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Supporting information

Exploring the Blue Luminescence Origin of the Nitrogen-Doped Carbon Dots by Controlling Water Amount in Synthesis

Hui Ding and Huan-Ming Xiong*

Department of Chemistry, Fudan University, Shanghai 200433, P. R. China.

E-mail: hmxiong@fudan.edu.cn

Experimental

1.1 MTT assay

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were from Sigma-Aldrich. Dulbecco's Modified Eagle's medium (DMEM, High Glucose), fetal bovine serum (FBS), and trypsinase were from GIBCOBRL (USA). HeLa cells were seeded in a 96-well cell culture plate in DMEM at a density of 5×10^4 cells/mL with 10% fetal bovine serum (FBS) and 5% CO_2 at 37 °C for 24 h. Afterwards, the culture medium was replaced by 200 μL of DMEM containing the carbon dots at different doses and cultured for another 48 h. Then, 20 μL of 5 mg/mL MTT solution was added to each cell well. The cells were further incubated for 4 h, followed by removal of the culture medium with MTT, and then 150 μL of DMSO was added. The resulting mixture was shaken for 15 min at room temperature. The absorbance of MTT at 492 nm was measured on an automatic ELISA analyzer (SPR-960). The control experiments were carried out without CDs. Each experiment was conducted by 5 times and the average data were presented.

1.2 Cell imaging

Cellular fluorescent images were recorded using a Leica Tcs sp5 Laser Scanning Confocal Microscope. HeLa cells were seeded in 6-well culture plates at a density of 10^5 per well in DMEM containing 10% fetal bovine serum (FBS) at 37 °C in a 5% CO_2 incubator for 24 h. After removing DMEM, the mixture of N,S-CDs (100 μg/mL) in the DMEM medium was added into each well for 1 h of incubation. Finally, the

cells were washed twice by phosphate buffer solution (PBS) to remove extracellular CDs and then fixed with 4% paraformaldehyde.

Results

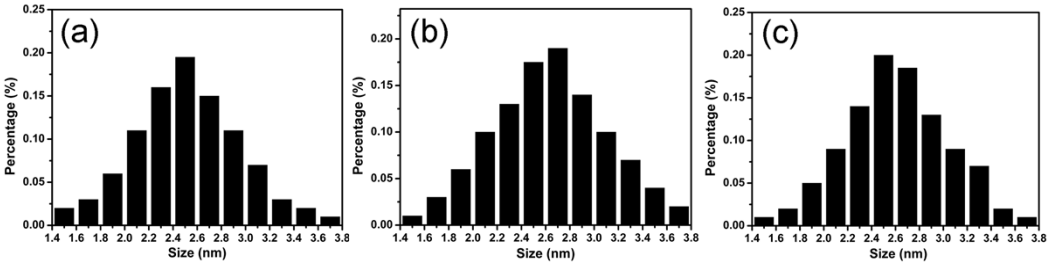

Fig. S1 Size distribution histograms of the obtained (a) N-CDs-1, (b) N-CDs-2 and (c) N-CDs-3 counted by TEM.

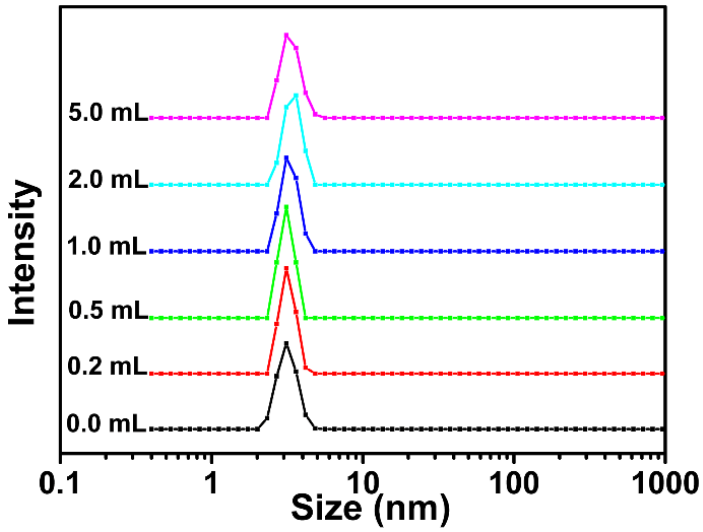

Fig. S2 DLS results of all N-CDs samples produced with adding different amount of water.

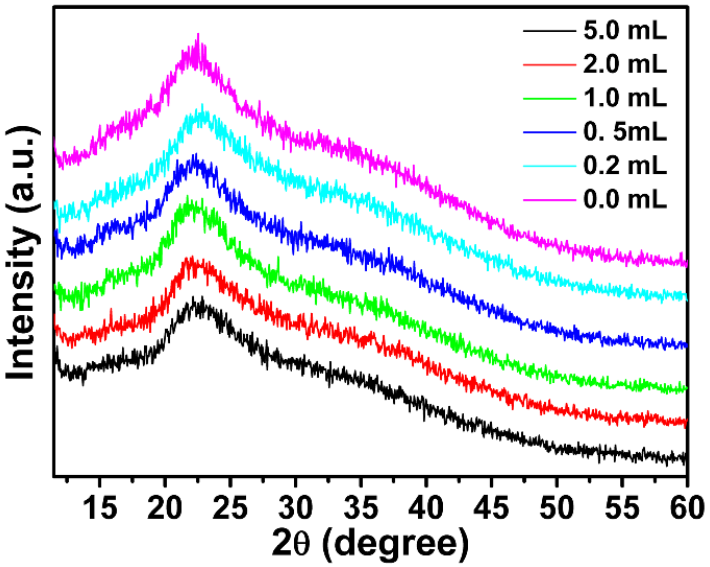

Fig. S3 XRD patterns of all N-CDs samples prepared with adding different W.

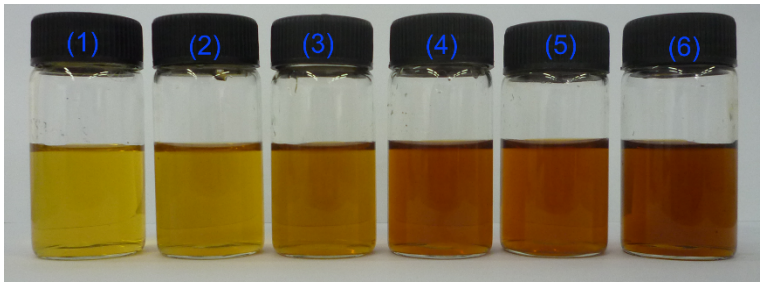

Fig. S4 Photograph of the obtained sample dissolved in 10 mL of water in daylight. Samples 1 to 6 represent the N-CDs prepared with W = 0.0, 0.2, 0.5, 1.0, 2.0 and 5.0 mL respectively.

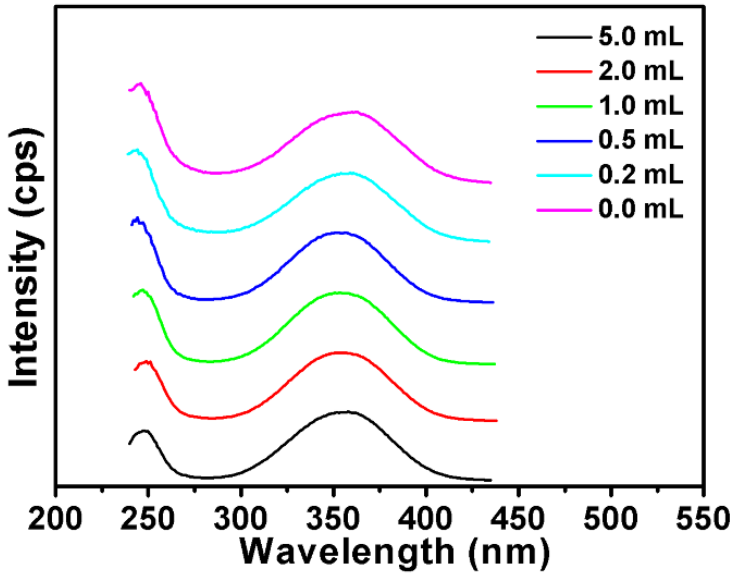

Fig. S5 The excitation spectra of N-CDs obtained by adding different W. The excitation wavelength is 452 nm.

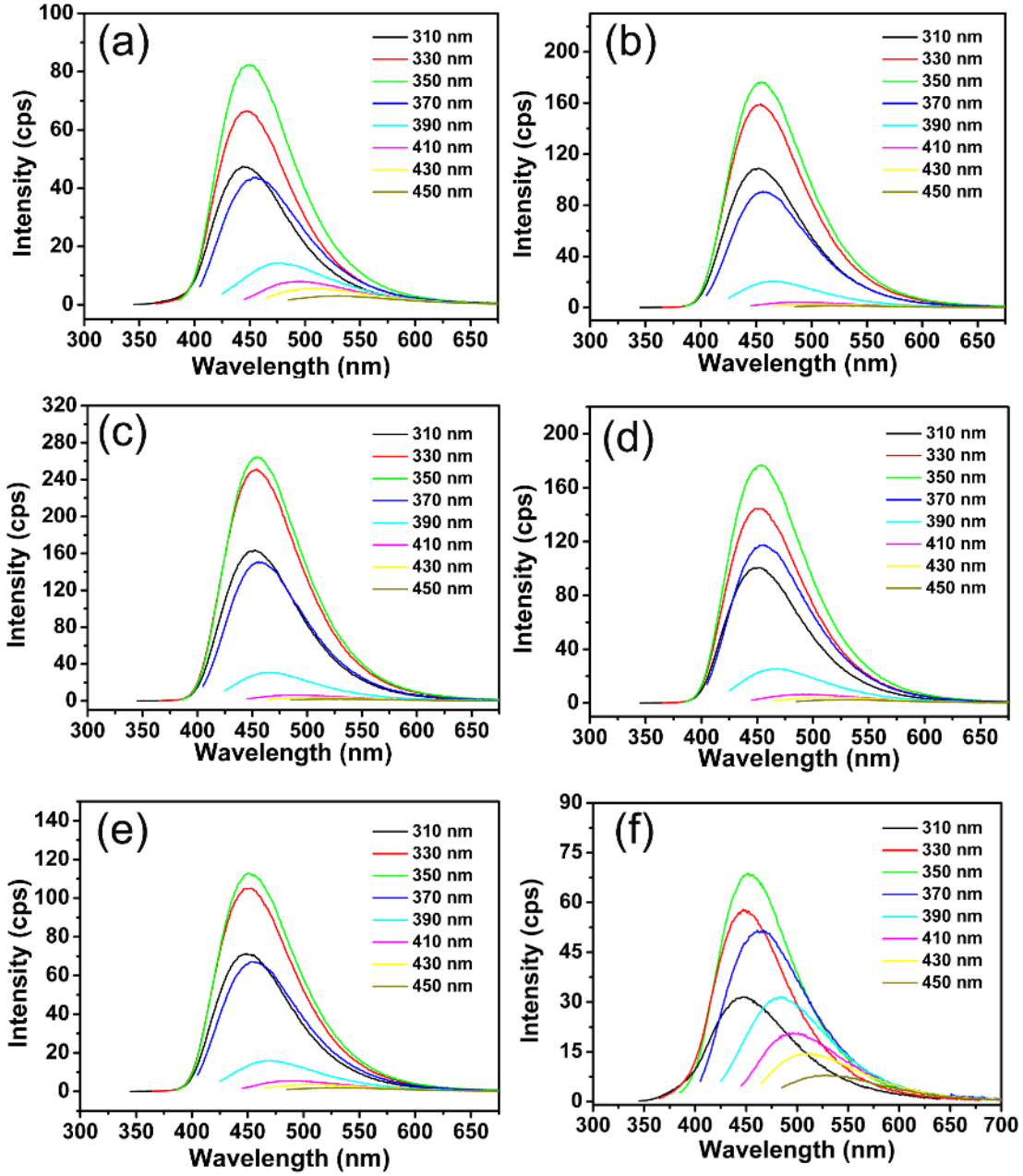

Fig. S6 PL spectra of N-CDs prepared with adding (a) 0.0, (b) 0.2, (c) 0.5, (d) 1.0, (e) 2.0 and (f) 5.0 mL of water, respectively.

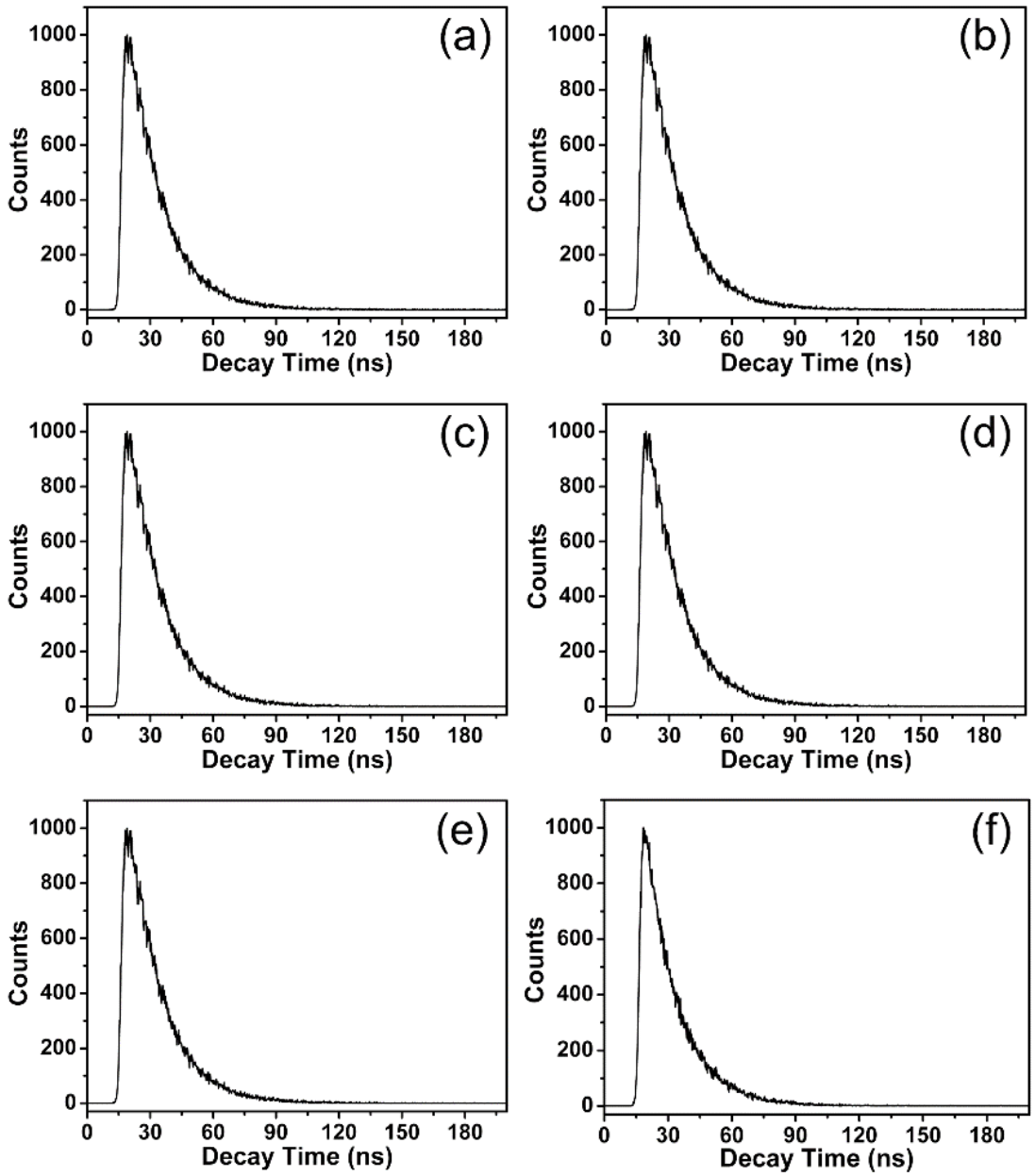

Fig. S7 PL decay curves (a to f) of N-CDs prepared with adding 0.0, 0.2, 0.5, 1.0, 2.0, and 5.0 mL of water, respectively.

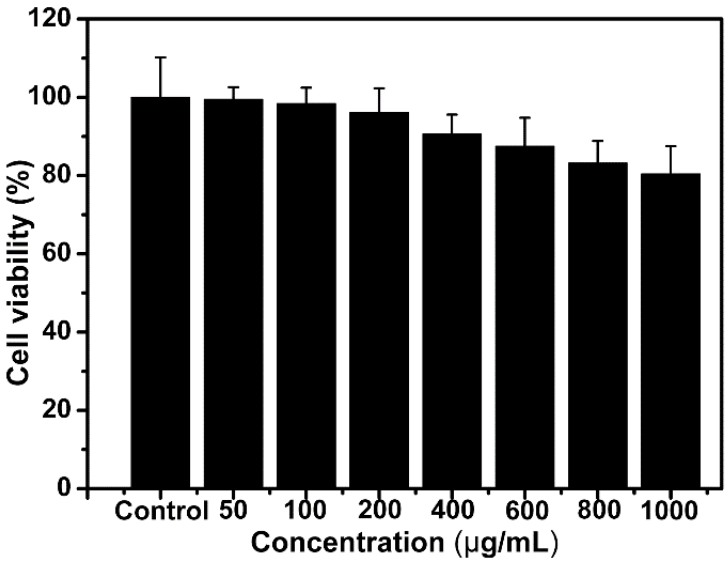

Fig. S8 Cytotoxicity of the N-CDs-2 toward HeLa cells from an MTT assay.

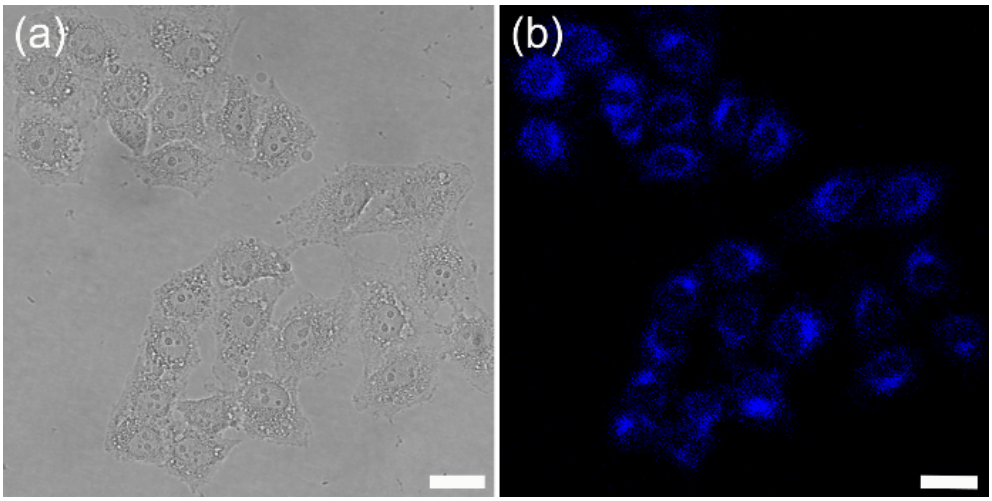

Fig. S9 Confocal fluorescence images of HeLa cells after incubation with N-CDs-2 (100 μg/ml) for 1h under (a) bight field, and (b) 405 nm excitation.

The bioimaging properties of such N-CDs was also assessed due to their high QY. The cytotoxicity toward HeLa cells was performed by the conventional MTT assay (Fig. S8), in which HeLa cells had a high viability of 80% even after incubation with 1 mg/mL N-CDs for 48 h. Before the confocal laser imaging, HeLa cells were incubated with 100 μg/mL N-CDs for 1 h, and then washed by buffers. Under 405 nm excitation (Fig. S9), these cells showed bright blue fluorescence in the cytoplasm, suggesting the potential applications in bioimaging and biosensing.

Table S1 The I_D/I_G ratios of the as-prepared N-CDs.

Sample	(1)	(2)	(3)	(4)	(5)	(6)
Volume of water (mL)	0.0	0.2	0.5	1.0	2.0	5.0
Ratio value	1.07	1.12	1.16	1.23	1.31	1.35

Table S2 Lifetime data from the time-resolved decay profiles of different N-CDs.

Sample	Water (mL)	λ_{ex} (nm)	λ_{em} (nm)	τ (ns)	Percentage (%)	χ^2
①	0.0	352	450	15.24	100	1.177
②	0.2	352	450	14.94	100	1.021
③	0.5	352	450	15.28	100	1.117
④	1.0	352	450	14.73	100	1.138
⑤	2.0	352	450	14.67	100	1.053
⑥	5.0	352	450	14.99	100	1.011