# Supplementary Materials for:

## Orange emissive carbon nanodots for fluorescent and colormetric bimodal discrimination of $Cu^{2+}$ and pH

Lihong Shi [a,*], Yuejing Bao[a], Yan Zhang[a], Caihong Zhang[a], Guomei Zhang[a], Chuan Dong[a], Shaomin Shuang[a,*]

[a]*College of Chemistry and Chemical Engineering, Shanxi University, Taiyuan 030006, PR China*

**Corresponding author. E-mail address: shilihong@sxu.edu.cn, smshuang@sxu.edu.cn*

### Materials

The citric acid, 5-aminosalicylic acid, Tris, alanine, phenylalanine, methionine, glutamicacid, arginnine, lysine, tyrosine, leucine, proline, threonine, asparagine, valine, isoleucine, histidine were obtained from Shanghai Aladdin Reagent Co, Ltd. (Shanghai, China). $CuCl_2$, $CoCl_2$, $BiCl_3$, $CaCl_2$, $CdCl_2$, $HgCl_2$, KCl, $AlCl_3$, $BaCl_2$, $MgCl_2$, $MnCl_2$, NaCl, $PbCl_2$ and $ZnCl_2$ were purchased from Beijing Chemical Corp (Beijing, China). Distilled deionized (DDI) water was obtained from a Millipore Milli-Q-RO4 water purification system with a resistivity of 18.2 MΩ $cm^{-1}$ (Bedford, MA, USA). Dialysis membranes with a MWCO of 500–1000 Da were obtained from Beijing Solarbio Science & Technology Co., Ltd (Beijing, China).

### Apparatus

The transmission electron microscopy (TEM) images were obtained on a JEOL JEM-2100 transmission electron microscope (Tokyo, Japan) at an accelerating voltage of 200 kV. Atomic force microscope (AFM) images were obtained using an AFM Bruker MultiMode 8 in the contact mode. The UV-vis absorption spectrum of O-CDs was measured by Puxi TU-1901 UV–vis absorption spectrophotometer. Fluorescent properties were measured on a Hitachi F-4500 spectrophotometer (Tokyo, Japan). The X-ray photoelectron spectroscopy (XPS) was done on an AXISULTRA DLD X-ray photoelectron spectrometer (Kratos, Tokyo, Japan) with Al Kα radiation operating at 1486.6 eV. Spectra were processed by the Case XPS v.2.3.12 software using a peak-fitting routine with symmetrical Gaussian–Lorentzian functions. Fourier Transform

infrared (FTIR) spectrum was recorded on Thermo Scientific Nicolet iS50 FTIR Spectrometer. Nanosecond fluorescence lifetime experiments were performed using a FLS 920 time-correlated single-photon counting (TCSPC) system under right-angle sample geometry. All fluorescence images were collected with Zeiss LSM880 confocal laser-scanning microscope.

**Fluorescence QY measurements**

The relative fluorescence QY ($\Phi$) of the O-CDs was calculated using the equation: $\Phi_x = \Phi_{std} I_x A_{std} \eta_x^2 / (I_{std} A_x \eta_{std}^2)$. The optical densities were measured using a Puxi TU-1901 UV–vis absorption spectrophotometer. In the equation, $I_x$ and $I_{std}$ are the fluorescence intensities of the O-CDs and the standard, respectively. $A_x$ and $A_{std}$ denote the optical densities (OD) of the O-CDs and the standard, respectively. Rhodamin 6G in ethanol was chosen as a standard with a QY of 0.94 at 488 nm. $\eta_x$ and $\eta_{std}$ denote the refractive indices of the O-CDs and the standard, 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.

**MTT assay**

For the cell cytotoxicity text, HeLa cells were first plated on a Costar 96-well tissue-culture cluster and cultured at 37$^o$C with 5% $CO_2$ in air for 3 h to adhere cells onto the surface. The well without cells and treatment with O-CDs was taken as a zero set. The medium was then changed with 100 μL of fresh DMEM supplemented with 10% FBS containing O-CDs, and the cells were allowed to grow for another 24 h. At least five parallel samples were performed in each group. Cells without treatment with N-CDs were taken as a control. After adding 20 μL of 5.0 mg/mL MTT reagent into individual well, the cells were further incubated for 4 h, followed by removing the culture medium with MTT, and then 150 μL of DMSO was added. The resulting mixture was shaken for ca. 10 min at room temperature. The OD of the mixture was measured at 490 nm with a SunRisemicroplate reader (Tecan Austria GmbH, Grödig, Austria).The cell viability was estimated using the equation of Cell Viability (%) = $(OD_{Treated}/OD_{Control}) \times 100\%$, where $OD_{Control}$ and $OD_{Treated}$ were obtained in the absence and presence of O-CDs, respectively.

**Cell imaging**

In a 5% $CO_2$ incubator, HeLa 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 Hela cells were treated with mixture of O-CDs and DMEM for 2 h. The extracellular O-CDs were removed by rinsing twice with Tris-Hcl buffer solutions (pH=7.2), and then cells were incubated with 1 mL Tris-Hcl buffer (0.01 M) containing a range of concentration of $Cu^{2+}$. Immediately, fluorescence images were captured on laser scanning confocal microscope (LSCM).

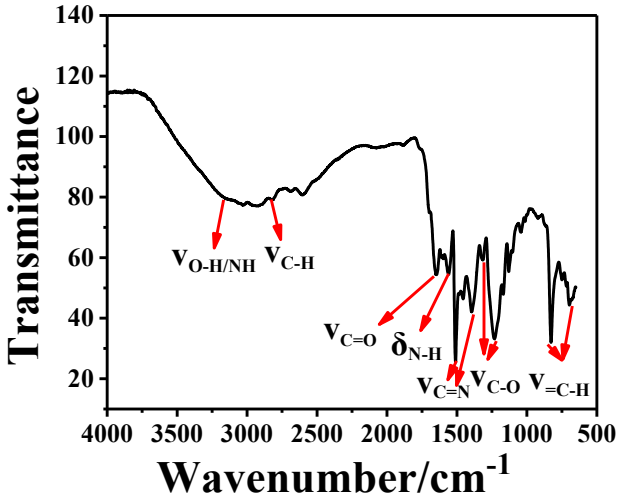


**Fig. S1** FTIR spectrum of O-CDs.

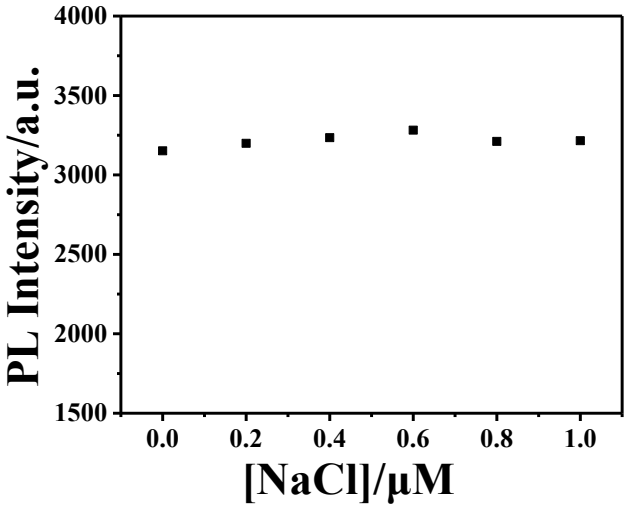


**Fig. S2** Effect of NaCl concentration on fluorescence intensity of O-CDs.

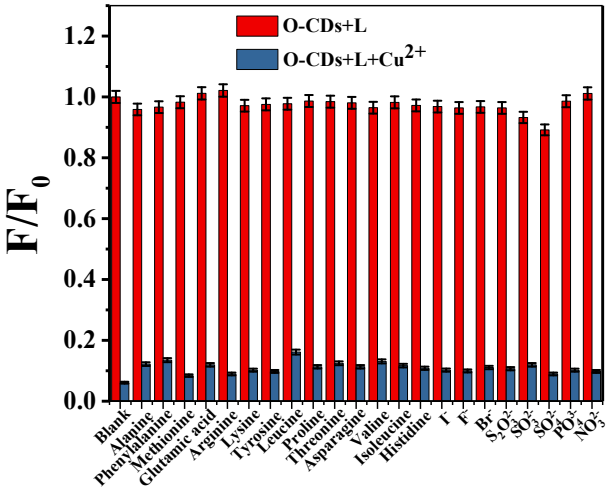


**Fig. S3** Selectivity of O-CDs for $Cu^{2+}$ against amino acids and anion under pH 7.2 conditions at 550 nm excitation.

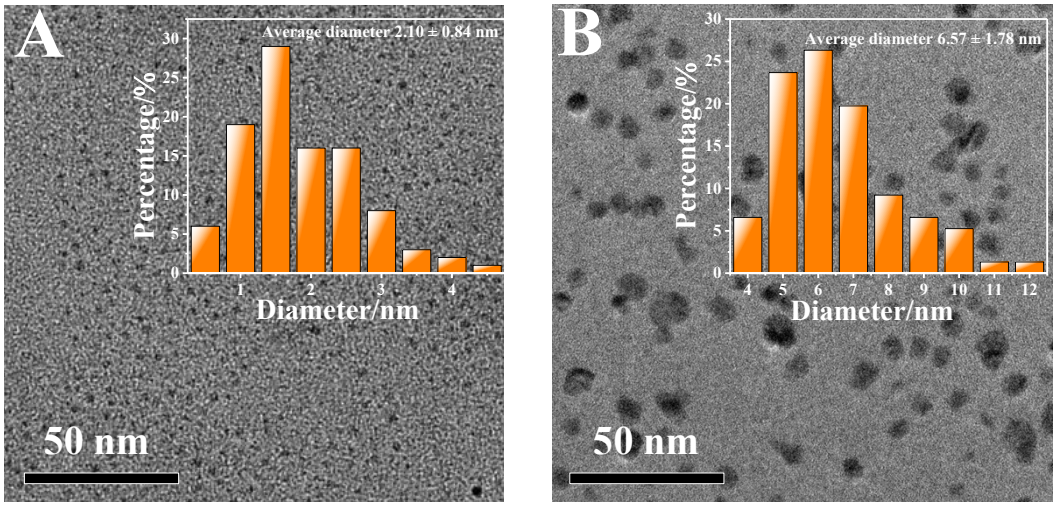


**Fig. S4** TEM images of O-CDs (A) and O-CDs/$Cu^{2+}$ (B).

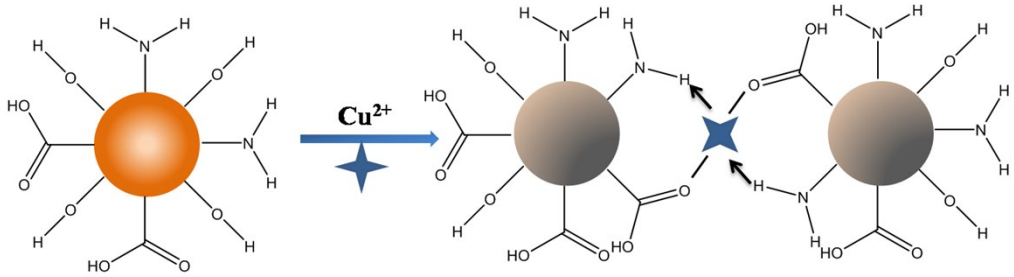


**Fig. S5** The schematic diagram of the quenching process of O-CDs coordinating with $Cu^{2+}$.

Table S1 Comparison of different fluorescent CDs based probes for $Cu^{2+}$ detection.

| Starting material of synthesis | linear scope | Detection limit | Ref. |
|---|---|---|---|
| citric acid and ure | 1–10 μM | 40 nM | [1] |
| radish | 1–5 μM | 160 nM | [2] |
| m-phenylenediamine and sulfamide | 2–60 μM | 290 nM | [3] |
| PEI and l-cysteine | 0–200 μM | 38 nM | [4] |
| 1-carboxyethyl-3-methyl imidazole chloride gel | 0–40 μM | 125 nM | [5] |
| lemon juice and l-arginine | 0–300 μM | 47 nM | [6] |
| citric acid and 5-aminosalicylic acid. | 0–300 μM | 28 nM | This work |

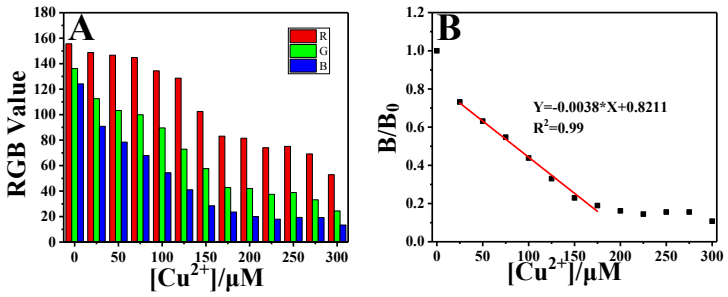


**Fig. S6** (A) RGB values versus the $Cu^{2+}$ concentration. (B) $B/B_0$ versus the $Cu^{2+}$ concentration.

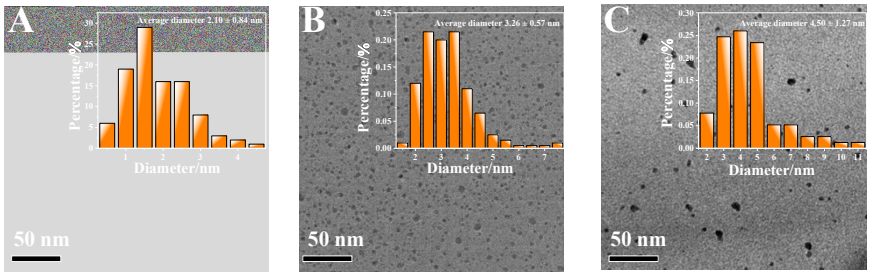


**Fig. S7** TEM images of O-CDs under different pH: (A) 7.2; (B) 8.2; (C) 9.2.

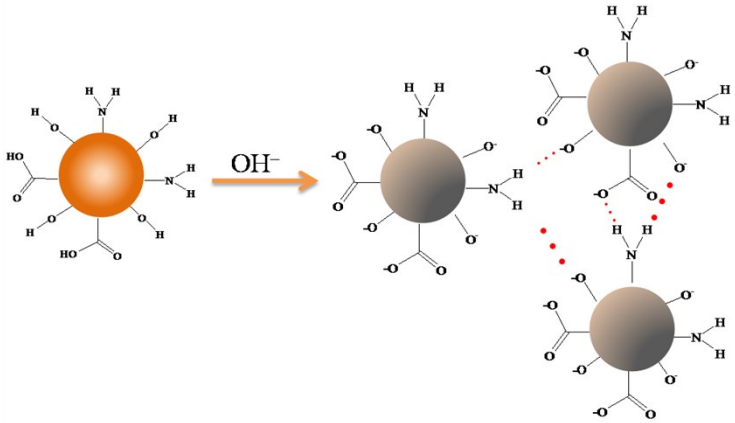


**Fig. S8** PL quenching mechanism of O-CDs under alkaline media.

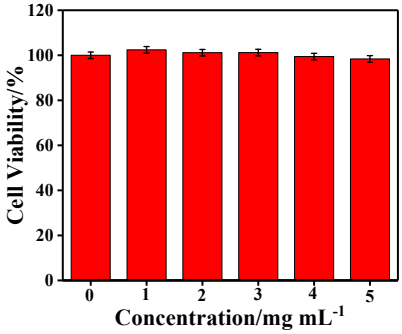


**Fig. S9** Cytotoxic effect of obtained O-CDs on HeLa cells.

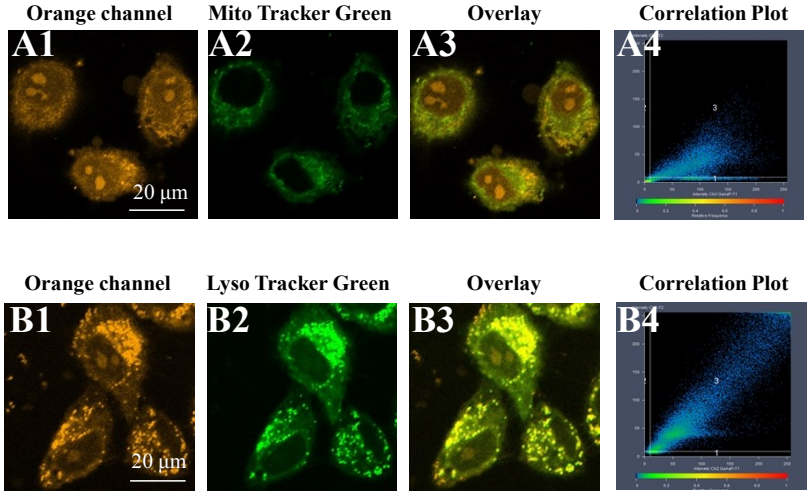


**Fig. S10** Colocalization of O-CDs in HeLa cells. (A) O-CDs and MitoTracker Green colocalization imaging. (B) O-CDs and Lyso Tracker Green colocalization imaging.

## References

1 C. D. Chen, M. Xu, W. W. Wu, and S. C. Li, *J. Alloys Compd*, 2017, **701**, 75–81.

2 J. Praneerad, N. Thongsai, P. Supchocksoonthorn, S. Kladsomboon, and P. Paoprasert, *Spectrochim. Acta A Mol. Biomol. Spectrosc*, 2018, **211**, 59–70.

3 Y. Y. Dai, Z. C. Liu, Y. F. Bai, Z. Z. Chen, J. Qin, and F. Feng, *RSC Adv.*, 2018, **8**, 42246–42252.

4 S. S. Wei, T. H. Li, X. Y. Zhang, H. Y. Zhang, C. H. Jiang, and G. Y. Sun, *Anal. Methods*, 2020, **12**, 5110–5119.

5 B. G. Wang, H. Tan, T. L. Zhang, W. M. Duan, and Y. Q. Zhu, *Analyst*, 2019, **144**, 3013–3022.