

## Supporting Information

### **Ultrasensitive fluorometric determination of iron(III) and inositol hexaphosphate in cancerous and bacterial cells by using carbon dots with bright yellow fluorescent**

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## Chemicals and instruments

Reagent grades of *o*-phenylenediamine, KCl, CaCl<sub>2</sub>, NaCl, FeCl<sub>3</sub>, CdCl<sub>2</sub>, MgCl<sub>2</sub>, ZnCl<sub>2</sub>, FeCl<sub>2</sub>, MnCl<sub>2</sub>, CuCl<sub>2</sub>, CoCl<sub>2</sub>, NiCl<sub>2</sub>, inositol hexaphosphate were purchased from Aldrich Ltd. (Shanghai, China, <https://www.sigmaaldrich.com/china-mainland.html>). All the other chemicals were of analytical grade and used without further purification. All solutions were prepared with Ultra-pure water from the Mill-Q-RO<sub>4</sub> water purification system (Millipore).

## Characterizations

High-resolution transmission electron microscopy (HRTEM) images were obtained on a JEM-2100 (JEOL, Japan) electron microscope operating at 200 kV. X-ray photoelectron spectra (XPS) were recorded with a KRATOS XSAM800 X-ray photoelectron spectrometer (Kratos Analytical Ltd, Manchester), using Mg as the exciting source. Raman spectra was collected on an alpha300 WiTec Raman microscope (Witec Inc., Ulm, Germany). Fourier transform infrared (FTIR) spectra were obtained on a Thermo FTIR spectrophotometer. UV-vis absorption spectra were recorded on an Avaspec-2048 UV-vis spectrophotometer. Excitation and emission spectra were measured using a fluorescence spectrophotometer (F-7000, Hitachi). Powder X-ray diffraction (PXRD) spectra were obtained on PANalytical B.V. (Netherlands) X'PERT PXRD. The CCK-8 assay was performed using a microplate reader (Spectra MAX 340, Molecular Devices Co., Sunnyvale, CA, U.S.A.). PL lifetime and quantum yields were measured using FL3-111 (HORIBA Instruments). Confocal optical micrographs were captured using a confocal laser scanning microscope (CarlZeiss LSM710, Oberkochen, Germany). IVIS SpectrumCT Small-animal Imaging System (Caliper Life Sciences, Hopkinton, MA) was used to record the fluorescence images. Fluorescence imaging physical photos excited by blue lamp at 450 nm (LUYOR-3260RB, Shanghai LUYOR Instrument Co., Ltd).

## Bacterial culture and imaging

*Escherichia coli* (*E. coli*) was cultured in LB medium (10 mg·mL<sup>-1</sup> NaCl, 10 mg·mL<sup>-1</sup> tryptone, and 5 mg·mL<sup>-1</sup> yeast extract). The turbidity of the bacteria was adjusted to 1 (3×10<sup>8</sup> CFU·mL<sup>-1</sup>), centrifuged at 5000 rpm for 3 min, and washed twice with PBS.

Resuspend in PBS and heat the bacteria to 60 °C to kill the bacteria. The bacterial was collected and cultured with YN-CDs (50  $\mu\text{g}\cdot\text{mL}^{-1}$ ). The bacterial was incubated at 37 °C at 120 rpm. After incubation for 12 h, the bacterial was collected by centrifugation at 5000 rpm for 3 min, and washed with PBS for 3 times. Then using confocal laser scanning microscopy (CLSM) imaged.

### **Cell culture and imaging**

The hepatocellular carcinoma (Hep G2) was obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were grown in DMEM medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin in a humidified atmosphere containing 5% CO<sub>2</sub> at 37 °C. Hep G2 cells were plated into 4-well plates at a density of 10<sup>5</sup> cells/cm<sup>2</sup>, and allowed to adhere by incubation for 12 h at 37 °C. The safety of the YN-CDs cells was evaluated by the CCK-8 method before starting the cell imaging experiment. YN-CDs (after being filtered with a 0.22  $\mu\text{m}$  filter, 50  $\mu\text{g}\cdot\text{mL}^{-1}$ ) solution was separately added into 500  $\mu\text{L}$  of DMEM, then the YN-CDs were incubated with Hep G2 cells. After growing for 12 h, PBS was used to wash the samples three times. Then, the samples were ready for imaging and the staining was visualized by a confocal microscope. The PL intensity of each sample was analyzed using the ZEN software (Carl Zeiss AG., Oberkochen, Germany).

### **In vivo imaging**

To evaluate the effect of in vivo imaging of YN-CDs, an aqueous solution of YN-CDs (100  $\mu\text{L}$ , 50  $\mu\text{g}\cdot\text{mL}^{-1}$ ) was subcutaneous injection in mice. IVIS Spectrum CT Small-animal Imaging System was used to record the fluorescence images at 465 nm excitation and emission at 620 nm.

### **Detection of IP6 in food samples**

An IP6 (500 nM) standard was prepared in ultrapure water. Typically, 50  $\mu\text{M}$  of Fe quenching fluorescence was added to 500  $\mu\text{L}$  of YN-CDs (50  $\mu\text{g}\cdot\text{mL}^{-1}$ ) spot solution, then different amounts of IP6 were added and finally brought to 1 mL with water. After 2 min of reaction at room temperature, the spectra were recorded under excitation at 420 nm. All experiments were repeated at least three times and the recovery was calculated as follows:

$$\text{Recovery} = (C_{\text{measured}} - C_{\text{initial}}) / C_{\text{added}}$$

### **Nematode maintenance and imaging**

Wild-type nematode N2 was used as the tested nematode in this study. The food was *Escherichia coli* OP50, and N2 was maintained at 20 °C inoculation culture on a nematode growth medium (NGM) plate in which food was present<sup>1</sup>. An age-synchronized population of L1-larvae was obtained. Nematodes were incubated with YN-CDs (final concentration: 50 µg·mL<sup>-1</sup>) at 20 °C for 12 h in 12-well sterile tissue culture plates. Confocal observation was carried out by treatment with 0.5 mM sodium azide.

### **Figure captions**

**Figure S1** Fluorescence decays of the YN-CDs in the absence or presence of  $\text{Fe}^{3+}$  (Ex: 425 nm, Em: 575 nm).

**Figure S2** The Photo-stability of YN-CDs in aqueous solution under 420 nm.

**Figure S3** Fluorescence of YN-CDs after adding  $\text{Fe}^{3+}$  at different reaction times.

**Figure S4** The Stern-Volmer equation of YN-CDs and the concentration of  $\text{Fe}^{3+}$ .

**Figure S5** Confocal results of quenching effects of different  $\text{Fe}^{3+}$  concentrations on YN-CDs in Hep G2 cells.

**Figure S6** FT-IR spectra of YN-CDs with  $\text{Fe}^{3+}$  ion.

**Figure S7** UV spectra of YN-CDs with  $\text{Fe}^{3+}$ ,  $\text{Na}^+$ ,  $\text{Cd}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Al}^{3+}$  ions.

**Figure S8** The selectivity of YN-CDs with  $\text{Fe}^{3+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Al}^{3+}$  ions.

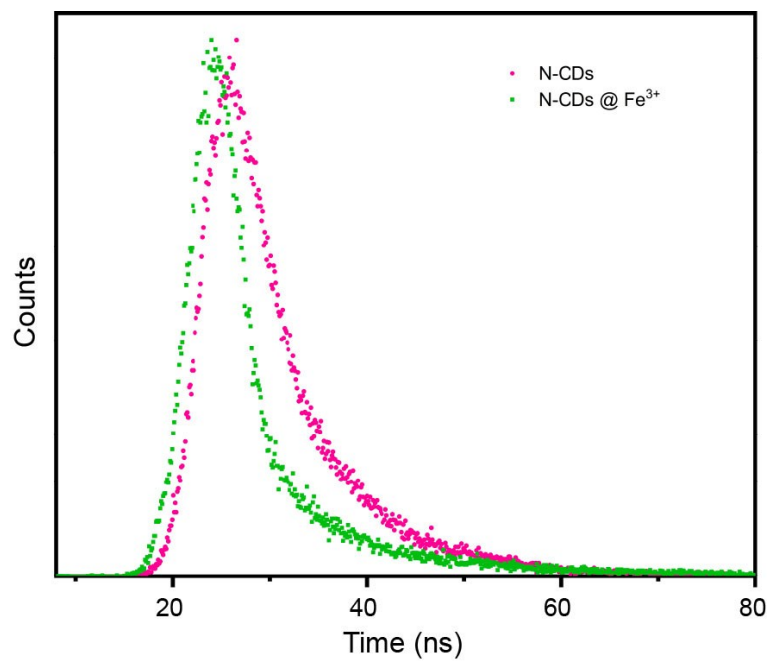
**Figure S9** The selectivity of YN-CDs for  $\text{Fe}^{3+}$  and IP6 in different pH value.

## **Table captions**

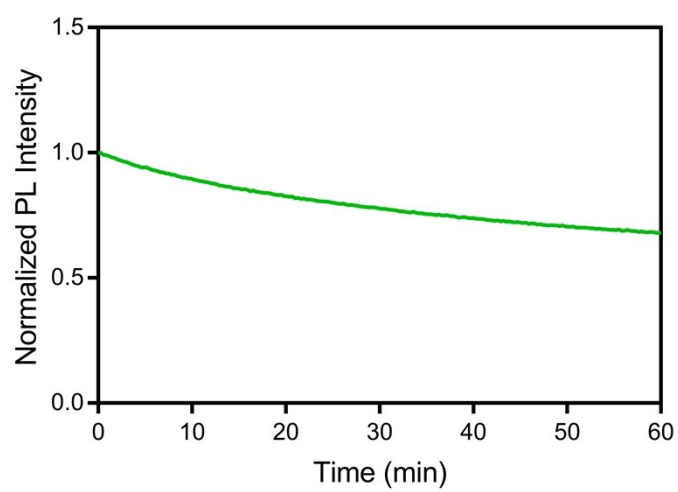
**Table S1** Comparison of Different Fluorescent Probes for  $\text{Fe}^{3+}$  Detection.

**Table S2** Results of determination of IP6 in food samples by this method and HPLC.

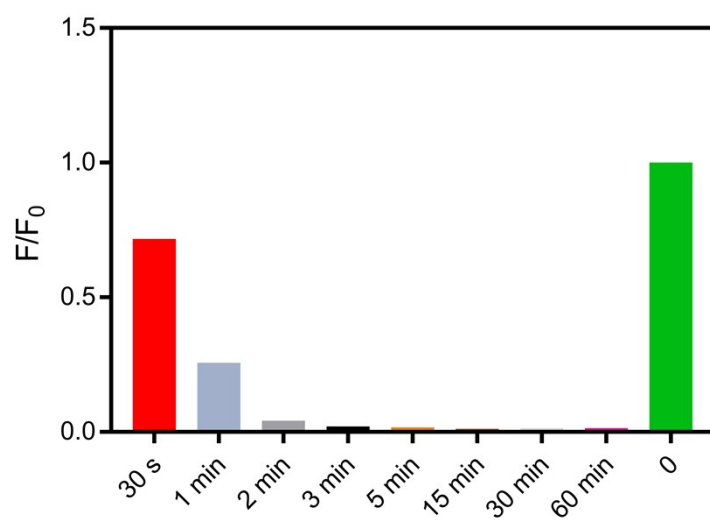
**Figure S1** Fluorescence decays of the YN-CDs in the absence or presence of  $\text{Fe}^{3+}$  (Ex: 425 nm, Em: 575 nm).



**Figure S2** The Photo-stability of YN-CDs in aqueous solution under 420 nm.

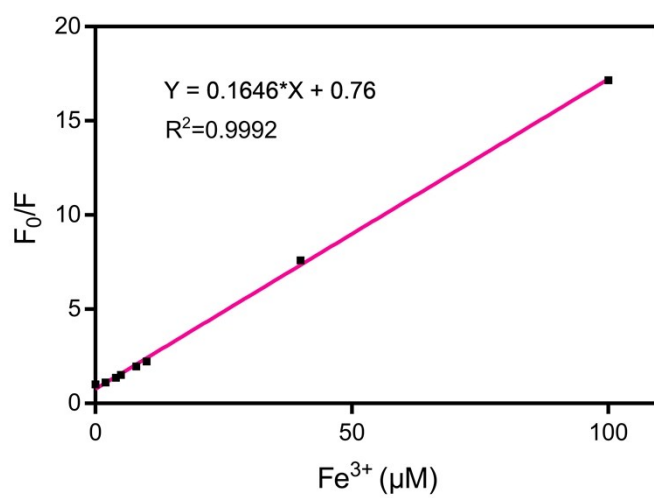


**Figure S3** Fluorescence of YN-CDs after adding  $\text{Fe}^{3+}$  at different reaction times.

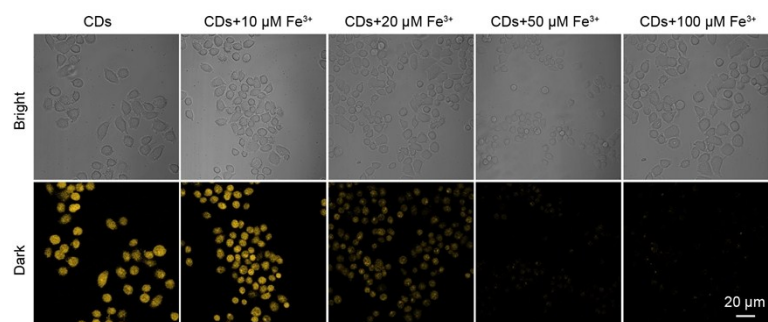




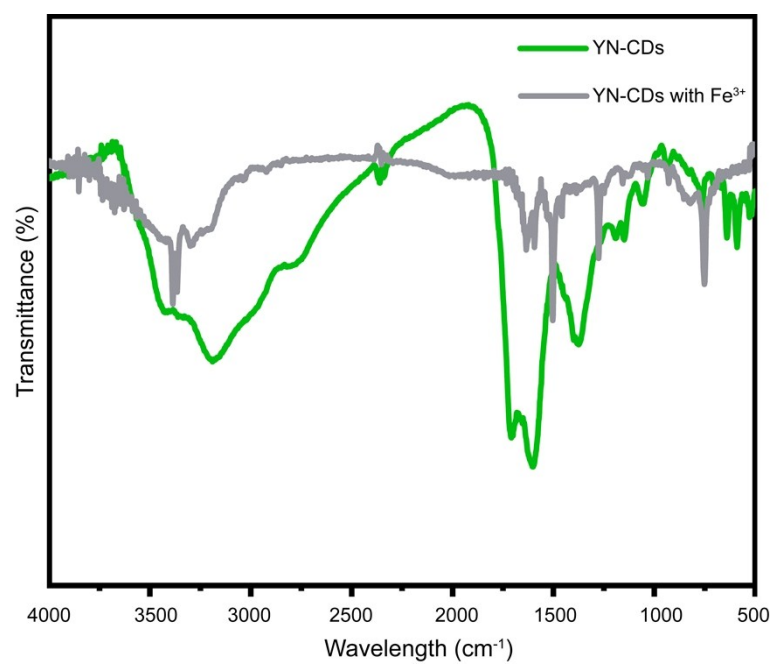
**Figure S4** The Stern-Volmer equation of YN-CDs and the concentration of  $\text{Fe}^{3+}$ .



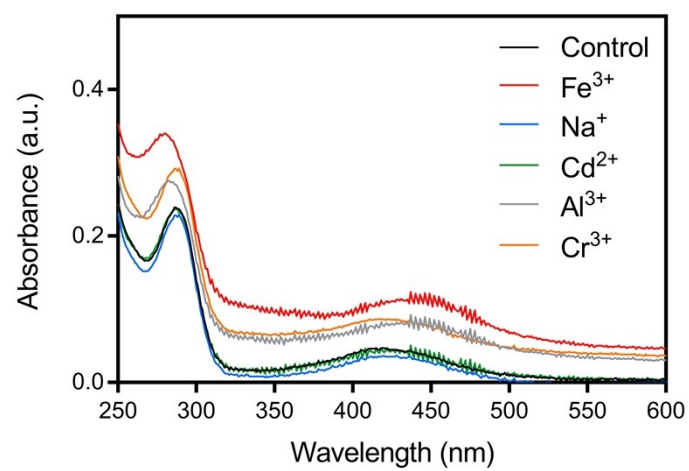
**Figure S5** Confocal results of quenching effects of different  $\text{Fe}^{3+}$  concentrations on YN-CDs in Hep G2 cells.



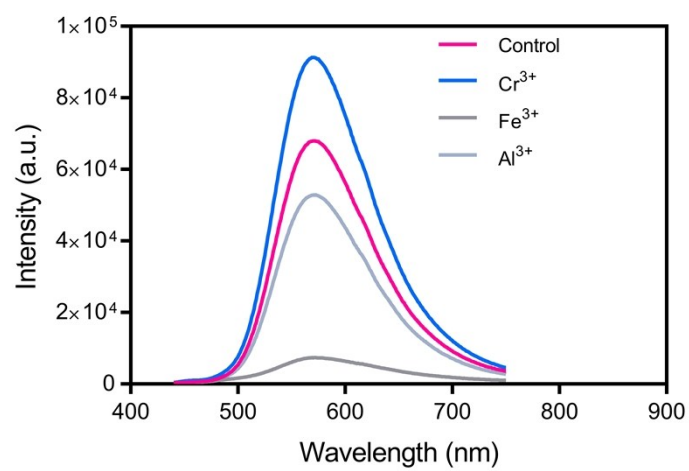
**Figure S6** FT-IR spectra of YN-CDs with  $\text{Fe}^{3+}$  ion.



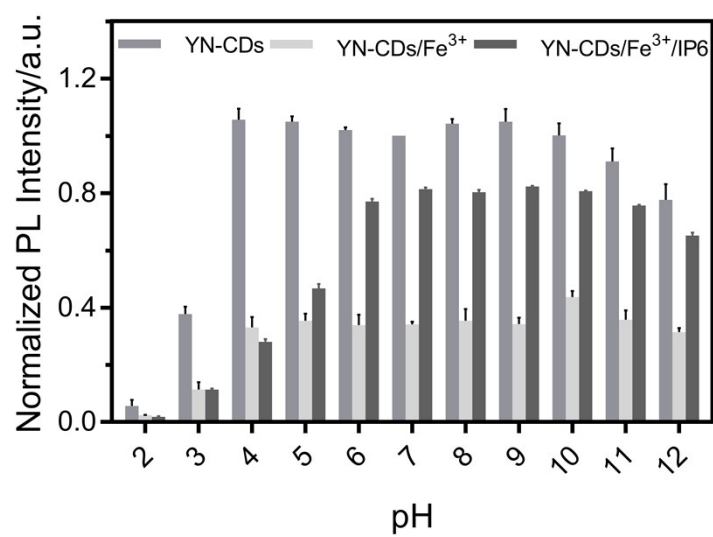
**Figure S7** UV spectra of YN-CDs with  $\text{Fe}^{3+}$ ,  $\text{Na}^+$ ,  $\text{Cd}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Al}^{3+}$  ions.



**Figure S8** The selectivity of YN-CDs with  $\text{Fe}^{3+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Al}^{3+}$  ions.



**Figure S9** The selectivity of YN-CDs for  $\text{Fe}^{3+}$  and IP6 in different pH value.



**Table S1** Comparison of Different Fluorescent Probes for Fe<sup>3+</sup> Detection.

Material	Detection limit (μM)	References
aminoantipyrine	0.211	2
N-doped carbon dots	4.67	3
carbon dots	0.20	4
B-doped carbon dots	0.242	5
2,5-diphenylfuran and 8 hydroxyquinoline	0.97	6
N and P codoped carbon dots	0.33	7
graphene oxides nanosheets	17.9	8
S-doped carbon dots	0.10	9
N-doped carbon dots	0.0337	This work

**Table S2** Results of determination of IP6 in food samples by this method and HPLC.

Food sample	Detected (nM)	Spiked (nM)	Found (nM)	Recovery (%)	RSD (%)	Found by HPLC (nM)
Apple	ND	5.00	4.99	99.80	1.05	4.97
		10.00	10.01	100.10	2.10	10.04
		100.00	99.20	99.20	0.88	99.44
Celery	ND	5.00	5.06	101.20	1.92	5.02
		10.00	10.04	100.40	1.16	10.07
		100.00	102.10	102.10	2.05	100.27
Rice	21.60	5.00	26.22	92.30	1.48	26.57
		10.00	30.77	91.70	2.62	31.70
		100.00	116.80	95.20	1.13	122.03
Wheat	78.30	5.00	83.17	97.30	0.82	83.42
		10.00	87.45	91.50	2.12	88.24
		50.00	125.65	94.70	0.97	128.53
Bread	111.40	5.00	115.87	89.30	2.51	116.45
		10.00	120.61	92.10	1.03	121.56
		20.00	129.18	88.90	1.10	131.58

ND: Not detectable.

### References

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