Supporting Information

Synthesis and Analytical Applications of Photoluminescent Carbon Nanodots

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Materials and methods

Chemicals. Insulin, polybrene, quinine sulfate, sodium dodecyl sulfate (99%), sodium hydroxide, HPLC grade solvents were purchased from Aldrich (Milwaukee, WI, USA). Sodium chloride, sodium phosphate monobasic, anhydrous sodium phosphate dibasic, trisodium phosphate, and tris(hydroxymethyl) aminomethane were obtained from J.T. Baker (Phillipsburg, NJ, USA). Boric acid was purchased from RiedeldeHaën (Buchs, Switzerland). RPMI-1640, fetal bovine serum (FBS), antibiotic-antimycotic, L-glutamine, and non essential amino acids were obtained from Biowest (Lewes, UK). Ultrapure water (18.2 MΩ/cm) from a Milli-Q ultrapure system was used in this study. The pH values of phosphate buffer solutions are 3.0–10.0. Phosphate buffered saline (PBS; 1x, 1 L, pH 7.4) contained NaCl (8 g), KCl (0.2 g), Na_2HPO_4 (1.44 g), and KH_2PO_4 (0.24 g).

Synthesis of C-dots. The used coffee grounds (Blue Mountains, Taiwan) were dried in an oven at 110°C prior to being subjected to grinding into fine powders. After autoclave calcination at 300°C for 2 h in air, black carbonized powders (~3 mg) were cooled to 20°C and dispersed into ethanol (5 mL). We noted that further increases in calcination temperature from 300 to 600°C did not produce greater amounts of C-dots, which was supported by the slight decrease in the fluorescence of C-dots solutions that had same PL profiles. The solution was then centrifuged at 3000 rpm for 10 min to remove large or agglomerated particles. The supernatant containing C-dots was filtered through a 0.22-μm membrane to further remove large particles.

The quantum yield (ϕ) of C-dots was calculated by comparing the integrated PL intensities (excited at 365 nm) and the absorbance values of C-dots at 365 nm with those of the reference quinine sulfate.[1-2] Quinine sulfate (literature ϕ_f = 54%) was dissolved in 0.1 M H_2SO_4 (refractive index (η) of 1.33), and C-dots were dissolved in ethanol (η = 1.36). To minimize reabsorption effects, the optical densities of the two

solutions in the 10 mm cuvettes were kept under 0.1 at the excitation wavelength. Excitation and emission slit widths of 5.0 nm were set to excite the two solutions and record their PL spectra, respectively.

Characterization. The size and shape of the as-prepared C-dots was measured using a JSM-1200EX II TEM system (JEOL Ltd., Tokyo, Japan) and FEI Tecnai-G2-F20 TEM. Prior to TEM and HRTEM measurements, the synthesized C-dots were concentrated 10-fold by removing ethanol in vacuum. The purified C-dots were carefully deposited onto 400-mesh C-coated Cu grids and the excess solvents were evaporated at ambient temperature and pressure. An energy dispersive X-ray (EDX) system (Philips, Roanoke, VA, USA) was used to confirm the compositions of C-dots. For XRD measurement, a PANalytical X'Pert PRO diffractometer (Almelo, Netherlands) in conjunction with Cu Kα radiation (λ = 0.15418 nm) was used. UV–Vis spectra of C-dots in ethanol solution were recorded using a GBC Cintra 10e double-beam spectrometer (Victoria, Australia). PL spectra were recorded using a Cary Eclipse PL spectrophotometer (Varian CA, USA) operated at excitation wavelengths in the range 350–500 nm. The photostability of C-dots was investigated under continuous illumination of the Xe lamp in a PL spectrophotometer (Varian, CA, USA). A Raman microscopy system with 50x objective (Dongwoo KyungGiDo, Korea) was used to analyze air-dried C-dots on a silica wafer. A diode-pumped solid-state laser operating at λ = 532 nm was used as the excitation source with a power of 100 mW and an accumulation time of 200 s. The precipitated cells were washed thrice with PBS before being used for bright field and PL imaging measurements by using an Olympus IX71 (Tokyo, Japan) fluorescence microscope with a DP70 digital camera. Owing to the limit of the microscopic system, the excitation wavelengths were set in a range 510–530 nm.

Cell labeling. Renal proximal tubular LLC-PK1 cells (American Type Culture

Collection) were maintained in the RPMI-1640 medium supplemented with FBS (10%), antibiotic-antimycotic (1%), L-glutamine (2 mM), and non-essential amino acids (1%). Prior to measurement, cells were seeded in 96-well plates at an initial cell density of 1×10^4 cells/mL. A concentrated aqueous C-dots solution (10x, 12 mg/mL) was mixed with polybrene solution (80 ng/mL) and the mixture was equilibrated at 37°C for 30 min. Aliquots (100 μL) of aqueous C-dots solution were added to each well and the mixtures were incubated for 24 h. Cell number and viability were then determined by the trypan blue exclusion method and the Alamar Blue cell viability test, respectively. The precipitated cells were suspended in PBS (1x, pH 7.4). The process was repeated two more times, and finally, the cells were suspended in PBS for imaging. The PL images were recorded using a confocal microscope (Leica DMIRM, Germany). PL was focused by a tube lens (200 mm focal distance) and then detected by a charge-coupled device (Andor, model DV 412, San Mateo, CA, USA) with an acquisition time 0.5 s, and a single-photon avalanche diode (AQR-14, Perkin Elmer, Waltham, Massachusetts, USA) with a 175-μm active area created by a beam splitter. An optical fiber was used as a pinhole to reject out-of-focus light in the system. An emission bandpass filter (HQ420, Chroma) was positioned in front of the optical fiber to reject scattered light. The excitation source was a pulsed laser at 375 nm operated with a 10-MHz repetition rate and 280-ps pulse width (PDL800, PicoQuant GmBH, Berlin, Germany). The excitation power on the sample is 1 W cm^{-2}.

SALDI-MS. Aliquots of insulin or angiotensin I solution (100 μM, 50 μL), ultrapure water (350 μL), and 500 mM ammonium citrate solution (100 μL, pH 4.0) were mixed. Aliquots of the mixtures (0.8 μL) and C-dots (7.2 mg/mL, 0.8 μL) were separately pipetted onto a stainless-steel 96-well MALDI target (Bruker Daltonics). After mixing, the mixtures were dried in air at 20°C for 30 min prior to SALDI-MS analysis. A Microflex MALDI–TOF mass spectrometer (Bruker Daltonics, Bremen,

Germany) was operated in the linear positive ion mode. The samples were irradiated with a 337-nm nitrogen laser at 10 Hz. Ions produced by laser desorption were stabilized energetically during a delayed extraction period of 200 ns and then accelerated through the time-of-flight instrument in the linear mode prior to entering the mass analyzer. The available accelerating voltages range from +20 to –20 kV. To obtain good resolution and high signal-to-noise ratios, the laser fluence was adjusted to slightly higher than the threshold and each mass spectrum was generated by averaging over 400 laser pulses.

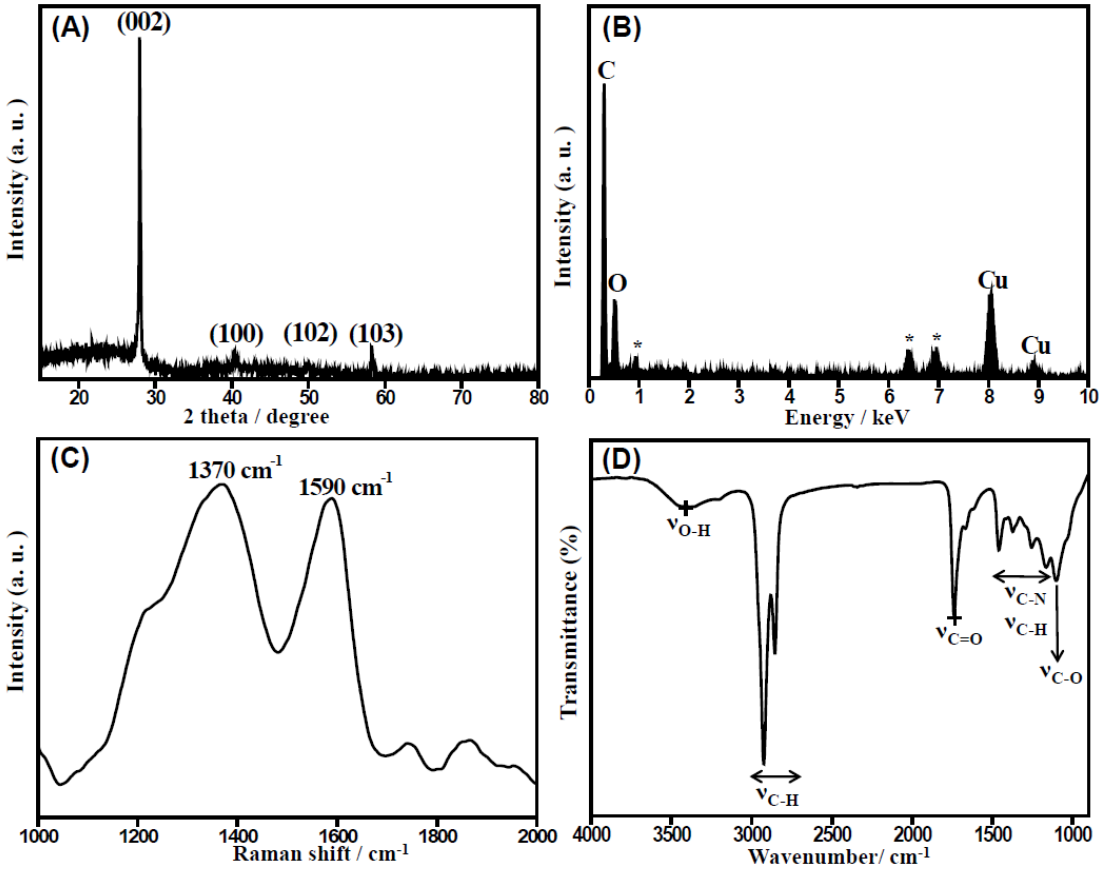

Fig. S1 (A) XRD pattern, (B) EDX pattern, (C) Raman spectrum, and (D) FT-IR spectrum of C-dots. (B) * is background from the system.

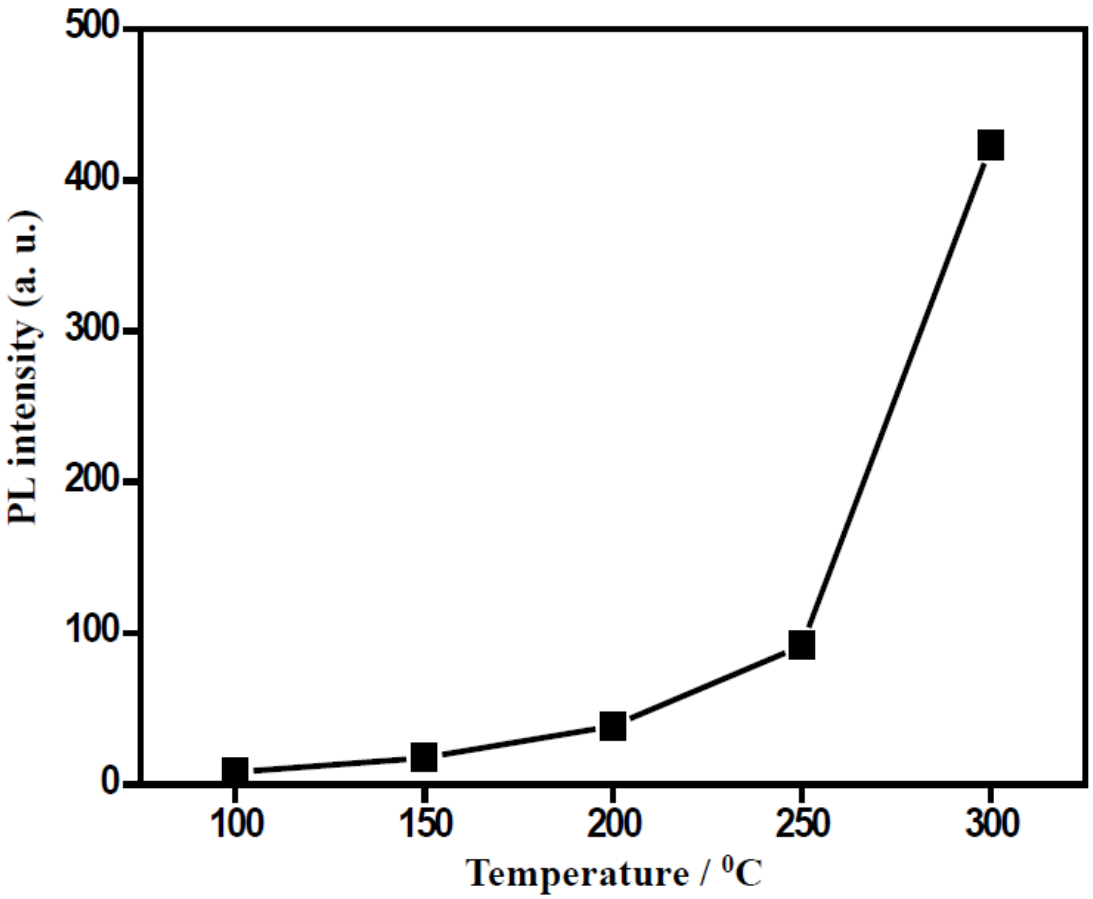

Fig. S2 Effect of calcination temperature of photoluminescence intensity on C-dots. PL wavelength at 440 nm (λ_{ex} = 365 nm). C-dots were prepared in phosphate buffers (5 mM, pH 7.4).

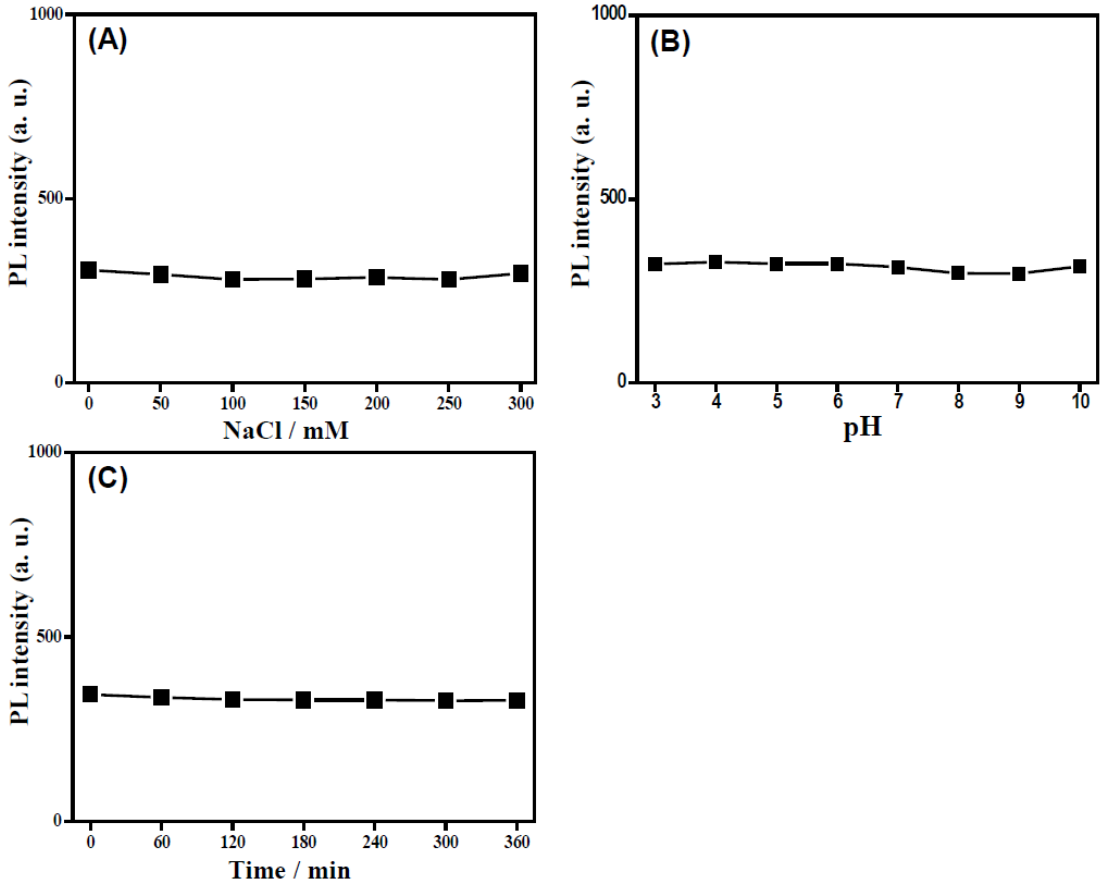

Fig. S3 Effect of (A) NaCl concentration, (B) pH, and (C) time-dependence with a Xe lamp of photoluminescence intensity on C-dots. PL wavelength at 440 nm (λ_{ex} = 365 nm). (A) C-dots were prepared in phosphate buffers (5 mM, pH 7.4) containing various concentrations of NaCl. (B) C-dots were prepared in phosphate buffers (5 mM) at various pH values. (C) C-dots were prepared in phosphate buffer (5 mM, pH 7.4). The photoluminescence intensities are plotted in arbitrary units (λ_{em} = 440 nm).

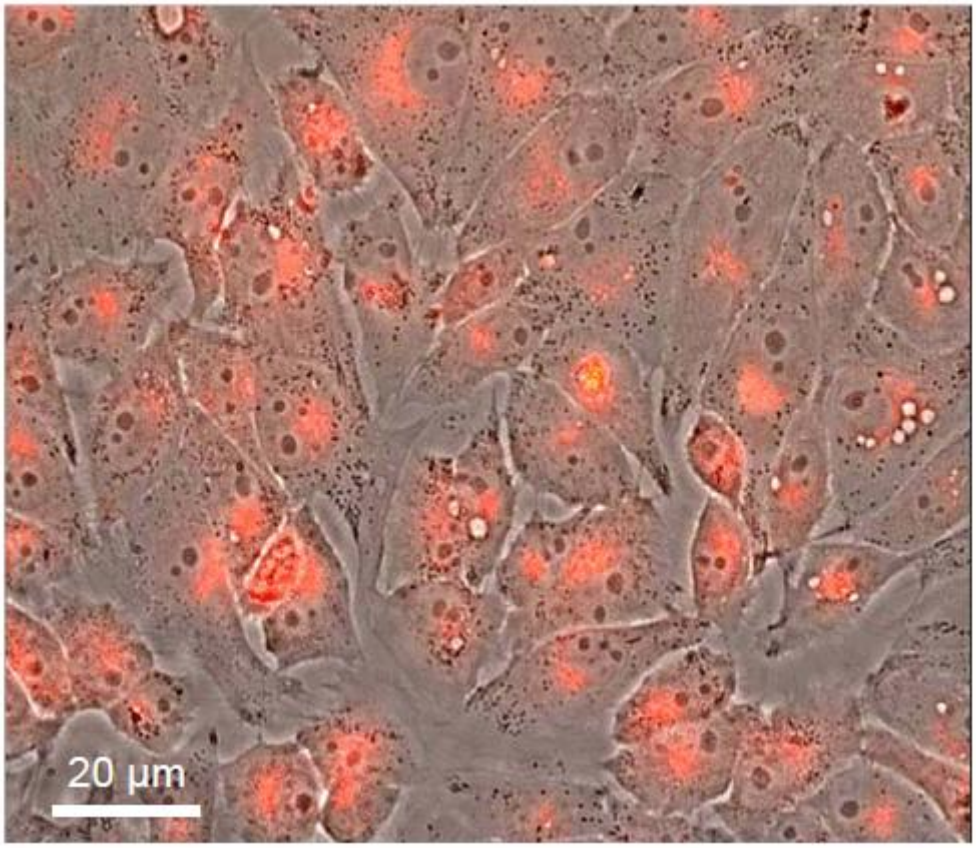

Fig. S4 A representative PL image of LLC-PK1 cells incubated with C-dots (1.2 mg/mL) for 24 h. The cells were excited at 510–530 nm. The grey channel shows transmission images, while the intensity coded channel (red) shows the PL of C-dots.

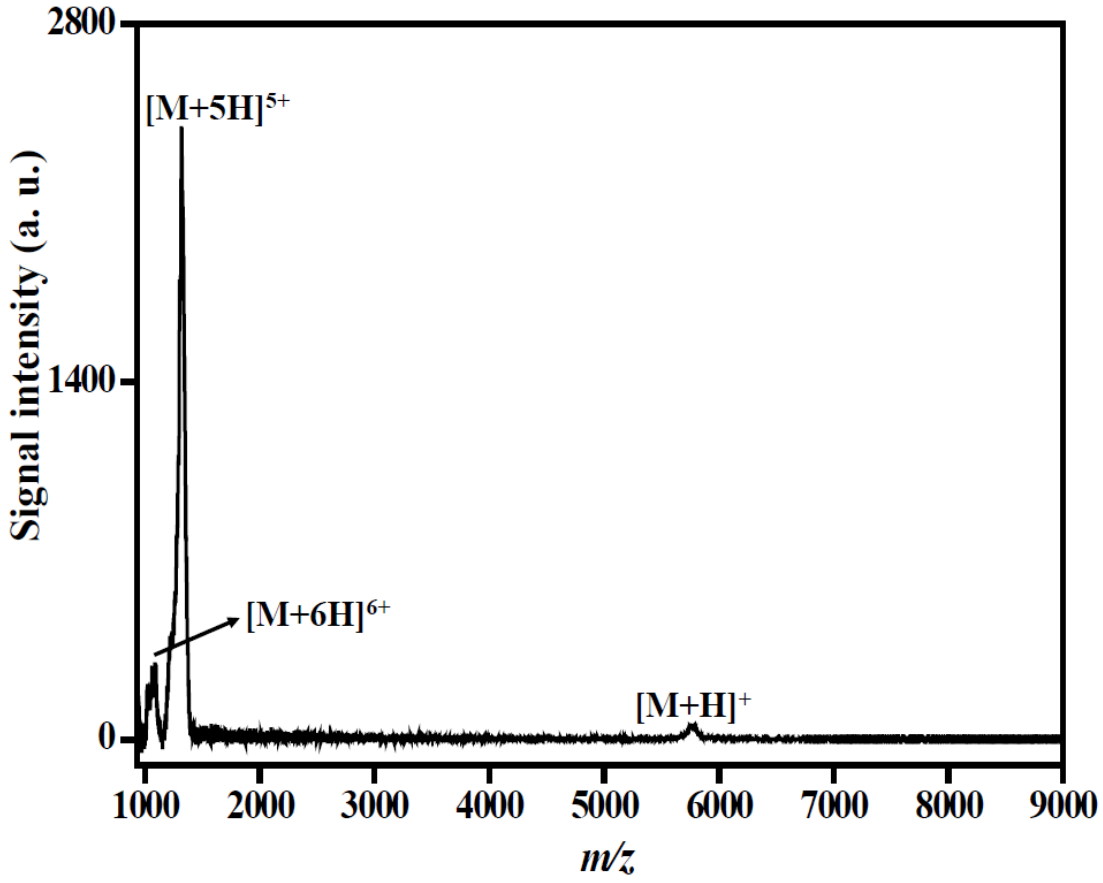

Fig. S5 SALDI–MS spectra of insulin (8 pmol) using C-dots (7.2 mg/mL). SALDI-MS was performed in the linear mode. A total of 400 pulsed laser shots were applied under a laser pulse energy set at 70 μJ. The signals at m/z at 5758, 1318, and 1087 represent the $[M+H]^+$, $[M+5H]^{5+}$, and $[M+6H]^{6+}$ adducts of insulin.

References

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2. H. Liu, T. Ye and C. Mao, *Angew. Chem. Int. Ed*., 2007, **46**, 6473-6475.