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Electronic Supplementary Information (ESI)

Strongly green-photoluminescent graphene quantum dots for bioimaging applications

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1. Preparation of graphene oxide.

GO was synthesized from natural graphite powder (<150 micron, Aldrich) by the modified Hummers method.[1] Before the GO preparation, an additional preoxidation procedure was performed. The graphite powder (8 g) was put into an 80 °C solution of concentrated H_2SO_4 (30 mL), $K_2S_2O_8$ (10 g), and P_2O_5 (10 g). The resultant dark mixture was thermally isolated and cooled to room temperature over 12 hours. The mixture was then carefully diluted with distilled water, filtered, and washed until the rinse water became neutral. The product was dried at ambient temperature over 24 hours. This preoxidized graphite was then put into cold (0 °C) concentrated H_2SO_4 (184 mL). $NaNO_3$ (4 g) was then added. Next, $KMnO_4$ (24 g) was

added gradually with stirring and the temperature of the mixture was kept below 10 °C, and the mixture was stirred for another 1 hour. The mixture was then stirred at 35 °C (±3 °C) for 6 h and stayed overnight. Distilled water (368 mL) was added, the temperature reached over 90 °C, after 15 min, the reaction was terminated by adding a large amount of distilled water (1120 mL) and 30% H_2O_2 solution (20 mL), after which the color of the mixture changed to bright yellow. The mixture was filtered and washed with a 1:10 HCl solution (1000 mL) to remove metal ions, then washed with a large amount of distilled water. The GO product was suspended in distilled water to produce a brown, 2% dispersion, which was subjected to dialysis to completely remove metal ions and acids (7 days, changed DI-water every 12 hours). The solid GO was obtained by freeze-drying (done on a heto lyolab 3000) or vacuum drying at 45 °C.

2. Preparation of GQDs.

GO was dissolved in DMF with a concentration of 270 mg/10 ml. The GO/DMF solution was under ultrasonication for 30 minutes (120 W, 100 kHz). The GO/DMF solution was then transferred to a poly(tetrafluoroethylene) (Teflon)-lined autoclave (30 mL) and heated at 200℃ for 5 h. After this reaction, the reactors were cooled to room temperature by water or naturally. The product contained brown transparent suspension and black precipitates, and the black precipitates were discarded. The yield of GQDs was about 1.6%. The GQDs can be obtained by evaporating the solvents under reduced pressure, and the GQDs have excellent solubility in many polar organic solvents or water with different pH values.

The residue was purified by column chromatography on silica gel using water as eluent to provide the desired product.

3. Cellular imaging and cellular toxicity test.

Cellular imaging: MG-63 (Human osteosarcoma) cells (10^4 cells/150 μL) were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (DMEM) using a 96-well plate. Suspensions (2.5 mg/mL) of GQDs from the stock solution were prepared with Dulbecco's phosphate buffer saline (DPBS). After sonication for 10 min to ensure complete dispersion, an aliquot (typically 0.01 mL) of the suspension was added to the well of a chamber slide containing the cells cultured for 24 h. The chamber slide was then incubated at 37 ℃ in a CO_2 incubator for 12 h for GQDs uptake (only 10 μg of GQDs to 150 μL of culture medium (10^4 cells) was added). Prior to the

fixation of the cells on the slide for inspection with a confocal fluorescence microscope, the excess GQDs were removed by washing 3 times with warm DPBS.

MC3T3 cells were performed using a similar method.

Cellular toxicity test: The cells were first cultured for 24 h in an incubator (37 ℃, 5% CO_2), and another 24 h after the culture medium was replaced with 100 μL of DMEM containing the GQDs at different doses (0, 10, 20, 50, 100, 200, 400 μg). Then, 20 μL of 5 mg/mL MTT solution was added to every well. The cells were further incubated for 4 h, followed by removing the culture medium with MTT, and then 150 μL of DMSO was added. The resulting mixture was shaken for ca. 5 min at room temperature. The optical density (OD) of the mixture was measured at 490 nm. The cell viability was estimated according to the following equation:

Cell Viability [%] = ($OD_{treated}$ / $OD_{control}$) × 100 %

(where $OD_{control}$ was obtained in the absence of GQDs, and $OD_{treated}$ was obtained in the presence of GQDs, each experiment was performed 5 times and the average data was presented.)

4. Quantum Yields (QY) Measurement.

9,10-Bis (phenylethynyl) anthracene in cyclohexane (QY=1) was chosen as the standard. The quantum yields of GQDs (in water) were calculated according to:

$$\phi_x = \phi_{st}(I_x / I_{st})(\eta_x^2 / \eta_{st}^2)(A_{st} / A_x)$$

Where φ is the quantum yield, I is the integrated emission intensity, η is the refractive index of the solvent, and A is the optical density. The subscript *"st"* refers to standard with known quantum yield and *"x"* refers in particular to the sample. To minimize re-absorption effects, absorption in the 10 mm fluorescence cuvette was kept below 0.10 at 360 nm.

Table S1. Quantum yields of GQDs using 9,10-bis (phenylethynyl) anthracene as a reference.

Sample	Integrated emission intensity *(I)*	Abs. at 425 nm *(A)*	Refractive index of solvent *(η)*	Quantum Yields *(φ)*
9,10-Bis (phenylethynyl) anthracene	20198.1	0.069	1.4264	1 (known)
GQDs	2183.5	0.057	1.33	0.114

5. Instruments and characterization.

TEM was conducted using a Hitachi H-800 electron microscope at an acceleration voltage of 200 kV with a CCD cinema. AFM images were recorded in the tapping mode with a Nanoscope IIIa scanning probe microscope from Digital Instruments under ambient conditions. Fluorescence spectroscopy was performed on a Shimadzu RF-5301 PC spectrophotometer. The excitation wavelength was 375 nm. UV-vis absorption spectra were obtained using a Shimadzu 3100 UV-vis spectrophotometer. The confocal microscopy images were taken on Olympus Fluoview FV1000. IR spectra were collected on a Nicolet AVATAR 360 FT-IR spectrophotometer. Raman spectra were measured with a Renishaw Raman system model 1000 spectrometer with radiation at 514.5 nm. A TA Instruments TGA Q-500 thermogravimetric analyzer (TGA) with a heating rate of 10 ℃ min^{-1} up to 1000 ℃ was used for the thermal degradation study of the GO under nitrogen. X-ray Photoelectron Spectroscopy (XPS) was investigated by using ESCALAB 250 spectrometer with a mono X-Ray source Al Kα excitation (1486.6 eV). Binding energy calibration was based on C1s at 284.6 eV. Zeta potential measurement was performed using a Zetasizer Nano-ZS (Malvern Instruments). Each sample was measured 5 times and the average data was presented.

Figure S1. TEM of graphite and graphene oxide, and AFM image of graphene oxide. A) TEM of graphite. B) TEM of graphene oxide. C) AFM of graphene oxide. D) Height profile along the line in (C), and the height of graphene oxide was about 0.875 nm.

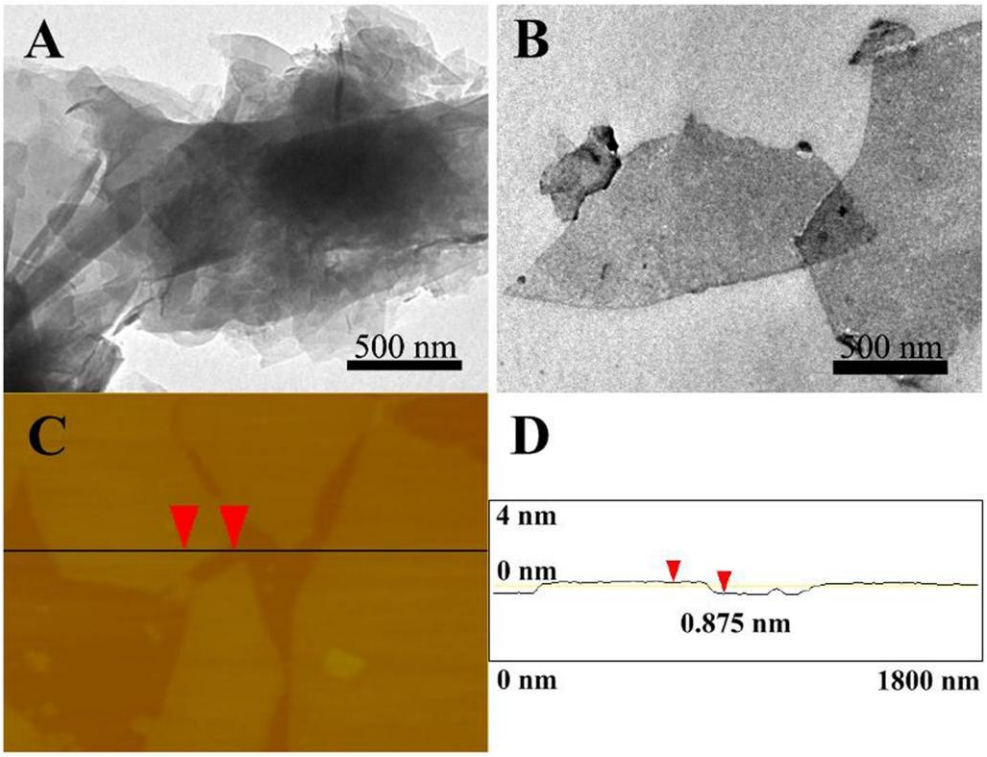


Figure S2. FTIR spectra of the graphene oxide. It contained -COOH, epoxyl and -OH groups.

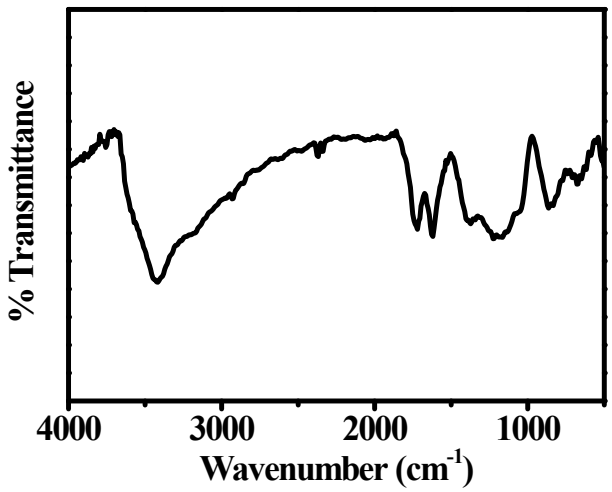


Figure S3. Raman spectra of the graphene oxide. G band at 1598 cm^{-1} and a D band at 1350 cm^{-1} were observed.

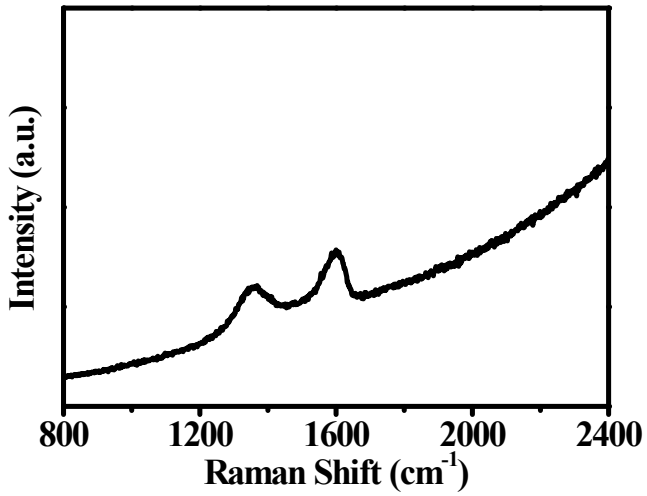


Figure S4. TGA of graphene oxide, and the decomposed temperature was 200 ℃.

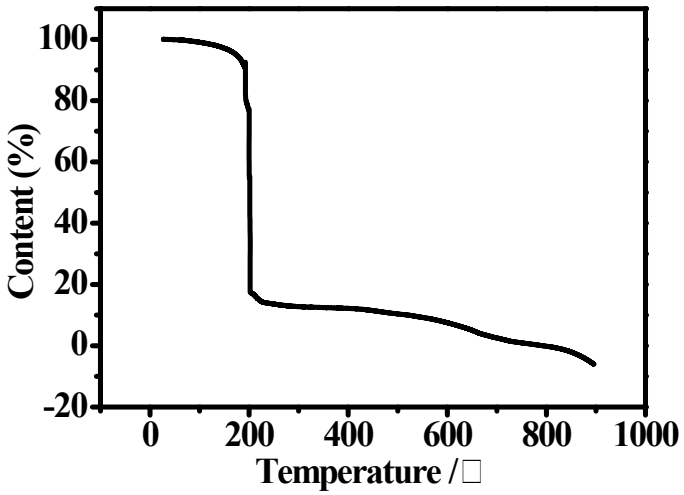


Figure S5. FTIR spectra of GQDs. It contained the -OH, epoxy/ether, C=O and -CO-NR_2 groups.

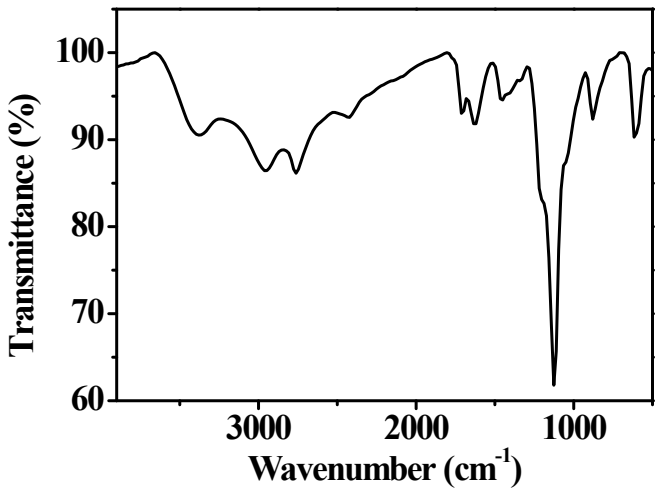


Figure S6. PL spectra of the GQDs under 320 nm excitation.

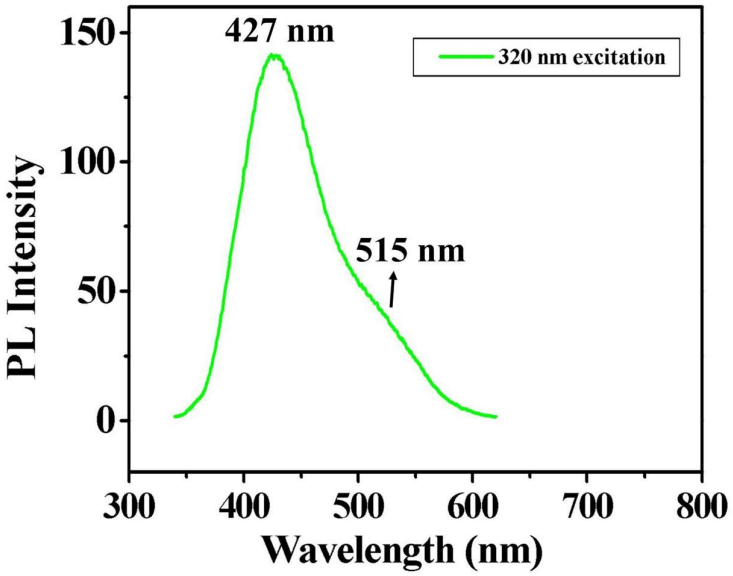


Figure S7. Stability of GQDs. a) Dependence of fluorescence intensity on excitation time for GQDs in DI water (1000 W high-power mercury (arc) lamp). b) Effect of ionic strengths on the fluorescence intensity of GQDs (ionic strengths were tuned by various concentrations of KCl). c) Effect of pH on the fluorescence intensity of GQDs (three different batches).

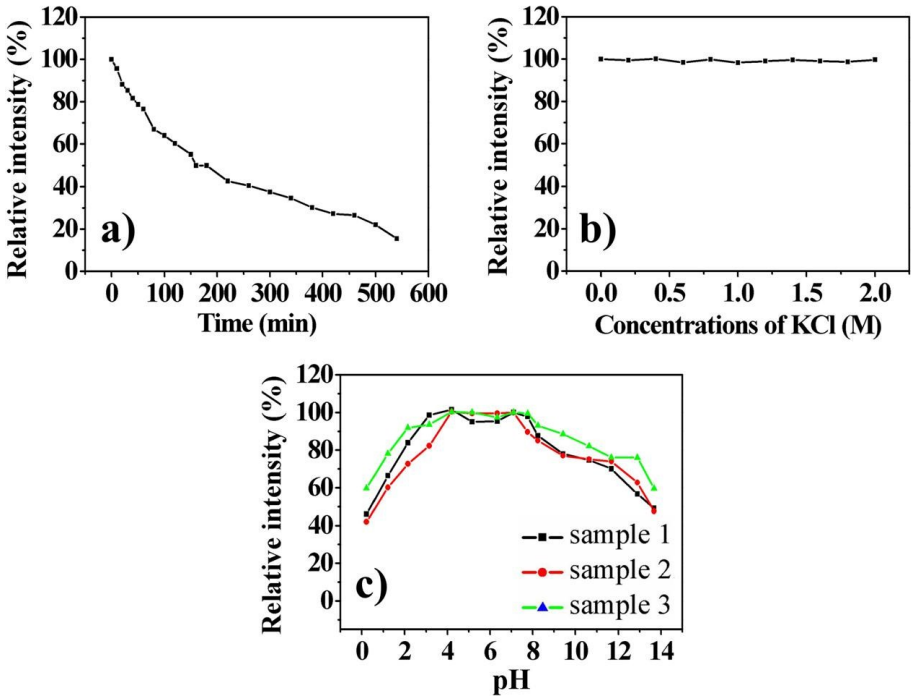
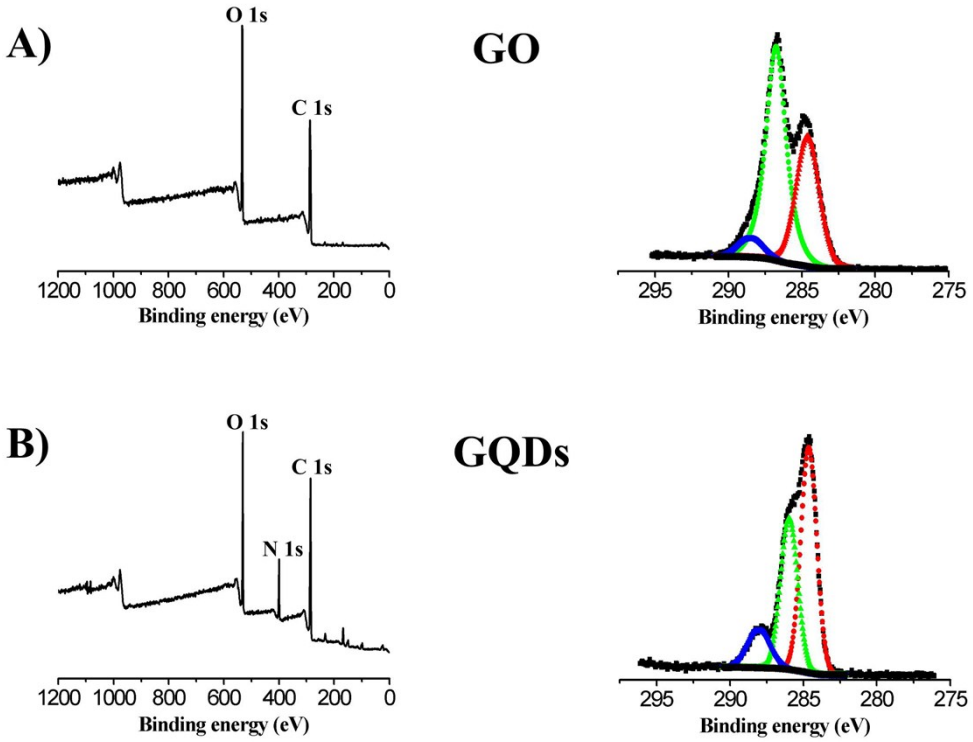


Figure S8. XPS of graphene oxide and GQDs.



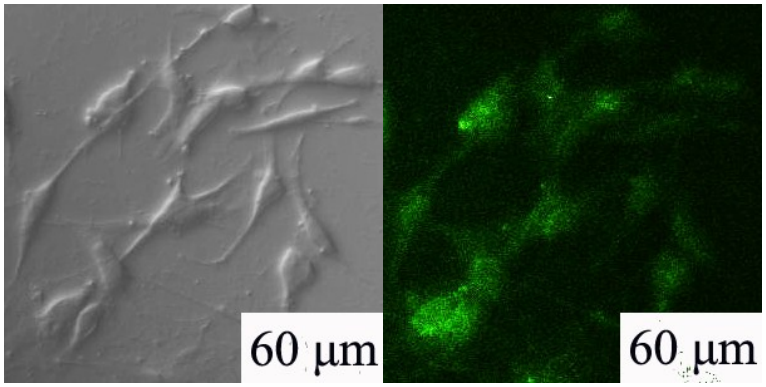
Note: GQDs aqueous solution was dripped on Silicon wafers and dried for XPS testing, so there were some impurities in binding peaks (Si signals).

Table 2. XPS analysis of GO and GQDs (Sample 1, 2 and 3).

	Peak Binding Energy	GO (%)	GQDs (%)
C=C	284.6(±0.2)	36.65	51.32
Oxygenated C and Nitrous C	285.5-288.5	63.35 (1)	48.68 (2)

(1) Only the oxygen-containing carbon (it may include carbon-hydroxy groups (C-OH), epoxy/ether groups (C-O), carbonyl groups (C=O) and carboxylate carbon group (O-C=O)).
(2) Oxygenated C (C-OH, C=O, C-O, O-C=O) and Nitrous C.

Figure S9. MC3T3 cells (GQDs uptake) imaged under bright field and 405 nm laser, respectively.



References

1 (a) W. S. Hummers and R. E. Offeman, *J. Am. Chem. Soc.,* 1958, **80**, 1339; (b) Q. Liu, Z. Liu, X. Zhang, L. Yang, N. Zhang, G. Pan, S. Yin, Y. Chen and J. Wei, *Adv. Funct. Mater.,* 2009, **19**, 894.