Electronic Supplementary Information (ESI)

Protein as the source for synthesizing fluorescent carbon dots by one-pot hydrothermal route

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Experimental Section

Chemicals

4, 7, 10-trioxa-1, 13-tridecanediamine (TTDDA) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Bovine serum albumin (BSA) was obtained from Shanghai Chemical Factory (Shanghai, China). Other reagents were of analytical grade and were used as received without further purification. All aqueous solutions were prepared with Milli-Q water (18.2 MΩ.cm).

Apparatus

Transmission electron microscopy (TEM) images were obtained with a TECNAI G_2 high-resolution transmission electron microscope (Holland) with an accelerating voltage of 200 kV. UV-vis spectra were obtained on a UV5800 spectrophotometer. Photoluminescence spectra were acquired with a Hitachi F7000 Luminescence spectrophotometer. Fourier-transformed infrared spectroscopy (FTIR) study was conducted with a VERTEX 70 FTIR (KBr wafer technique). The photostability of C-dots was performed with a 6W light source at a wavelength of 365 nm. The light source is about 5 mm above the sample.

Synthesis of C-dots

In a typical preparation, 1 g of BSA was dispersed in 20 mL of Milli-Q water with stirring. Then 3 mL TTDDA was added in and the mixture was kept stirring at room temperature for 0.5 h. The mixture was transferred to a Teflon-lined stainless steel autoclave for hydrothermal reaction at 180 °C for 12 h. After the hydrothermal treatment was completed, the autoclave was cooled and then the light yellow product dialyzed against water through a dialysis membrane (MW cutoff 3500) for 24 hours.

Fluorescence Imaging Experiments

The cellular uptake of C-dots was determined by Nikon Ti 2000 Microscope. SW1116 cells were cultured in 2 mL Dulbecco's modified Eagle's medium (high Glucose, DMEM, Sigima) containing 10% fetal bovine serum (FBS, Gibco) and 1% Antibiotic-Antimycotic (Gibco) at 37 °C in a 5% CO_2/95% air incubator. After an incubation of 3 h with 400 μg/mL of C-dots, the medium was removed and the cells were washed three times with PBS (pH 7.4, containing 1.8 mM KH_2PO_4, 10.1 mM Na_2HPO_4, 2.7 mM KCl, and 140 mM NaCl).

Cell viability

For the cell viability study, the HCT116 cells cultured in the presence of C-dots were determined by 3-[4, 5-dimethylthiazol-2-yl]-diphenyltetrazolium bromide (MTT, Sigma) assay. The HCT116 cells were first grown by using a 96 well plates (1000 cells/well) and then cultured in culture media with various concentration of C-dots (0, 12.5, 25, 50, 75, 100 and 125μg/mL) at 37 °C in a 5% CO_2/95% air incubator. All samples had five parallel wells. After culturing for 24 hours, the culture medium was replaced by 90μL serum-free appropriate medium and 10μL MTT solution (5 mg / mL) and then incubated at 37□ for 4h. The reaction solution was carefully aspirated; and 150μL DMSO was added to dissolve the formazan crystals. The optical density value (Absorbance, Abs) was read by the Multi-Detection Microplate Reader (Power Wave XS2, Bio-Tek Instrument Inc, USA).

Quantum Yield Measurements

Quantum yield was measured according to established procedure (Lakowicz, J. R. *rinciples of Fluorescence Spectroscopy*, 2nd Ed., **1999**, Kluwer Academic/Plenum Publishers, New York). The optical densities were measured on UV-vis spectra were obtained on a UV5800 Spectrophotometer. Quinine sulfate in 0.1 M H_2SO_4 (literature quantum yield 0.54 at 360 nm) was chose as a standard. Absolute values are calculated using the standard reference sample that has a fixed and known fluorescence quantum yield value, according to the following equation:

$$\varphi_x = \varphi_{std} \frac{I_x}{A_x} \frac{A_{std}}{I_{std}} \frac{\eta^2{}_x}{\eta^2{}_{std}}$$

Where φ is the quantum yield, I is the measured integrated emission intensity, and A is the optical density, and η is the refractive index. The subscript "std" refers to the reference fluorophore of known quantum yield. In order to minimize re-absorption effects absorbencies in the 10 mm fluorescence cuvette were kept under 0.1 at the excitation wavelength (360 nm).

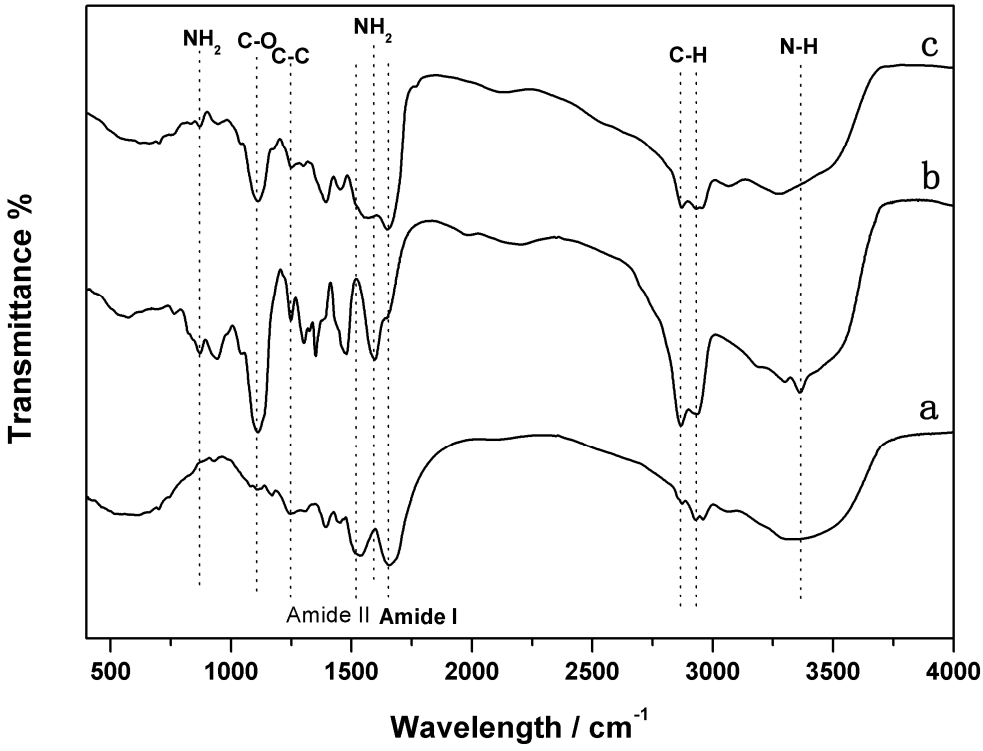

Fig. S1 FT-IR spectra of BSA (a), TTDDA (b), and C-dots (c).

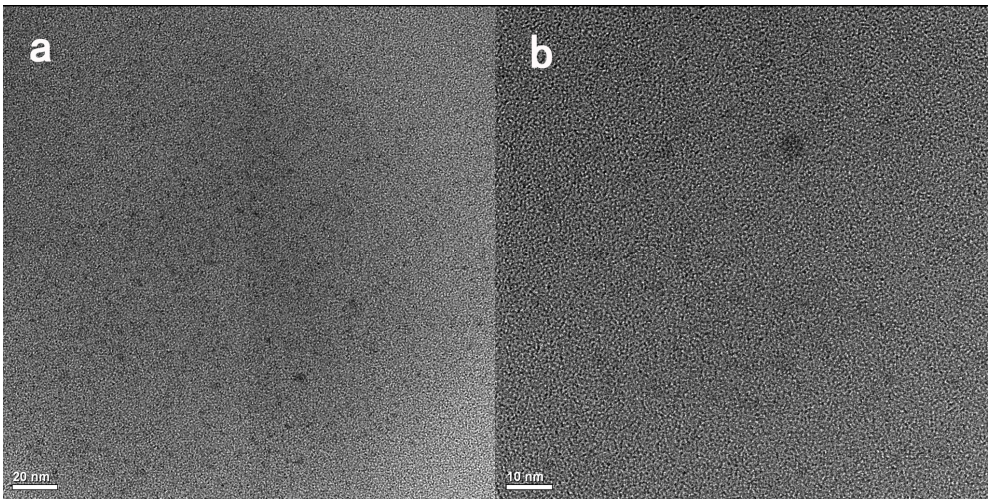

Fig. S2 TEM images of C-dots at different magnifications.

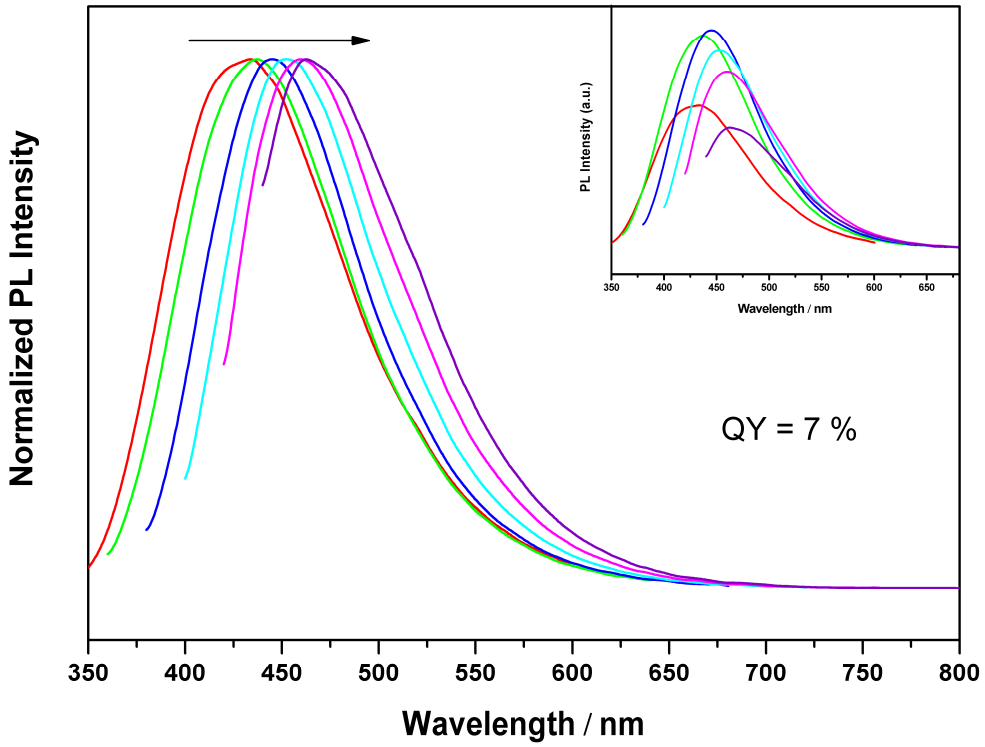

Fig. S3 Normalized PL spectra at excitation wavelengths from 320 nm to 420 nm on the left in 20 nm increment. Inset: PL emission spectra of C-dots. The sample was synthesized at 180 °C for 12 h with 1 mL TTDDA.

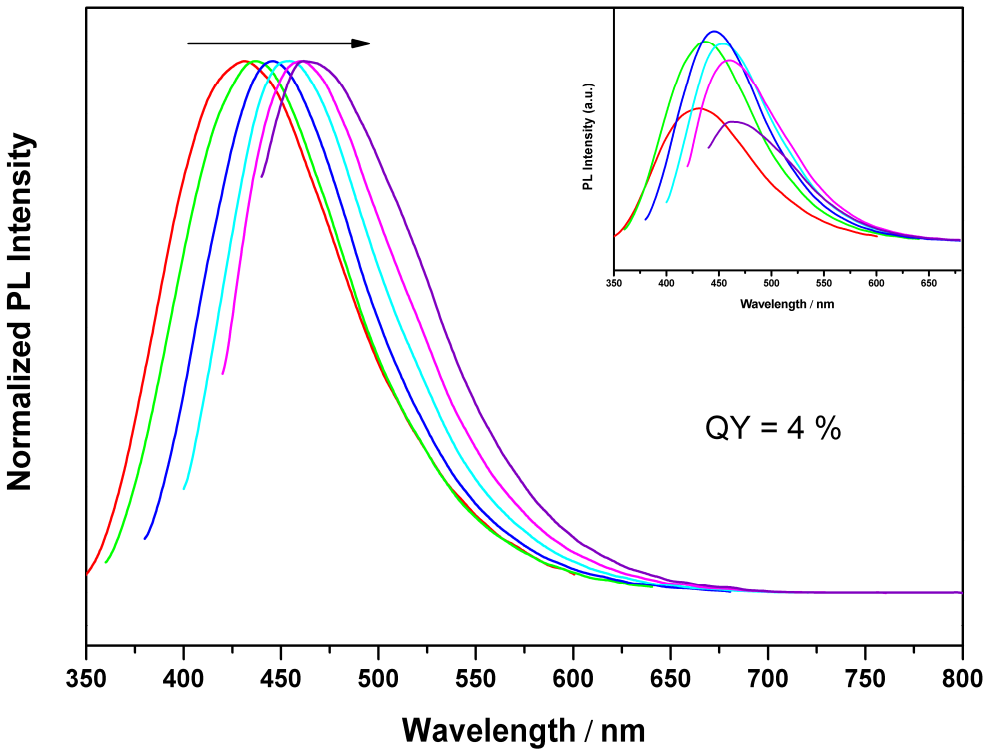

Fig. S4 Normalized PL spectra at excitation wavelengths from 320 nm to 420 nm on the left in 20 nm increment. Inset: PL emission spectra of C-dots. The sample was synthesized at 200 °C for 4 h with 3 mL TTDDA.

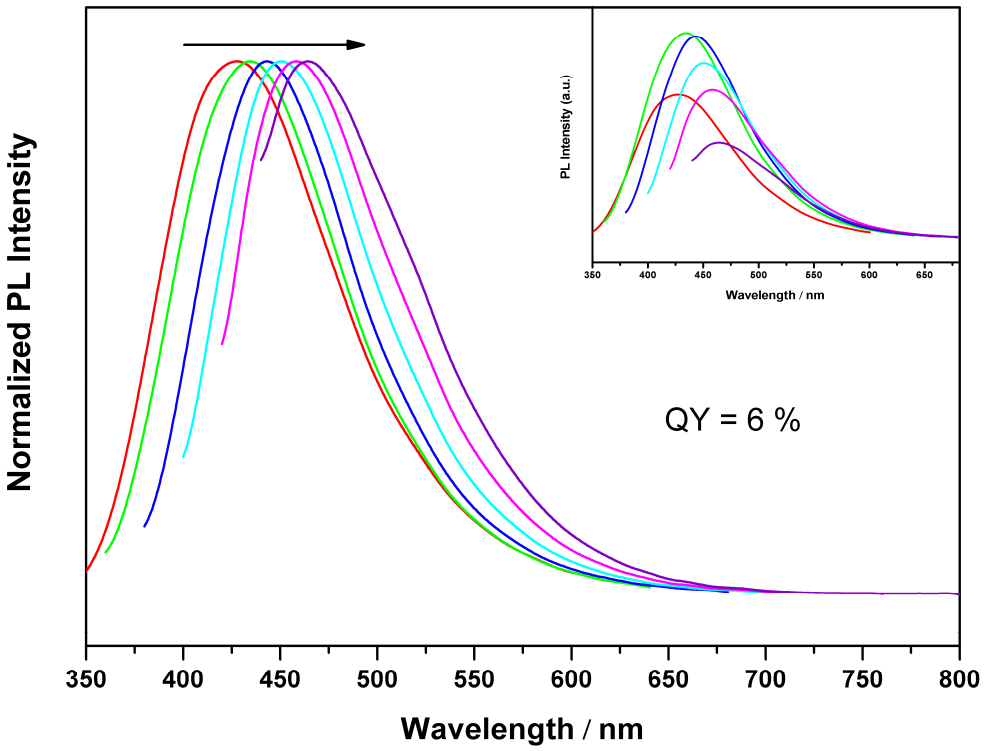

Fig. S5 Normalized PL spectra at excitation wavelengths from 320 nm to 420 nm on the left in 20 nm increment. Inset: PL emission spectra of C-dots. The sample was synthesized at 200 °C for 8 h with 3 mL TTDDA.

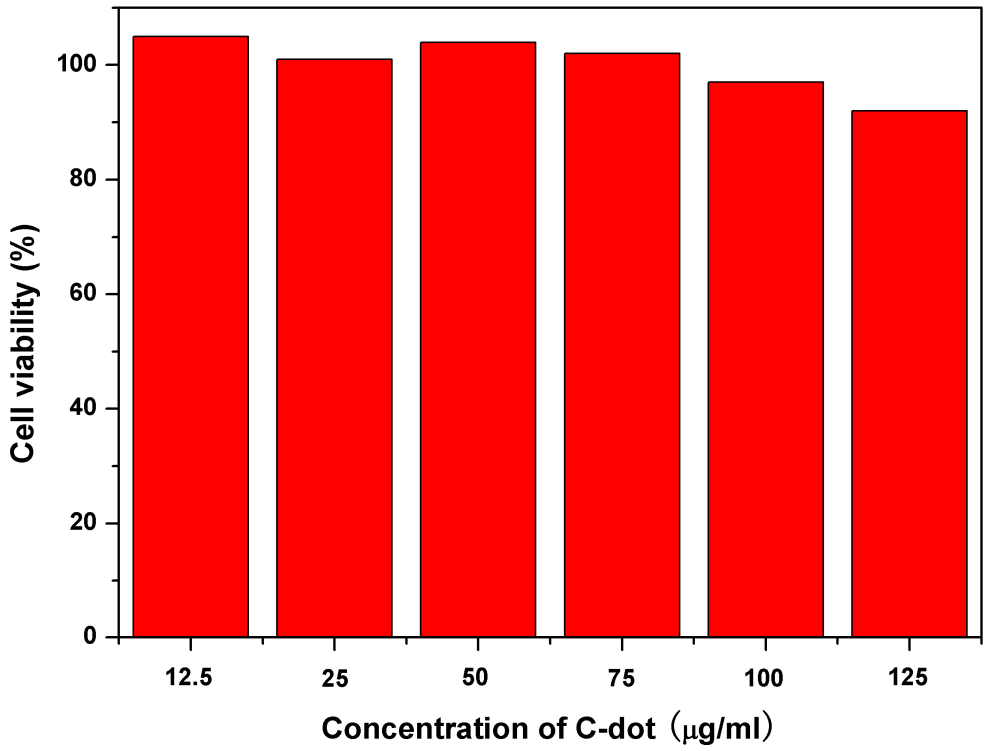

Fig. S6 Cell viability of HCT116 cells cultured at different concentrations of C-dots and tested by MTT assay.

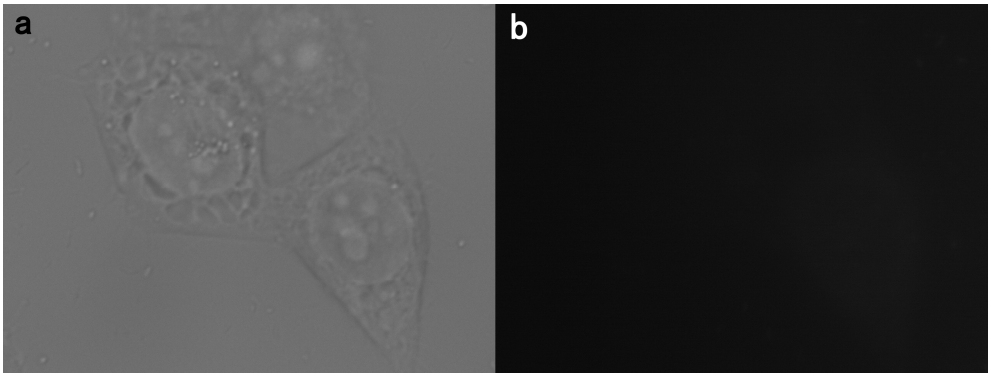

Fig. S7 (a) Bright field and (b) fluorescence microscopic images of SW1116 cells cultured in the absence of C-dots under UV excitation.