Electronic Supplementary Information (ESI)

One-pot hydrothermal synthesis of highly luminescent nitrogen-doped amphoteric carbon dots for bio- imaging from bombyx mori silk – natural proteins †

Zhu Lian Wu[a], Pu Zhang[b], Ming Xuan Gao[a], Chun Fang Liu[a], Wei Wang[b], Fei Leng[a], Cheng Zhi Huang*[ab]

[a] *Education Ministry Key Laboratory on Luminescence and Real-Time Analysis, School of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, China*

[b] *College of Pharmaceutical Science, Southwest University, Chongqing 400716, China*

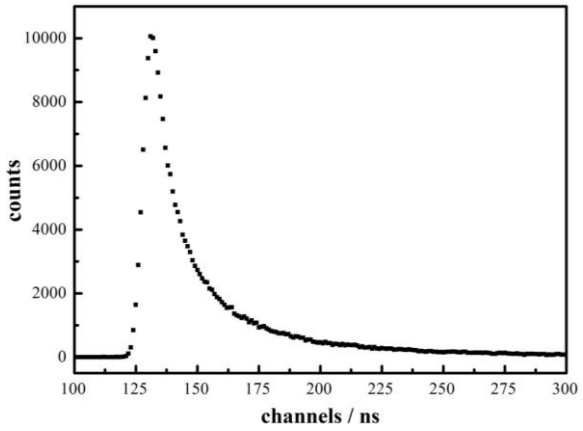
**Corresponding author: chengzhi@swu.edu.cn*

Table.1 Results of the orthogonal experiment.

Factors	*t* (h)	c_{NaOH} (mol.L^{-1})	*T* (^{o}C)	QY (%)	Excitation wavelength(nm)	Emission wavelength(nm)
1	2	0. 1	190	7. 2	374	453
2	2	0. 15	220	1. 67	422	502
3	2	0. 2	250	1. 41	456	507
4	2	0. 25	280	1. 32	453	515
5	2. 5	0. 1	220	4. 56	400	475
6	2. 5	0. 15	190	2. 93	399	477
7	2. 5	0. 2	280	0. 79	450	522
8	2. 5	0. 25	250	1. 38	423	508
9	3	0. 1	250	8. 13	393	461
10	3	0. 15	280	2. 46	451	486
11	3	0. 2	190	4. 11	390	470
12	3	0. 25	220	0. 98	373	462
13	3. 5	0. 1	280	9. 33	440	488
14	3. 5	0. 15	250	3. 56	396	464
15	3. 5	0. 2	220	1. 47	422	505
16	3. 5	0. 25	190	0. 63	470	538

We designed the orthogonal experiment based on the single factor analysis and the condition for the hydrothermal preparation was optimized through orthogonal design software as following: the concentration of NaOH was 0.1 mol / L, time and temperature for reaction were 3h, 190 ^{o}C, respectively. Under relatively lower temperature, in less time, the silk may react incompletely, so the PL quantum yield and the content of

nitrogen were low. However, when the temperature was relatively higher and the silk reacted for a longer time, the nitrogen may lose relatively more, so the PL quantum yield and the content of nitrogen were low, too. At the same time， we can get different excitation wavelengths and emission wavelengths under different conditions, which results from of CDs with different size and different content of amine-group under different conditions. (Rf.23b in manuscript)



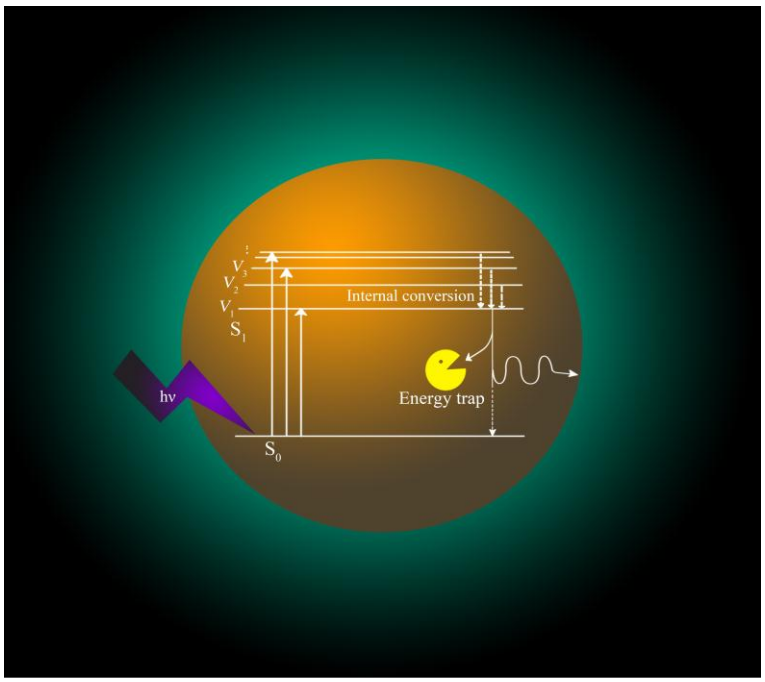
τ_i / ns	A_i / %
4.27	46.25
1.05	27.21
15.1	26.54

Fig.S1 Fluorescence lifetime intensity decay of CDs in aqueous solution (excitation at 370 nm, emission at 450 nm).

According to Equation (1)

$$\bar{\tau} = \frac{A_1\tau_1 + A_2\tau_2 + A_3\tau_3}{A_1 + A_2 + A_3} \quad (1)$$

the average fluorescence lifetime of CDs was calculated as 4.23 ns, wherein A_i is the fractional contributions of time-resolved decay lifetime of τ_i.



Scheme S1The possible luminescent mechanism of CDs from bombyx mori silk.

The possible mechanism of luminescence resembling that of the organic dye is that the electrons in CDs are excited by illuminant in the external environment, jumping from a fundamental electronic state S_0 to the various vibrational energy level of the first excited singlet state S_1 (marked as V_1, V_2 and V_3…), then through internal conversion, the electrons come back to S_1. That some energy is absorbed by the energy trap and some is emitted in the form of photons is following, in the meanwhile, the protons return S_0.

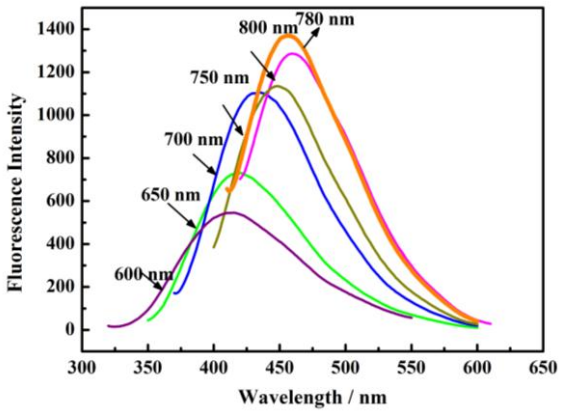


Fig.S2 Upconversion photoluminescence spectra of CDs at different excitation wavelengths as indicated.

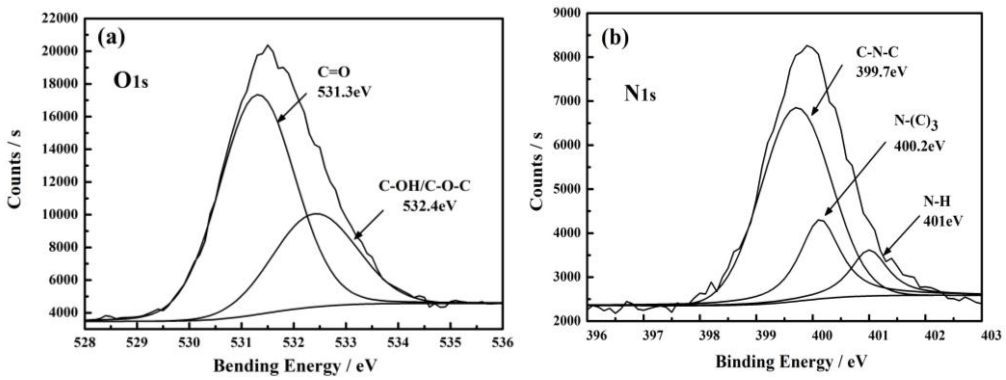


Fig.S3 (a) O_{1s}, (b) N_{1S} spectrum of CDs.

Quantum Yield Calculations

The quantum yield (Φ) of CDs was calculated using Fluorescein sodium as reference. Fluorescein sodium (literature[1] Φ = 0.79) was dissolved in 0.1 M NaOH (refractive index (η) of 1.33) while the CDs was dissolved in ultra-pure water (η = 1.33). Then the quantum yield of CDs was calculated by comparing the integrated photoluminescence intensities (excited at 370 nm) and the absorbance values (at 370 nm) of CDs with the references Fluorescein sodium. The data was plotted (Figure. S7) and the slopes of the sample and the standards were determined. The data showed good linearity.

The quantum yield was calculated using the below equation:

$$\Phi x = \Phi_{ST} (m_x / m_{ST}) (\eta_x^2 / \eta_{ST}^2)$$

Where Φ is the quantum yield, m is slope, η is the refractive index of the solvent, ST is the standard and X is the sample. The quantum yield for CDs is 13.9%.

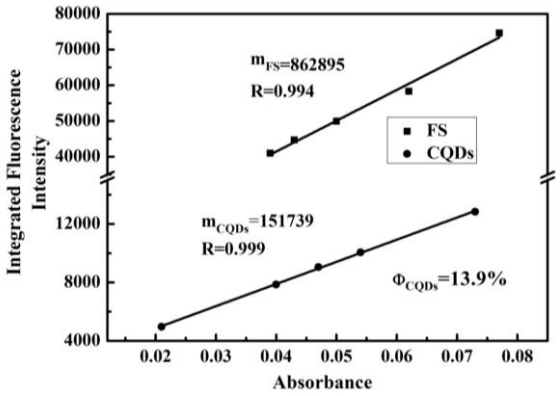


Fig.S4 Plot of integrated photoluminescence intensity vs. absorbance of the CDs and Fluorescein sodium.

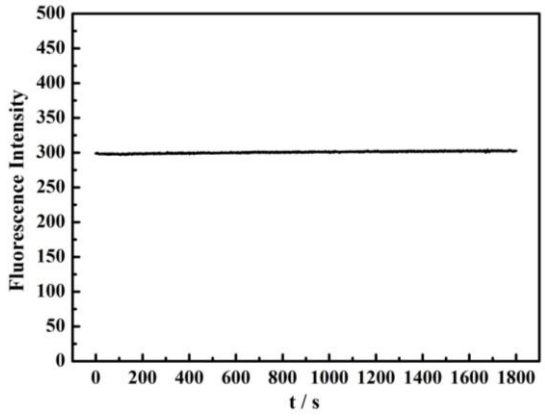


Fig.S5 Effect of time on the fluorescence intensity of CDs.

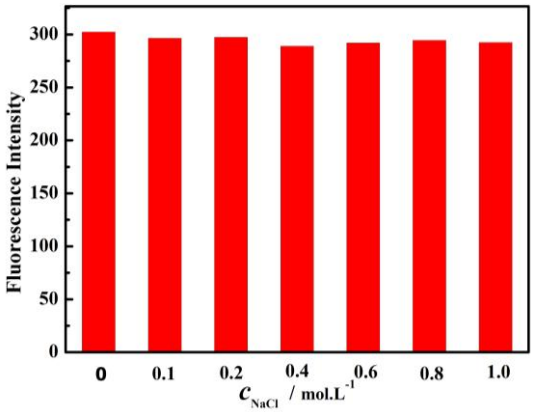


Fig.S6 Effect of ionic strengths on the fluorescence intensity of CDs (ionic strengths were controlled by various concentrations of NaCl).

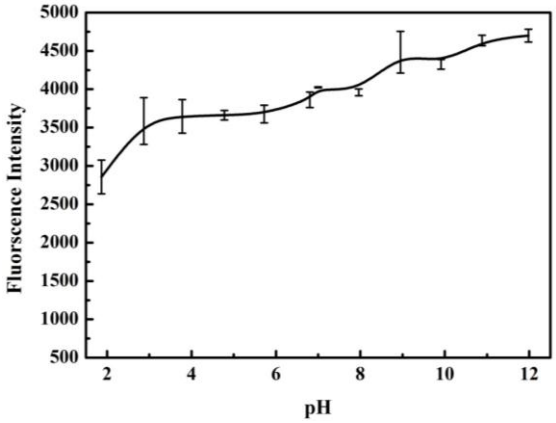


Fig.S7 Effect of pH on the fluorescence intensity of CDs.

Table.2 Zeta potentials vary from different pH.

pH	Zeta potentials
1.89	5.37
5.72	-16.8
7	-18.7
9.91	-25.5
11.98	-28.1

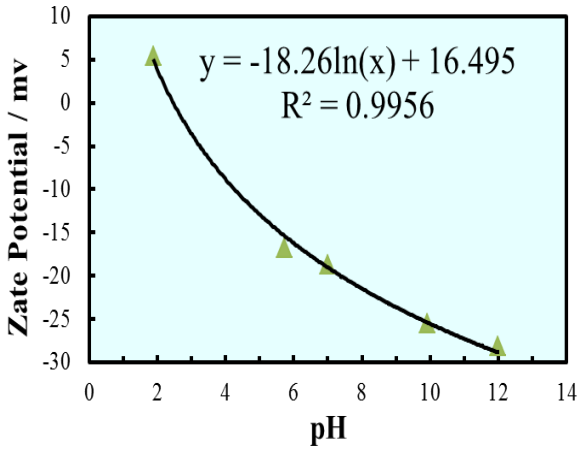


Fig.S8 Plot of the relationship between pH and zate potential.

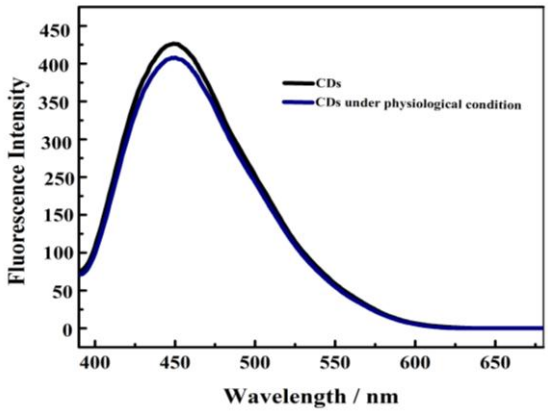


Fig.S9 The fluorescence intensity change of CDs under physiological condition (pH 7.4, 10 mM tris-HCl，0.15 mM NaCl).

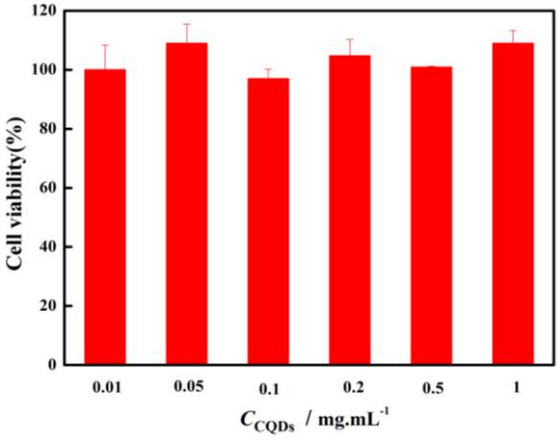


Fig.S10 Cellular toxicity of CDs. (Cell viability by CKK-8 assay)

Ref:

1. B. Jacob, Q. Umberger, V. Lamer, *J. Am. Chem. Soc.*, **67**, 1945, 1099-1109.