Supplementary Materials for:

Hg²⁺ detection, pH sensing and cell imaging based on bright blue-

fluorescent N-doped carbon dots

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Materials

Folic acid and citric acid were obtained through Shanghai Aladdin Reagent Co., Ltd. (Shanghai, China). AlCl₃, BaCl₂, CaCl₂, CdCl₂, CoCl₂, CuCl₂, FeCl₃, HgCl₂, MgCl₂, MnCl₂, NaCl, NiCl₂, PbCl₂ and ZnCl₂ were obtained through Beijing Chemical Corp (Beijing, China). Dialysis membranes with a MWCO of 500–1000 Da were obtained through Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). HepG-2 cells were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China).

Apparatus

Transmission electron microscopy (TEM) studies were carried out in a JEOL JEM-2100 instrument operating at an accelerating voltage of 200 kV. Samples for TEM measurements were obtained via placing a drop of colloidal solution on a carbon coated copper grid and then drying at room temperature. The UV-vis absorption spectrum was recorded through HITACHI U-2910 UV. The fluorescence spectrum was operated with a Hitachi F-4500 fluorescence spectrophotometer (Tokyo, Japan). Fourier transform infrared (FTIR) spectra were recorded on a Bruker tensor 2 spectrometer at a resolution of 4 cm⁻¹. A 1 mg sample (ratio 1:200) diluted in KBr was pressed into the pan.

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Cell imaging

In a 5% CO₂ incubator, HepG-2 cells in the exponential phase were seeded into 15 mm glass culture dishes at an initial density of 1×106 cells/mL with DMEM containing 10% FBS and incubated at 37°C. After incubation, the medium was discarded and HepG-2 cells were treated with mixture of N-CDs (200 µg/mL) and DMEM for 40 min. The extracellular N-CDs were removed by rinsing twice with phosphate buffer solutions (pH =7), and then cells were incubated with PBS buffer (0.01 M) containing a range of concentration of Hg²⁺. Immediately, fluorescence images were captured on laser scanning confocal microscope (LSCM). For intracellular pH sensing, under the above conditions, HepG-2 cells was cultured by taking the place of pH 8.

QY measurements

Quinine sulfate in 0.1 M H₂SO₄ was selected as a reference with a QY of 0.54 at 360 nm. The relative fluorescence QY (Φ) of as-prepared N-CDs was calculated using the equation: $\Phi_x = \Phi_{std}I_xA_{std}\eta_x^2/(I_{std}A_x\eta_{std}^2)$. In the equation, I_x and I_{std} denote the fluorescence intensities of obtained N-CDs and reference, respectively. A_x and A_{std} represent the optical densities of obtained N-CDs and reference, respectively. η_x and η_{std} represent the refractive indices of obtained N-CDs and reference, respectively. The absorbances of all the samples in a 1.0 cm cuvette were kept under 0.1 at the excitation wavelength to minimize re-absorption effects.



Fig. S1 CIE chromaticity coordinate from the PL spectra of N-CDs.



Fig. S2 (A) Influence of storage times on the PL intensity of N-CDs. (B) $E \square$ ect of ionic strength on the PL intensity of N-CDs



Fig.S3CytotoxicitytestofN-CDs.

Fluorescent probes	Raw material	Linear range	Detection limit	Ref.
CDs	citric acid and	0 μΜ–8 μΜ	0.24 µM	[1]
	polyethyleneimine			[0]
CDs	Musk melon	1 μΜ–25 μΜ	0.33 μM	[2]
N, S-CDs	glycerol and	1 μM–75 μM	0.50 μΜ	[3]
	cystine			
CDs	citric acid and	0 μM–30 μM	0.20 µM	[4]
	diethylenetriamine			
CDs	adipic acid and	4 μM–18 μM	2.47 μM	[5]
	triammonium			
	citrate			
N-CDs	citric acid and	2 μM–14 μM	0.44 µM	[6]
	melamine			
N-CDs	sodium citrate and	0.001 μM-5 μM	0.65 μM	[7]
	urea		·	
CDs	cellulose	6 μM-80 μM	1.6 µM	[8]
N-CDs	citric acid and	50 μM–400 μM	0.124 μM	This
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	folic acid			

Table S1 Comparison of different fluorescent N-CDs based probes for Hg²⁺ detection.

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