Supplementary Material

Microwave-assisted Synthesis, Characterization, Cell Imaging of Fluorescent Carbon Dots Using L-Asparagine as Precursor

Xi Wang,[a] Tian Gao,[a] Mian Yang,[c] Jie Zhao,[a] Feng-Lei Jiang[b] and Yi Liu[a,b,c]*

a. State Key Laboratory of Virology & Key Laboratory of Analytical Chemistry for Biology and Medicine, College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, P. R. China

b. College of Chemistry and Material Science, Guangxi Teachers Education University, Nanning 530001, P. R. China

c. School of Chemistry and Chemical Engineering, Wuhan University of Science and Technology, Wuhan 430081, P. R. China

Corresponding Authors

*E-mail: yiliuchem@whu.edu.cn. Tel: +86-27-68753465 (Y.L.).

1. Theoretical calculated result of relative content of N, C, O in the 20 amino acids.

Table S1 Relative contents of C, O, N in the 20 amino acids.

Atomic content/%	N	C	O	S
Alanine ($C_3H_7NO_2$)	17.18	43.93	38.99	
Arginine ($C_6H_{14}N_4O_2$)	35.00	45.01	19.99	
Asparagine ($C_4H_8N_2O_3$)	22.73	38.73	38.70	
Aspartic acid ($C_4H_7NO_4$)	11.12	38.12	50.76	
Cysteine ($C_3H_7NO_2S$)	12.29	31.62	14.04	28.14
Glutamic acid ($C_5H_9NO_4$)	10.15	43.50	46.35	
Glutamine ($C_5H_{10}N_2O_3$)	20.60	44.14	35.26	
Glycine ($C_2H_5NO_2$)	20.01	34.30	45.69	
Histidine ($C_6H_9N_3O_2$)	28.77	49.33	21.90	
Isoleucine ($C_6H_{13}NO_2$)	11.87	61.05	27.08	
Leucine ($C_6H_{13}NO_2$)	11.87	61.05	27.08	
Lysine ($C_6H_{14}N_2O_2$)	21.21	54.56	24.23	
Methionine ($C_5H_{11}O_2NS$)	10.14	43.48	23.17	23.21
Phenylalanine ($C_9H_{11}NO_2$)	9.09	70.15	20.76	
Proline ($C_5H_9NO_2$)	13.21	56.63	30.16	
Serine ($C_3H_7NO_3$)	14.29	36.75	48.96	
Threonine ($C_4H_9NO_3$)	12.73	43.66	43.61	

Tryptophan ($C_{11}H_{12}N_2O_2$)	14.58	68.76	16.66
Tyrosine ($C_9H_{11}NO_3$)	8.24	63.55	18.81
Valine ($C_5H_{11}NO_2$)	13.21	56.63	30.16

2. Photos of A-CDs under powder and solution condition.

Yellow powders of A-CDs were got by freeze drying (Figure S1a). And dissolving these yellow powders into deionized water, a light yellow solution was obtained (Figure S1b). Put the A-CDs solution under a 365 nm UV irradiation lamp, it emits a bright blue photoluminescence.

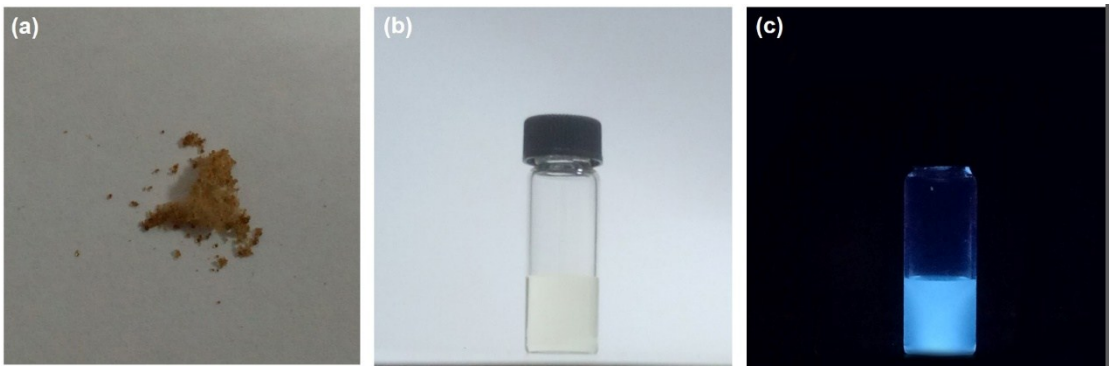

Fig. S1 Photos of A-CDs. (a) The powder of A-CDs after vacuum freezing drying. (b) The A-CDs solution in white light. (c) The A-C solution under 365 nm excitation.

3. Dynamic Light Scattering result of A-CDs

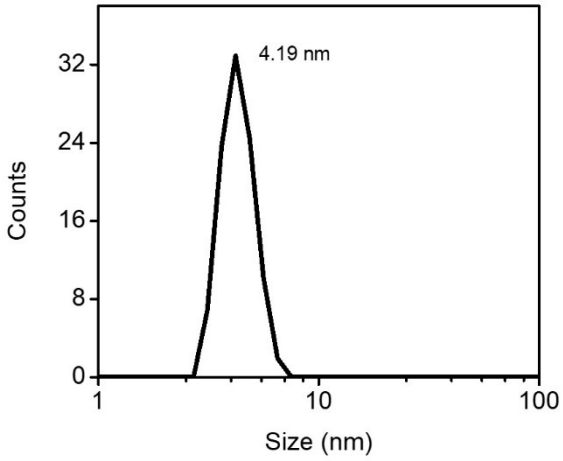

Figure. S2 The Dynamic Light Scattering result of A-CDs.

4. Elemental content of A-CDs.

The relative elemental contents of A-CDs were determined by XPS. The relative contents of C, O, N were 58.03%, 32.53%, 9.44%, indicating the doping of nitrogen atoms.

Table S2 Relative contents of C, O, N.

Atomic content/%	C	O	N
	58.03	32.53	9.44

5. Possible structure of A-CDs.

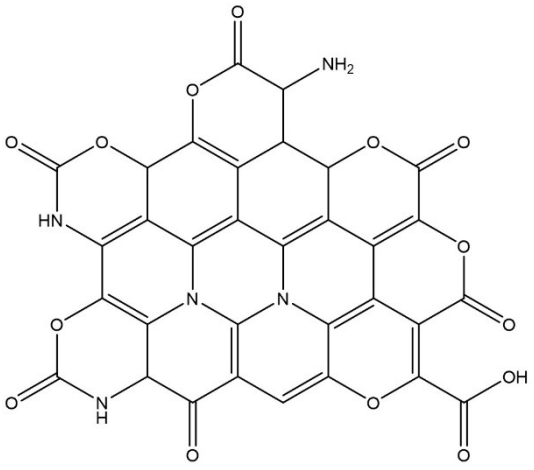

Fig. S3 Possible structure of A-CDs.

6. PL lifetime of A-CDs.

The average lifetimes were calculated using the equation below:

$$\tau_{average} = \sum_{i=1}^{n} a_i \cdot \tau_i^2 \Big/ \sum_{i=1}^{n} \tau_i \quad (1)$$

In the equation, τ means the decay lifetime, α is the fractional contribution of decay lifetime, *n* stands for total number of fractions, and i represents each fraction.

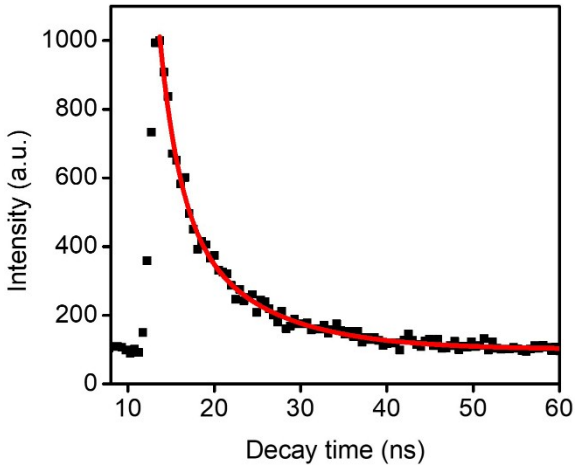

Fig. S4 The time-correlated single-photon counting (TCSPC) of C-dots (360 nm excitation, decay time at 450 nm).

7. Quantum yield calculation of A-CDs.

The quantum yield of A-CDs was measured by reference method, using quinine sulfate (QS) in 0.1 M H_2SO_4 as reference. The quantum yield calculation of A-CDs was according to the equation below:

$$Q = Q_R \cdot \frac{I}{I_R} \cdot \frac{A_R}{A} \cdot \frac{n^2}{n_R^2} \quad (2)$$

In the equation, *Q* represents the quantum yield, *I* means the integrated emission intensity measured by FL emission spectroscopy, *A* is the UV-vis absorbance at PL

excitation wavelength, *n* stands for the refractive index, and *R* is the reference.

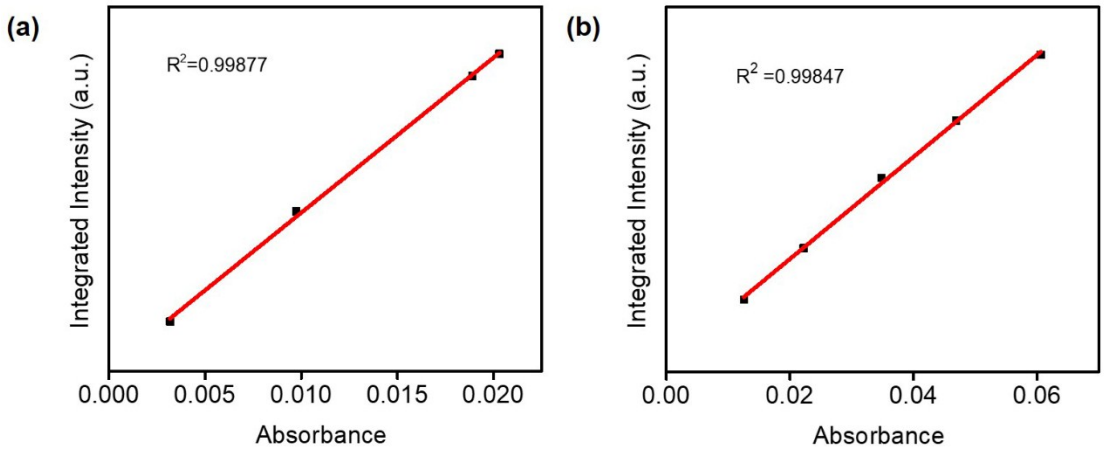

Fig. S5 Liner fitting of integrated emission intensity and absorbance of Quinine Sulfate (a) and A-CDs (b).

Table S3 Calculation of the quantum yield of A-CDs.

Sample	Integrated emission Intensity (*I*)/ Absorbance (*A*)	Refractive index of solvent (*n*)	Quantum yield (*Q*)
Quinine Sulfate	2975870	1.34	55.7%
A-CDs	419330	1.34	7.8%

More than four groups of data were used to obtain the liner fitting of each sample (Figure S3). Results were shown in Table S2, the quantum yield of A-CDs was 7.8%, using Quinine Sulfate as standard.

8. Photos and PL emission spectrum of A-CDs after stored for over 6 months, and antiphotobleaching property of A-CDs.

The A-CDs could still emit bright blue photoluminescence, as shown in Figure S5 inset photograph. And the fluorescent intensity of A-CDs remains over 80%, after being irradiated under 365 nm UV lamp for 2 hours.

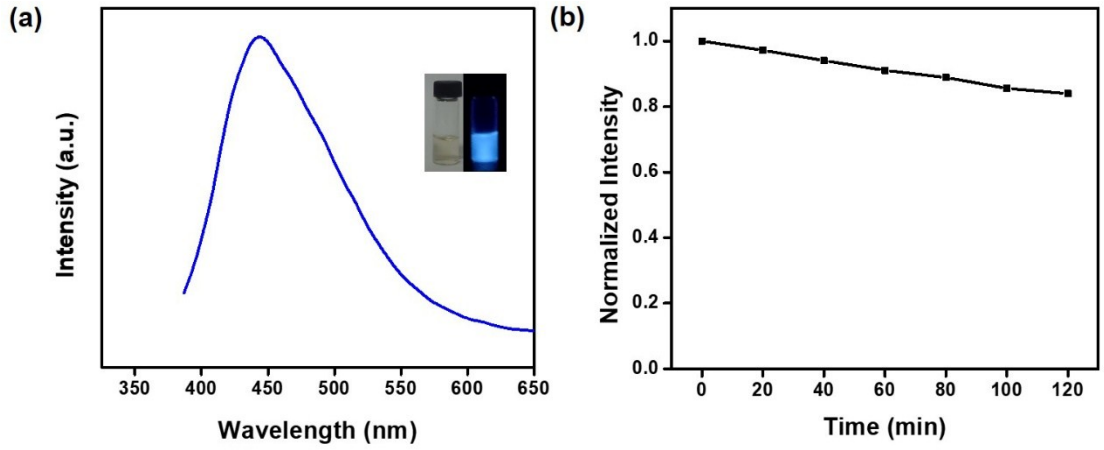

Fig. S6 (a)The PL emission spectrum of A-CDs under maximum excitation. Inset: photographs of A-CDs solution in room light (left) and in 365 nm UV irradiation lamp (right). (b) Antiphotobleaching property of A-CDs under irradiation of a 365 nm UV lamp.

9. Stability of A-CDs in various conditions.

There was no obvious PL intensity change of A-CDs under various conditions including of persistent excitation, different pH solutions, different ionic strengths, and different incubation time in DMEM culture.

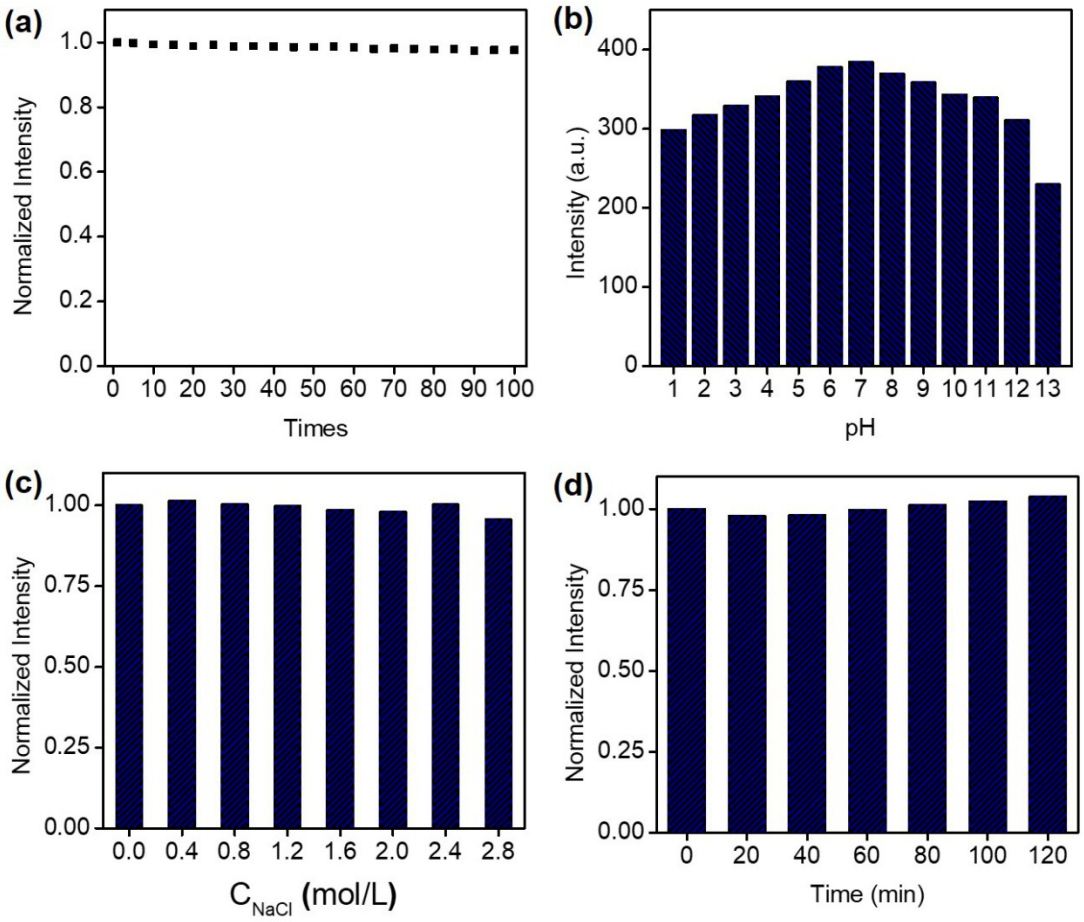

Fig. S7 Stability of A-CDs. (a) Dependence of fluorescence intensity on persistent excitation times for the A-CDs. (b) Effect of pH on the fluorescence intensity of the A-CDs. (c) Effect of ionic strengths on the fluorescence intensity of the A-CDs (ionic strengths were controlled by various concentrations of NaCl). (d) Dependence of A-CDs PL emission intensity on incubation time in DMEM culture.

10. Influence of various ions on A-CDs in PBS buffer.

As illustrated in Figure S8, there is nearly no influence on the PL intensity of the A-CDs when the A-CDs' solution mix with various ions (up to 400 μM) in PBS buffer.

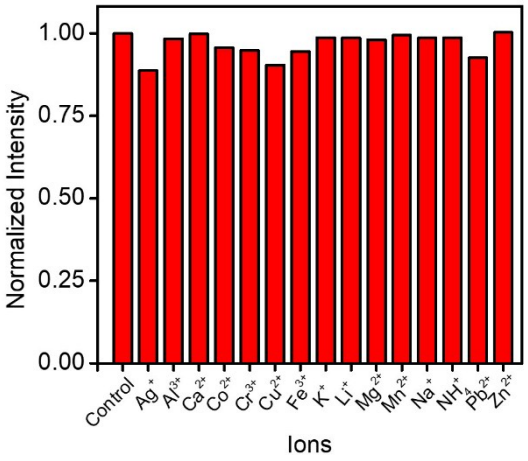

Fig. S8 Normalized PL emission intensity of A-CDs in PBS buffer with 400 μM ions.

References

1 X. Dong, L. Wei, Y. Su, Z. Li, H. Geng, C. Yang, Y. Zhang, Efficient long lifetime room temperature phosphorescence of carbon dots in a potash alum matrix, *J. Mater. Chem. C*, 2015, **3**, 2798-2801.

2 J. W. Eastman, Quantitative spectrofluorimetry - The fluorescence quantum yield of quinine sulfate, *Photochem. Photobiol.*, 1967, **6**, 55-72.