

Supporting information:

Well-defined organic fluorescent nanomaterials with AIE characteristics for colorimetric/UV-vis/fluorescent multi-channel recognition Zn^{2+} and multiple applications in plant cell and zebrafish

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1. Materials and Instruments

Unless otherwise noted, all reagents and solvents were purchased from commercial suppliers in analytical grade and were used as received. Chemicals and solvents during the experiment were of analytical grade and used without purification. The solution of metal ions was prepared from their chloride or nitrate salts. Zebrafish were provided by the Biotechnology company (Shanghai).

The ultrapure water was obtained from a water ultra-purification system in lab. ^1H -NMR spectra were recorded on a Bruker AV-600 spectrometer. Mass spectrometry (MS) was performed with an Agilent 1100 Series. UV-vis absorption spectra were acquired on a SHIMADZU UV-1800 instrument. Fluorescence spectra were measured by Agilent Cary Eclipse Fluorescence spectrophotometer. The morphology of the sensor was assessed by scanning electron microscope S-3400. FV1000 confocal laser scanning microscope was served to collect the fluorescence images.

2. Preparation of all real samples

The distilled water (bottle water, tap water and lake water) and beverage (pure milk, coffee, cola) samples were prepared. Bottle water from the drink *Wahaha*, Tap water were obtained from university chemistry lab, the lake water from local lake, beverage (pure milk, coffee, cola) samples were bought from local shop. All those water samples were used without further purification after simple filtration and static placement for 12 h.

3. Calculation of fluorescence quantum yield

The fluorescence quantum yield of the probe was determined by following equation^[1]:

$$\phi = \frac{F_x}{F_s} \cdot \frac{A_s}{A_x} \cdot \frac{n_x^2}{n_s^2}$$

(ϕ is the quantum yield, F is the area under the emission spectra, A is the absorbance at the excitation wavelength, n is the refractive index of the solvent used, x subscript

denotes unknown, and s means standard. The rhodamine B solution ($\Phi_s = 0.96$ in EtOH) was chosen as the standard substance).

4. Calculation of detection limit

The detection limit was calculated from the fluorescence titration data based on the equation^[2].

$$DOL = \frac{3\sigma}{K}$$

(σ is the standard deviation of blank experiment and K is slope of the calibration curve).

5. Gaussian simulation

All electronic structure calculations were conducted using Gaussian 16 program^[3]. The geometry optimizations were performed at the CAM-B3LYP/6-31G(d) level^[4]. On the basis of 6-311+G(d, p), the electron excitation energy and emission energy were calculated in the assumed nonequilibrium and equilibrium solvation, respectively. To be consistent with the experiment, the mixed solvent Water/EtOH = 1:1 was considered, and the SMD solvation effects model were chosen. The electron excitation characteristic was analyzed by the *hole-electron*^[5] approach, performed by Multiwfn and visualized by VMD^[6].

6. Blood compatibility test

The whole sheep blood is used as the test sample. For erythrocyte preparation experiment, PBS solution (5.0 mL) is added into the sheep blood (2.0 mL) and then shake well and centrifuged at 1000 r/ min for 15 min and liquid supernatant is removed. Furthermore, above precipitate is washed for three times by using PBS solution. Then, the remained red blood cells are centrifuged in order to remove the supernatant and putted into PBS solution to make the cell suspension of red blood, then the sample is prepared for next the experiment. In hemolysis assays, the supernatants of NC (500 μ L) are collected and mixed with red blood cell suspension in 12-well plates and oscillation

for an hour with medium speed. The sample solution in the microporous plate is removed and centrifuged at 8000 rmp for 10 min, then **BC** solution (150 μ L) is added to the 96-well plate, and the absorbance value is measured at 440 nm. Hemolysis ratio (HR) is calculated by using the flow equation:

$$\text{Hemolysis ratio} = \frac{(A_s - A_n)}{(A_p - A_n)} \times 100\%$$

where A_s , A_p , and A_n stand for the absorbance of samples, positive control (PBS solution), and negative control (0.1% triton) at 425 nm, respectively.

7. Figures

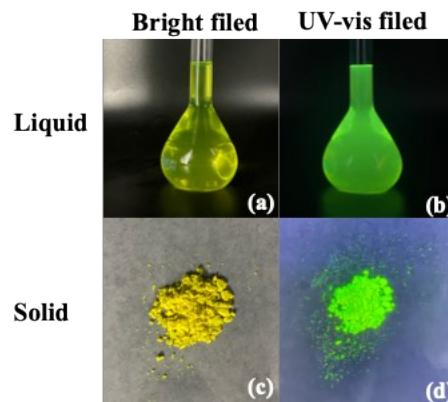


Fig. S1 Color of NC in pure ethanol solution and the solid state under sunlight and green fluorescence under ultraviolet lamp (365 nm).

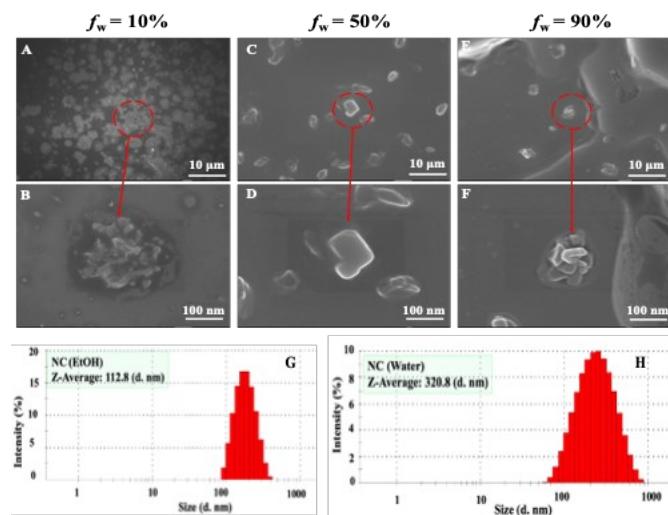


Fig. S2 Scanning electron microscope (SEM) analysis and dynamic light scattering

(DLS) measurements of NC (10 μM) in different f_w (10, 50, 90%) solution.

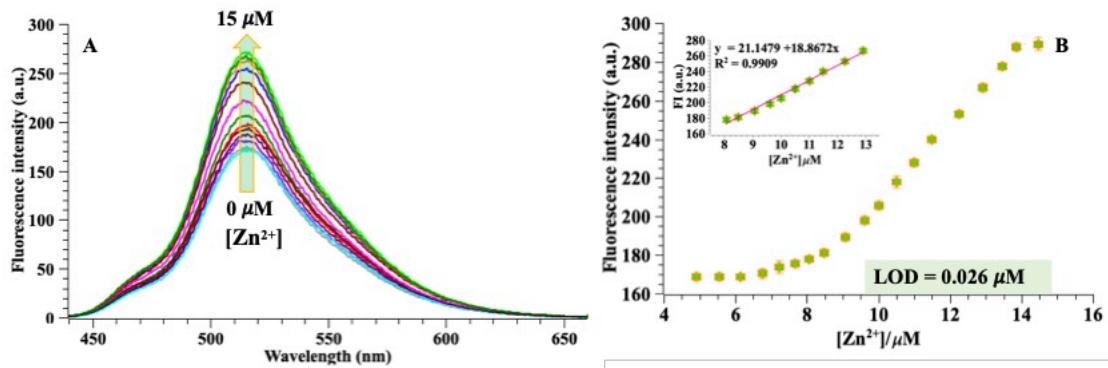


Fig. S3 (A) Fluorescence titration experiments of NC (10 μM) after addition of different concentration of Zn²⁺ (0-15 μM). (B) Scatter diagram and linear relationship of fluorescence intensity of NC (10 μM) after addition of different concentration of Zn²⁺ (0-15 μM). ($\lambda_{\text{ex}} = 425 \text{ nm}$, slit: 15/10 nm).

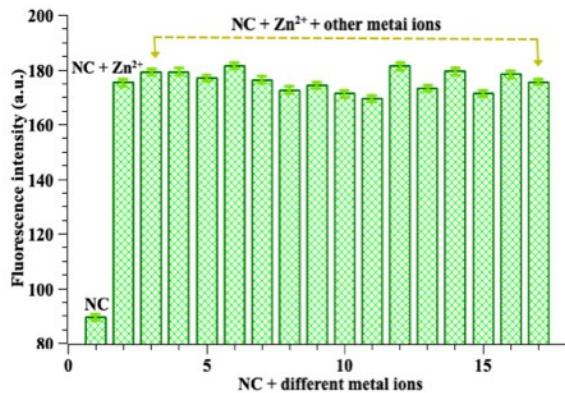


Fig. S4 Fluorescence intensity change of other metal ions (15 μM) (K⁺, Ca²⁺, Na⁺, Mg²⁺, Ba²⁺, Cu²⁺, Hg²⁺, Cd²⁺, Al³⁺, Co²⁺, Cr³⁺, Ag⁺, Ni²⁺) were added into the NC (10 μM) + Zn²⁺ mixtures. ($\lambda_{\text{ex}} = 425 \text{ nm}$), slit: 15/ 10 nm.

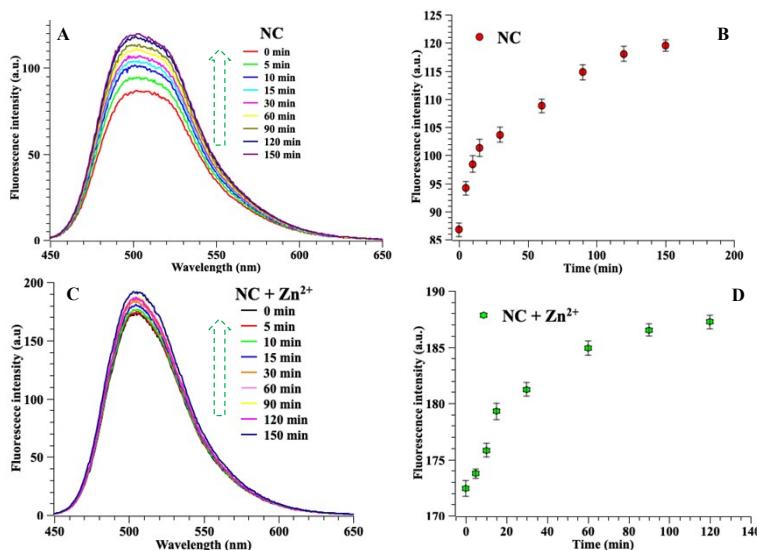


Fig. S5 Within 2 h of the test time, the change of fluorescence intensity (time stability)

of free NC (10 μ M) and NC + Zn²⁺. ($\lambda_{\text{ex}} = 425$ nm), silt: 15/ 10 nm.

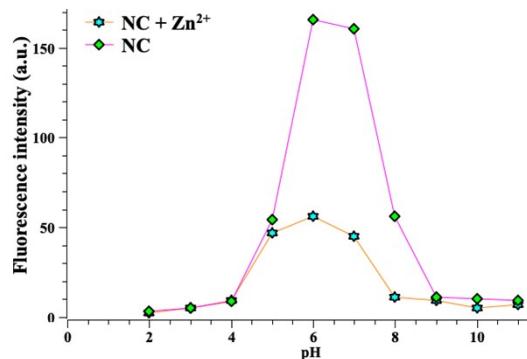


Fig. S6 Effect of different pH (2.0 -11.0) on the detection of Zn²⁺ (15 μ M) by using NC (10 μ M). ($\lambda_{\text{ex}} = 425$ nm), silt: 15/ 10 nm.

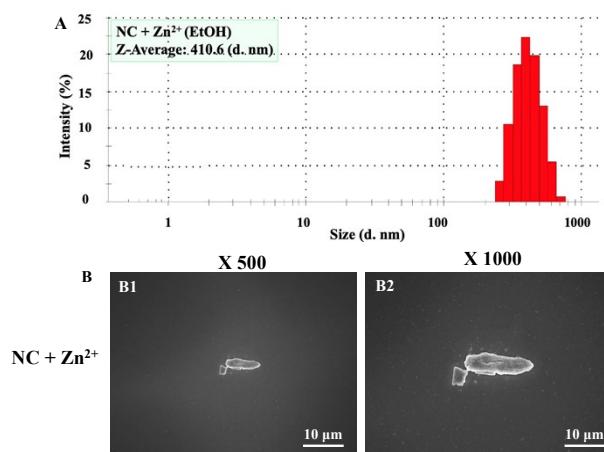


Fig. S7 Scanning electron microscope (SEM) analysis and dynamic light scattering (DLS) measurements of NC (10 μ M) with Zn²⁺ (15 μ M). Scale bar: 10 μ m.

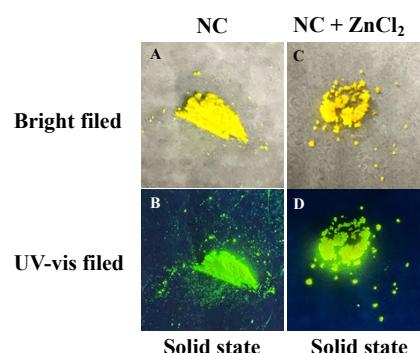


Fig. S8 Color change of solid NC and recognition solid ZnCl₂ under sunlight and ultraviolet lamp (365 nm).

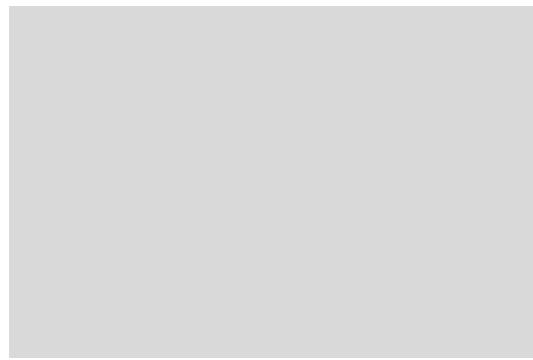


Fig.S9 Job's plot experiment of NC (10 μ M) and Zn^{2+} (15 μ M).

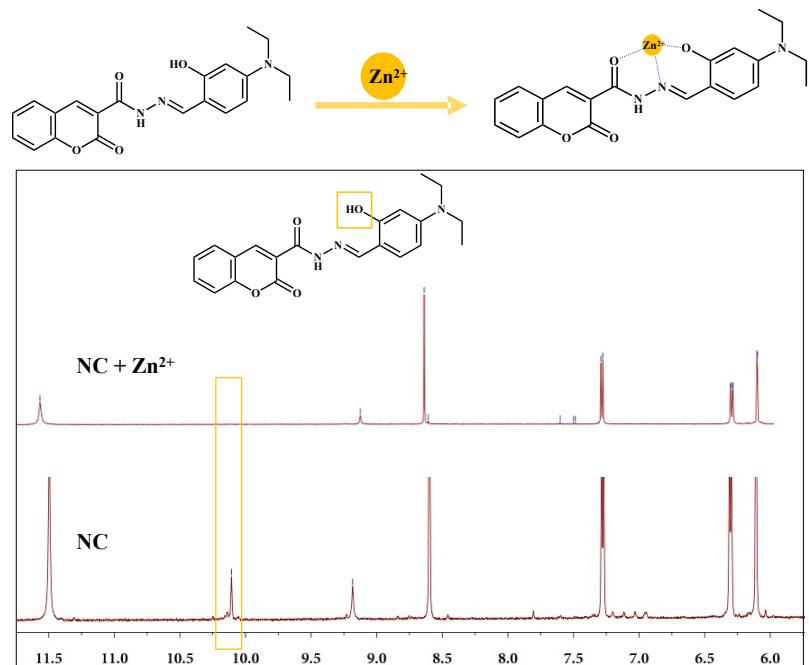


Fig.S10 Possible combinations of NC and Zn^{2+} and 1H -NMR titration of NC (10 μ M) and Zn^{2+} in DMSO.

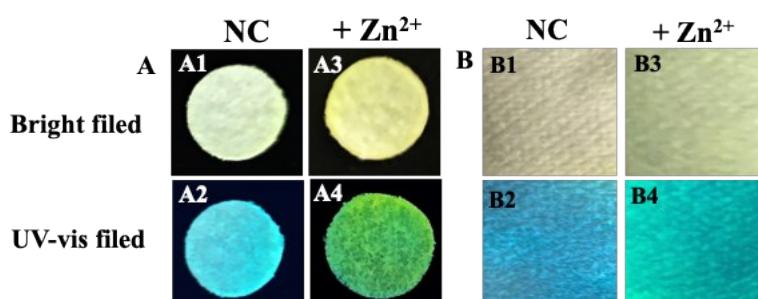


Fig.S11 (A) Color change of test strip and (B) polymer film (PU) for NC detection Zn^{2+} .

Table 1 NC is used to measure the recovery rate and error of Zn^{2+} in actual sample

Samples	Added (μM)	Found ($x^a \pm SD^b$) (μM)	Recovery (%)	RSD (%)
Bottled water	2.0	2.11± 0.52	105.5	0.45
	4.0	4.39± 0.44	109.7	0.34
	6.0	5.88± 0.12	98	0.21
Tap water	2.0	2.01± 0.16	100.5	0.12
	4.0	4.23± 0.05	102	0.31
	6.0	6.12± 0.44	101.3	0.22
Lake river	2.0	1.91± 0.13	95.5	0.17
	4.0	4.54± 0.17	113.5	0.32
	6.0	5.92± 0.09	98.6	0.51

^a Mean of three determination

^b SD: Standard deviation

Table 2 NC is used to measure the recovery rate and error of Zn²⁺ in actual sample

Samples	Added (μM)	Found ($x^a \pm SD^b$) (μM)	Recovery (%)	RSD (%)
Pure milk	2.0	1.98± 0.12	99	0.12
	4.0	3.99± 0.32	99.7	0.29
	6.0	6.21± 0.71	103.5	0.33
Coffee	2.0	2.11± 0.54	105.5	0.12
	4.0	3.98± 0.62	99.5	0.14
	6.0	6.01± 0.35	100.1	0.23
Cola	2.0	2.21± 0.19	110.5	0.71
	4.0	4.09± 0.04	102.5	0.32
	6.0	6.23± 0.43	103.8	0.24

^a Mean of three determination

^b SD: Standard deviation

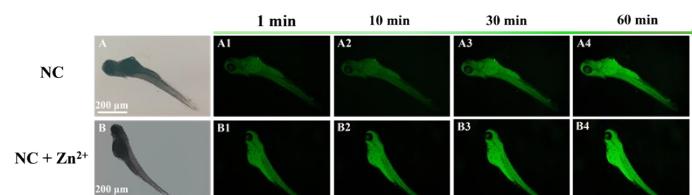


Fig.S 12 Fluorescence images of NC and NC + Zn²⁺ in zebrafish under different periods of time.

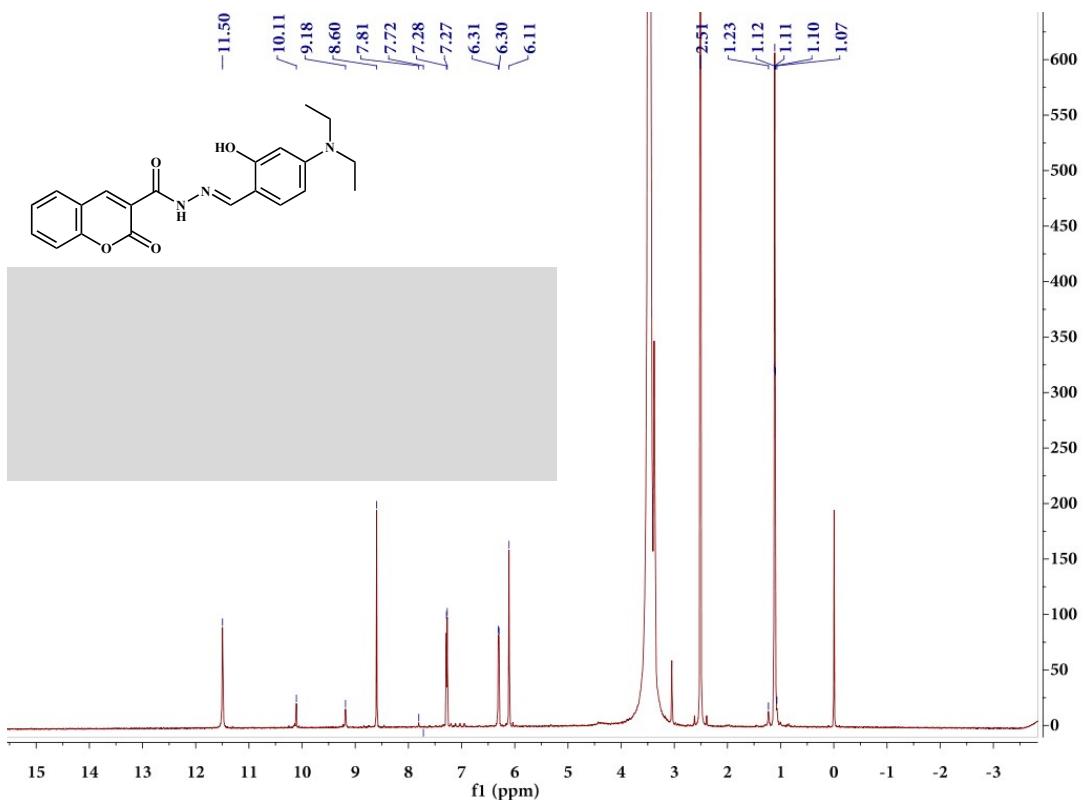


Fig.S8 ^1H -NMR of NC

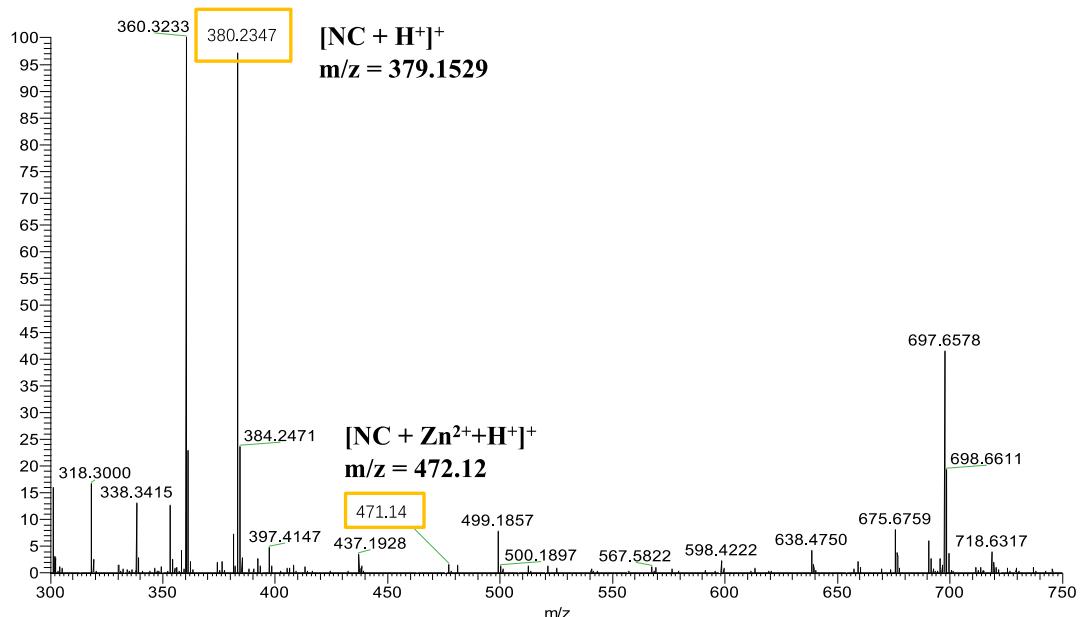


Fig.S9 HRMS of NC and NC + Zn²⁺

References:

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