# Turning Waste into Wealth: Facile and Green Synthesis of Carbon Nanodots from Pollutants and Applications to Bioimaging

Weifeng Chen,[a,1] Jialu Shen,[a,1] Zuo Wang,[b,1] Xiang Liu,[a]* Yanyi Xu,[b] Haiyu Zhao[b]* and Didier Astruc[c]*

a. College of Materials and Chemical Engineering, Key Laboratory of Inorganic Nonmetallic Crystalline and Energy Conversion Materials, China Three Gorges University, Yichang, Hubei 443002, P. R. China, E-mail: xiang.liu@ctgu.edu.cn

[b] Gansu Key Laboratory of Biomonitoring and Bioremediation for Environmental Pollution, School of Life Sciences, Lanzhou University, Lanzhou, Gansu, 730000, P. R. China, E-mail: zhaohy@lzu.edu.cn

[c] ISM, UMR CNRS N° 5255, Univ. Bordeaux, 351 Cours de la Libération, 33405 Talence Cedex, France. E-mail: didier.astruc@u-bordeaux.fr

[1] These authors have equally contributed to this work and should be considered as co-first authors.

## Table of Content

## 1. Chemical reagents and Synthesis of CNDs

All commercial materials were used without further purification, unless indicated. The deionized water was prepared in the laboratory. Reactive Red 2 (RR2) was purchased from (Shanghai Macklin Biochemical Co., Ltd).

**Synthesis of CNDs**: The CNDs were synthesized using a hydrothermal method. In a typical procedure, 0.15 g of Reactive Red 2 and 34 mL of deionized water were added into a 50 mL Teflon-lined autoclave and heated at 200 ˚C for 14 hours. After cooled naturally, the solution was centrifuged at 10000 r/min for 30 min to remove the sediments. Next, the solution was filtered by the 0.22 μm membrane filter. Finally, the CNDs solution was obtained and the yield was calculated to be 88.4 %.

**Quantum Yield Measurements**: Rhodamine B in ethanol (QY=69.0 %) was used as a standard for CNDs. The quantum yield of CNDs was calculated to be 15.7 % by using the following formula (**Fig. S1**):

$$QY_S = QY_R \times \frac{\eta_S^2}{\eta_R^2} \times \frac{A_R}{A_S} \times \frac{D_S}{D_R}$$

S is sample, R is reference, D is the area of the spectral integration of the correction fluorescence emission, A is absorbance under 365 nm, and η is refractive index of solvent.

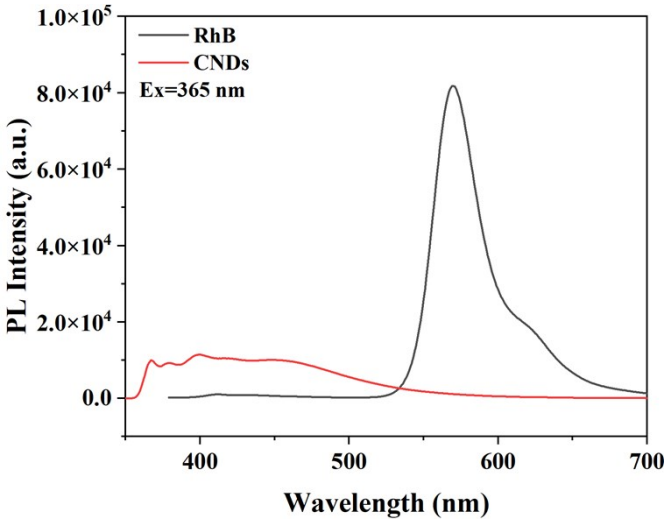


**Figure S1**. The PL spectra of CNDs and RhB.

## 2. Characterizations of the CNDs

Transmission electron microscope (TEM) and High-resolution TEM (HRTEM) were utilized to observe the CNDs on JEOL JEM-2100 F. Atomic force microscopy (AFM) images were obtained on BRUKER Dimension Icon. Raman spectrum was measured on Thermo Scientific DXR. Powder X-ray diffraction (XRD) was performed on a Rigaku Ultima IV (Cu Kα radiation, 3kW). X-ray photoelectron spectroscope (XPS) was performed on a Thermo ESCALAB 250XI. Fourier transform Infrared spectrum (FT-IR) was acquired on PE Fourier Transform. UV-vis. spectra were

obtained on a UV-1900 (Shimadzu, Japan). PL emission spectra were measured using an RF-6000 (Shimadzu, Japan).

**3. Animal Model and Ethics Statement**

All zebrafish husbandry and experimental procedures were performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals and were reviewed and approved by the Animal Care Committee of Lanzhou University (Ethic approval ID No. EAF2020007). All efforts were taken to minimize animal suffering.

Zebrafish wild-type AB strains were raised according to standard husbandry protocols[1] at 28 °C and under the photoperiod of 14:10 h (light: dark). The zebrafish were fed twice daily with newly-hatched brine shrimp. Zebrafish embryos were obtained from the adults that segregated by sex in mating tanks overnight. Spawning was induced in the next morning by light stimulation. Fertilized healthy embryos were collected and washed with the standard zebrafish E3 culture medium (pH=7.3). The embryos at 4 hours post-fertilization (hpf) were examined under a stereomicroscopy (Olympus), normally developed embryos were selected for further experiments (Zebrafish Developmental Toxicity Assays and Zebrafish Fluorescence Imaging).

**4. Zebrafish Developmental Toxicity Assays**

Zebrafish embryos were exposed to RR2 or CNDs at various concentrations (50, 100, 200, 300 and 400 mg/L) suspended in the E3 medium at 28.5 °C, and observation was continuously conducted at different developmental stages ranging from 3 hpf to 120 hpf. The groups without exposure to RR2 or CNDs were used as control. The medium was refreshed daily during the entire exposure period. Phenotypic development was recorded and analyzed under a stereomicroscopy (Olympus). The control and treatment of each group was carried out using 50 embryos per condition and each experiment was repeated for at least three times.

**Zebrafish survival:** During the first 24 h postfertilization (hpf), zebrafish mortality was identified by coagulation of the embryos and the failure to perform appropriate cell division using the method described previously.[2] After 24 hpf, survival was defined by the continued cardiac contractility. Mortality rates were calculated within each treatment group at different developmental stages as the percentage of dead embryos out of the total number of embryos.

**Hatching and malformations:** The hatching and morphological malformations (e.g., malformed yolk sacs, tail malformations, delayed development, and oedema in the heart and body cavity) of the larvae were observed and recorded by stereomicroscopy along with a digital camera. The hatching rates were calculated within each treatment group as the percentage of hatched embryos out of the total number of embryos.

**Body length:** Body length of zebrafish larvae was measured at 4 dpf under the stereomicroscope with a digital camera. Treated larvae and controls were placed in petri dishes with E3 culture medium, the body length was measured using scale tools of the microscope (n = 30).

**Behavior test:** Spontaneous movement of 24 hpf zebrafish embryo (before hatching) was recorded manually under the stereomicroscope in a 1 min window, water temperature was kept stable at 28 °C by a thermo-plate coupled. The behavior of zebrafish larvae (6 dpf) was monitored using a DanioVision Video-Tracking system (Noldus, Netherlands). Larval swimming behavior was monitored in response to light-dark stimulations (alternating light/dark, every 10 minutes) and typing stimulations (every 30 seconds). The behavior parameters like distance traveled, frequency of movements, total duration of movements etc. were recorded and analyzed using the EthoVision XT behavioural analysis system.

**5. Zebrafish Fluorescence Imaging**

Zebrafish embryo and larvae at different developing stages were treated by exposure in a gradient concentration of CNDs, and the fluorescence excited by CNDs was observed with the fluorescence microscope in bright field, UV (380–400 nm), blue (470–490 nm) and green (530-550 nm) fluorescence field, respectively.

**6. Cell Culture and Cell Viability Assay**

The human cervical cancer cells (HeLa) were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with FBS (10%) and 1% Gibco® antibiotic-antimycotic solution in 5% $CO_2$ at 37 °C. A colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, Sigma Aldrich) assay was performed to detect cell viability. Specifically, Hela cells were plated as $2 \times 10^3$ cells / well in 96-well plates. After treatment with gradient concentrations of CNDs (0, 50, 100, 200, 400, 600, 800, 1000 mg/L) for 24 hours, cells were washed with PBS and incubated with 0.5 mg/mL MTT in DMEM medium at 37°C for 3 hours. Subsequently, the medium was removed and DMSO was used to dissolve crystals. After 5 min incubation at room temperature the relative quantity of living cells was assessed with an automatic microplate reader (Tecan Switzerland), using a test wavelength of 450 nm and a reference wavelength of 490 nm. The cell viability was calculated using the following equation: Cell viability (%) = (optical density of the cells treated with RR2 or CNDs/ optical density of the control) × 100%.

**7. Cellular Fluorescence Imaging**

The potential for bioimaging with CNDs was tested by using the HeLa cells. CNDs were initially dissolved in ultrapure water at a concentration of 40 mg/mL before use. Approximately $2 \times 10^3$

cells were deposited on each confocal petri dish (14 mm glass center) to form a sparsely distributed layer of cells to ensure good exposure to the CNDs. HeLa cells were cultured in DMEM growth medium with fetal bovine plasma (10%) at 37 °C under 5% $CO_2$. After 24 hours incubation of 0.1 mg/mL CNDs, the cells were washed three times with PBS (pH = 7.4) to remove extracellular CNDs and then fluorescence images were obtained using a confocal fluorescence microscope (LSM 880, Carl Zeiss ZEISS).

**8. Statistical Analysis**

Data were analyzed by using GraphPad Prism (Version 7.0) or OriginPro 8 (OriginLab). All the results were expressed as means ± SEM. Student's t test or analysis of variance (ANOVA) were used to determine significant differences followed by Sidak's multiple comparison post-test. P values < 0.05 were considered statistically significant. Statistical differences of $p < 0.05$, $p < 0.01$, $p < 0.001$ are represented by *, ** or ***, respectively.

**9. DLS and Zeta Potential Analysis of CNDs (Fig. S2)**

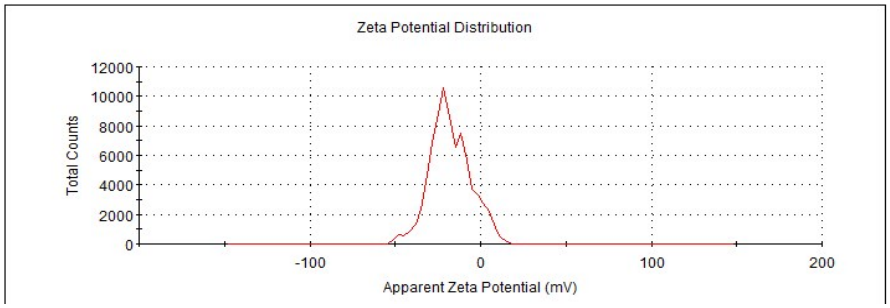


**Figure S2**. The zeta potential analysis of CNDs

**10. References**

[1] M. Westerfield, The Zebrafish Book, University of Oregon Press, Corvallis, USA, 2000.

[2] C. B. Kimmel, W. W. Ballard, S. R. Kimmel, B. Ullmann, T. F. Dev. Dyn. 1995, 203, 253-310.