Electronic Supplementary Information for

Nitrogen-Doped Carbon Dots from Plant Cytoplasm as Selective and Sensitive Fluorescent Probes for Detecting P-Nitroaniline in Both Aqueous and Soil Systems

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Experimental section

Materials: fresh camphor leaves, lake water and dry soil were collected in campus of Wuhan University of Technology. Anhydrous ethanol, dinitrotoluene (DNP), P-nitrobenzoic acid (P-NBA), M-nitrobenzoic acid (M-NBA), P-nitroaniline (PNA), P-nitrophenol (PNP) from Sinopharm Chemical Reagent Co., LTD and M-nitroaniline (MNA), O-nitroaniline (ONA) from Aladdin Reagent Co., LTD are all of analytical grade. Deionized water was prepared in our lab.

Lake water was filtrated with filtration membrane with 220 nm pore size before use.

Preparation of W-N-CDs:

Typically, 2 g fresh camphor leaves were washed with deionized water and then directly added into 50 mL PTFE sealed autoclave, followed by addition of 30 mL H_2O. Subsequently, the autoclave was transferred into muffle furnace, of which the work temperature and time for carbonization was set at 250 °C and 150 min. After cooled down to room temperature, a bright yellow solution was found inside the autoclave and also the black leaves that retained the original shape. Aqueous solution W-N-CDs can be acquired with a further filtration using filtration membrane with pore size of 220 nm.

Preparation of E-N-CDs:

Pure W-N-CDs was obtained by freeze-drying process. With sequential addition of 30 mL anhydrous ethanol, sonication for 10 min and final centrifugation for 15 min at 10,000 rpm, ethanol solution of E-N-CDs was obtained.

Sensitivities of W-N-CDs and E-N-CDs for detecting PNA:

10 mL Aqueous solutions of PNA with concentration of 1×10^{-6}、2×10^{-6}、1×10^{-5}、2×10^{-5}、4×10^{-5}、8×10^{-5}、1.2×10^{-4}、1.6×10^{-4}、2×10^{-4}、4×10^{-4}、6×10^{-4} and 1×10^{-3} M,

were added into those vessels containing 10 mL 0.04 mg/mL W-N-CDs. After gently stirred for few minutes, the solutions were applied for fluorescence test, and the emissions at 370 nm excitation were collected.

The test of E-N-CDs follows the same procedure yet using ethanol as the solvent.

Selectivity of W-N-CDs and E-N-CDs for detecting PNA:

8.7 mg PNA, 6.7 mg DNT, 8.7 mg PNP, 10.5 mg 4-NBA and 10.5 mg 3-NBA were dissolved in five beakers containing 125 mL H_2O with stirring. Then 10 mL of each solution was added into 10 mL aqueous solution of W-N-CDs. And the mixed solution was employed for collecting emissions under 370 nm excitation.

The test of E-N-CDs follows the same procedure yet using ethanol as the solvent.

Detection of PNA In lake water: Simply filtrated water was used as solvents to prepare 0.04 mg/mL solution of W-N-CDs and 6×10^{-5}, 10×10^{-5} and 14×10^{-5} M solutions of PNA. Then 10 mL of each PNA solution was added into 10 mL solution of the fluorescent probe. The results collected from emission spectra were applied for calculation of PNA concentration with the stern-volmer equation in the main text, for the purpose of evaluating the accuracy of the present method.

Detection of PNA In soil sample: 8.3, 13.8 and 19.3 mg were first mixed with three pieces of 100 g dry soil by extensive milling. Typically, 100 mL ethanol added to extract PNA from the mixture. After shaking for 10 min and centrifugation at 8000 rpm for few minutes, 1 mL of the resultant upper liquid was collected and further diluted to 10 mL with ethanol. Then it is added into 10 mL 0.04 mg/mL ethanol solution of E-N-CDs. The results collected from emission spectra were further applied for calculation of PNA concentration with the stern-volmer equation in the main text.

Characterization

Transmission electron microscopy (TEM) images were acquired with a Joel JEM-2001F at acceleration voltage of 200 kV. FL spectra were obtained with a Shimadzu RF-5301PC spectrophotometer. Fourier transform infra-red (FTIR) spectra were obtained using Thermo Nicolet Nexus FTIR spectrometer. X-ray photoelectron spectra (XPS) were carried out with a Kratos XSAM800 X-ray photoelectron spectrometer (MgKα, 1253.6 eV).

Supplementary results

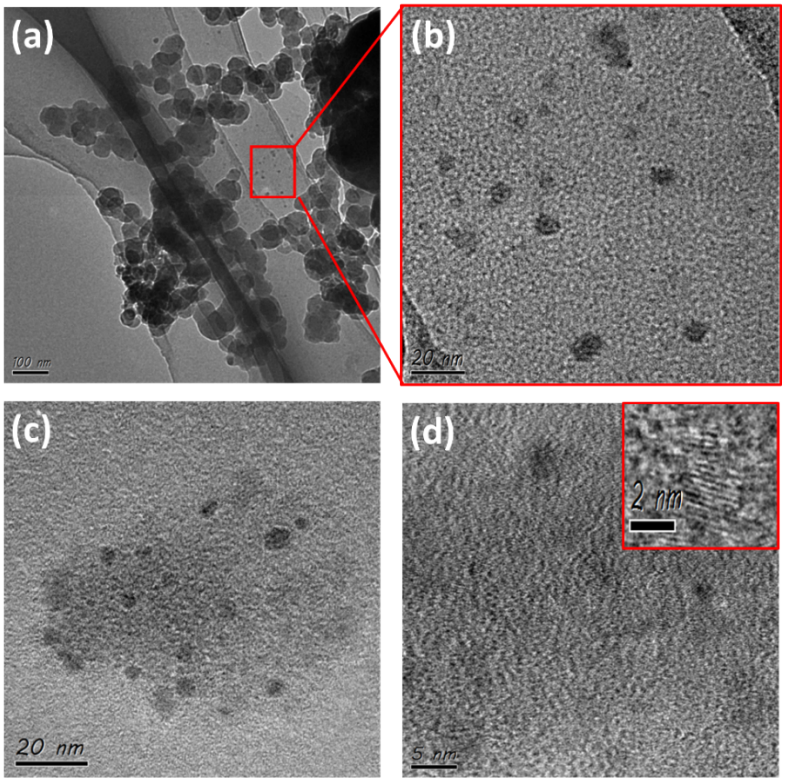

Fig. S1. TEM images of (a, b) W-N-CDs and (c, d) E-N-CDs.

Table S1 Quantum yields of W-CL-CDs (in aqueous) and E-CL-CDs (in ethanol) at 370 nm excitation

Sample	*I*	*A*	*η*	Ψ_{370}
Quinine Sulphate	50664.108	0.009	1.33	0.54
W-CL-CDs	12131.544	0.090	1.33	0.024
E-CL-CDs	14265.483	0.089	1.36	0.030

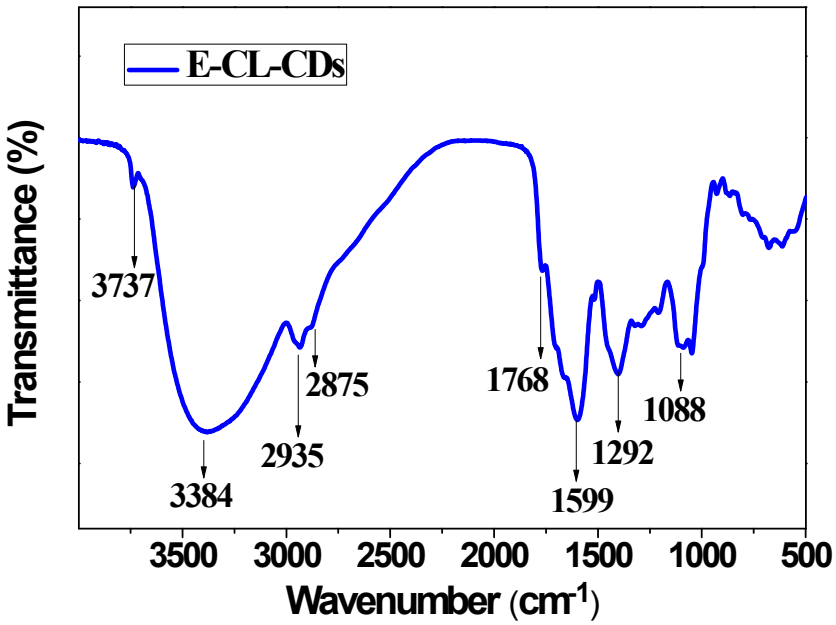

Fig. S2. Fourier Transform Infrared spectrum of E-N-CDs

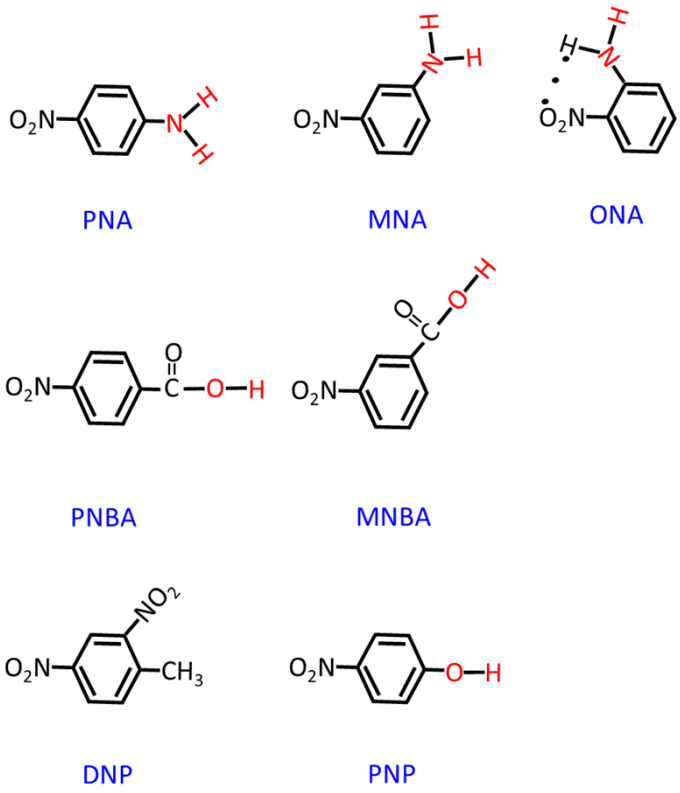

Fig. S3. Possible active sites (marked by red colour) of the nitro-aromatics for hydrogen bonding formation.

Table S2. Lists of the possible factors influencing fluorescence quenching of carbon dots by different nitro-aromatics.

Nitro-aromatic compound	Number of available active sites for formation hydrogen bonding	Other possible factors
P-nitroaniline (PNA)	3	-
M-Nitroaniline (MNA)	3	➢ Decreased molecular polarity due to asymmetrical distribution of amino and nitro
O-Nitroaniline (ONA)	2	➢ Intramolecular hydrogen bond ➢ Decreased molecular polarity due to asymmetrical distribution of amino and nitro
P-nitrophenol (PNP)	2	-
P-nitrobenzoic acid (PNBA)	2	➢ Larger distance for electron transferring ➢ Hindrance of electron transferring by C=O
M-nitrobenzoic acid (MNBA)	2	➢ Larger distance for electron transferring ➢ Hindrance of electron transferring by C=O
Dinitrotoluene (DNP)	0	-

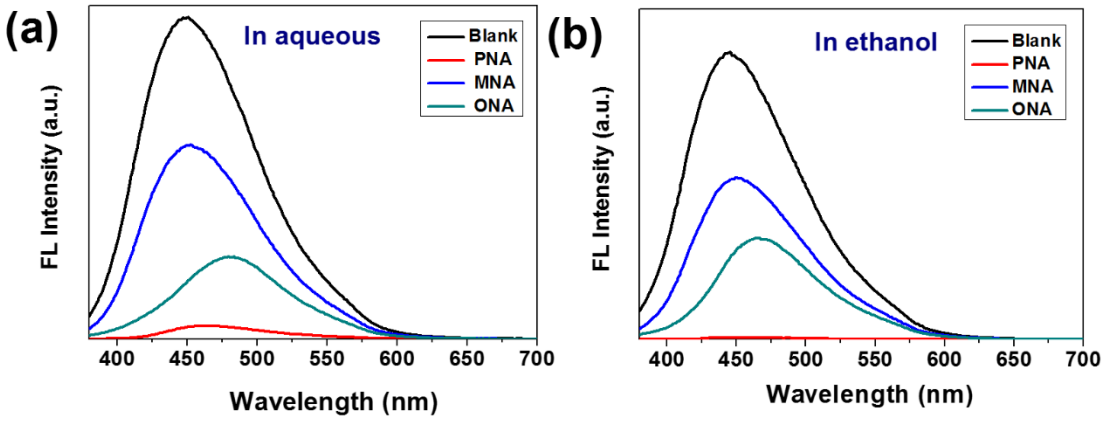

Fig. S4. Fluorescence spectra of (a) W-N-CDs in aqueous and (b) E-N-CDs in ethanol upon the addition of three nitroanilines (PNA, MNA and ONA).

Discussions over the difference between quenching behaviours of these nitroaromatics (see Fig. 4 in main text) from a structural point of view:

PNA and DNP: The latter one is free of any hydroxyl-like groups that could form hydrogen bonds with the CDs, thus revealed no quenching capacity.

PNA, PNBA and MNBA: Carboxyl groups did be able form hydrogen bonding with CDs,

yet there is a larger distance for the subsequent electron transferring, and the C=O may have also served as a structural hindrance that blocked that path for this process, accordingly, the fluorescence from solutions added with PNBA and MNBA only slightly decreased.

PNA and PNP: These two have quite similar structures. Moreover, both are able to form hydrogen bonding with CDs and did led to the fluorescence quenching. However, one amino group could offer three active sites and hydroxyl group have only two, therefore, the former showed much stronger quenching capacity than the latter.

PNA, MNA and ONA: Nitro group was electron-drawing while amino group was electron-pushing, and therby both contribute to the molecular polarity of nitroaniline molecules, namely the capacity of drawing the excited electrons from CDs. Especially, it is very important to mention that such polarity would gradually decrease as distribution of the two groups went from para- to meta- and finally otho-position. Besides, the intramolecular hydrogen bonding may exhaust one active site of ONA, thus here PNA exhibited the strongest fluorescence quenching capacity, and MNA should have revealed a stronger capacity than that of ONA, but it did not. On the other hand, it was observed that the emission peaks from solutions added with ONA revealed an obvious redshift by approximately 16 nm. It is likely that the otho-position may lead to a closer distance between nitro groups and light-emitting sites of CDs, and thus enabled a direct interference with the radiative process, resulting in the unusual quenching behaviour. Anyway, the finding here was really inspiring as it offered quite facile a method for simultaneous identification of three nitroanilines.

On the whole, it is clear that both the W-N-CDs and E-N-CDs can serve as excellent probes for selective detection of PNA.