Supporting Information

A High Yield and Versatile Synthesis Method of Carbon Dots for Bio-imaging Applications

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1. X-ray diffraction measurement:

The average size of the CA-CDs was calculated using the Debye-Scherrer formula $D = \frac{K\lambda}{\beta \cos\theta}$, where D is the particle size, λ is the wavelength of the radiation, θ is the angle of the considered Bragg reflection, β is the the width on a 2θ scale, and K is a constant close to unity.

The calculation results show that $2\theta = 26°$, *FWHM*=15.827°, *D*=0.5 nm.

2. Quantum yields measurement：

The quantum yield (QY) was calculated by a relative method. Quinine sulfate (QY=54% in 0.1 M H_2SO_4) was used as a reference. The QY of the CDs was calculated using the following equation (1):

$$\phi = \phi' \times \frac{A'}{I'} \times \frac{I}{A} \times \frac{n^2}{n'^2} \quad (1)$$

The terms ϕ, *I*, *A* and *n* are the parameters of the CDs, which indicate the QY, integrated emission intensity, optical density and refractive index of CDs, respectively. The symbol (′) refers to the quinine sulfate of known QY. The optical absorbance values of the CDs and quinine sulfate were kept below 0.1 during the measurement process.

3. Preparation of Gd-CA-CDs and calculation of Gd^{3+} chelated rate :

Gd-CA-CDs were obtained by adding fresh $GdCl_3 \cdot 6H_2O$ solution (3.7 mg/mL) to the CA-CDs (2 mg/mL) dropwise with magnetic stirrer at 40 ℃ for 1 h, and then centrifuged at 4000 rpm for 30 min in a Millipore ultrafiltration centrifuge tube (MW = 3000) for at least 5 times to remove the unchelated Gd^{3+}. The chelating rate of Gd^{3+} was calculated using the following equation (2):

$$\boldsymbol{\theta = \frac{m(Gd)}{m(CDs)} \times 100\%}$$

The term $m_{(Gd)}$ refers to the actual chelated mass, which was calculated by ICP-AES measurement. The $m_{(CDs)}$ refers to the mass of the CA-CDs.

4. Relaxmetric measurement:

The T1-weighted images and the longitudinal relaxation rate (r1) of Gd-CA-CDs were measured and compared with commercial MRI agent Gd-DTPA with the same concentration of Gd(III). Furthermore, the Gd-CA-CDs dispersion with various Gd(III) concentration (0, 0.04, 0.08, 0.12, 0.16, 0.20 mM) to investigate the relationship between Gd(III) concentration and MR response (i.e., brightness of T1-weighted images, longitudinal relaxation times T1). To calculate the r1 of the Gd-CA-CDs, T1 was measured with a 3.0 T MRI scanner (Trivo, Siemens). T1-weighted images were obtained with two SE sequence (TR/TE=300 ms/12 ms, TR/TE=100 ms/12 ms and Matrix 256×256 pixel, FOV 60 mm×24 mm, slice thickness of 1.5 mm). The T1 value was calculated by SIEMENS T1 software for three times, and the average value was taken as the T1 value of the solution. Relaxivity values of Gd-CA-CDs were obtained from the slope of the linear fitting of 1/T relaxation time (s^{-1}) versus Gd concentration (mM).

5. Gd^{3+} leakage experiment:

In vitro leakage tests of Gd-CA-CDs were performed using a dialysis method in the PBS (pH = 5.5 and pH = 7.2). Gd-CA-CDs solution (1 mL, 500 μg/mL CA-CDs with chelating rate of 45%) put into a dialysis membrane tubing (MWCO 3000) and the membrane bags were immersed in 10 ml of PBS with disposable scintillation vials. At predetermined time intervals, 1 mL of PBS was withdrawn as samples and replaced with the same volume of fresh PBS. The leakage Gd^{3+} content was measured using the UV-vis spectrophotometer.

6. Intracellular metabolism measurement:

Hela cells were seeded in 35 mm cell culture plates at a density of 10^4 cells per well in DMEM/F12 culture media. The cells were incubated with Gd-CA-CDs solution at a concentration of 200 $\mu g \cdot mL^{-1}$ for 4 h, and then the cell medium was replaced with fresh medium and incubated for another 6, 12, 24, 48 h to test the in vitro cellular metabolism of the Gd-CA-CDs by using a confocal microscope under bright-field illumination and three different laser channels.

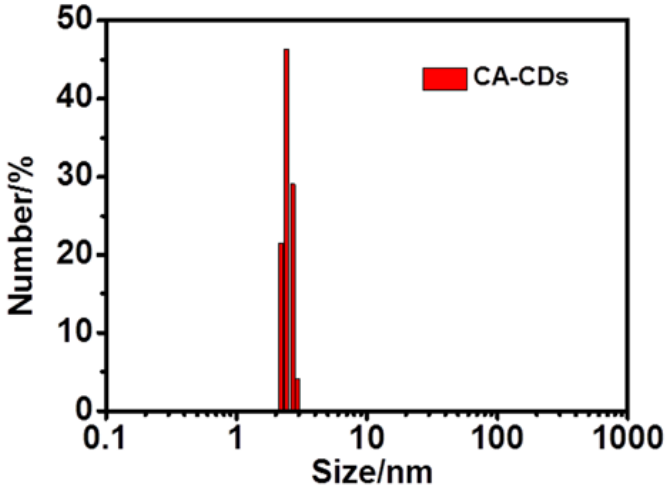

Figure S1. DLS size dispersion of the CA-CDs.

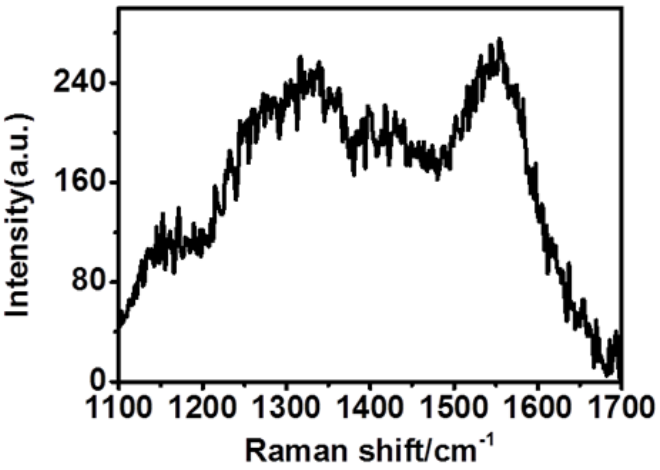

Figure S2. Raman spectrum of CA-CDs under 638 nm excitation.

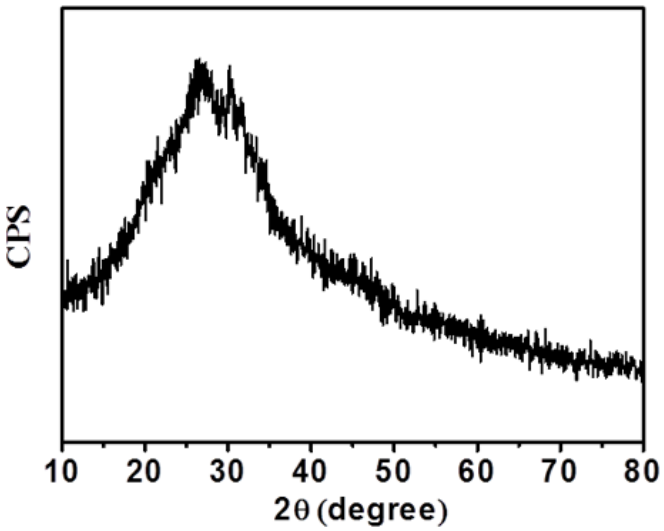

Figure S3. XRD pattern of CA-CDs.

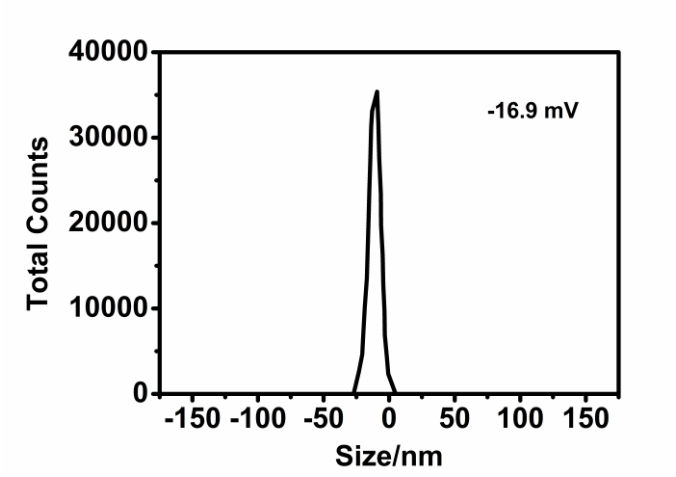

Figure S4. Zeta potential of the CA-CDs.

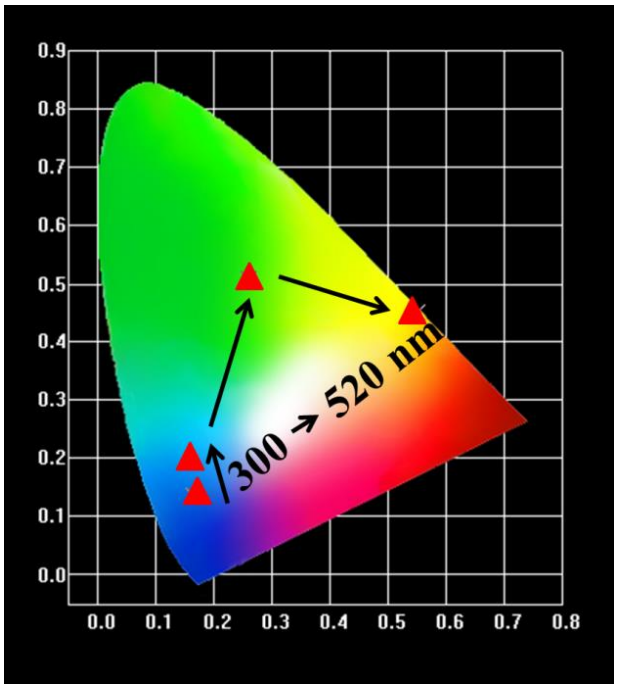

Figure S5. CIE chromaticity diagram of the CA-CDs at 300, 340, 400, and 520 nm excitation wavelengths

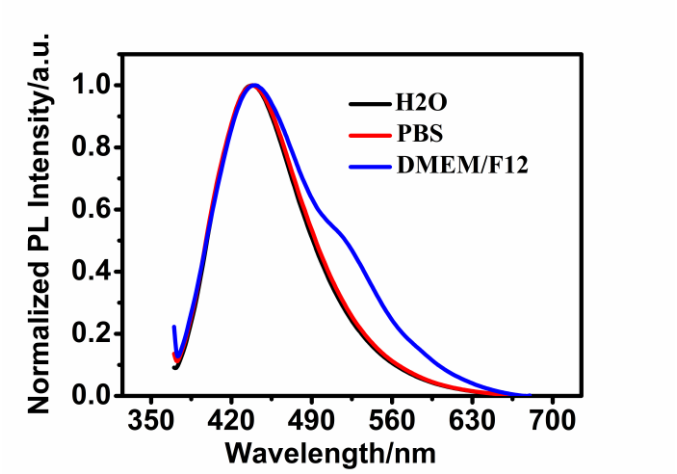

Figure S6. Fluorescence emission spectra of the CA-CDs (20 ppm) in DI water, PBS, and DEME/F12 culture media

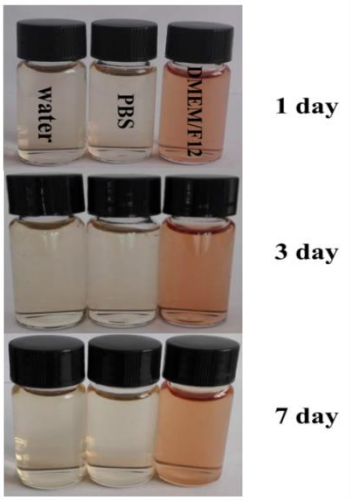

Figure S7. Photographs of CA-CDs (200 μg/mL) in water, PBS, and DMEM/F12.

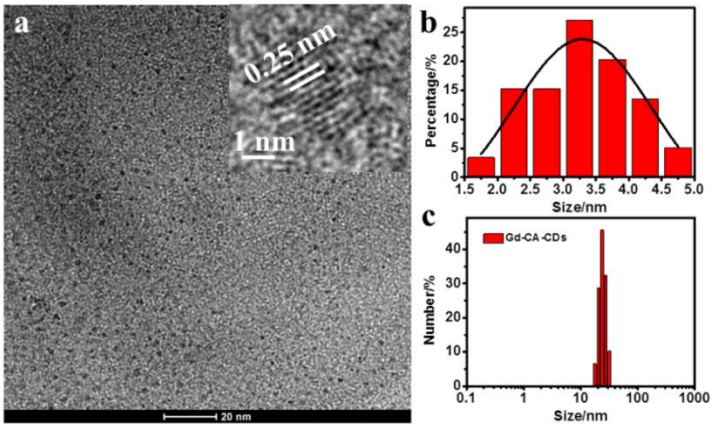

Figure S8. TEM image of the Gd-CA-CDs (Insert is the High-resolution TEM image of one nanoparticle); (b) the size distribution and (c) DLS size dispersion of the Gd-CA-CDs.

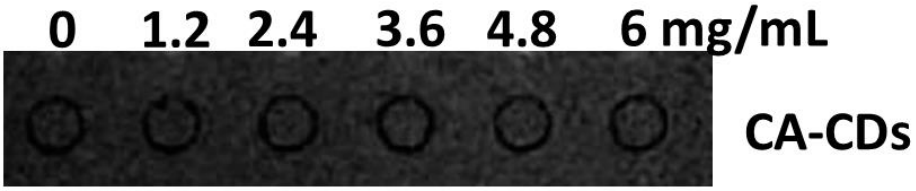

Figure S9. T1-weighted MR images of as-prepared CA-CDs with various concentrations (0, 1.2, 2.4, 3.6, 4.8, and 6 mg/mL).

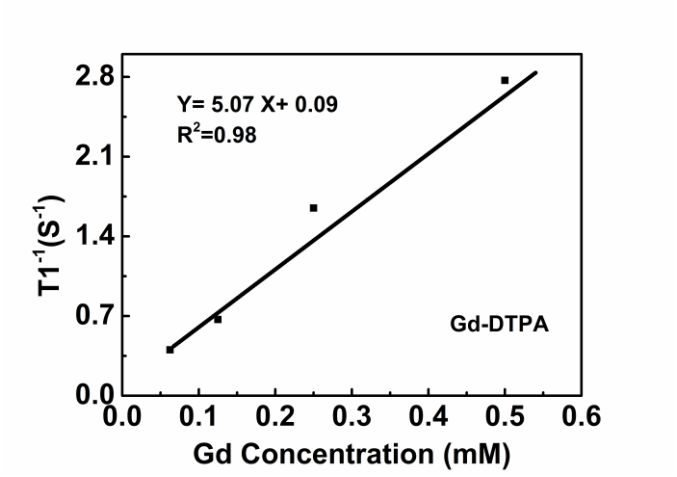

Figure S10. In vitro MR images of Gd-DTPA prepared using various Gd concentrations: 0, 0.04, 0.08, 0.12, 0.16, and 0.2 mM.

Figure S11. Photographs of Gd-CA-CDs (200 μg/mL CA-CDs with chelating rate of 45%) in water, PBS, and DMEM/F12.

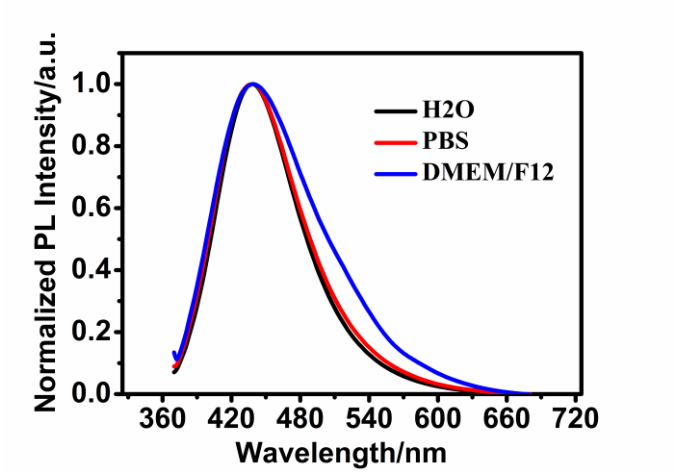

Figure S12. Fluorescence emission spectra of the Gd-CA-CDs (20 ppm) in DI water, PBS, and DEME/F12 culture media

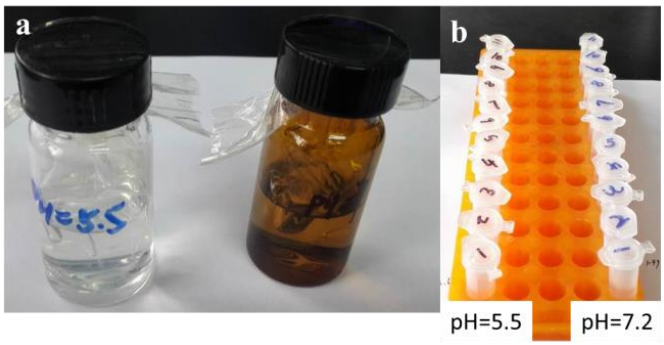

Figure S13. The photograph of (a) dialysis of the Gd-CA-CDs; (b) the samples collected from dialysate at 0, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, 48 h.

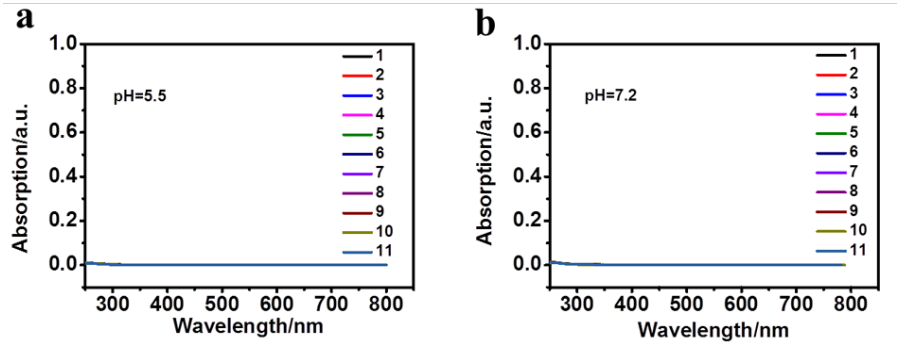

Figure S14. The UV-visible spectra of (a) the acidic (pH=5.5) and (b) the neutra (pH=7.2) PBS solution out of the dialysis bag suspended with Gd-CA-CDs (1 mL, 500 μg/mL CA-CDs with chelating rate of 45%).

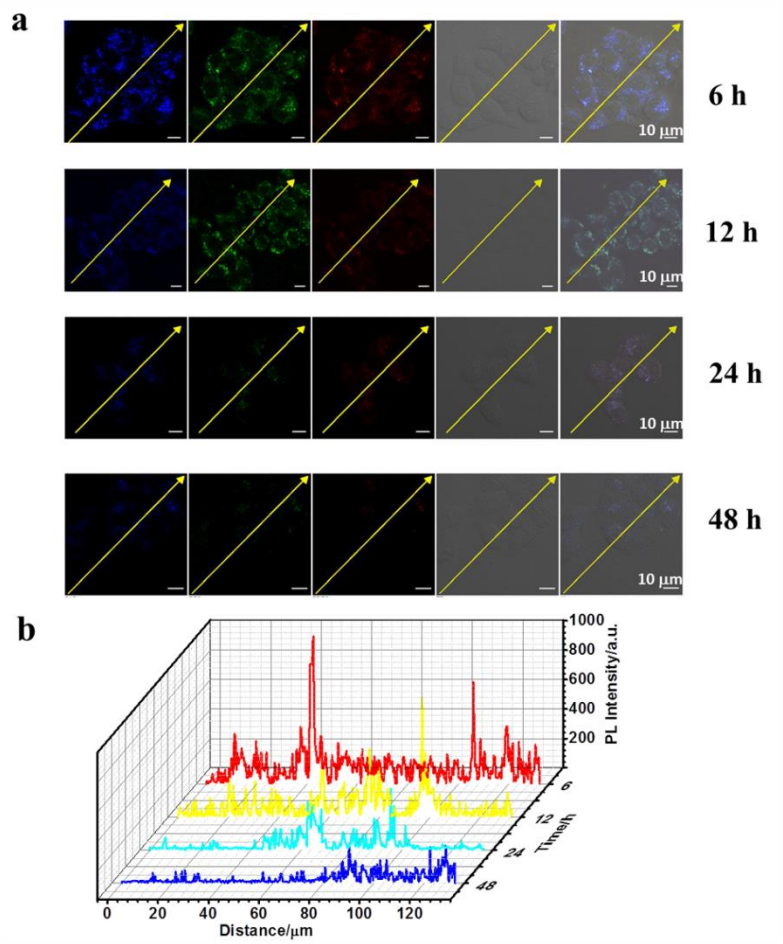

Figure S16. (a) The confocal fluorescence images of Hela cells with the Gd-CA-CDs under 405, 488, and 543 nm laser excitation and the overlay and the dark-field image of the Gd-CA-CDs; (b) quantification of the fluorescence intensity of the Gd-CA-CDs treated living cells under 405 nm excitation.

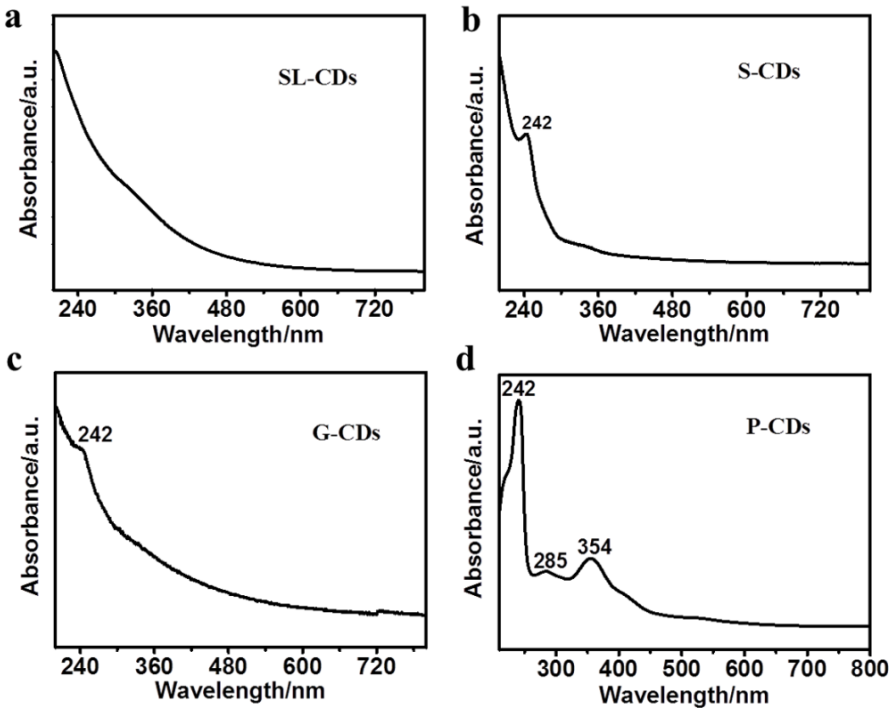

Figure S17. UV-visible absorption spectra of SL-CDs, S-CDs, G-CDs, and P-CDs.

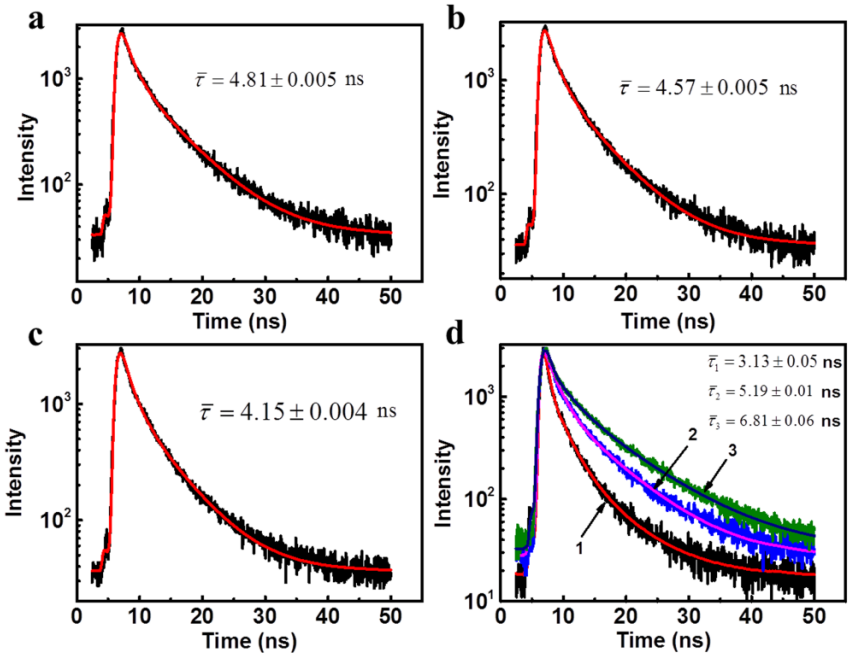

Figure S18. Time-resolved PL decay curves of (a) SL-CDs, (b) S-CDs, (c) G-CDs and (d) P-CDs (Insert: $\bar{\tau}_1$ and $\bar{\tau}_2$ stand for PL lifetime of 419 and 605 nm excited at 320, and $\bar{\tau}_3$ stands for the PL lifetime of 605 nm excited at 500 nm).

Table S1. The QY of the CA-CDs under excitation wavelength of 340 nm.

Sample	Integrated area	absorbance	QY
Quinine sulfate	379395.7	0.068	54 %
CA-CDs	158612	0.074	20.8 %

Table S2. The QY of the Gd-CA-CDs under excitation wavelength of 340 nm.

Sample	Integrated area	absorbance	QY
Quinine sulfate	387190	0.081	54 %
Gd-CA-CDs	24359	0.021	13.1 %

Table S3. The QY of four as-prepared CDs.

Sample	Integrated area	absorbance	excitation wavelength/nm	QY
Quinine sulfate	84546.0	0.022	370	54.0 %
SL-CDs	23066.1	0.034	370	9.5 %
S-CDs	59192.3	0.072	370	11.6 %
G-CDs	30032.3	0.053	370	8.0 %
Rhodamine B	6996.9	0.017	505	64.0 %
P-CDs	1412.1	0.042	505	5.3 %