Electronic Supplementary Information (ESI)

A novel high-fluorescent S, N, O co-doped carbon dots for biosensing and bioimaging of copper ions in live cells

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Fig. S1. Plots of integrated fluorescence intensity against absorbance of Rhodamine 6G, S, N, O-CDs and undoped CDs at 460 nm.

Fig. S2. (A) XPS survey spectrum of S, N, O-CDs. (B) High resolution XPS survey spectrum of C 1s. (C) High resolution XPS survey spectrum of N 1s. (D) High resolution XPS survey spectrum of O 1s.

Fig. S3. FT-IR spectrum of S, N, O-CDs.

Fig. S4. The fluorescence quenching of S, N, O-CDs after addition of 100 μM Cu²⁺ for different times.

Fig. S5. UV-Vis spectra of m-PD, Cu²⁺, S, N, O-CDs and the mixture of S, N, O-CDs and Cu²⁺ (S, N, O-CDs/Cu²⁺).

Fig. S6. Fluorescence response of S, N, O-CDs towards common anion.

Fig. S7. Comparison of S, N, O-CDs after adding various amino acids.

Fig. S8. Cytotoxicity testing results of S, N, O-CDs on Hela cells viability. The values represent percentage cell viability (mean% ±SD, n=6).

Fig. S9. The fluorescence of mixture of S, N, O-CDs and 100 μM Cu²⁺ after addition of 200 μM PPI for different times.

Fig. S10. Fluorescence response of S, N, O-CDs towards different potential interferences in the presence of 100 μM Cu²⁺. The concentrations of PPI and potential interferences were 200 μM and 0.1 M, respectively. F₀ and F represent the fluorescence of S, N, O-CDs in the absence of PPI and in the presence of PPI or potential interferences.

Fig. S11. The fluorescence of mixture of S, N, O-CDs, 100 μM Cu²⁺ and 200 μM PPI after addition of 160 U/L ALP for different times.

Fig. S12. Fluorescence response of S, N, O-CDs towards different potential interferences in the presence of 100 μM Cu²⁺ and 200 μM PPI. The concentrations of ALP or potential interferences was 160 U/L, respectively. F₀ and F represent the fluorescence of S, N, O-CDs in the absence of ALP and in the presence of ALP or potential interferences.

Table S1. Comparison of fluorescence quantum yield of different carbon dots.
Table S2. Comparison of limit detection of CDs-based Cu²⁺ probes.
Table S3. Detection of PPI and ALP in human urine samples or serum samples.
Fluorescence Quantum Yield ($\Phi_s$) Measurement: Rhodamine 6G is chosen as a standard (Photochemistry and photobiology, 1969, 9: 439-444). The optical densities were measured on a Lambda 35 UV-vis spectrometer spectrophotometer. Absolute values are calculated using the standard reference sample that has a fixed and known fluorescence quantum yield value, according to the following equation:

$$\Phi_X = \Phi_{ST} \left( \frac{M_X}{M_{ST}} \right) \left( \frac{\eta_X}{\eta_{ST}} \right)^2$$

Where the subscripts ST and X denote standard and determinand samples respectively, $\Phi$ is the quantum yield, M is the gradient of integrated fluorescence vs. absorbance, and $\eta$ is the refractive indices of the solvents.
**Fig. S1.** Plots of integrated fluorescence intensity against absorbance of Rhodamine 6G, S, N, O-CDs and undoped CDs at 460 nm.
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Fig. S11. The fluorescence of mixture of S, N, O-CDs, 100 μM Cu$^{2+}$ and 200 μM PPi after addition of 160 U/L ALP for different times.
**Fig. S12.** Fluorescence response of S, N, O-CDs towards different potential interferences in the presence of 100 μM Cu$^{2+}$ and 200 μM PPI. The concentrations of ALP or potential interferences was 160 U/L, respectively. F$_0$ and F represent the fluorescence of S, N, O-CDs in the absence of ALP and in the presence of ALP or potential interferences.
Table S1. Comparison of fluorescence quantum yield of different carbon dots *.

| Carbon dots          | Standard Reference   | Quantum Yield | Reference |
|----------------------|----------------------|---------------|-----------|
| S,N,O codoped        | Quinine Sulfate      | 42.8%         | S1        |
| S,N,O codoped        | Quinine Sulfate      | 21.6%         | S2        |
| N-doped              | Rhodamine 6G         | 18.7%         | S3        |
| S,N,O codoped        | Quinine Sulfate      | 73%           | S4        |
| N-doped              | Rhodamine B          | 47%           | S5        |
| S-doped              | Quinine Sulfate      | 5.77%         | S6        |
| S-doped              | Quinine Sulfate      | 21.4%         | S7        |
| S,N,O codoped        | Quinine Sulfate      | 63.8%         | S8        |
| S,N,O codoped        | Rhodamine 6G         | 78.6%         | this work |

* The quantum yields (QY) of quinine sulfate (0.1 M H$_2$SO$_4$) at 350 nm and 366 nm excitation wavelength are 57.7% and 53%, respectively. Similarly, the QY of Rhodamine 6G in ethanol at 488 nm excitation wavelength is 94%. The QY of Rhodamine B using ethanol as a solvent are 69% and 89% under 365 nm and 495 nm excitation wavelength, respectively.
**Table S2.** Comparison of limit detection of CDs-based Cu\(^{2+}\) probes.

| Fluorescent carbon dots (CDs)                                      | Limit of detection | Reference |
|--------------------------------------------------------------------|--------------------|-----------|
| B, N-CDs                                                           | 0.3 µM             | S9        |
| Calcium phosphate/CDs hybrid composites                            | 9.82 µM            | S10       |
| CDs                                                               | 4.8 µM             | S11       |
| CDs                                                               | 0.76 µM            | S12       |
| CDs/polymeric matrix                                              | 0.71 µM            | S13       |
| Cyclam-functionalized CDs                                         | 0.1 µM             | S14       |
| Branched-polyethylenimine (BPEI)-capped CDs                       | 0.115 µM           | S15       |
| S, N, O-CDs                                                       | 0.29 µM            | this work |
Table S3. Detection of PPi and ALP in human urine samples or serum samples

| Real samples   | Added  | Found   | Recovery (%) | RSD (n=3, %) |
|----------------|--------|---------|--------------|--------------|
| Urine samples  |        |         |              |              |
| (PPi)          | 20 µM  | 38.78 µM| 107.84       | 3.12         |
|                | 40 µM  | 56.42 µM| 97.28        | 4.44         |
|                | 80 µM  | 100.89 µM| 104.54      | 5.31         |
| Serum samples  |        |         |              |              |
| (ALP)          | 20 U/L | 18.95 U/L| 94.73        | 5.13         |
|                | 60 U/L | 62.41 U/L| 104.02      | 4.04         |
|                | 100 U/L| 104.72 U/L| 104.72      | 4.51         |
|                | 140 U/L| 147.58 U/L| 105.40      | 5.03         |

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