# Electronic Supporting Information

## Development of carbon quantum dots-based fluorescent $Cu^{2+}$ probe suitable for living cell imaging†

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**Note added after first publication:** This supplementary information file replaces that originally published on 11 April 2012. Figure S4 shows the reaction rate between CQD–TPEA and $Cu^{2+}$ at pH 7.0. In the original file, the figure showed data obtained at pH 7.4 in error. The authors have repeated the experiments at the correct conditions of pH 7.0 and these corrected data are shown in this updated version.

## 1. Experimental section

***Reagents and Chemicals.*** Graphite rods and sodium cyanoborohydride (95%) were bought from Alfa Aesar. All the amino acids (99%) and metal salts, ethanol, dichloromethane, methanol, petroleum ether, ethanol, ethyl acetate, acetonitrile, ethyl acetate, dichloromethane, chloroform, diethyl ether, tetrahydrofuran, and silica gel (200-300 meshes) were purchased from Sinopharm. Hydrazine hydrate (98%) was obtained from Tokyo Chemical Industry (TCI). Ethylenediamine (99%), chloroform-d (99.8%), N-(2-bromoethyl)phthalimide, pyrrolidine dithiocarbamate (PDTC), methyl thiazolyl tetrazolium (MTT) and N-(2-bromoethyl)phthalimide were purchased from Sigma.

***Preparation of CQDs.*** The preparation of CQDs were based on the previously reported electrochemical method.[S1] Briefly, 0.3g NaOH was dissolved in 100 mL ethanol/$H_2O$ (99.5:0.5, v:v). By using graphite rods (diameter 0.5 cm) as both anode and cathode, CQDs were obtained in the electrolyte with a current intensity 40 mA $cm^{-2}$. The as-prepared CQDs were separated by centrifugation and column chromatography. CQDs solution was then centrifuged at 4000 rpm to remove large particles. A suitable amount of $MgSO_4$ was mixed with the CQDs solution and stirred for 20 min, and then stored for 24 h to remove the salts and waters. Afterwards the purified CQDs solution was separated by silica-gel chromatography with a mixture of dichloromethane and ethanol as the developing solvent.

***Synthesis of AE-TPEA.*** TPEA was synthetized accordingly.[S2] Then, TPEA (4.31g, 12.9 mmol), N-(2-bromoethyl) phthalimide (3.28 g, 12.9 mmol), anhydrous $K_2CO_3$ (4 g, 28.9 mmol), and KI (0.215 g, 1.29 mmol) were mixed in 40 mL acetonitrile then the solution was stirred and heated to reflux for 24 h under a nitrogen atmosphere. The solution was evapored in a vacuum distillation system. The collected substance was further purified by alumina column chromatography. The obtained solid product and hydrazine hydrate (0.625 mL, 12.9 mmol) were added in 35 mL anhydrous ethanol and then stirred and heated to reflux for under nitrogen, in this process white substance separated out. After the solution was cooled to room temperature, the white substance was filtered off with hydrochloric acid (10.75 mL, 129 mmol) and the ethanol was removed by vacuum distillation. Sodium hydroxide was added to neutralize the unreacted hydrochloric acid. An aqueous solution of the residue was extracted with $CH_2Cl_2$. Concentration of the $CH_2Cl_2$ extracts gave 1.94 g AE-TPEA.

***Synthesis of CQD-TPEA.*** CQDs and AE-TPEA was added to dichloromethane using 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydcrochloride (EDC) as catalyst. After stirring for 2 h, insoluble substance was removed by negative-pressure filtration. CQD-TPEA was separated by centrifugation at 12000 rpm and dissolved in pure water.

***Instruments and Measurements.*** Optical absorption spectrum was recorded using a 8453 Agilent spectrophotometer. Fluorescence was obtained with a F-2700 Hitachi fluorescence spectrophotometer. TEM images were taken on a JEOL 2100 operated at an acceleration voltage of 200 KeV. AFM images were recorded by Picoscan 2100 MI, USA. The cell images were taken by a confocal laser scanning microscope (Leica TCS SP5-II) with a PMT voltage of 174V.

***MTT Assay.*** The cellular cytotoxicity of CQDs-TPEA was tested on HepG2 cells and evaluated by "MTT assay", which is a colorimetric assay for measuring the activity of enzymes that reduce MTT to purple formazan dyes in living cells, thus allowing to assess the viability of cells. HepG2 cells were first seeded in a 96 well plate and maintained in the culture medium for 24 h at 37°C. Then, after the addition of 10 μL of 1 mM $CuCl_2$ solution to each well containing 100 μL of the culture medium, 1 μL of the CQD-TPEA solution with various concentrations was added to each well and the cells were incubated for 20 h. After being washed with the culture medium, 100 μL of the new culture medium containing MTT (10 μL, 5 mg/mL) was added to each well followed by incubation for 4 h to allow the formation of formazan dye. Finally, the supernatant was removed before 150 μL of DMSO was added to each well and the plate was shaken for 10 min. By measuring the absorbance at 490 nm using an enzyme-labeling instrument (EX-800 type) in quintuplicate, the cell viability values were determined (at least three times) according to the following formula: cell viability (%) = the absorbance of experimental group / the absorbance of blank control group × 100%.

***In Vivo Experiments.*** Cells were cultured in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 units $mL^{-1}$ penicillin and 100 μg $mL^{-1}$ streptomycin. The day before imaging, cells were passaged and plated on a 35-mm Petri dish in culture medium. For the in vivo imaging studies, CQD-TPEA probes (0.4 mg $mL^{-1}$) were added to the Petri dish and were incubated in 1×PBS (pH=7.0) for ~30 min at 37°C. After being washed with fresh 1×PBS (pH=7.0)

three times, the cells were imaged on a confocal microscope. The excitation wavelength was 405 nm, and the fluorescence emission between 460 nm and 600 nm was collected for cell imaging. Then, 100 μM $CuCl_2$ and 100 μM PDTC were added and incubated for ~30 min before imaging.

**References**:

S1. Li, H.; He, X.; Kang, Z.; Huang, H.; Liu, Y.; Liu, J.; Lian, S.; Tsang, C. H. A.; Yang, X.; Lee, S. *Angew. Chem. Int. Ed* **2010**, 49, 4430-4434.

S2. (a) Ambundo, E. A.; Yu, Q.; Ochrymowycz, L. A.; Rorabacher, D. B. *Inorg. Chem*. **2003**, 42, 5267-5273. (b) Qian, F.; Zhang, C.; Zhang, Y.; He, W.; Gao, X.; Hu, P.; Guo, Z. *J. Am. Chem.Soc.* **2009**, 131, 1460-1468.

## 2. NMR Data

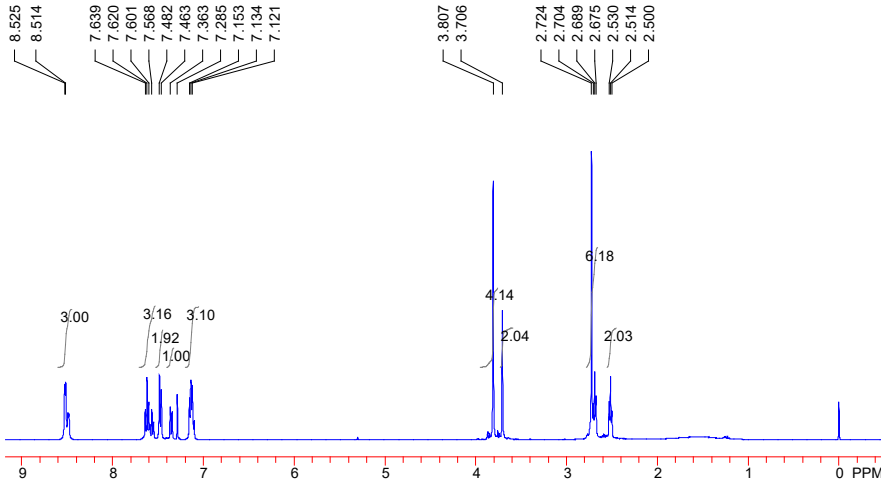


***Figure S1A.*** $^{1}$H NMR spectrum (400 MHz) of AE-TPEA in $CDCl_3$.

$^{1}$H NMR (400 MHz, $CDCl_3$): δ 8.53-8.51 (m, 3H, $C_5H_4N$), 7.64-7.12 (m, 9H，$C_5H_4N$), 3.81 (m, 4H, $CH_2$), 3.71 (s, 2H, $CH_2CH_2$), 2.71 (m, 6H, $CH_2CH_2$), 2.51 (t, 2H, $CH_2CH_2$).

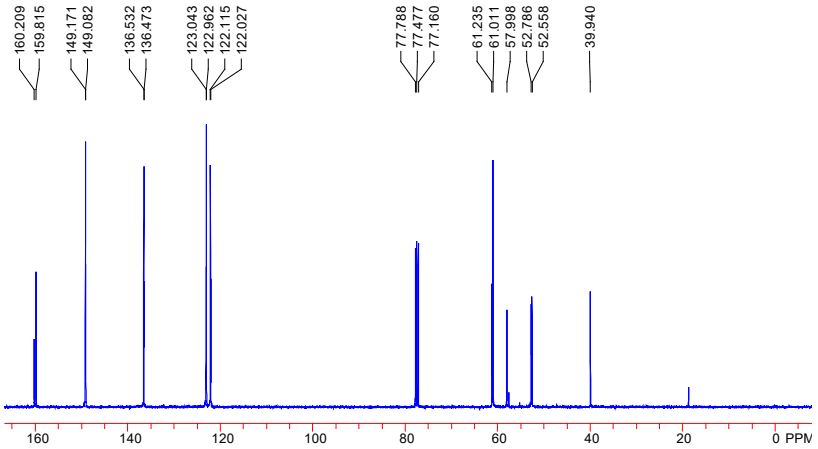


***Figure S1B.*** $^{13}$C NMR spectrum (100 MHz) of AE-TPEA in $CDCl_3$.

$^{13}$C NMR (100 MHz, $CDCl_3$): δ 160.21, 159.81, 149.17, 149.08, 136.47, 122.96, 122.11, 61.24, 61.01, 57.99, 52.79, 52.56, 39.94.

TOF MS EI+: calculated for $[M + H]^+$ 376.2375, found 376.2377.

## 3. Bare CQDs toward Metal Ions

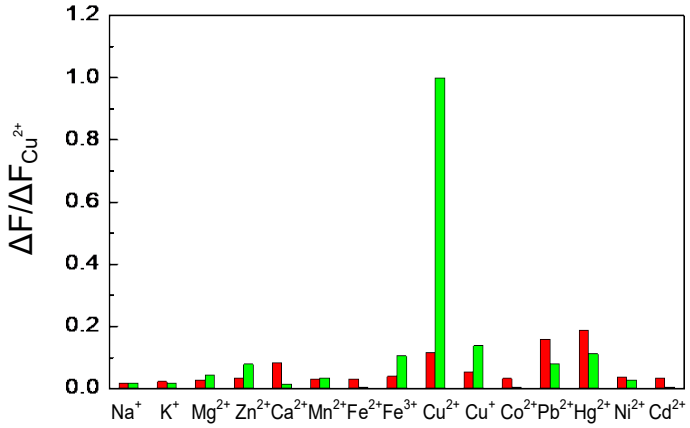


***Figure S2.*** The ratio between the fluorescence changes of 0.04 mg mL$^{-1}$ bare CQDs (red bars) or CQD-TPEA (green bars) toward different metal ions (100 μM for $K^+$ $Na^+$ $Ca^{2+}$, 5 μM for other metal ions) and that for 5 μM $CuCl_2$ in 10 mM PBS (pH 7.0).

## 4. Optimized ratio of CQD and TPEA

***Table S1.*** Analytical performance of CQD-TPEA toward 50 μM $Cu^{2+}$ based on different ratio of CQD and TPEA.

| CQD:TPEA (W:W) | $\Delta F/F_0$ (50 μM $Cu^{2+}$) | Linear range (M) | Detection limit (M) |
|---|---|---|---|
| 1:20 | 0.23 | $1\times10^{-7}$-$5\times10^{-5}$ | $8.5\times10^{-8}$ |
| 1:40 | 0.63 | $1\times10^{-6}$-$1\times10^{-4}$ | $1.1\times10^{-8}$ |
| 1:60 | 0.65 | $1\times10^{-6}$-$1\times10^{-4}$ | $1.3\times10^{-8}$ |

## 5. Different Copper Salts

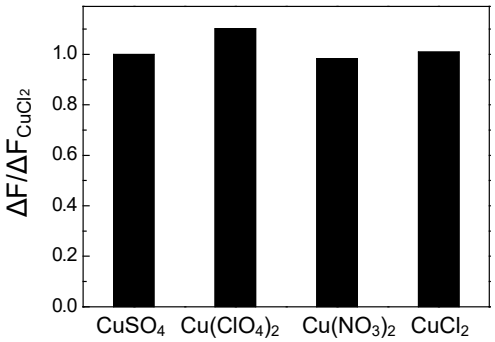


***Figure S3.*** The ratio between the fluorescence changes of 0.04 mg mL$^{-1}$ CQD-TPEA after the addition of 5 μM of different kinds of copper ($Cu^{2+}$) salts and that for $CuCl_2$.

## 6. Time dependence

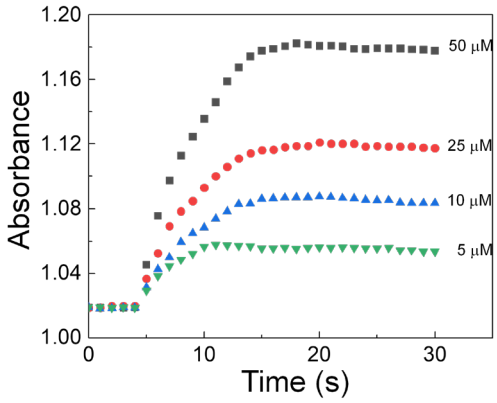


***Figure S4.*** Time dependence of absorbance changes with the addition of 5, 10, 25, and 50 μM $Cu^{2+}$ in 10 mM PBS (pH 7.0).

## 7. Photostability comparison

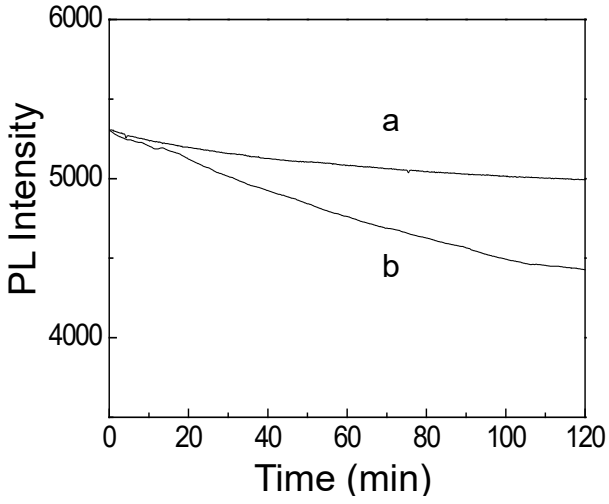


***Figure S5.*** Time courses of 0.08 mg $mL^{-1}$ CQD-TPEA (a) and 0.12 μM rhodamine B (b) in 400 μL $H_2O/C_2H_5OH$ (9:1, v/v) solution (pH 7.0) by fluorescence spectrophotometer ($\lambda_{ex}$ = 420 nm, $\lambda_{em}$ = 500 nm for CQD-TPEA and 574 nm for rhodamine B).

## 8. Localization study

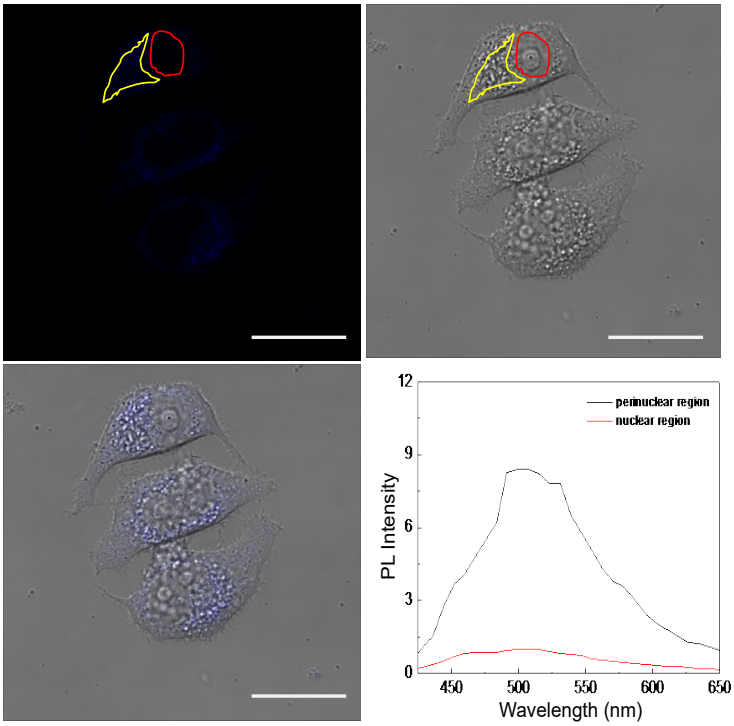


***Figure S6.*** Confocal fluorescence (A), bright-field (B) and overlay of fluorescence and bright-field image (C) of Hela cells stained with 0.04 mg $ml^{-1}$ CQD-TPEA for

~30 min at 37°C. Panel D are the fluorescence emission scans in the perinuclear region (black curve) and nuclear region (red curve) of Hela Cells. The scanned perinuclear region and nuclear region of Hela cells are within range of the yellow line and red line, respective, in Panel A and B. The scale bar is 20 μm. ($\lambda_{ex}$ = 405 nm, the blue fluorescence image was produced by integrating the spectral region from 460 to 600 nm)

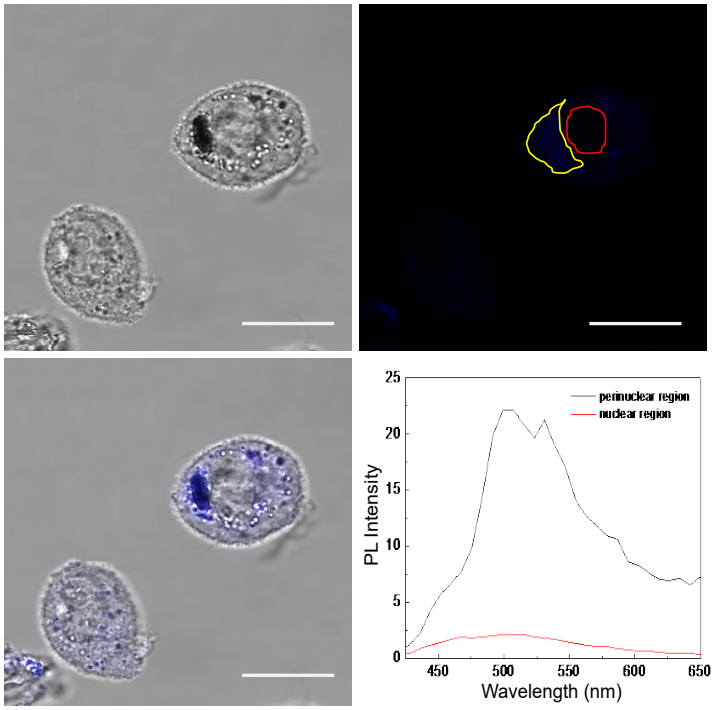


***Figure S6.*** Confocal fluorescence (A), bright-field (B) and overlay of fluorescence and bright-field image (C) of HepG2 cells stained with 0.04 mg $ml^{-1}$ CQD-TPEA for ~30 min at 37°C. Panel D is the fluorescence emission scan in the perinuclear region (black curve) and nuclear region (red curve) of HepG2 Cells. The scanned perinuclear region and nuclear region of HepG2 cells are within range of the yellow line and red line, respective, in Panel A and B. ($\lambda_{ex}$ = 405 nm, the blue fluorescence image was produced by integrating the spectral region from 460 to 600 nm)