Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Microwave-assisted synthesis of fluorescent carbon quantum dots from A2/B3 monomer set

Ari Chae,[a] Yujin Choi,[a] Seongho Jo,[a] Nur'aeni,[a] Peerasak Paoprasert,[d] Sung-Young Park[a,c*] and Insik In[a,b*]*

[a] *Department of IT Convergence (Brain Korea PLUS 21), Korea National University of Transportation, Chungju 380-702, South Korea.*

[b] *Department of Polymer Science and Engineering, Korea National University of Transportation, Chungju 380-702, South Korea.*

[c] *Department of Chemical and Biological Engineering, Korea National University of Transportation, Chungju 380-702, South Korea.*

[d] *Department of Chemistry, Faculty of Science and Technology, Thammasat University, Pathumthani 12121, Thailand.*

1. Experimental Section

Materials and Characterization

Both SA (ACS reagent, ≥99.0%) and TAEA (96%) were purchased from Sigma-Aldrich Corp. A household microwave oven (700 W) (Daewoo Electronics, South Korea) was used throughout the experiments. Dialysis was carried out by using a molecular weight cut-off (MWCO) membrane (3,500 Da) of Spectrum Laboratories, Inc.

UV-Vis spectra were measured by an Optizen Alpha UV-Vis Smart spectrometer of (Mecasys, South Korea). The photoluminescence spectra of the CQD solutions were examined by using a FS-2 Fluorescence Spectrometer (Scinco Corp., South Korea) with a xenon lamp excitation source (150 W). HRTEM images were taken on a TECNAI F20 system (Philips) at an operating voltage of 200 kV. AFM images were acquired by a Multimode-N3-AM nanoscope three-dimensional (3D) scanning probe microscopy (SPM) system (Bruker Corp.). FT-IR spectra were acquired on a Nicolet iS10 FT-IR spectrometer (Thermo Scientific). Raman spectroscopy measurements were performed on an ARAMIS Raman spectrometer (Horiba Jobin Yvon, France) by using 514.5 or 785 nm laser radiation. XRD measurements of the powder samples were recorded on a D8-Advance X-ray powder diffractometer (Bruker) using Cu Kα radiation (λ = 1.5406 Å). The XPS spectra were recorded with a Scientific Sigma Probe spectrometer (Thermo VG). Fluorescence microscopy images of CQD-labelled cells were obtained using LSM 510 confocal laser scanning microscope (ZEISS).

For the microwave-assisted synthesis of CQDs from SA and TAEA, 3 g (1 eqv.) of SA and 5.572 g (1.5 eqv.) of TAEA were completely dissolved in 10 mL of deionized water in a 250 mL flat-bottomed flask. After loosely wrapping the flask with polyethylene wrapping film, the mixture solution was treated inside a microwave oven for 5 min (**CAUTION!** ***Carry out the whole experiment inside a fume hood because of the emission of organic volatiles during the microwave treatment***). After the microwave treatment and complete cooling, dark-brown

solids were obtained, indicating the formation of CQDs. Extraction of the CQDs was carried out by adding 100 mL of deionized water to the solids and subsequent ultrasonication in a bath sonicator (500 W) for 30 min. Centrifuging at 4000 rpm for 30 min was carried out to remove any insoluble precipitates or agglomerates from the CQD solution. Finally, 1.48 g of brown CQD2 powders was obtained after freeze-drying of the CQD solution obtained after dialysis using 3,500 Da MWCO membranes for 3 days.

Cell Viability Test and Bioimaging

The cell viability of the CQDs was measured using the MTT assay method. 200 μL of MDA-MB-231 or MDCK cells at a density of 1×10^5 cells/mL was placed in each well of a 96-well plate. Afterwards, the cells were incubated for 24 h at 37 °C in a humidified 5% CO_2 atmosphere. To determine the cellular viability, a stock solution of CQD2 was dissolved in RPMI medium at a concentration of 1 mg/mL and the stock solution was diluted up to 0.001 mg/mL. The media was removed and the cells were treated with different concentrations of the CQD2solution. Then, the cells were incubated for another 24 hrs. The media containing CQD2 were replaced with 180 mL of fresh medium and 20 μL of a stock solution containing 15 mg of MTT in 3 mL of PBS and incubated for another 4 h. Finally, the medium was removed and a 200 μL solution of an MTT solubilizing agent was added to the cells and accurate shaking was performed for 15 min. The optical absorbance was measured at a wavelength of 570 nm using a microplate reader (Varioskan® Flash, Thermo Electron Corporation). The relative cell viability was measured by comparing the samples with the 96-well control plate containing only cells. After cell viability measurements, confocal images were obtained to confirm the stained cells by using a laser scan microscope (CLSM) at 10× magnification) with excitation wavelengths of 405, 488, and 543 nm.

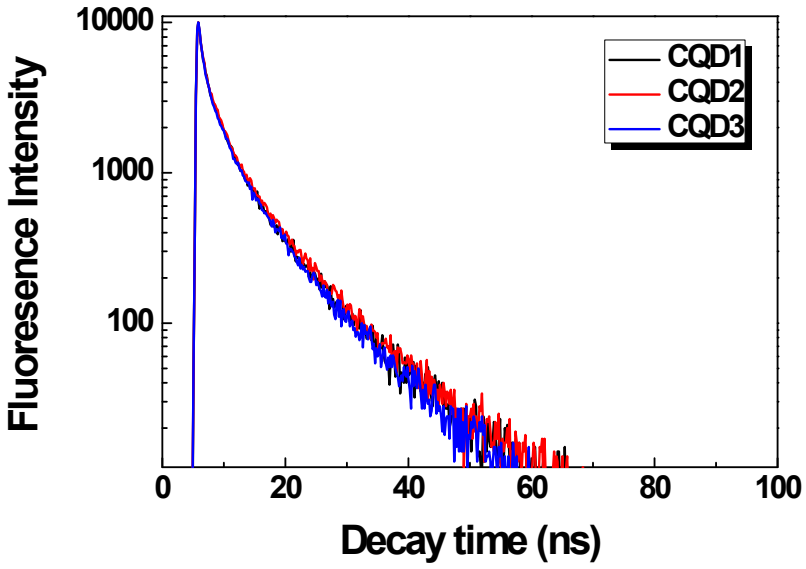
2. Supporting Table and Figures

Table S1. Summary of the mass synthesis of lysine-based CQDs through microwave pyrolysis

Starting Materials	Microwave time (min/W)	Mass Yield[a] (wt.-%)	PLQY[b] (%)	Ref.
SA : TAEA (3:1)	5/700	20.6	0.7	**CQD5** In this work
SA : TAEA (3:2)		45.0	1.3	**CQD4** In this work
SA : TAEA (1:1)		3.57	24.0	**CQD1** In this work
SA : TAEA (1:1.5)		17.3	49.9	**CQD2** In this work
SA : TAEA (1:2)		3.13	43.5	**CQD3** In this work
SA : TAEA (1:2.5)		negligible	not measured	This work
SA : TAEA (1:3)		negligible	not measured	This work
SA : TAEA (1:3.5)		negligible	not measured	This work
Citric acid : Urea (1:3.2)	4–5/750	not reported	14	**Ref 36** *Angew. Chem. Int. Ed.* **2012**, *51*, 12215
Glucose	1–11/280–700	not reported	7–11	**Ref 45** *ACS Nano* **2012**, *6*, 5102
Glucose : PEG 1500 (2g : 4 g)	10/700	not reported	not reported	**Ref 46** *Nanoscale* **2013**, *5*, 2655
Carbohydrate (glucose etc. + inorganic ion)	14/750	not reported	3.2–9.5	**Ref 47** *J. Mater. Chem.* **2011**, *21*, 2445

[a] Mass yield = (mass of purified CQD / mass of lysine)×100%

[b] PLQY of CQDs (0.1 mg/mL) measured from the relative PL emission compared with that of quinine sulfate (0.1 mg/mL in 0.1 M H_2SO_4)



	Relaxation time (ns)	Percent (%)
τ_1	0.705	52.3
τ_2	3.30	36.1
τ_3	10.1	11.6
τ_{avg}	2.73	

Fig. S1. Fluorescence decay profiles of prepared CQDs (the emission at 470 nm with the excitation of 375 nm light).

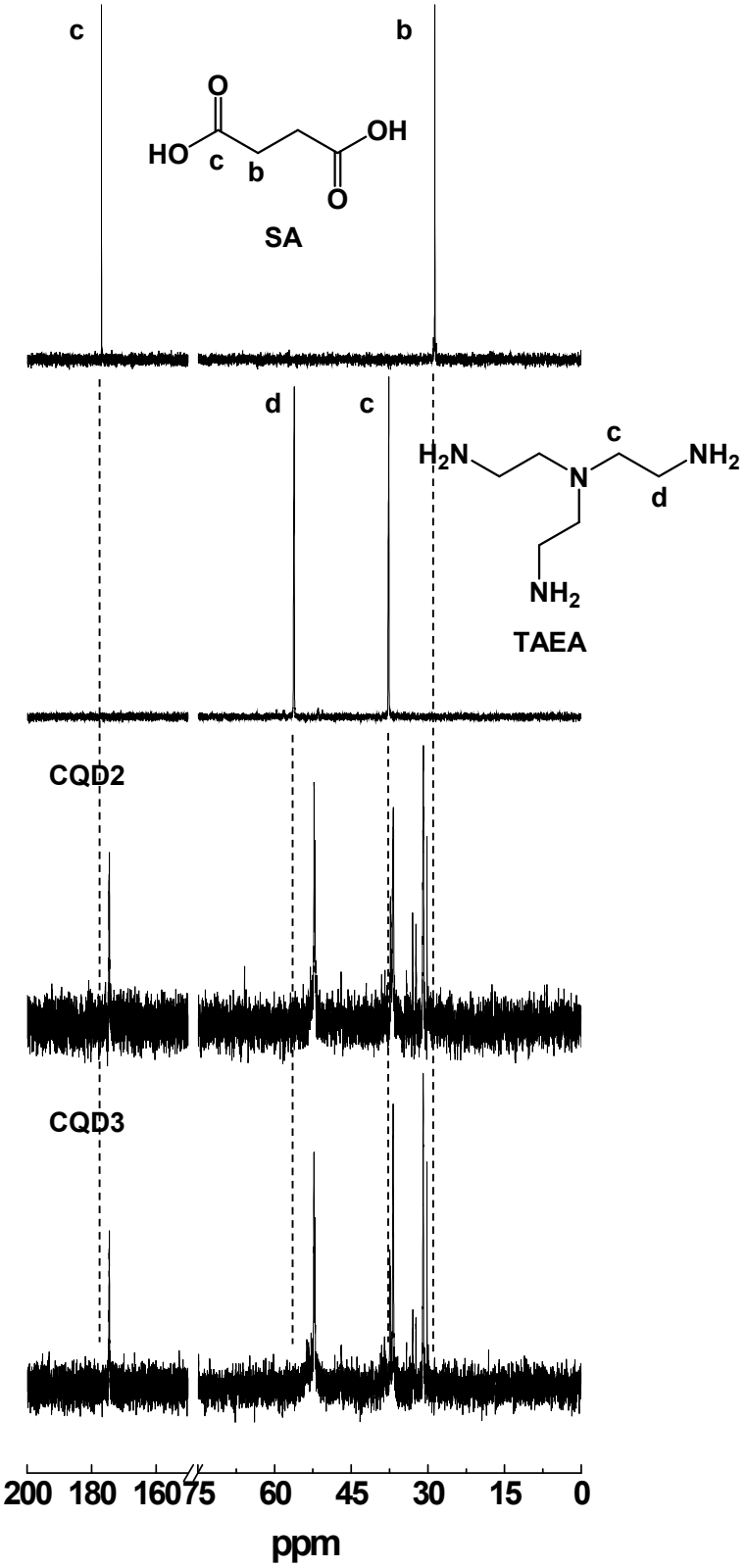


Fig. S2. ^{13}C-NMR spectra of profiles of SA, TAEA, CQD2, and CQD3 in D2O.

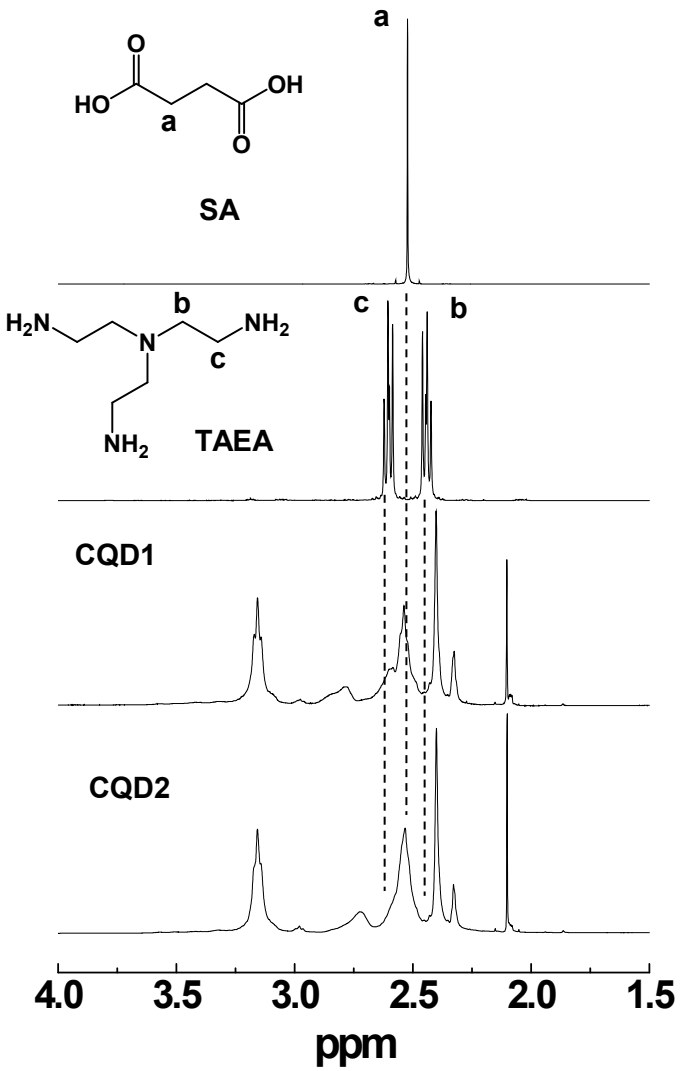


Fig. S3. ^{1}H-NMR spectra of profiles of SA, TAEA, CQD1, and CQD2 in D2O.

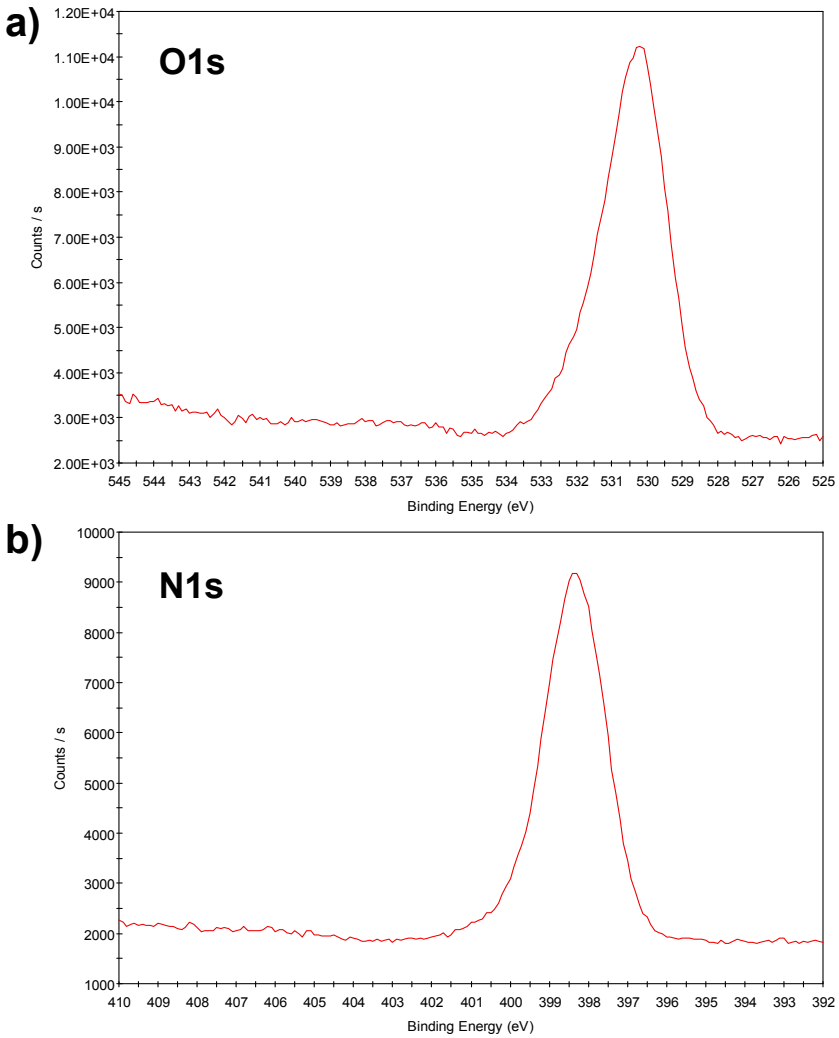


Fig. S4. XPS high resolution O1s and N1s binding peaks of CQD2.

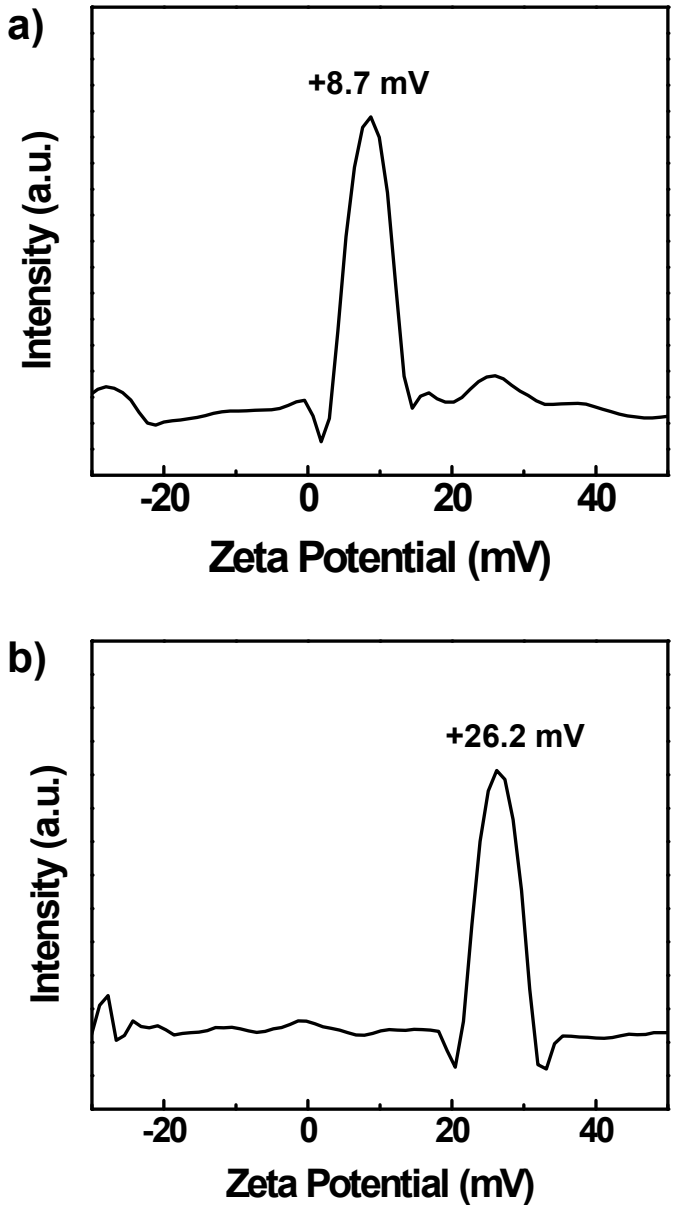


Fig. S5. Zeta-potential profile of the aqueous solution (0.1 mg/mL) of lysine-based CQD prepared from microwave pyrolysis for 4 min.

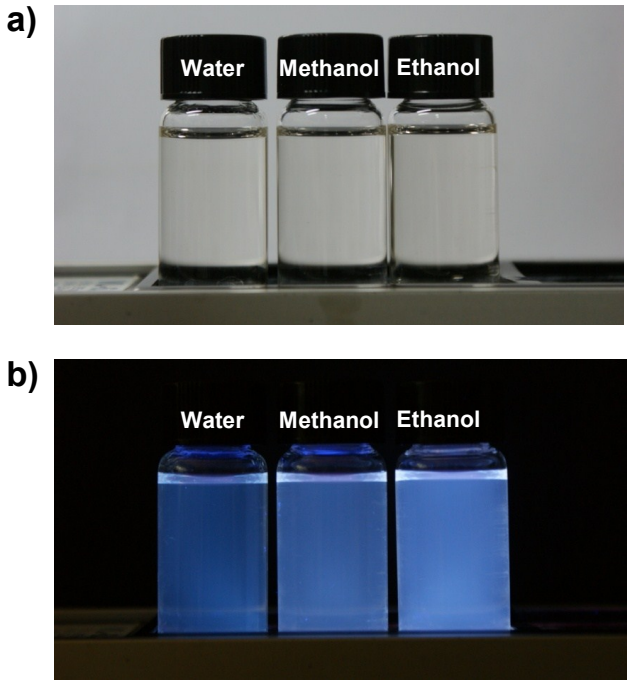


Fig. S6. Photo images of CQD2 solutions (0.1 mg/mL) in water, methanol, and ethanol under a) day light and b) dark with the 365 nm UV irradiation.

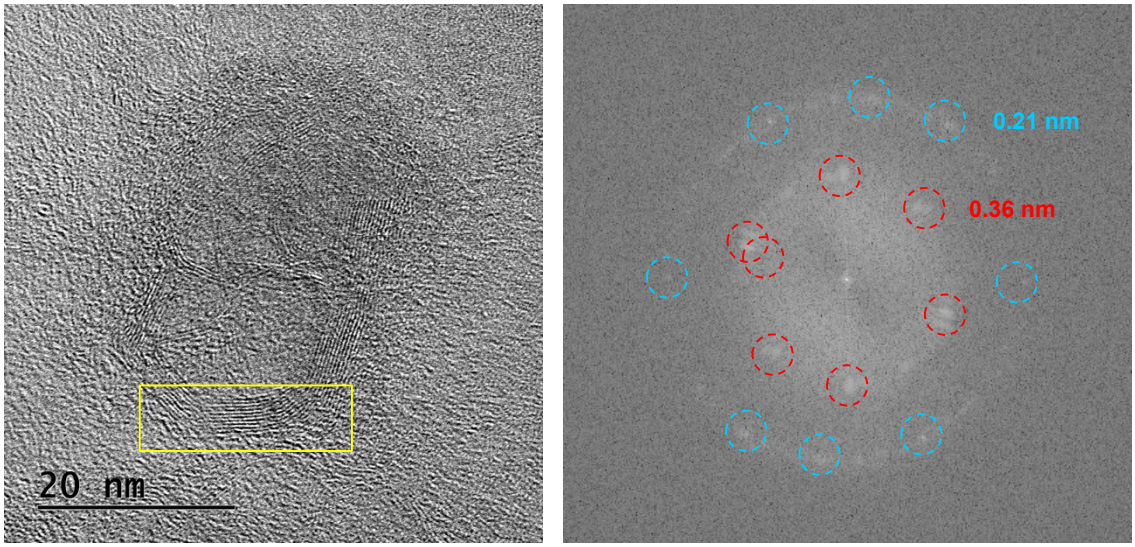


Fig. S7. HRTEM image of CQD2 (left) and Fourier transform image of selected area. d-spacing of 0.36 nm (red circles) is much clearer compared with weaker d-spacing of 0.21 nm (blue circles).

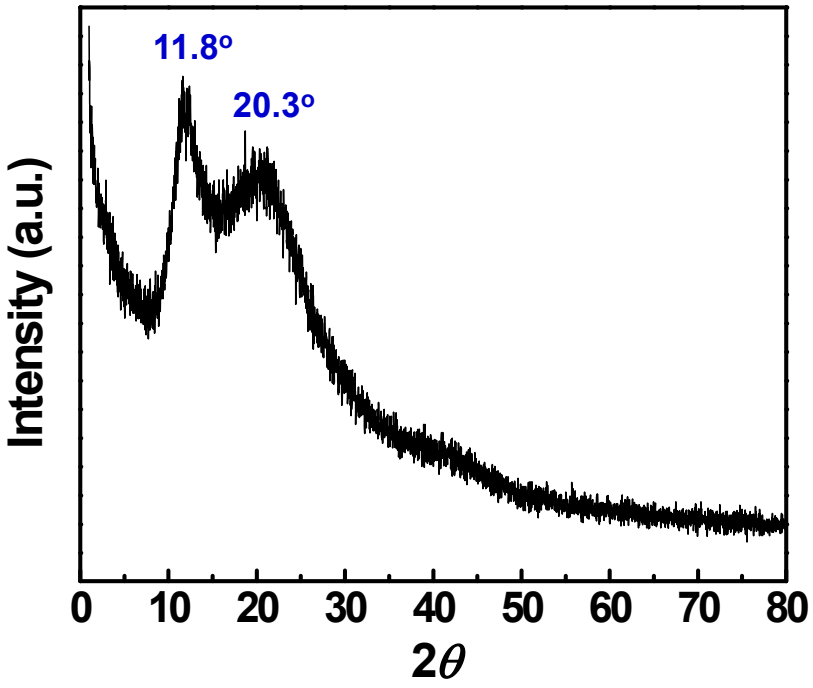


Fig. S8. XRD spectrum of CQD2.

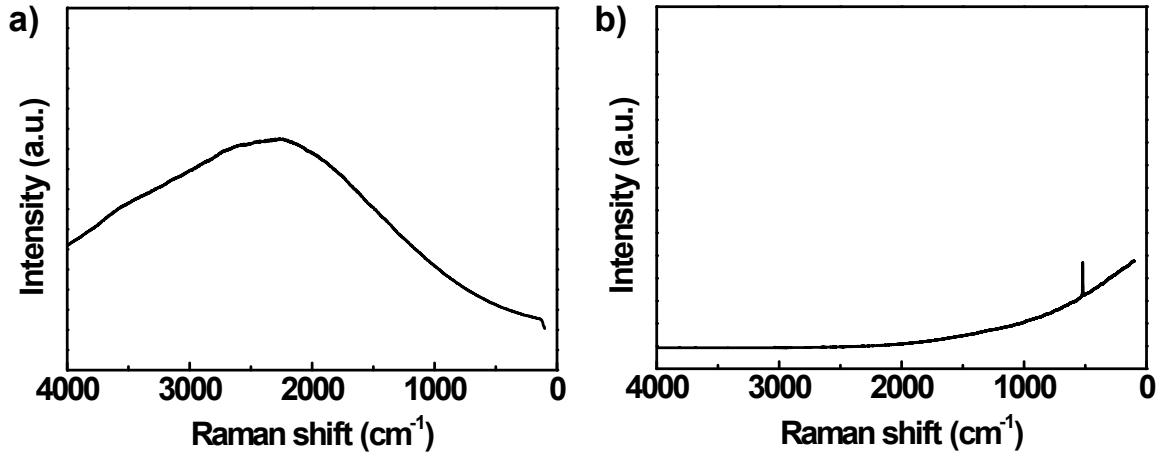


Fig. S9. Raman spectra of CQD2 on Si wafer with the excitation of a) 514 nm laser and b) 785 nm laser (the sharp peak in the right spectrum is Raman peak of underlying Si wafer.

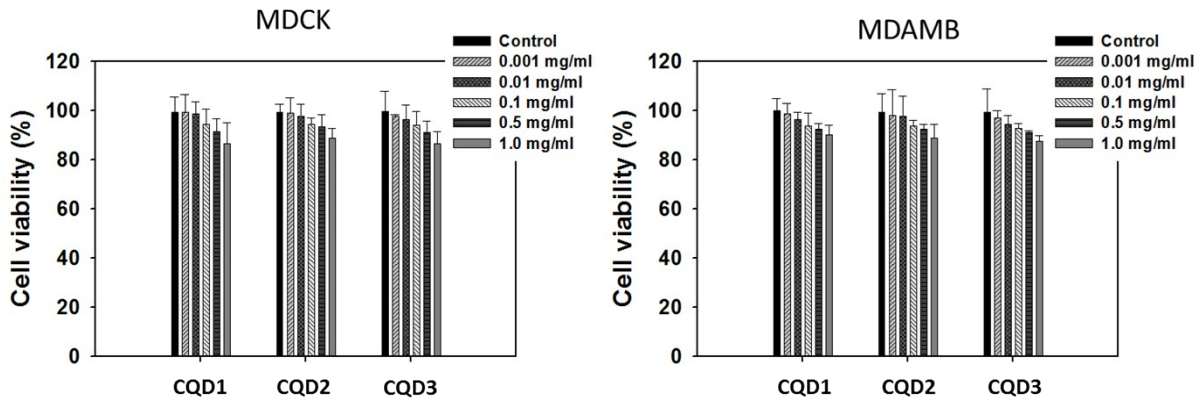


Fig. S10. LDH cell viability tests of CQDs with different CQD concentrations.

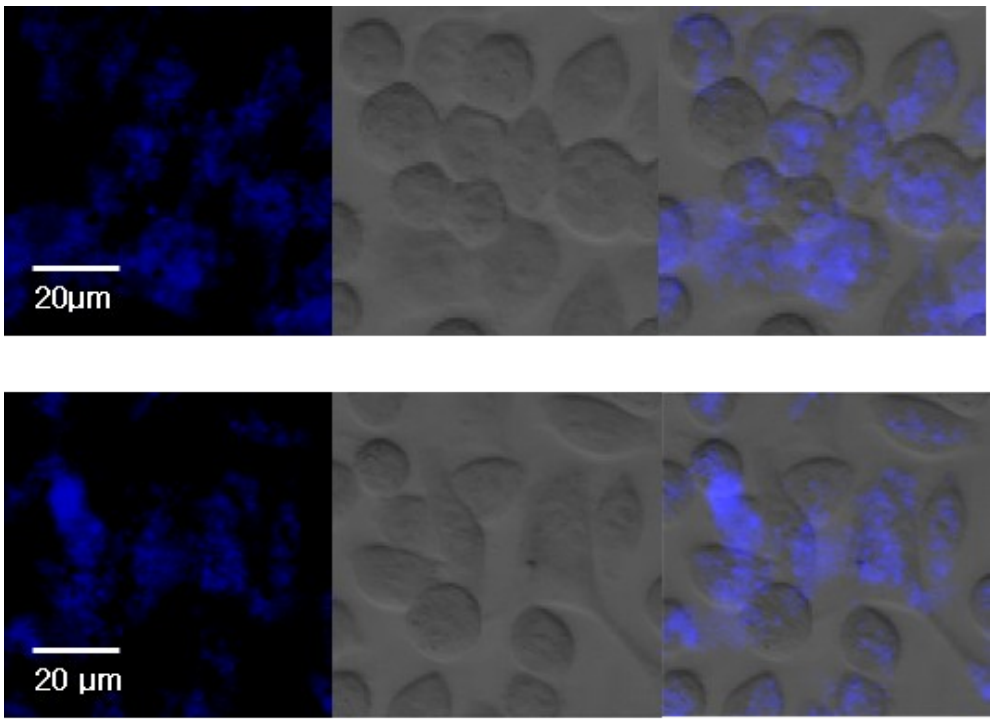


Fig. S11. Blue fluorescence images (from left to right : PL, bright, and merged cell images) of MDAMB (above) and MDCK (bottom) cells after incubation with CQD2 (excitation wavelength of 405 nm).