Supplementary Information (SI) for RSC Advances.
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Supplementary Information

Efficient preparation of highly fluorescent dual-emission carbon dots and their application in berberine hydrochloride detection

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1. Materials and instruments.

All reagents used in this work showed below were purchased from commercial suppliers without further purification. L-arginine (L-Arg, $C_6H_{14}N_4O_2$, CAS: 74-79-3), 1-pyrenecarboxaldehyde ($C_{17}H_{10}O$, CAS: 3029-19-4), aluminum chloride ($AlCl_3$), calcium chloride ($CaCl_2$), cupric sulfate ($CuSO_4$), ferric chloride hexahydrate ($FeCl_3$•$6H_2O$), magnesium sulfate ($MgSO_4$), zinc sulfate heptahydrate ($ZnSO_4$•$7H_2O$), potassium bromide (KBr), potassium chloride (KCl), potassium carbonate (K_2CO_3), sodium fluoride (NaF), potassium iodide (KI), potassium phosphate (K_3PO_4), sodium sulfate (Na_2SO_4), glutathione (GSH, $C_{10}H_{17}N_3O_6S$), glucose ($C_6H_{12}O_6$, CAS: 50-99-7), L-glycine (L-Gly, $C_2H_5NO_2$), Berberine hydrochloride (BH, $C_{20}H_{18}ClNO_4$), L-glutamic acid (L-Glu, $C_5H_9NO_4$), L-histidine (L-His, $C_6H_9N_3O_2$), ascorbic acid (AA, $C_6H_8O_6$), nicotinamide ($C_6H_6N_2O$), citric acid (CA, $C_6H_8O_7$), urea (CH_4N_2O). Berberine hydrochloride capsule and tablet were obtained from the local pharmacy.

Fluorescence spectra were measured by Hitachi F7000 with the slit width of 5/5 nm. Other instruments employed in this work are UV-Visible spectrophotometer (Thermo Evolution 260 Bio), pH Meter (FiveEasy Plus FE28), Transmission electron microscope (TF20), X-ray powder diffractometer (Bruker D8 Advance), X-ray photoelectron spectrometer (Thermo ESCALAB 250), Fourier transform infrared spectrometer (Perkin Elmer Frontier), Steady-state/transient fluorescence spectrometer, Zeta potential analyzer.

2. Optimization of the hydrothermal reaction conditions for DE-CDs.

L-arginine (L-arg, 0.3-0.9 g) and 1-pyrenecarboxaldehyde (CAS: 3029-19-4, 0.01-0.03 g) were mixed in pure water (12.5 mL) and sonicated for 5 min to dissolve. Then, the mixture was sealed into a 25 mL stainless autoclave lined with Teflon and heated at 180-200 °C for 3-5 h. After that, the mixture was naturally cooled to room temperature and the DE-CDs were obtained by centrifugation at 15,000 rpm for 15 min. The obtained DE-CDs supernatant was stored at room temperature and directly employed as stock solution. The DE-CDs stock solution (500 μL) was diluted with pure water to 5.0 mL, and then the fluorescence intensity was measured as shown in Table S1.

Table S1 Optimization of the hydrothermal reaction conditions for DE-CDs.

Entry	L-arg (g)	1-pyrenecarbo-xaldehyde (g)	Temperature (℃)	Time (h)	λ_{max}^{em} (nm)	Fluorescence intensity (a.u.)
1	0.9	/	190	4	401	259.4
2	0.9	0.02	190	4	378/398	2162/1997
3	0.3	0.02	190	4	383/402	1709/1433
4	0.6	0.02	190	4	380/398	1501/1468
5	0.9	0.01	190	4	380/399	1536/1586
6	0.9	0.03	190	4	381/399	1589/1742
7	0.9	0.02	180	4	381/399	1253/1362
8	0.9	0.02	200	4	379/398	1603/1464
9	0.9	0.02	190	3	380/399	1287/1389
10	0.9	0.02	190	5	379/398	1426/1302

3. XRD pattern of DE-CDs.

For the preparation of DE-CDs samples for XRD, commonly used dialysis and freeze drying methods were employed. The DE-CDs stock solution (10 mL) was firstly dialyzed (2000 Da) and then dried under vacuum at -20 °C for 12 h. The obtained powder was directly used for the XRD determination.

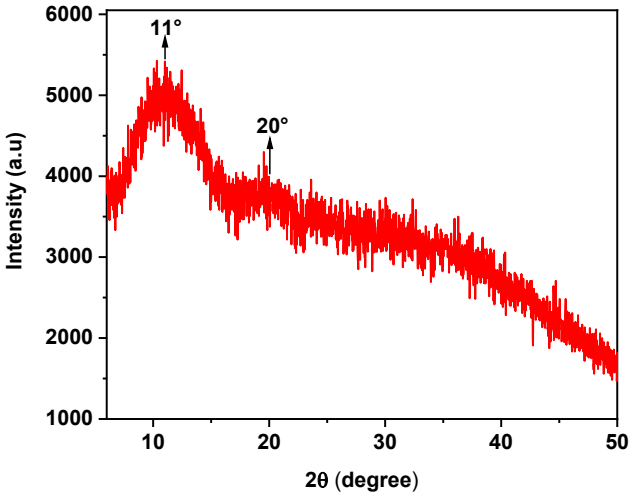


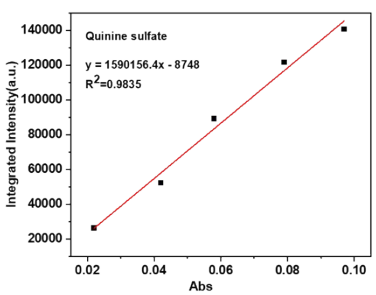
Fig. S1 XRD pattern of DE-CDs.

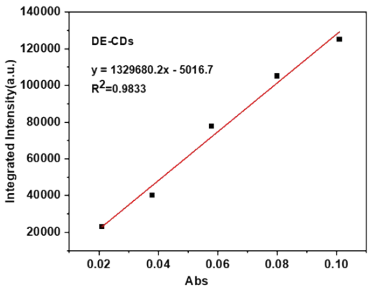
4. Calculation of fluorescence quantum yield of DE-CDs.

The fluorescence quantum yield (FLQY) of DE-CDs was determined by a common method according to litterateurs using quinine sulfate in pure water as a reference (FLQY = 55%). The FLQY value of DE-CDs was calculated as follows:

$$FLQYt = FLQYr \times (It/Ir) \times (Ar/At) \times (\eta t/\eta r)^2$$

The subscript "t" and "r" refer to the DE-CDs and quinine sulfate. A is the optical density, I is the integrated emission intensity, and ŋ is the refractive index of the solvent. To get more reliable results, the absorption of the two solutions were adjusted to less than 0.1 to prevent the reabsorption effect.





	Quinine sulfate					DE-CDs				
Absorbance	0.022	0.042	0.058	0.079	0.097	0.021	0.038	0.058	0.08	0.101
Integrated Intensity	26347.05	52292.67	89183.01	121611.7	140692	23064.44	40221.06	77730.08	105109.5	125036.8
Excitation (nm)	340					340				
Slope	1590156.4					1329680.2				
QY (%)	55					46				

Fig. S2 Plots of integrated intensity of Quinine sulfate and DE-CDs.

5. The sensing property of DE-CDs.

For sensing selectivity: The DE-CDs stock solution (500 μL) was first diluted with pure water (3.0 mL) and 100 μM ions and compounds (Al^{3+}, Ca^{2+}, Cu^{2+}, Fe^{3+}, K^{+}, Mg^{2+}, Na^{+}, Zn^{2+}, Br^{-}, Cl^{-}, CO_3^{2-}, F^{-}, I^{-}, NO_3^{-}, PO_4^{3-}, SO_4^{2-} and glutathione (GSH), glucose, L-Glycine (L-Gly), L-arginine (L-Arg), L-glutamic acid (L-Glu), L-Histidine (L-His), ascorbic acid (AA), nicotinamide, citric acid (CA), urea and berberine chloride (BH) (50 μM)) were added. Then the solutions were further diluted to 5.0 mL, and the fluorescence intensity of the solutions were measured. All the procedure was repeated three times.

For anti-interference ability: The DE-CDs stock solution (500 μL) was first diluted with pure water (3.0 mL) and 100 μM ions and compounds (Al^{3+}, Ca^{2+}, Cu^{2+}, Fe^{3+}, K^{+}, Mg^{2+}, Na^{+}, Zn^{2+}, Br^{-}, Cl^{-}, CO_3^{2-}, F^{-}, I^{-}, NO_3^{-}, PO_4^{3-}, SO_4^{2-} and glutathione (GSH), glucose, L-Glycine (L-Gly), L-arginine (L-Arg), L-glutamic acid (L-Glu), L-Histidine (L-His), ascorbic acid (AA), nicotinamide, citric acid (CA) and urea) were added. Then berberine chloride (BH) (50 μM) were added to those solutions in the presence of ions and compounds. The solutions were further diluted to 5.0 mL, and the fluorescence intensity of the solutions were measured. All the procedure was repeated three times.

For sensing sensitivity: The DE-CDs stock solution (500 μL) was first diluted with pure water (3.0 mL) and different amounts of BH (0-200 μM) were added. Then the solutions were further diluted to 5.0 mL, and the fluorescence intensity of the solutions were measured. All the procedure was repeated three times.

For all the measurements, the excitation and emission slits of the fluorescence spectra were both 5.0/5.0 nm, the excitation wavelengths were 340 nm, and the emission wavelengths were recorded at 378/398 nm.

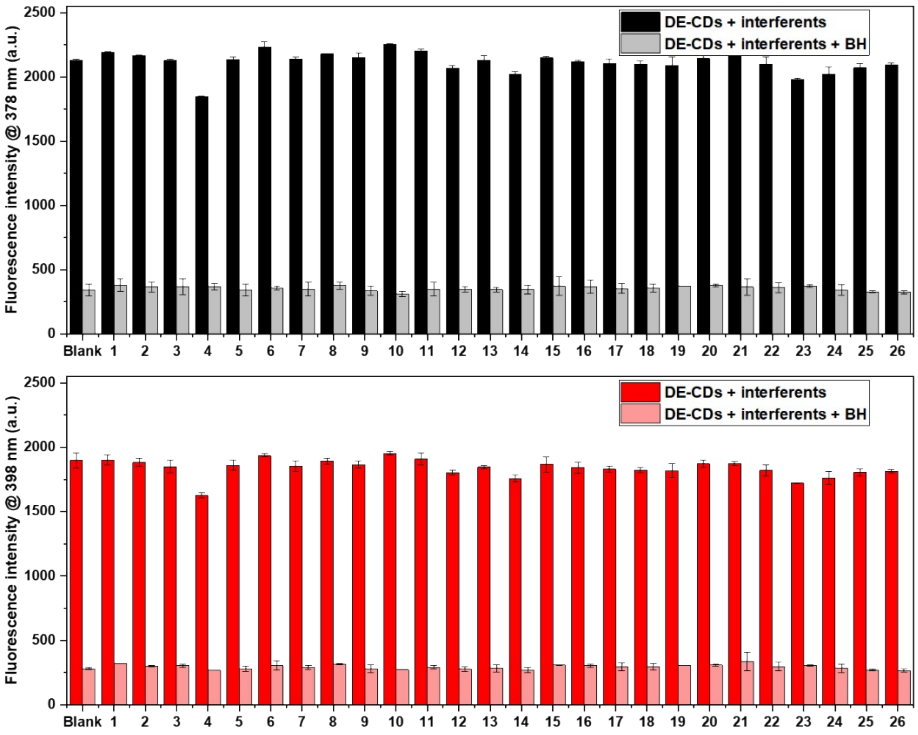


Fig. S3 Fluorescence intensities of DE-CDs (black@378 nm and red@398 nm) with 100 μM different ions and compounds (1-26: Al^{3+}, Ca^{2+}, Cu^{2+}, Fe^{3+}, K^{+}, Mg^{2+}, Na^{+}, Zn^{2+}, Br^{-}, Cl^{-}, CO_3^{2-}, F^{-}, I^{-}, NO_3^{-}, PO_4^{3-}, SO_4^{2-} and glutathione (GSH), glucose, L-Glycine (L-Gly), L-arginine (L-Arg), L-glutamic acid (L-Glu), L-Histidine (L-His), ascorbic acid (AA), nicotinamide, citric acid (CA) and urea) in the absence (black/red)/presence (gray/pink) of BH (50 μM).

6. Response time of DE-CDs to BH.

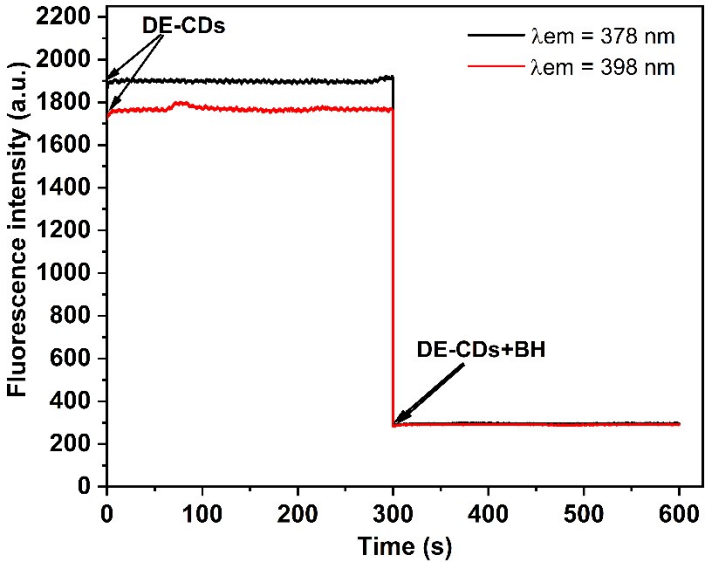


Fig. S4 Fluorescence intensity of two emission peaks (378/398 nm) of DE-CDs before and after addition of BH (50 μM).

7. Fluorescence intensity and standard deviation.

Table S2 Fluorescence intensity and standard deviation of DE-CDs.

No.	Fluorescence intensity at 378 nm (a.u.)	F/F_0@378		Fluorescence intensity at 398 nm (a.u.)	F/F_0@398
1	2088	1.0024		1836	1.0022
2	2070	0.9938		1819	0.9929
3	2081	0.9991		1832	1.0000
4	2104	1.0101		1847	1.0082
5	2095	1.0058		1844	1.0066
6	2074	0.9957		1824	0.9956
7	2079	0.9981		1829	0.9984
8	2083	1.0000		1834	1.0011
9	2079	0.9981		1829	0.9984
10	2076	0.9967		1826	0.9967
	σ	**0.0047**			**0.0045**

8. Actual sample preparation procedure.

The berberine hydrochloride powders from berberine hydrochloride tablet and capsule were dissolved in 20 mL of purified water with the assistance of sonication. Then the solutions were centrifuged at 8000 rpm for 20 min and filtered through 0.22 μM membrane. The sample solutions were prepared by diluting the obtained supernatant with purified water to maintain the concentration within the linear range. Finally, different amounts of BH were added to the above solutions and the spiked recoveries and RSDs were calculated. All measurements were repeated three times.

9. Quenching mechanism exploration.

The occupancy of the IFE was explored at the stronger emission peaks of 378 nm of DE-CDs. A_{ex}, A_{em} and F_{obsd} are the absorbance and fluorescence intensities of DE-CDs at optimal excitation (340 nm) and emission (378 nm). F_{cor} (corrected fluorescence emission intensities of DE-CDs at 378 nm) was calculated according to Eq. S1 (the Parker's equation).

$$\frac{F_{cor}}{F_{obsd}} = \frac{2.3dA_{ex}}{1-10^{-dA_{ex}}} 10^{gA_{em}} \frac{2.3sA_{em}}{1-10^{-sA_{em}}} \quad \text{(Eq. S1)}$$

F_{cor} and F_{obsd} are the calibrated and measured fluorescence intensities of the DE-CDs (378 nm), d, g, and s are the geometric parameters of the quartz cuvette (Fig. S5) of 1.00, 0.25, and 0.50 cm, respectively, and A_{ex} and A_{em} are the absorptions of the DE-CDs at optimal excitation (340 nm) and emission (378 nm).

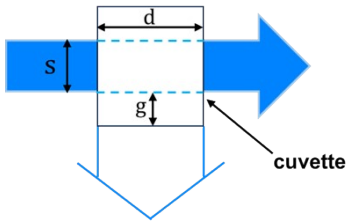


Fig. S5 Geometry of a quartz cuvette where d, g and s are 1.00, 0.25 and 0.50 cm respectively.

Then, *CF* (correction factor), E_{obsd} (observed fluorescence suppression efficiency), E_{cor} (corrected fluorescence suppression efficiency) and $F_{cor.o}/F_{cor}$ (the ratio of corrected fluorescence emission intensities of DE-CDs at 378 nm without/with BH) were calculated as shown in Table S3 (CF ≤ 3 to ensure the accuracy of the results). The IFE ratio was then calculated by (E_{obsd} - E_{cor})/ E_{obsd} to be 62.66% for BH.

Table S3 Parameters used to calculate the percentage of IFE of DE-CDs (λex = 340 nm, λem = 378 nm).

BH (μM)	A_{ex}	A_{em}	*CF*	F_{obsd}	F_{cor}	E_{obsd}	E_{cor}	$F_{cor.o}/F_{cor}$
0	0.42	0.09	1.73	2149	3718	0.00	0.00	1.00
2	0.51	0.12	1.94	1799	3491	0.16	0.06	1.06
5	0.59	0.11	2.06	1649	3393	0.23	0.09	1.10
10	0.72	0.13	2.37	1235	2927	0.42	0.21	1.27
15	0.87	0.14	2.72	1117	3033	0.48	0.18	1.23

$CF = F_{cor}/F_{obsd}$; $E_{obsd} = 1 - F_{obsd} / F_{obsd,0}$; $E_{cor} = 1 - F_{cor} / F_{cor,0}$; $F_{obsd,0}$ and $F_{cor,0}$ are measured and corrected fluorescence intensities without BH.

The ratio of corrected fluorescence emission intensities of DE-CDs at 378 nm without/with BH ($F_{cor.o}/F_{cor}$) versus the concentration of BH were plotted as shown in Fig S6. In the presence of BH, $F_{cor,0}/F_{cor}$ was not linearly related to BH and does not follow the Stern-Volmer equation (Eq. S2), which suggests that the static and dynamic quenching effects in this system are negligible.

$$F_{cor,0}/F_{cor} = 1 + K_{SV}[Q] \quad \text{(Eq. S2)}$$

$F_{cor,0}$ and F_{cor} are the corrected fluorescence intensities without and after addition of BH, respectively; Ksv is the Stern-Volmer constant; $[Q]$ is the concentration of BH.

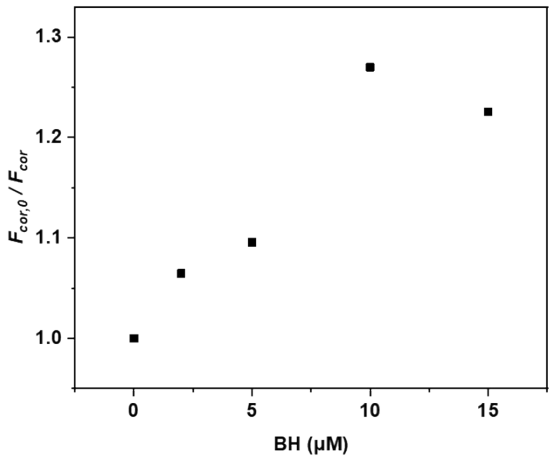


Fig. S6 Plot of corrected fluorescence emission intensity without/with BH ($F_{cor,o}/F_{cor}$) versus BH concentration.