Electronic Supplementary Material (ESI) for Biomaterials Science.


# Orange-emissive carbon quantum dots for ligand-directed Golgi apparatus-targeting and *in vivo* imaging

Ying Ying Wei,*[a] Lin Chen,[b] Xin Zhang,[b] Jing Lei Du,[a] Qiang Li,[a] Jing Luo,[a] Xu Guang Liu,[c] Yong Zhen Yang,[b] Shi Ping Yu,[a] and Yu Duan Gao*[d]

## Apparatuses and characterization

The morphology and particle size of L-CQDs was characterized by high resolution transmission microscope (TEM, JEOLJEM 2010); The crystallization properties of L-CQDs were analyzed by X-ray diffractometer (XRD) of Dandong Haoyuan DX-2700; Raman spectra of L-CQDs were measured by HORIBA HR800 Raman spectrometer with excitation wavelength of 325 nm; Surface functional groups of L-CQDs were further analyzed by German BRUKER TENSOR 27 Fourier Transform infrared spectrometer (FTIR); Surface structure of L-CQDs were analyzed by X-ray photoelectron spectroscopy (XPS, Kratos AXIS ULTRA DLD); The element composition and percentage of L-CQDs were tested by the elemental analyzer (Vario EL CUBE, Elementar Company, German, CHNS mode); Ultraviolet spectrophotometer (756MC, Jinghua Instruments, Shanghai) was used to test the ultraviolet-visible (UV-vis) absorption spectra and the absorption value of supernatant in hemolysis experiment; Fluoromax-4 fluorescence spectrometer (Horiba Jobin Yvon, France) was used to measure the fluorescence spectra of L-CQDs, including excitation and emission spectra; The fluorescence decay process and fluorescence lifetime of L-CQDs were measured by full functional steady-state/transient fluorescence spectrometer (EDINBURGH FLS980, British); The circular dichroism (CD) spectra of L-CQDs were analyzed by the CD spectrometer (J-1500, JASCO); The cytotoxicity was detected by Varioskan Flash multifunctional microplate reader from Thermo Fisher Technologies; The digital pathological section scanning system (Histech Pannoramic scan) was used for pathological analysis of L-CQDs; The confocal microscopy was used for fluorescent co-location imaging of HeLa cells (Leica TCS SP8, German); Live cell workstation (Nikon W1) and confocal microscope were used for dynamic imaging of live cells; Fluorescence imaging of mouse organs and living bodies were performed by Perkin Elmer IVIS Spectrum.

## Determination of fluorescence quantum yield

The absorbance value (A) of all solutions at 440 nm were measured by UV-Vis spectrophotometer. The emission spectra of all solutions were measured under excitation at 440 nm with fluorescence spectrometer, and the integral area value of the emission spectra in the range of 460−700 nm was recorded, which was the integral

fluorescence intensity. The plot was drawn in Origin 2017 using the absorbance value as the abscissa and the integrated fluorescence intensity as the ordinate. A linear line with an intercept of 0 was fitted to obtain the slope (Grad), and the quantum yield (QY) of L-CQDs was calculated according to Eq. (1):

$$Q_x = Q_{st}\left({Grad_x}/{Grad_{st}}\right)\left({\eta_x}/{\eta_{st}}\right)^2 \quad (1)$$

Where *Q* is QY, *Grad* is the slope of the fitted straight line, $\eta$ is the refractive index of the solvent. The subscript st is rhodamine 6G and x is L-CQDs. In this system, rhodamine 6G and L-CQDs are dissolved in absolute ethanol, $\eta_x/\eta_{st}=1$.

## Cell culture

Cells are cultured in culture flask containing 5 mL 1640 medium with 10% fetal bovine serum and 1% double antibody, and incubated in an incubator (Thermo Electronics Company, USA) at 37 °C with a certain humidity and 5% $CO_2$.

## Live cell workstation imaging

The uptake process of L-CQDs was evaluated by living cell workstation. Firstly, HeLa cells were digested and dispersed in the culture medium of 10% fetal bovine serum, then added to the imaging Petri dish (1 mL/ well), incubated in the incubator (37 ℃, 5% $CO_2$) for 24 h to make the cells adhere to the wall. Then supernatant was discarded, and the cells were washed with PBS buffer solution for 3 times. After that, the petri dish was placed in the living cell workstation, and the focal length was adjusted. Then 100 μL of L-CQDs sample medium (200 μg/mL) was added under the microscope. The fluorescence signal was collected at the excitation wavelength of 485 nm and photographed at a time interval of 2 s to observe the process of L-CQDs entering the cell.

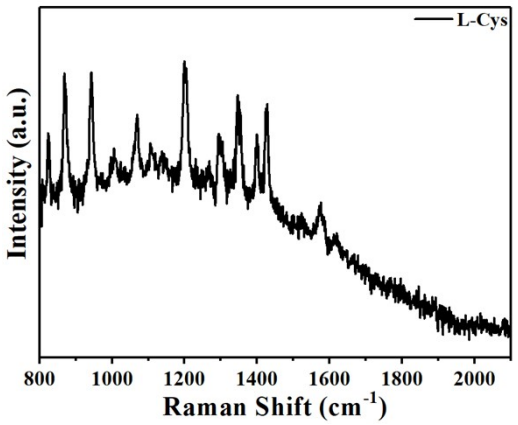


**Fig. S1** Raman spectra of L-Cys

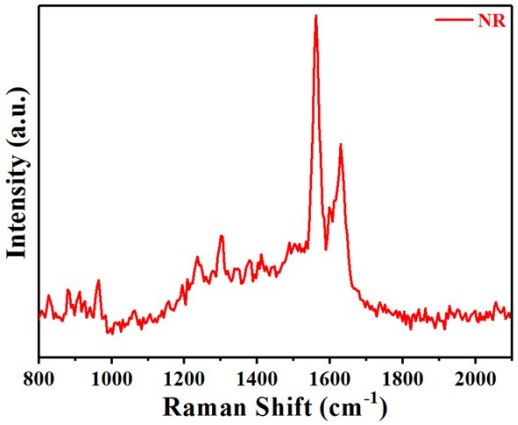


**Fig. S2** Raman spectra of NR

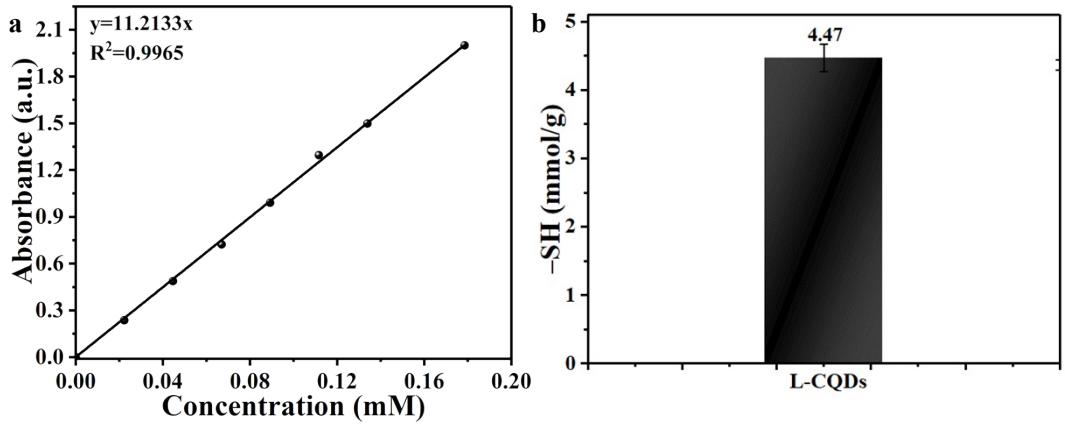


**Fig. S3** (a) Standard operating curve of L-Cys; and (b) Cys residue content of L-CQDs

**Table S1** Percentage of C, H, N, S, and O of L-CQDs

| Sample | C (%) | H (%) | N (%) | S (%) | O*(%) |
|---|---|---|---|---|---|
| **L-CQDs** | 34.93 | 5.35 | 9.92 | 19.76 | 30.04 |

*Represents the percentage of O calculated by subtraction

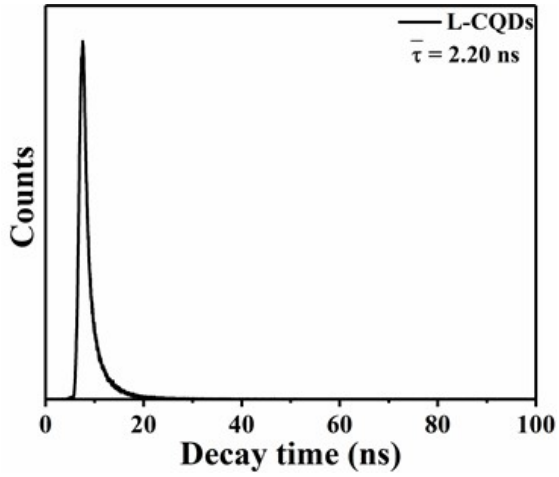


**Fig. S4** Fluorescence lifetime curve of L-CQDs

**Table S2** Fluorescence-decay lifetimes and fitting parameters of L-CQDs aqueous solution

| Sample | $B_1$ | $\tau_1$ (ns) | $\alpha_1$ (%) | $B_2$ | $\tau_2$ (ns) | $\alpha_2$ (%) | $\bar{\tau}$ (ns) |
|---|---|---|---|---|---|---|---|
| **L-CQDs** | 4185.74 | 0.98 | 50.43 | 1169.90 | 3.44 | 49.57 | 2.20 |

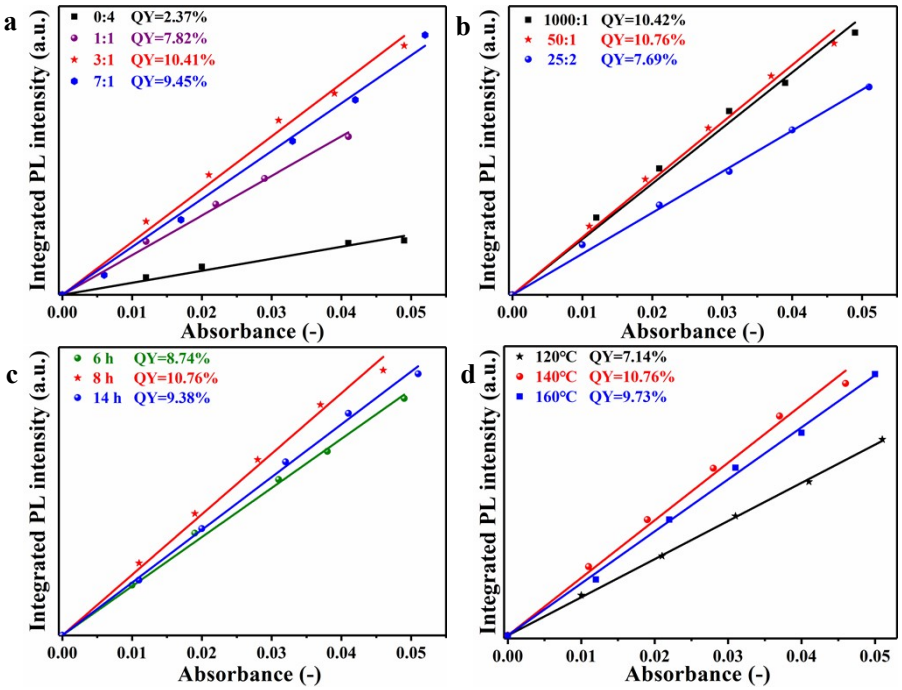


**Fig. S5** QY fitting lines of L-CQDs synthesized under different conditions. (a) different volume ratios of reaction solvent ethanol to water; (b) different molar ratios of L-Cys to neutral red; (c) different reaction time; and (d) different reaction temperature

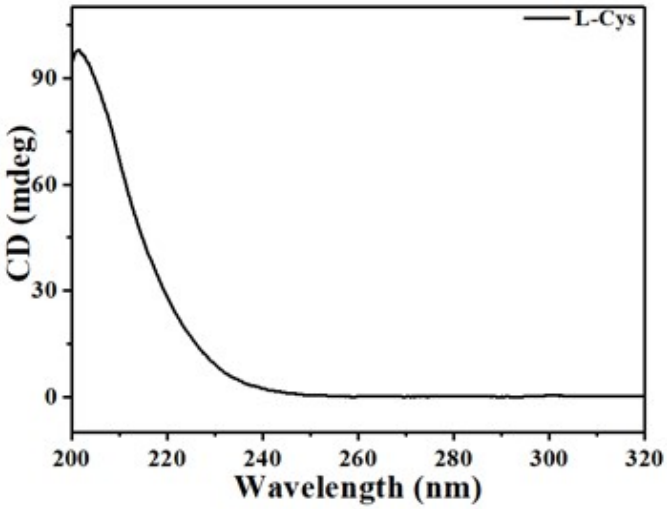


**Fig. S6** CD spectrum of L-Cys

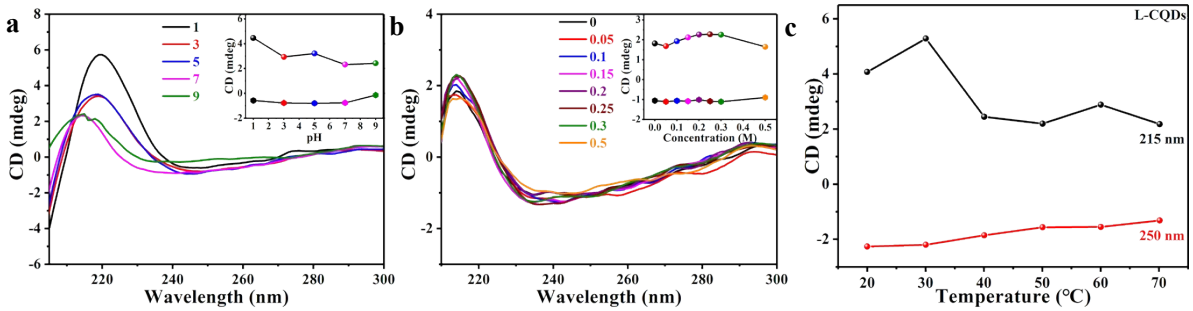


**Fig. S7** CD spectra of L-CQDs in solutions with different (a) pH and (b) concentrations NaCl; and (c) change curve of CD intensity with temperature at 215 and 250 nm

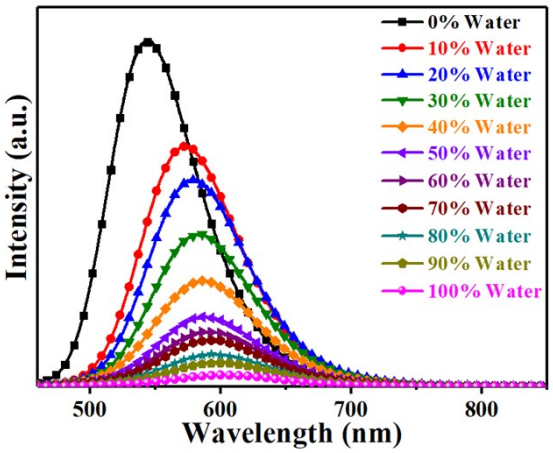


**Fig. S8** Emission spectra of L-CQDs in the water-dioxane binary system