

## *Supporting Information*

# Synthesis of High-Quality Carbon Nanodots from Hydrophilic Compounds: Role of Functional Groups

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## **Materials and methods**

**Chemicals.** Aniline, 1,2,4-benzenetricarboxylic acid, cadaverine, EDTA, glycine, polybrene, quinine sulfate, sodium hydroxide, sodium dodecyl sulfate (99%), sodium hydroxide, and hexanol were purchased from Aldrich (Milwaukee, WI, USA). Monobasic, dibasic, and tribasic sodium phosphate, as well as sodium chloride and TRIS, were obtained from J.T. Baker (Phillipsburg, NJ, USA). Boric acid was purchased from RiedeldeHaën (Buchs, Switzerland). RPMI-1640, alpha-modified minimum essential medium ( $\alpha$ -MEM), fetal bovine serum (FBS), an antibiotic-antimycotic solution, L-glutamine, and non-essential amino acids were obtained from Biowest (Lewes, UK). Ultrapure water (18.2 M $\Omega$ /cm) from a Milli-Q ultrapure system was used in this study. Phosphate-buffered saline (PBS; 1  $\times$ , 1 L, pH 7.4) contained NaCl (8 g), KCl (0.2 g), Na<sub>2</sub>HPO<sub>4</sub> (1.44 g), and KH<sub>2</sub>PO<sub>4</sub> (0.24 g). The pH values of phosphate solutions were 3.0–11.0.

**Synthesis of C-dots.** Each of the organic compounds of interest was mixed with water (1 M, 15 mL), and the mixtures were heated hydrothermally in a stainless steel autoclave at 300 °C for 2 h. Each of the resulting brown solutions was cooled to room temperature. The solutions were centrifuged at 3000 rpm for 10 min to remove small volumes of liquid and then filtered through a 0.22- $\mu$ m membrane to remove large or agglomerated particles. We observed that further increasing the calcination temperature from 300 to 500 °C did not produce a greater amount of carbon nanomaterials, which was supported by slight decreases in the PL intensity of the solutions that had the same PL emission wavelength (not shown). To further purify the C-dots, the pellets were added to water (5 mL), and the resulting mixtures were then subjected to dialysis against pure water through a membrane (MWCO = 3.5–5 kD, Float-A-Lyzer G2, Spectrum Laboratories, Rancho Dominguez, CA, USA) for 3 h. Finally, a clear, light yellow–brown aqueous solution containing surface

passivated C-dots was obtained. Aqueous solutions of the as-prepared C-dots at various concentrations were stable for at least 3 months. We estimated that 17.2, 36.4, 52, and 7.6 mg of C-dots were prepared from the glycine, TRIS, EDTA, and cadaverine solutions (1 M, 1 mL), respectively.

The QYs ( $\phi$ ) of the C-dots was calculated by comparing their integrated PL intensities (excitation at 365 nm) and absorbance values at 365 nm with those of quinine sulfate.<sup>1,2</sup> Quinine sulfate ( $\phi_f = 0.54$ ) was dissolved in 0.1 M H<sub>2</sub>SO<sub>4</sub> (refractive index: 1.33) and the C-dots were dissolved in water (refractive index: 1.33). To minimize reabsorption effects, absorbance values of the individual solutions in 10-mm cuvettes were maintained under 0.1 at the excitation wavelength. Excitation and emission slit widths were set at 5.0 nm when recording their PL spectra.

**Cell incubation.** MCF-7 and MCF-10A cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The human breast cancer MCF-7 cells were maintained in a RPMI-1640 medium supplemented with FBS (10%), antibiotic-antimycotic solution (1%), L-glutamine (2 mM), and non-essential amino acids (1%) in 5% CO<sub>2</sub> at 37 °C. The normal human mammary epithelial MCF-10A cells were maintained in  $\alpha$ -MEM supplemented with FBS (10%) and an antibiotic-antimycotic solution (1%) in 5% CO<sub>2</sub> at 37 °C. Prior to being measured, the cells were seeded in 96-well plates at an initial cell density of  $1 \times 10^4$  cells/mL. We used the C-dots prepared from glycine to conduct the cell experiments. A concentrated aqueous C-dot solution (10  $\times$ , 17.2 mg/mL, 0.9 mL) was mixed with a polybrene solution (8 mg/mL, 0.1 mL), and the resulting mixture was then equilibrated at 37 °C for 30 min. An aliquot (100  $\mu$ L) of the mixture of C-dots and polybrene was added to each well and incubated for 24 h. The cell number and viability of the cells in each well were then determined by the Trypan Blue exclusion method and Alamar Blue method, respectively. The precipitated cells were washed

three times with PBS before being used for bright-field and PL imaging measurements by an Olympus IX71 (Tokyo, Japan) fluorescence microscope with a DP70 digital camera. Owing to the limit of the microscopic system, the excitation wavelength was set in the range 360–380 or 460–480 nm.

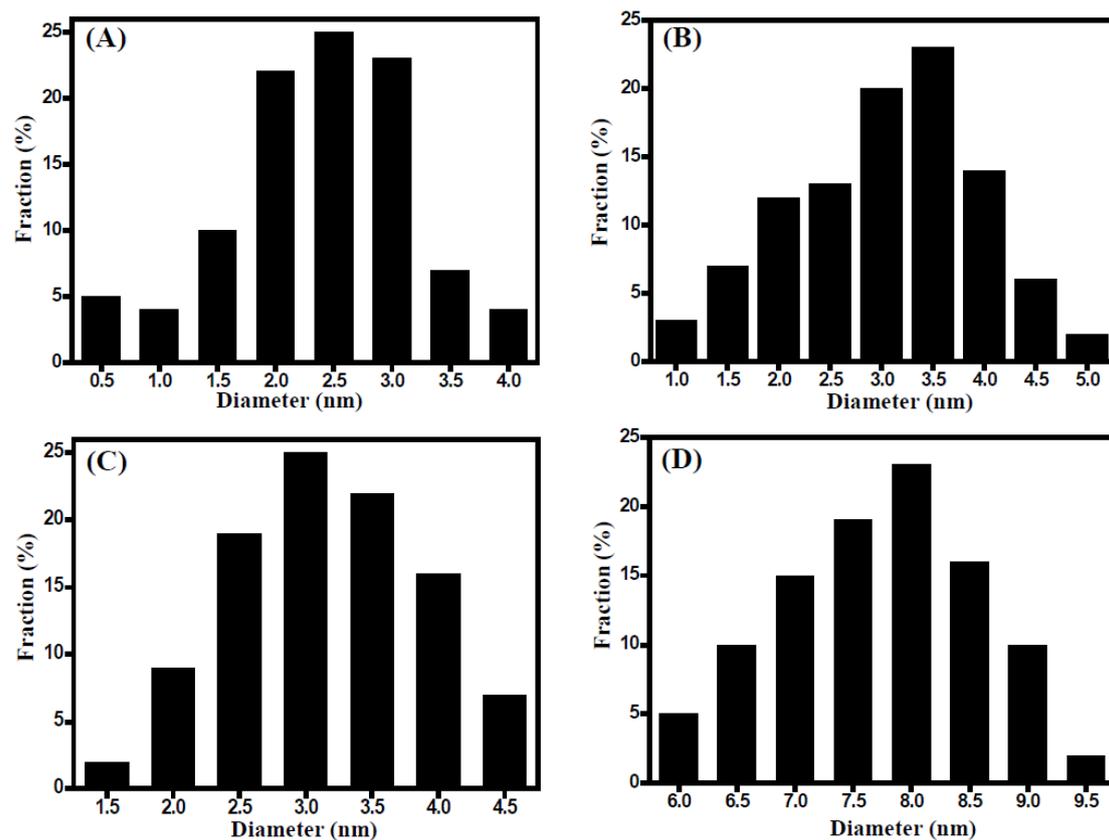
**Cytotoxicity assays.** Cell viability was determined using the Alamar Blue method.<sup>3</sup> Following the incubation of MCF-7 and MCF-10A cells ( $<10^4$  cells/mL/well) in a culture medium containing 5% CO<sub>2</sub> for 24 h at 37 °C, the culture medium was replaced with 100 μL of the medium containing the C-dots of different concentrations (0–1 ×, 0–1.72 mg/mL). The cells were then incubated for another 24 h. The cells were carefully rinsed with PBS three times followed by treatment with the Alamar Blue reagent (1 ×, 100 μL/well, BioSource International Inc., Camarillo, CA, USA) for 4 h. Fluorescence due to the reduction of the dye by live cells was measured by a microplate fluorometer (Synergy 4 Multi-Mode Microplate Reader, Biotek Instruments, Winooski, VT, USA) with an excitation wavelength at 545 nm and an emission wavelength at 590 nm. Because the optical intensity is directly correlated with cell quantity, cell viability was calculated by assuming 100% viability in the control set (media containing no C-dots).

**Characterization.** Prior to conducting transmission electron microscopy (TEM) and high-resolution TEM (HRTEM) measurements, the as-prepared C-dots were diluted 10-fold with water. The C-dots were carefully deposited on 400-mesh C-coated Cu grids, and excess solvents were evaporated at ambient temperature and pressure. To record the size and shape of the as-prepared C-dots, JSM-1200EX II (JEOL Ltd., Tokyo, Japan) and FEI Tecnai-G2-F20 TEMs were used. For X-ray diffraction (XRD) measurements, a PANalytical X'Pert PRO diffractometer (Almelo, Netherlands) in conjunction with Cu K $\alpha$  radiation ( $\lambda = 0.15418$  nm) was used. Prior to XRD measurements, C-dots were placed on quartz glass supports. A Raman

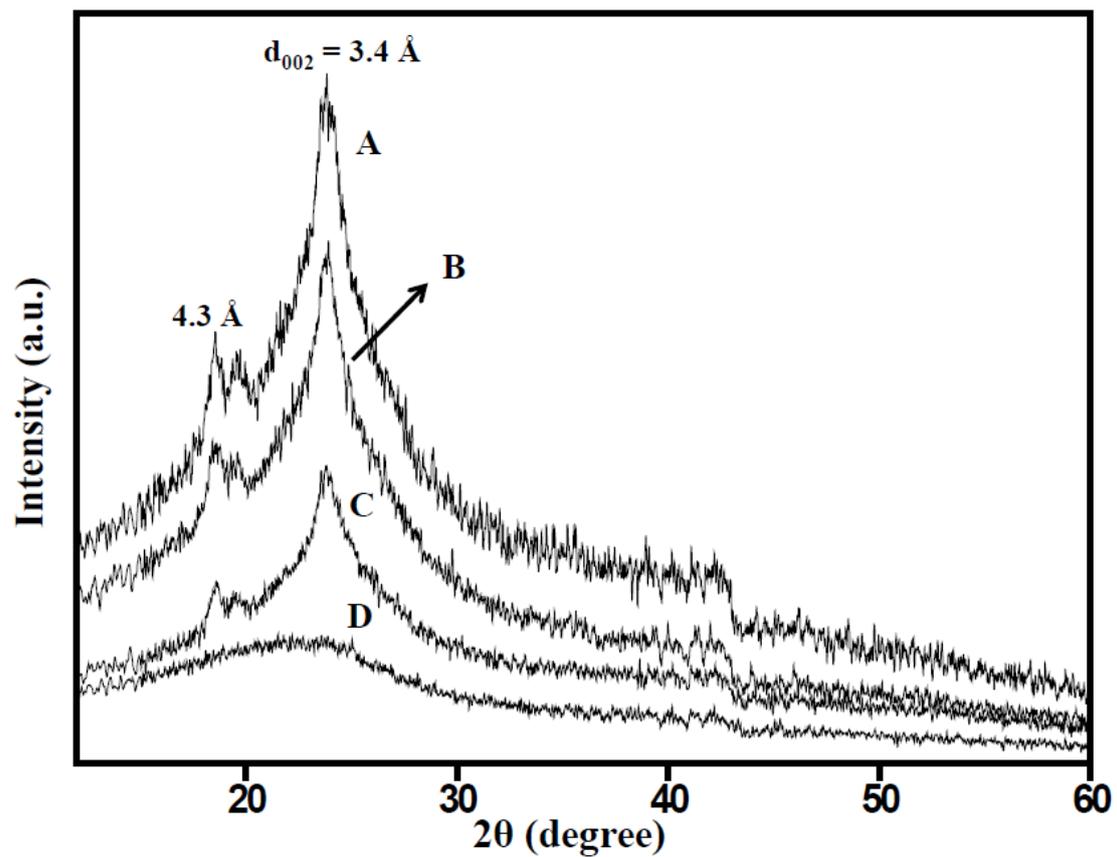
microscopy system with a 50 × objective (Dongwoo Optron Co. Ltd., KyungGiDo, Korea) was used to analyze air-dried C-dots on a silica wafer. A diode-pumped solid-state laser operating at 532 nm was used as the excitation source with a power of 100 mW and an accumulation time of 200 s. A GBC Cintra 10e double-beam UV–Vis spectrophotometer (Victoria, Australia) was used to record the UV–Vis spectra of the C-dots in ultrapure water. The PL spectra of the as-prepared C-dots were recorded on a Cary Eclipse PL spectrophotometer (Varian, CA, USA) that operated at excitation wavelengths in the range 340–500 nm. The photostability of the C-dots was investigated under continuous illumination of the Xe lamp in a PL spectrophotometer (Varian, CA, USA). The PL lifetime was measured by an Edinburgh FL 900 photo-counting system (Edinburgh Instruments Ltd., Livingston, UK), a 377-nm laser (Spectra Phys, Irvine, CA, USA) as an excitation source at a pulse rate of 6 ns, and a 440-nm narrow bandpass filter. A Varian 640 Fourier transform infrared (FTIR) spectrophotometer (Varian, USA) was used to analyze the as-prepared C-dots. The composition of the C-dots was further confirmed by conducting elemental analyses (Vario EL-III, GmbH, Hanau, Germany).

**Table S1.** Compositions of C-dots from four organic compounds from elemental analysis.

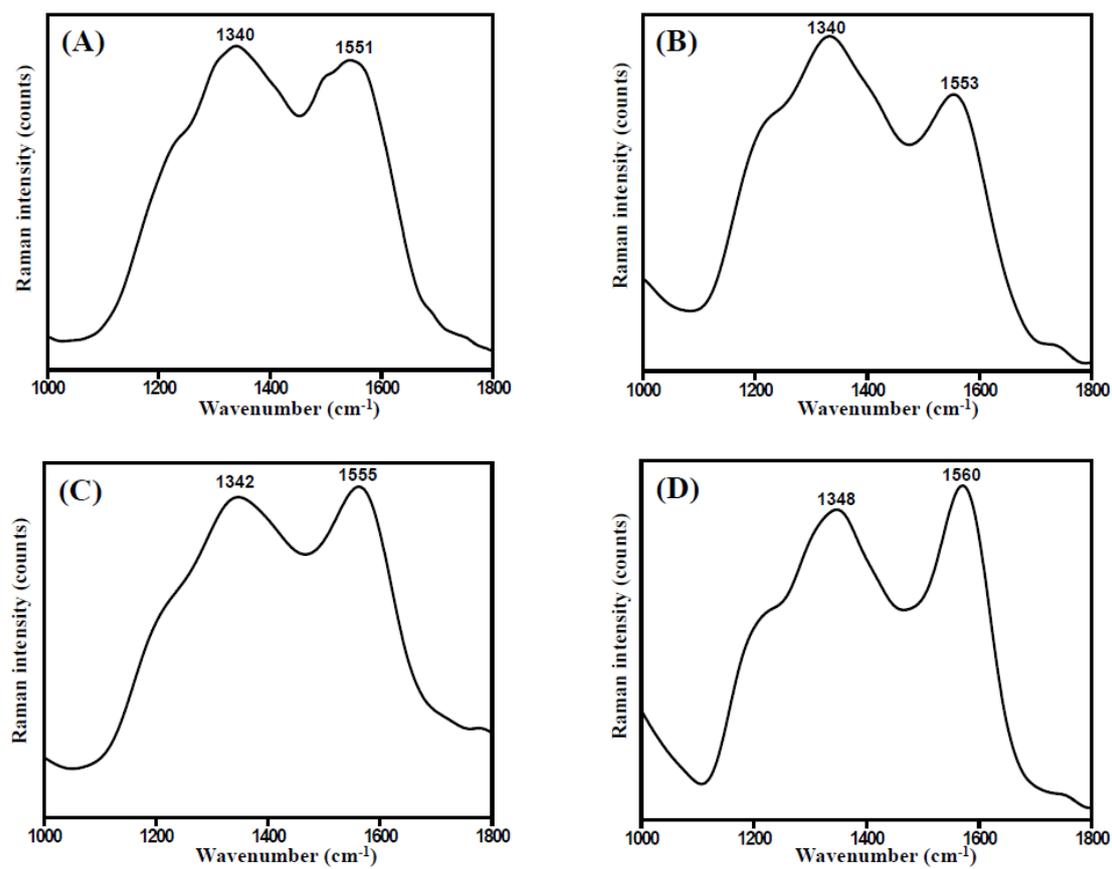
|                        | C %    | H %    | O %    | N %    |
|------------------------|--------|--------|--------|--------|
| C-dots from glycine    | 48.066 | 4.173  | 22.094 | 24.911 |
| C-dots from TRIS       | 63.427 | 5.985  | 13.035 | 13.908 |
| C-dots from EDTA       | 52.842 | 5.731  | 19.400 | 16.341 |
| C-dots from cadaverine | 79.438 | 14.283 | 4.107  | 5.554  |



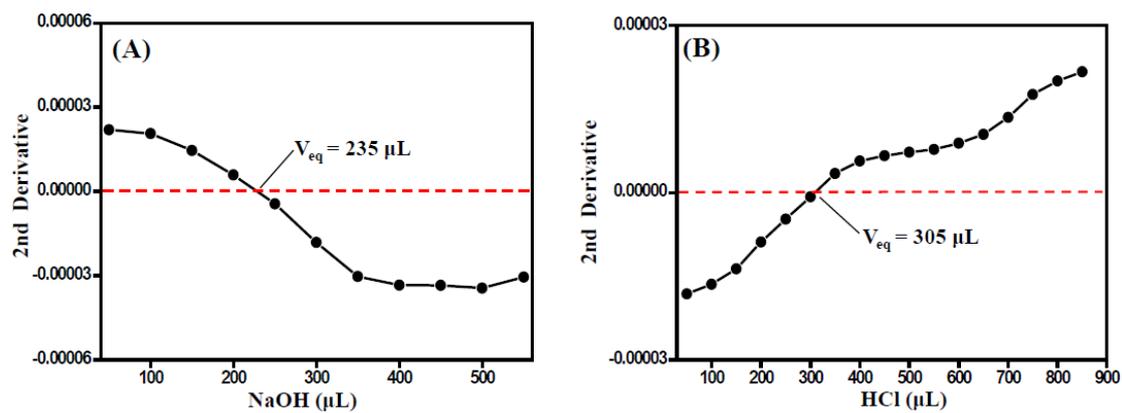
**Figure S1.** Histograms represent size distributions of the C-dots prepared from (A) glycine, (B) TRIS, (C) EDTA, and (D) cadaverine. 300 C-dots were counted from the corresponding TEM images.



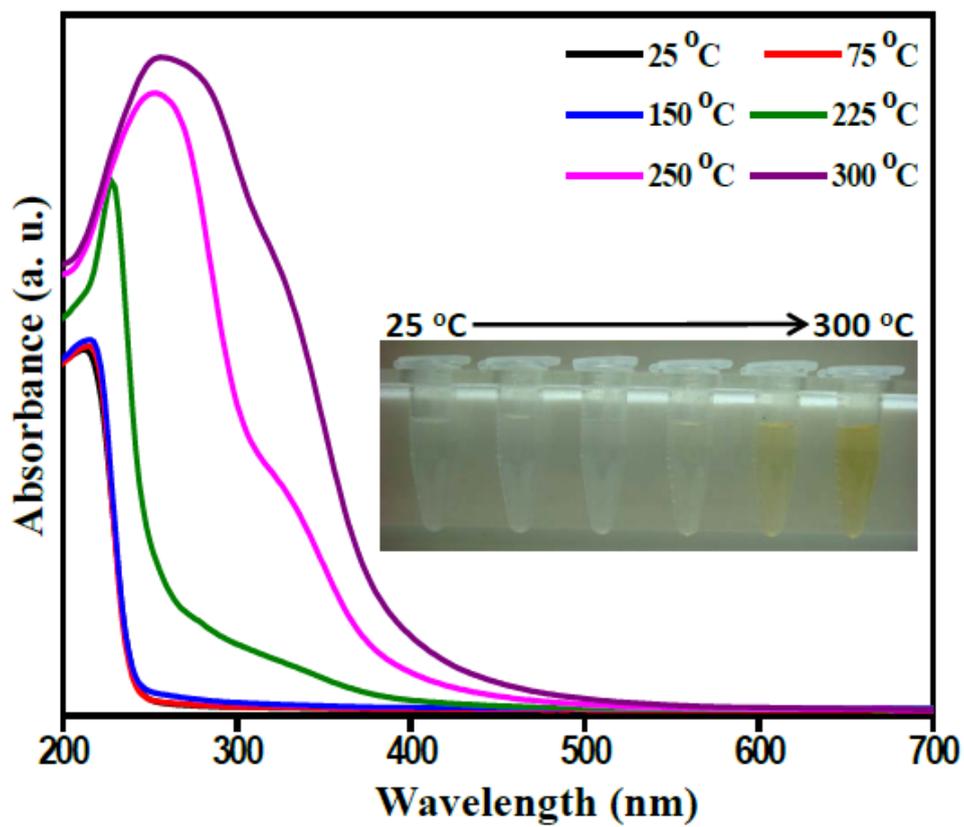
*Figure S2.* XRD patterns of C-dots prepared from (A) glycine, (B) TRIS, (C) EDTA, and (D) cadaverine.



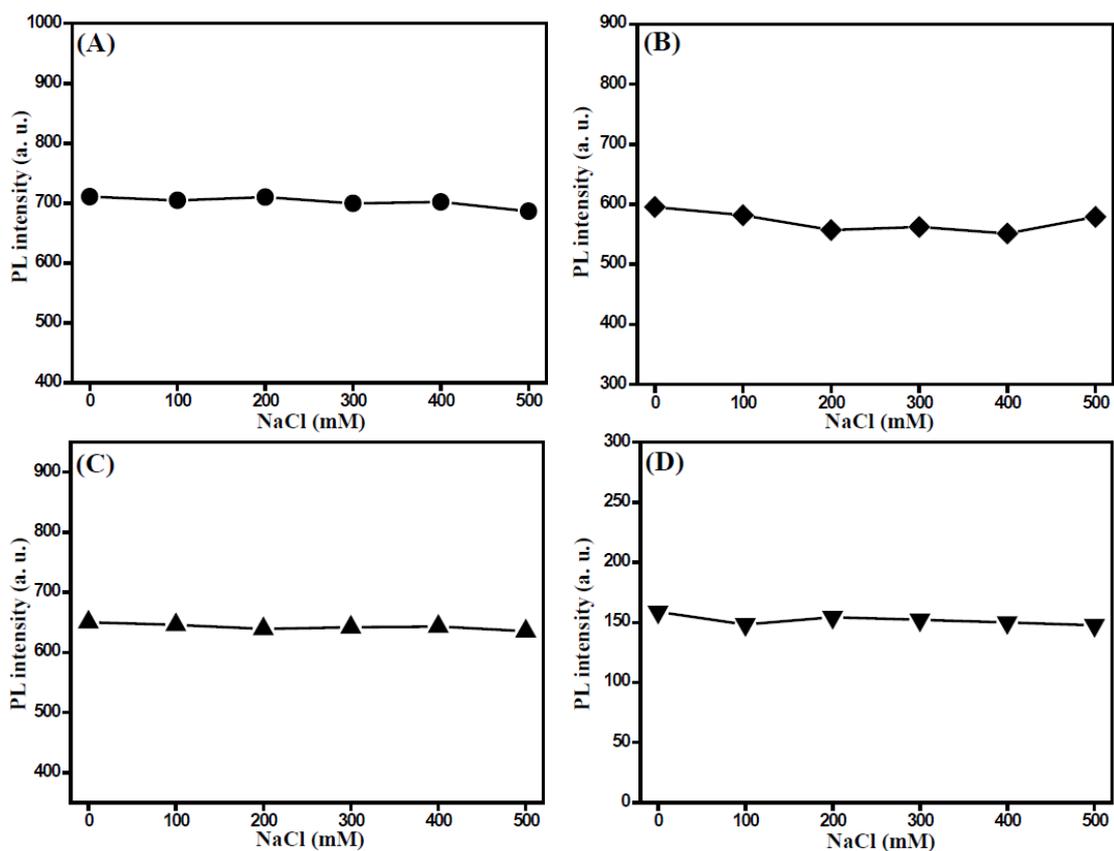
**Figure S3.** Raman spectra of C-dots prepared from (A) glycine, (B) TRIS, (C) EDTA, and (D) cadaverine.



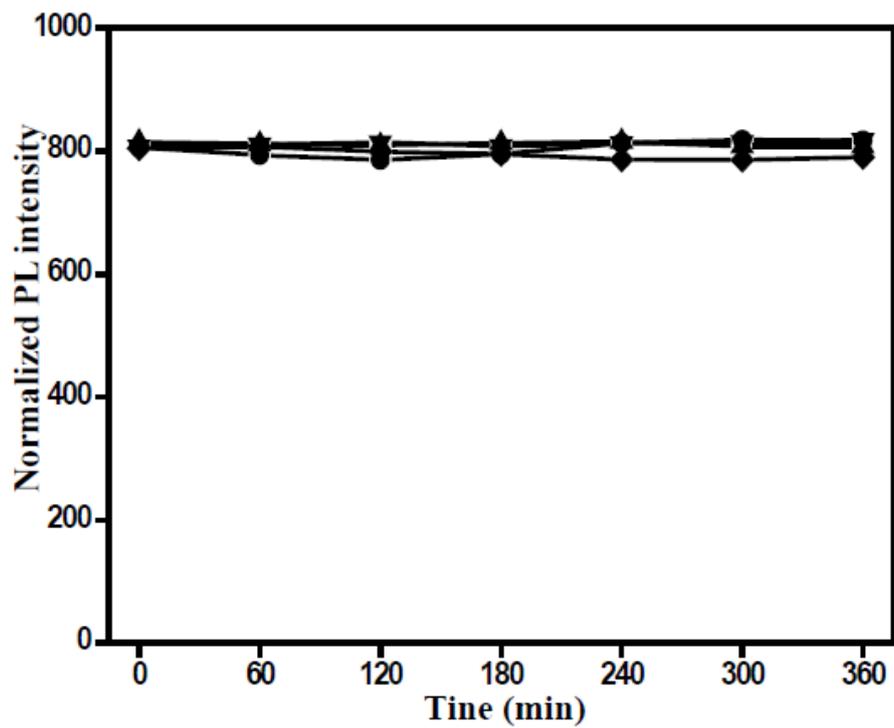
**Fig. S4.** A typical titration curve of C-dots, titrated with sodium hydroxide (A) and hydrochloric acid (B).



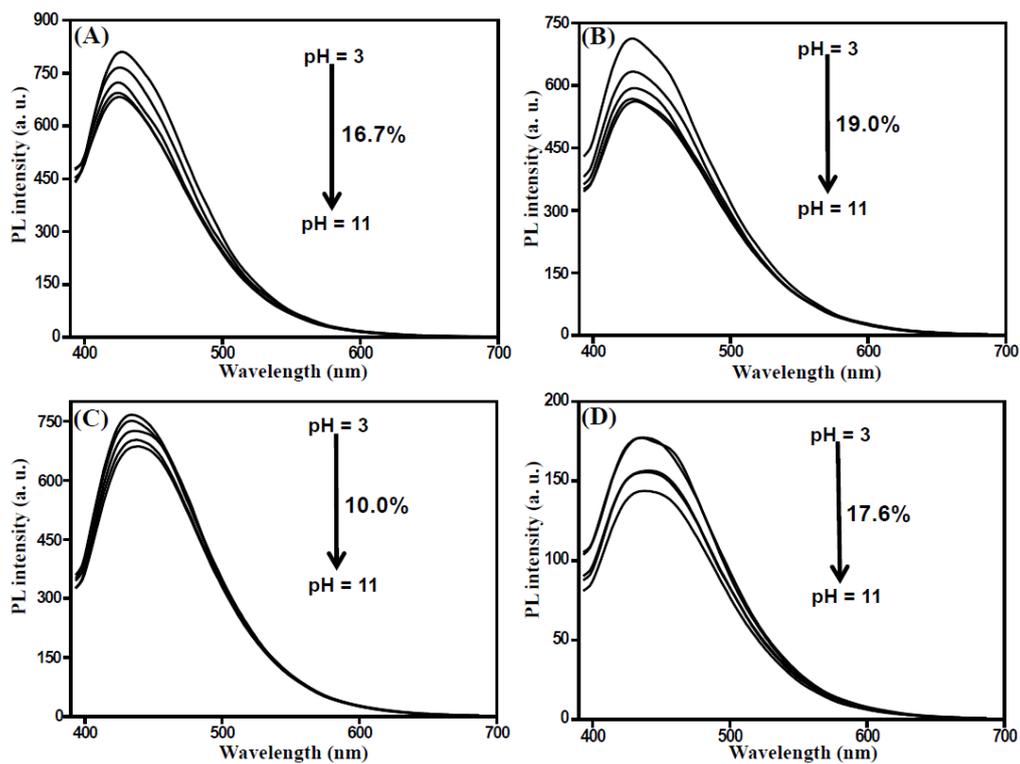
*Fig. S5.* Absorption spectra of glycine solutions (1 M, 15 mL) after being heated at various temperatures for 2 h. Inset: photograph of the glycine solutions.



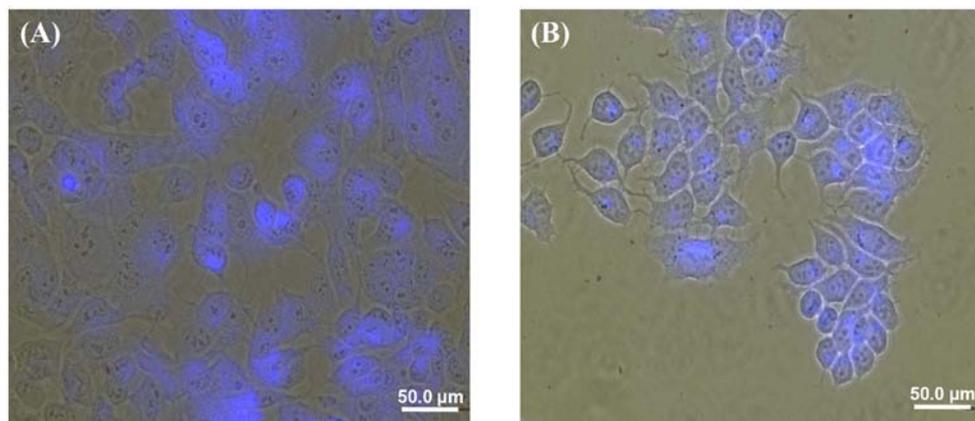
**Figure S6.** Effects of NaCl concentration on the stability of C-dots prepared from (A) glycine, (B) TRIS, (C) EDTA, and (D) cadaverine. Excitation and emission wavelengths were 365 and 450 nm, respectively. C-dots were prepared in phosphate buffers (5 mM, pH 7.4) containing various concentrations of NaCl.



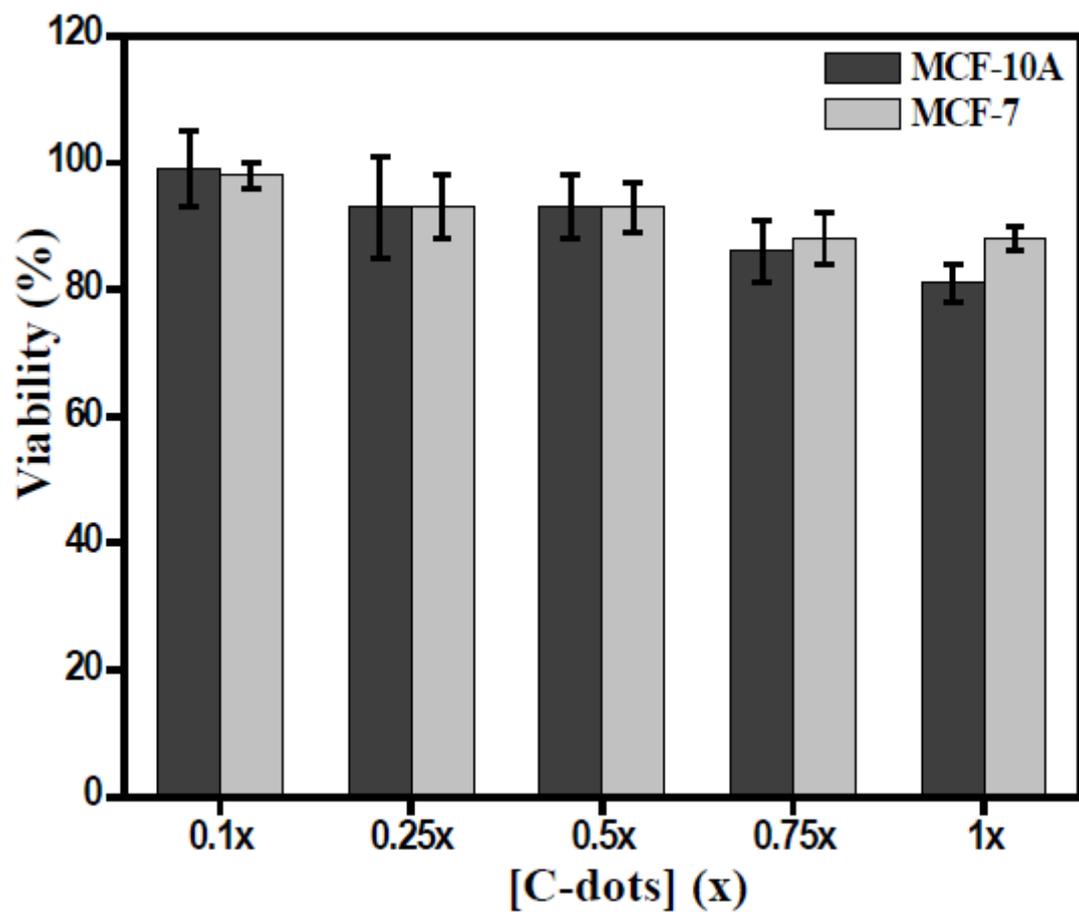
**Figure S7.** Photostability of C-dots prepared from glycine (—●—), TRIS (—◆—), EDTA (—▲—), and cadaverine (—▼—). C-dots were prepared in phosphate buffer (5 mM, pH 7.4).



**Figure S8.** Effects of pH on the PL of C-dots prepared from (A) glycine, (B) TRIS, (C) EDTA, and (D) cadaverine. C-dots were prepared in phosphate solutions (5 mM). The pH values decrease in two unit increments from 3.0 to 11.0.



**Fig. S9.** A representative PL image of MCF-10A cells (A) and MCF-7 cells (B) incubated with C-dots (0.86 mg/mL) for 24 h. The cells were excited at 360–380 nm. The grey channel shows transmission images, while the intensity coded channel (blue) shows the PL of C-dots.



**Figure S10.** Cytotoxicity of C-dots on MCF-10A and MCF-7 cells after 24 h. The values represent percentages of cell viability (mean  $\pm$  SD, n = 3). 1  $\times$  = 1.72 mg/mL.

### Supplementary References:

1. H. Liu, T. Ye, C. Mao, *Angew. Chem. Int. Ed.* **2007**, *46*, 6473-6475.
2. S. L. Hu, K. Y. Niu, J. Sun, J. Yang, N. Q. Zhao, X. W. Du, *J. Mater. Chem.* **2009**, *19*, 484-488.
3. S. Al-Nasiry, N. Geusens, M. Hanssens, C. Luyten, R. Pijnenborg, *Human Reproduction* **2007**, *22*, 1304-1309.