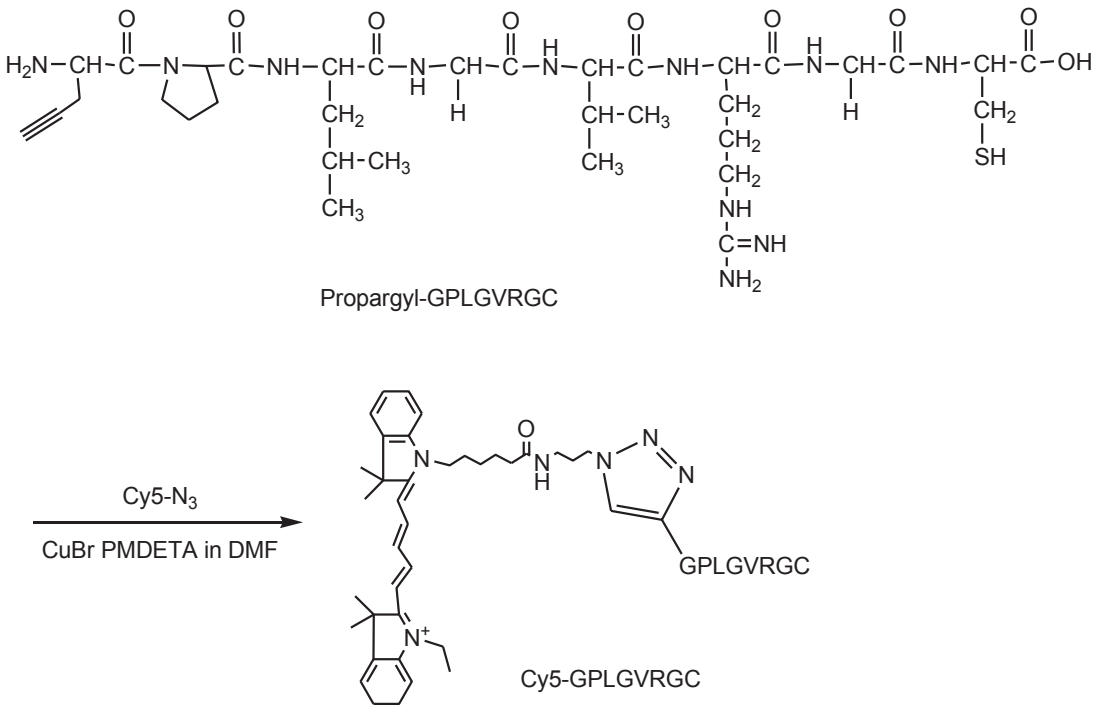

Figure S3. Synthetic scheme of Cy5-GPLGVRGC (p-Cy5).

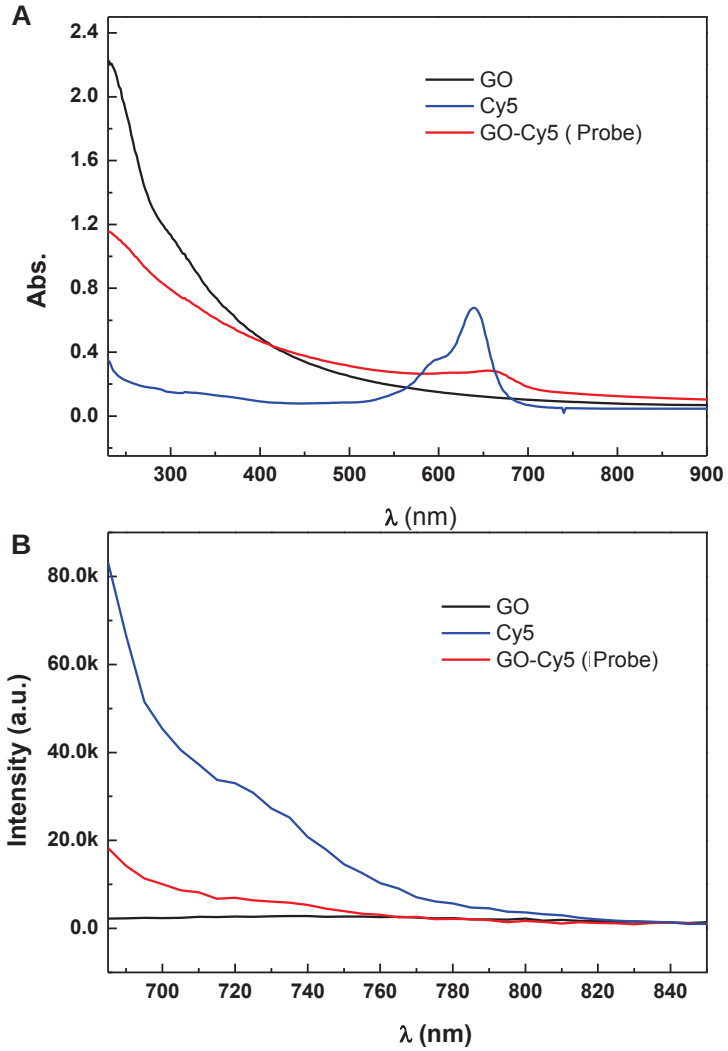

Figure S4. UV/Vis spectra (A) and emission spectra exited at 650 nm (B) of GO, Cy5 and GO-Cy5. UV/Vis spectra showed that the absorbance peak of Cy5 had a red shift after conjugation (from 640 nm to 660 nm). Emission spectra indicated that the Cy5 fluorescence was greatly quenched after grafted on the GO sheet.

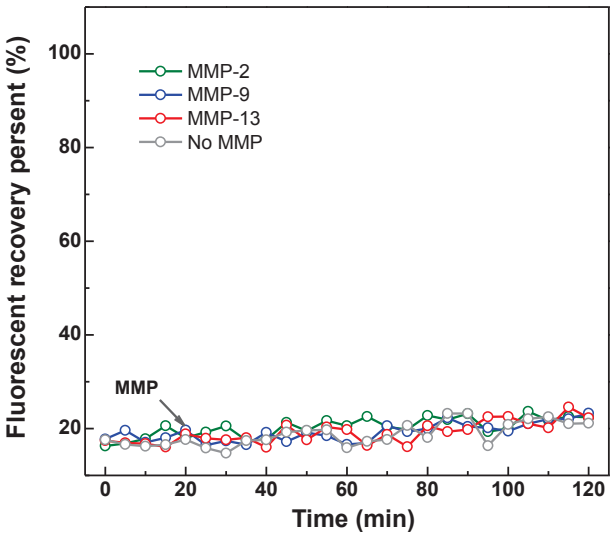

Figure S5. Fluorescence intensity kinetics of GO-scrambled peptide-Cy5 after exposed to different MMPs (100 nM). The probe without MMP was applied as a control. The fabricating method of GO-scrambled peptide-Cy5 was the same as that of probe, expect that the conjugate peptide was changed to a scrambled one (GIVGPLGC).

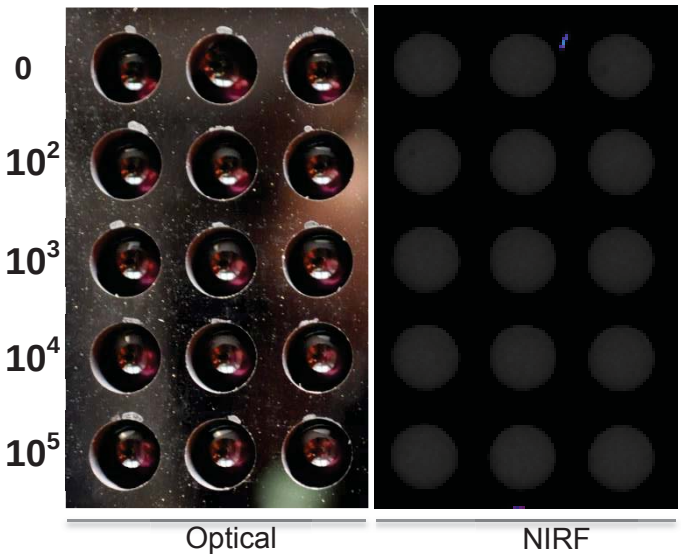

Figure S6. Optical and NIRF images of probe solution after 1 h incubation with cell culture media extracted from different numbers of HKC cell. In NIRF image, X-ray imaging was acquired for contouring the skeleton.

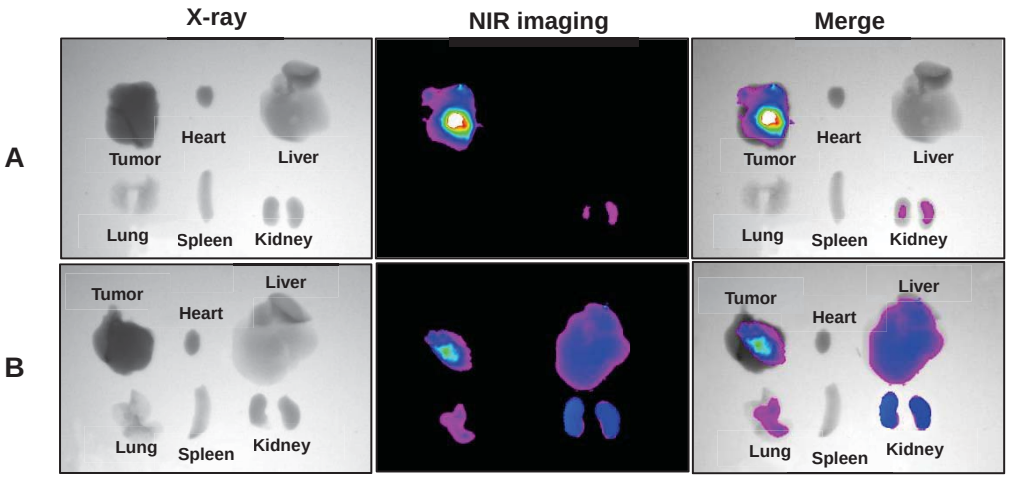

Figure S7. Representative NIRF images (merged with X-ray) of dissected main organs of LLC tumor-bearing mice sacrificed 6 h after intravenous injection of probe (A) and Cy5-RGD (B). After Cy5-RGD injected, although the tumor site showed some fluorescence, yet high background fluorescence could be detected in kidney, liver, and lung. In contrast, bright fluorescence can only be detected in tumor site when probe was injected.

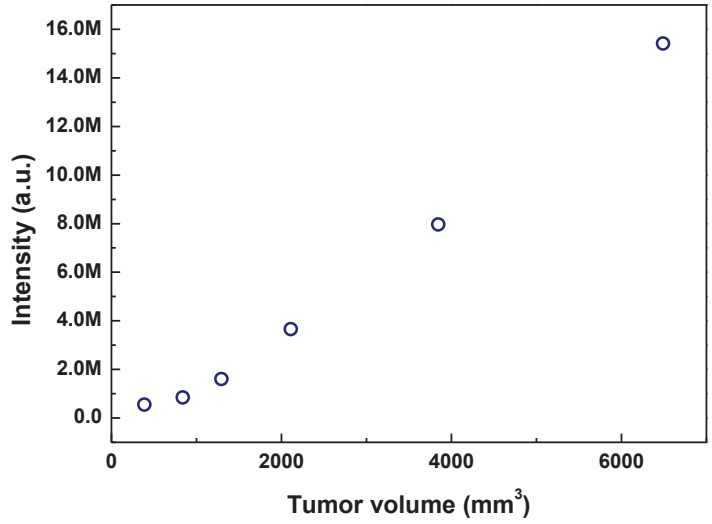

Figure S8. The relation of fluorescence intensity to tumor volume.

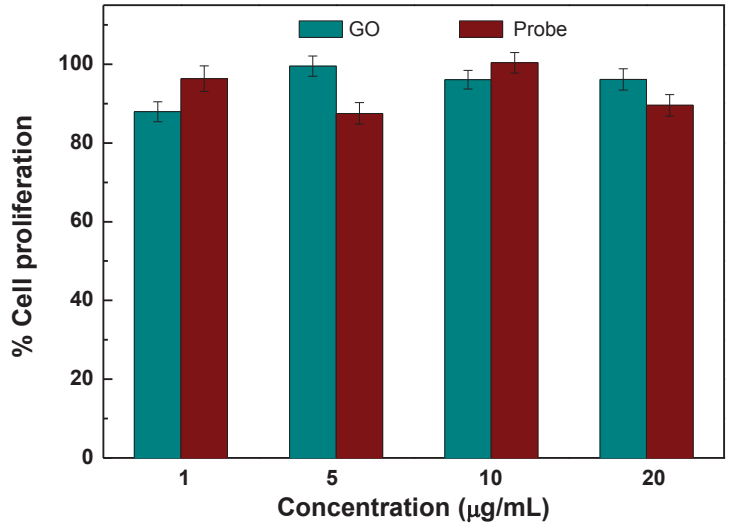

Figure S9. CCK8 assay of LLC cells after incubated with GO or probe for 24 h. Data present means ± SD (n = 3).

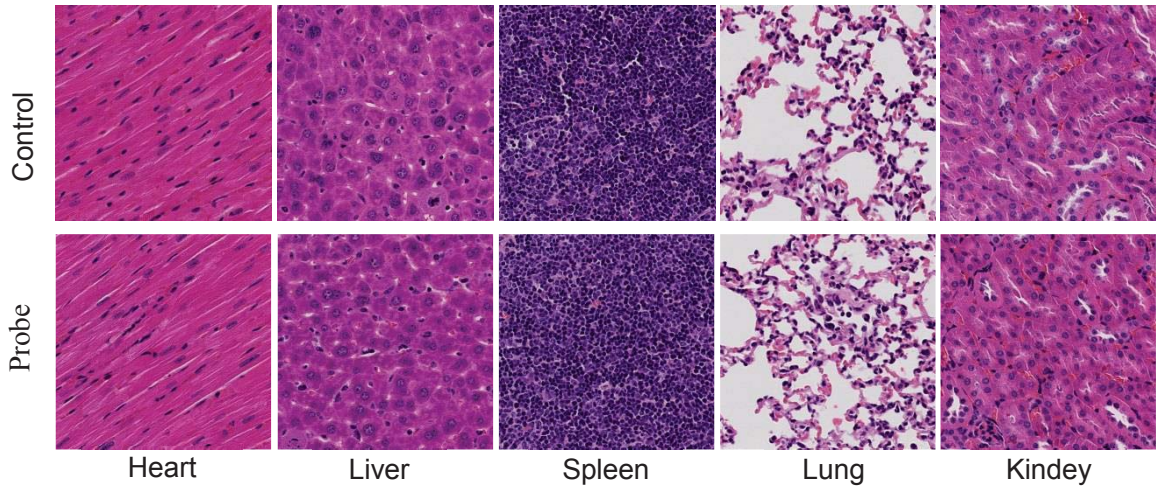

Figure S10. Histopathology of the main tissues (100×) in mice 7 d post injection of probe (0.05 mg/Kg) or PBS (100 μL).

Table S1. Blood cell counts after intravenous injection of probe (100 μL, 10 μg/mL) or PBS (100 μL) in LLC tumor-bearing C57BL/6 mice.

Groups	WBC (10^9/L)	RBC (10^{12}/L)	Platelets (10^{12}/L)
PBS	8.28 ± 1.62	8.56 ± 0.60	1093.71 ± 72.58
Probe	6.40 ± 1.27	7.13 ± 0.51	1136.14 ± 68.32
Normal range	3 ~ 15	5 ~ 9	700 ~ 1500

Blood sample was collected from mice on 7 d post injection. Data present means ± SD (n = 3).