

Electronic Supplementary Information

Fluorescent Graphene Oxide Logic Gates for Discrimination of Iron (3+) and Iron (2+) in Living Cells by Imaging

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EXPERIMENTAL SECTION

Materials and Apparatus. All reagents are of analytical reagent grade. Graphite flake (325 mesh) and n-butylamine were purchased from Alfa-Aesar. Thiazolyl blue tetrazolium bromide (MTT) was purchased from Sigma-Aldrich. FeCl₃·6H₂O, FeCl₂·4H₂O, NaCl, CuCl₂·2H₂O, CdCl₂·2H₂O, NiCl₂·6H₂O, CoCl₂·6H₂O, Pb(NO₃)₂, ZnSO₄·7H₂O, MgCl₂·6H₂O, hydrogen peroxide (H₂O₂), N,N'-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), tetrahydrofuran (THF), dichlorosulfoxide (SOCl₂), ascorbic acid (Vc) and glutathione were received from Shanghai Chemical Reagent Corporation (Shanghai, China). UV-vis absorption and fluorescence spectra were recorded at room temperature on a Shimadzu UV-2550

spectrometer and Perkin-Elmer LS-45 luminescence spectrometer, respectively. Fluorescence imaging of living cells was taken on a Leica DMI6000 fluorescence microscope.

Synthesis of Fluorescent Graphene Oxide (GO-C3Me). As we reported before,¹ the fluorescent graphene oxide (GO-C3Me) was synthesized as follows. The starting GO powder was prepared from natural graphite flakes by a modified Hummers method. 20 mg of the dried GO was dissolved in 2 mL of DMF and then refluxed in 20 mL SOCl₂ at 80 °C for 24 h. The resulting acyl chloride activated GO (GO-COCl) was collected by centrifugation and washing with anhydrous THF for two times. Then, 2 mL *n*-butylamine was mixed with GO-COCl and heated under nitrogen at 60 °C for 72 hours. After the reaction mixture was cooled down to room temperature, 20 mL of ultrapure water was added to extract the products (GO-C3Me). The GO-C3Me powder was obtained after the mixture was centrifuged at 8000 rpm for 10 min, and then dried by rotary evaporation. The powder was re-dispersed in 20 mL of ultrapure water to get light green suspension with a concentration of about 1.8 mg/mL.

Fluorescence Responses to Metal ions. The stock solutions of different metal ions were prepared in ultrapure water. 1 μL of GO-C3Me aqueous solution was first diluted into 3 mL of ultrapure water in a quartz cuvette, and then different amount of metal-ions stock solution was added gradually into the diluted GO-C3Me solution. All the fluorescence spectra were recorded using a 350-nm excitation wavelength at room temperature under ambient conditions. To discriminate the fluorescence quenching kinetics of Fe³⁺ and Fe²⁺, 1 μL of the prepared GO-C3Me aqueous solution was injected into 3 mL of ultrapure water and then 30 μL of the analyte (the final concentration is 0.1 mM) was added into the above solution. The fluorescence intensity was recorded every 2 min for a total time of 30 min to observe the quenching kinetics.

Cell Incubation and Imaging. The living human lung cancer cells A549 were provided by the Key Laboratory of Ion Beam Bioengineering, Institute of Plasma Physics, Chinese Academy of Sciences. The A549 cells were grown in MEM (modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum) at 37 °C and 5% CO₂. Before the fluorescent imaging experiments, the cells were washed with phosphate buffered saline (PBS), followed by incubating with 90 µg of GO-C3Me in 2 mL of medium for 24 h at 37 °C, and then washing with PBS three times. Experiments to detect Fe³⁺ in living cells were performed by incubating with 0.1 mM Fe³⁺ for 1 h, and followed by 0.1 mM H₂O₂ for another hour. To observe the transformation of Fe²⁺ to Fe³⁺, the A549 cells were first incubated with 0.1 mM Fe²⁺ for 1 h. Meanwhile, 2 mM ascorbic acid and 2 mM glutathione were added to keep the reductive environment.² After washing with PBS three times to remove the extracellular Fe²⁺, the cells were incubated with 0.1 mM H₂O₂ for 1 h. To identify the coexistence of different valence states of iron ions, 0.1 mM Fe³⁺ and 0.1 mM Fe²⁺ were first incubated into cells in the presence of ascorbic acid and glutathione, and followed by incubating with 0.1 mM H₂O₂ for 1 h. All the concentrations mentioned here are the final values. Cell imaging was then carried out on a Leica DMI6000 fluorescence microscope after the cells were washed with PBS three times.

MTT Assay. The cytotoxicity of GO-C3Me was examined by MTT assay. First, A549 cells were seeded in 96-well plates at a density of 3.25×10⁴ cells mL⁻¹. After 24 h incubation, the medium was then replaced by the medium containing GO-C3Me with various concentrations (30, 60, 90, 120, 150 µg/mL), and the cells were incubated for another 24 h. Next, the cells were washed three times with PBS, and freshly prepared MTT (0.5 mg/mL) solution in culture medium was added to each well. Last, the MTT medium solution was carefully removed after 4 h incubation, and DMSO was then added into each well. The plate was gently shaken for 10 min at

room temperature to dissolve all precipitates, and the absorbance of MTT at 570 nm was monitored.

References

1. Q. S. Mei, K. Zhang, G. J. Guan, B. H. Liu, S. H. Wang and Z. P. Zhang, *Chem. Commun.* 2010, **46**, 7319.
2. Y. M. Ma, H. D. Groot, Z. D. Liu, R. C. Hider and F. Petrat, *Biochem. J.* **2006**, 395, 49.

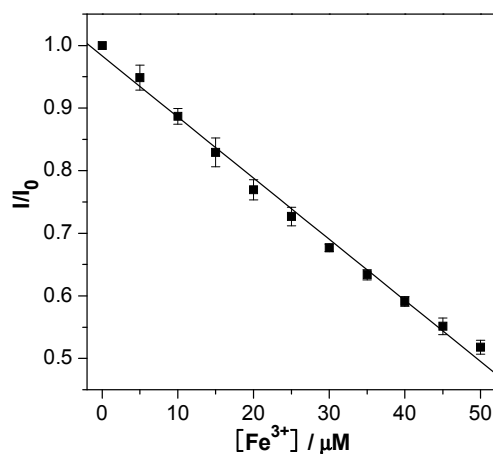


Figure S1. The plot of fluorescence quenching efficiency (I/I_0) of GO-C3Me vs the concentrations of Fe^{3+} .

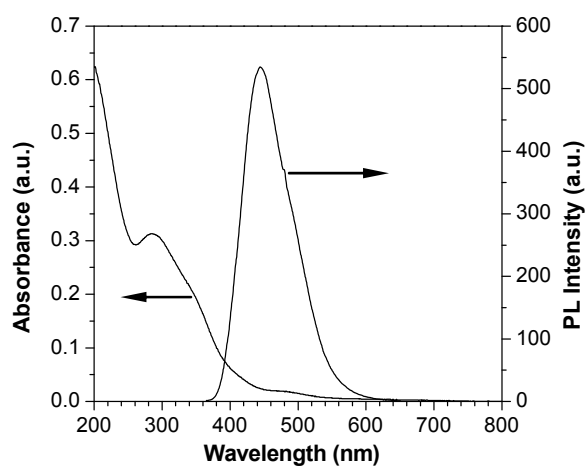


Figure S2. The absorption spectrum of Fe^{3+} (0.1 mM) and the emission spectrum of aqueous GO-C3Me solution (4.8 $\mu\text{g/mL}$). The results show that there is minimal spectral overlap between the emission and absorption spectra, indicating that resonance energy transfer should not be the dominant mechanistic pathway.

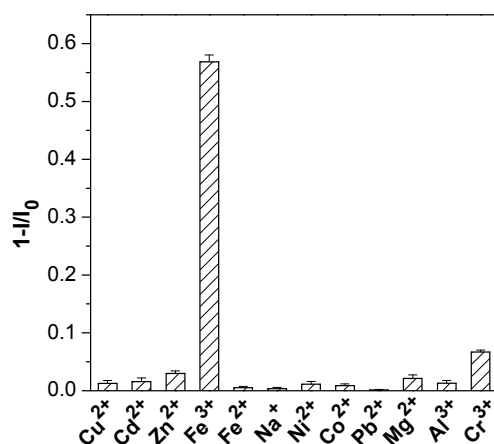


Figure S3. The fluorescence quenching selectivity of aqueous GO-C3Me solution toward various metal ions (0.1 mM).

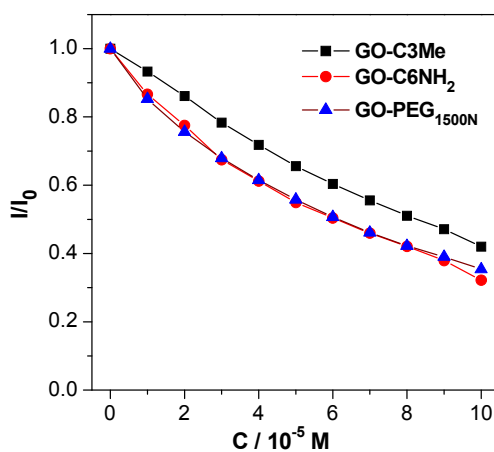


Figure S4. Fluorescence quenching efficiency (I/I_0) of different alkylamines modified graphene oxide upon the addition of Fe^{3+} .

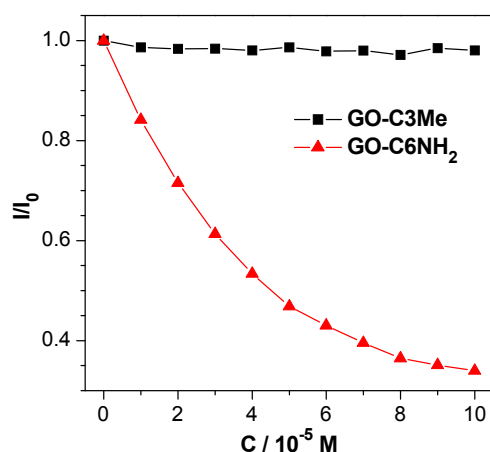


Figure S5. Fluorescence quenching efficiency (I/I_0) of different alkylamine modified graphene oxide vs the concentrations of Fe^{2+} .

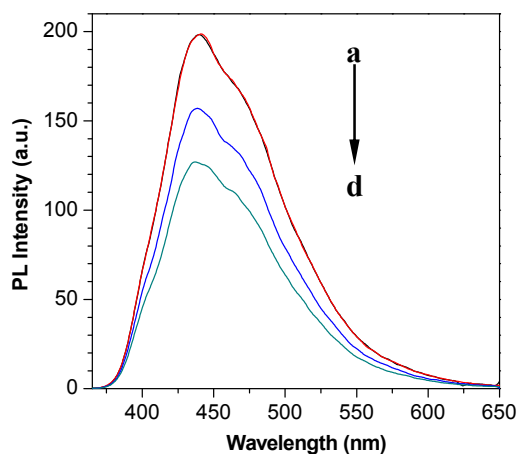


Figure S6. The fluorescence quenching of GO-C3Me upon the addition of hydroxyl radical (from a to d: blank, 0.1 mM Vc, mixture of 0.1 mM Vc, 0.1 mM Cu^{2+} and 0.1 mM H_2O_2 , mixture of 0.2 mM Vc, 0.2 mM Cu^{2+} and 0.2 mM H_2O_2). The results show that Vc and Cu^{2+} do not quench the fluorescence of GO-C3Me, while the mixture of Vc, Cu^{2+} and H_2O_2 can quench the fluorescence obviously. This should because that Cu^{2+} can reduce to Cu^+ in the presence of ascorbic acid, and Cu^+ further reacts with hydrogen peroxide to form the hydroxyl radicals. The fluorescence quenching should be attributed to the hydroxyl radicals.

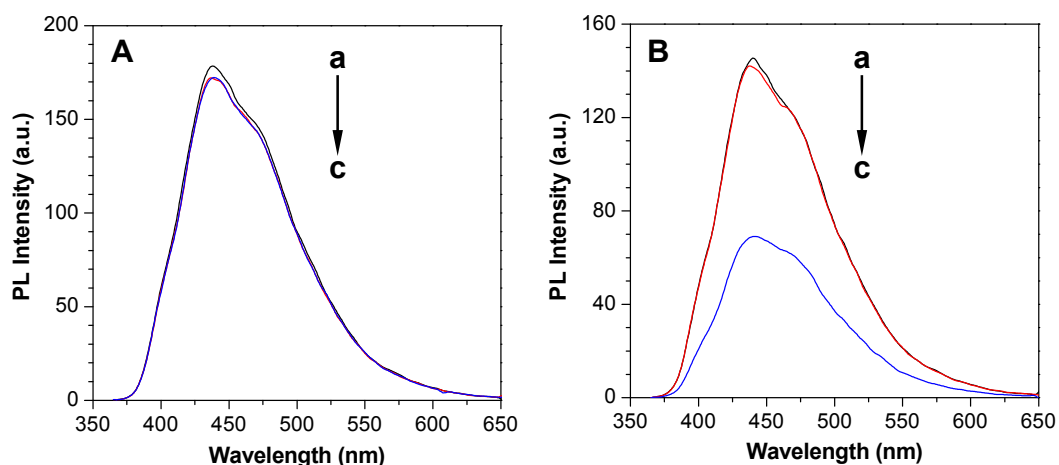


Figure S7. (A) The changes of fluorescence spectra of GO-C3Me solution with the addition of hydroxyl radicals and DMSO (from a to c: blank, mixture of 0.1% DMSO, 0.1 mM Vc, 0.1 mM Cu^{2+} and 0.1 mM H_2O_2 , mixture of 0.2% DMSO, 0.2 mM Vc, 0.2 mM Cu^{2+} and 0.2 mM H_2O_2). (B) The evolution of emission spectra of GO-C3Me upon the addition of different analytes (from a to c: blank, 0.1% DMSO, mixture of 0.1 mM Fe^{2+} , 0.1 mM H_2O_2 and 0.1% DMSO). The results show that DMSO has no obvious influence on the fluorescence of GO-C3Me, and the mixture of Vc, Cu^{2+} and H_2O_2 did not quench its fluorescence in the presence of DMSO. Meanwhile, the mixture of 0.1 mM Fe^{2+} and 0.1 mM H_2O_2 only quenched about 55% of fluorescence in the presence of DMSO, which is much smaller than that in the absence of DMSO. This implies that the addition of DMSO, a typical hydroxyl radical-scavenger, greatly suppresses the fluorescence quenching by hydroxyl radicals.

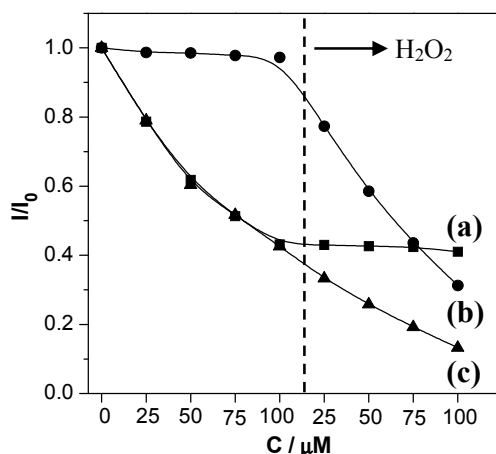


Figure S8. The fluorescence quenching efficiency (I/I_0) of GO-C3Me aqueous solution upon the addition of (a) Fe^{3+} , (b) Fe^{2+} , (c) the mixture of equal amount of Fe^{3+} and Fe^{2+} . Subsequently, different amounts of H_2O_2 were added into the solution, respectively.

In the living organisms, Fe^{3+} always coexists with Fe^{2+} because they can be easily transferred to each other in the processes of oxygen transportation and many enzymatic reactions. Thus, how to identify whether the Fe^{2+} is coexisted in the system of Fe^{3+} is very important. The fluorescence responses caused by Fenton reaction provide an excellent approach to identify the systems of Fe^{3+} , Fe^{2+} and mixing $\text{Fe}^{3+}/\text{Fe}^{2+}$ by the comparisons of fluorescence responses before and after the addition of H_2O_2 , as shown above. In the only presence of Fe^{3+} , the fluorescence was quenched and subsequently kept constant with the addition of H_2O_2 . On the contrary, only Fe^{2+} ions can not quench the fluorescence of GO-C3Me and subsequently the addition of H_2O_2 strongly quench the fluorescence. In the case of mixing $\text{Fe}^{3+}/\text{Fe}^{2+}$, the fluorescence of GO-C3Me continuously keeps quenched before and after the addition of H_2O_2 .

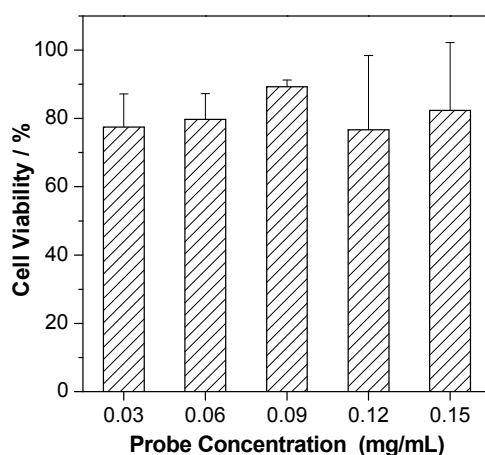


Figure S9. Metabolic viability of A549 cells after the incubation with GO-C3Me at different concentrations for 24 h. The results show that no obvious cytotoxic effects have been observed in the presence of GO-C3Me. When the concentrations range from 0.03 mg/mL to 0.15 mg/mL, about 80%-90% of the A549 cells are alive, indicating GO-C3Me is suitable for intracellular imaging studies.

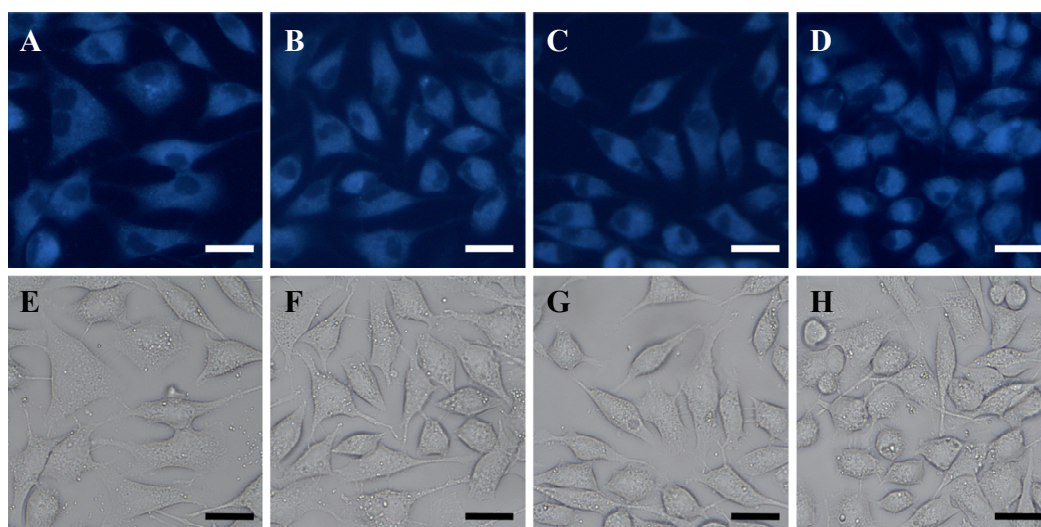


Figure S10. Fluorescence (A-D) and corresponding bright-field (E-H) images of A549 cells incubated with GO-C3Me for 24 h, washed with PBS three times, and then further incubated with different amount of H_2O_2 for 1 h (from left to right: 0, 10, 50, 100 μM).