**Electronic Supplementary Material**

**Nitrogen and** **chlorine co-doped carbon dots as probe for sensing and imaging in biological samples**

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**Fig S1.tif**

**Fig. S1** TEM images (a) of Orn-CDs (inset: the HRTEM image of the Orn-CDs),

and the corresponding SAED image (b) and XRD patterns of Orn-CDs (c).

1200 FTIR.tif

**Fig. S2** FTIR spectra of the prepared Orn-CDs.

XPS.tif

**Fig. S3** (a) XPS survey spectra, high resolution XPS spectra of C 1s (b), N 1s (c), O 1s (d) of the prepared Orn-CDs

**Figure S4.tif**

**Fig. S4** (a) The influence of pH value (3.0-8.0) of Tris-HCl solution (20 mmol L-1) on the Orn-CDs (0.15 mg mL-1) fluorescence quenching efficiency (Effq, black line) by 200 µmol L-1 Fe3+ and Orn-CDs (0.15 mg mL-1)/Fe3+ (200 µmol L-1) platform fluorescence recovering efficiency (Effr, red line) after addition of 50 µmol L-1 ascorbic acid. (b) The effect of concentrations of Orn-CDs (from left to right : 0.05, 0.10, 0.15, 0.20, 0.25 mg mL-1) on the fluorescence quenching efficiency (Effq, black line) by 200 µmol L-1 Fe3+ and Orn-CDs (0.15 mg mL-1)/Fe3+ (200 µmol L-1) platform fluorescence recovering efficiency (Effr, red line) after addition of 50 µmol L-1 ascorbic acid. (c) Time dependent FL response of 0.15 mg/ml Orn-CDs to 20 µmol L-1 Fe3+ in Tris-HCl solution at room temperature. (d) Time dependent fluorescence response of 0.15 mg mL-1 of Orn-CDs /200 µmol L-1 of Fe3+ to 10 µmol L-1 ascorbic acid in Tris-HCl solution at room temperature.

Table S1 Comparison of Orn-CDs and other carbon dots based probes for Fe3+ detection

|  |  |  |  |
| --- | --- | --- | --- |
| samples | linear range  (µmol L-1) | detection limit  (µmol L-1) | ref |
| water samples  human serum, urine | —  0.3-546 | 0.01  0.09 | [1](#_ENREF_1)  [2](#_ENREF_2) |
| water samples | 0.11-4.46 | 0.05 | [3](#_ENREF_3) |
| water samples  water samples  drinking and tap water  tap water,fruits | —  —  —  25-200 | 10  10  0.29  0.05882 | [4](#_ENREF_4)  [5](#_ENREF_5)  [6](#_ENREF_6)  [7](#_ENREF_7) |
| water, urine | 0.05-10.0 | 0.0137 | [8](#_ENREF_8) |
| human serum, urine | 0.3-50.0 | 0.0956 | this work |

Fe3+ selectively.tif

**Fig. S5** Selectivity of Orn-CDs toward different metal ions. The concentration of Orn-CDs is 0.15 mg mL-1 in Tris-HCl solution, 50 µmol L-1 for Fe3+ and 2 mmol L-1 for other metal ions.

on-off-on.tif

**Fig. S6** Photographs of Orn-CDs (5 mg mL-1) (left), Orn-CDs(5 mg mL-1) + Fe3+(10 mmol L-1 (middle), and Orn-CDs(5 mg mL-1) + Fe3+ (10 mmol L-1) + ascorbic acid (10 mmol L-1) (right).



**Fig. S7** Fluorescence spectra of 0.15 mg mL-1 of Orn-CDs before and after additon of ascorbic acid (10 µmol L-1).

Table S2 Comparison of Orn-CDs and other probes for ascorbic acid detection

|  |  |  |  |
| --- | --- | --- | --- |
| samples | linear range  (µmol L-1) | detection limit  (µmol L-1) | ref |
| fruits  human urine  juice  human serum  human blood plasma  water  human urine | 25-300  0.2-11.0  1-90  0.15-15.0  1.5-10  8-100  0.5-10.0 | 0.236  0.01  0.018  0.105  0.2  2.4  0.137 | [7](#_ENREF_7)  [8](#_ENREF_8)  [9](#_ENREF_9)  [10](#_ENREF_10)  [11](#_ENREF_11)  [12](#_ENREF_12)  this work |

LAA selectivity.tif

**Fig. S8** Selectivity of Orn-CDs/Fe3+ system toward ascorbic acid. The concentration of Orn-CDs is 0.15 mg mL-1 in Tris-HCl solution, 200 µmol L-1 of Fe3+and 10 µmol L-1 of ascorbic acid .The concentration of other interference analytes is 100 µmol L-1.

Absorbance spectra CDS Fe3+ LAA.tif

**Fig. S9** UV-vis spectra of the prepared Orn-CDs (0.15 mg mL-1, black line), 0.15 mg mL-1 Orn-CDs/200 µmol L-1 of Fe3+ (red line), and 0.15 mg mL-1 Orn-CDs/200 µmol L-1 Fe3+/AA (blue line for 10 µmol L-1 AA, green line for 20 µmol L-1 AA, purple line for 50 µmol L-1 AA respectively)

Fluorescence decay curves were performed in a time–correlated-single-photo- counting (TCSPC) system from FL980 spectrometer under excitation at 326 nm. Data were fit by using the bi-exponential function in equation (1).

  (1)

α1 and α2 were the fractional contributions of time-resolved decay lifetime of and.

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**Fig. S10** Fluorescence decays of the prepared Orn-CDs (0.15 mg mL-1, black curve) upon addition of 100 µmol L-1 Fe3+ (red curve) at excitation/emission wavelengths (λex/λem) of 326/404 nm.

**Table S3.** Double-exponential fitting of Orn-CDs and Orn-CDs /Fe3+ decay curves.

|  |  |  |
| --- | --- | --- |
| Sample name | Orn-CDs | Orn-CDs /Fe3+ |
| *τ1*(ns)/*A1* (%) | 3.5841/36.3 | 2.4074/21.24 |
| *τ2*(ns)/*A2* (%) | 12.4129/63.7 | 8.6186/78.76 |
| Average *τ* (ns) | 9.21 | 7.30 |

**Table S4**. Fe3+ determination results in human serum and urine samples.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sample | Added  (µmol L-1) | Found  (µmol L-1) | Recovery (%, n=3) | RSD (%, n=3) |
| Serum | 0.50 | 0.542 | 108.4 | 2.41 |
|  | 5.00 | 5.126 | 102.5 | 3.80 |
|  | 20.00  50.00 | 18.811  53.837 | 94.1  107.7 | 1.48  2.30 |
| Urine | 0.50 | 0.480 | 95.9 | 1.59 |
|  | 5.00 | 4.921 | 98.4 | 3.67 |
|  | 20.00  50.00 | 20.468  53.236 | 102.3  106.5 | 2.17  3.75 |

**Table S5**. Ascorbic acid determination results in human urine samples.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sample | Added  (µmol L-1) | Found  (µmol L-1) | Recovery (%, n=3) | RSD (%, n=3) |
| Urine 1 | 0.50 | 0.470 | 94.0 | 2.21 |
|  | 2.00 | 1.887 | 94.4 | 3.10 |
|  | 5.00  10.00 | 5.166  9.878 | 103.3  98.8 | 2.12  3.16 |
| Urine 2 | 0.50 | 0.474 | 94.9 | 2.07 |
|  | 2.00 | 2.136 | 106.8 | 2.78 |
|  | 5.00  10.00 | 4.831  10.490 | 96.6  104.9 | 3.47  2.66 |

**Cell viability assay**

The biocompatibility of Orn-CDs against A549 cells were investigated by MTT assay. After seeding A549 in 96-well plate at 1×104/well and cultured for 24 h at 37°C under 5% CO2, the supernatant was discarded and the cells were washed three times with PBS, subsequently, different concentration (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 mg mL-1 ) of the Orn-CDs in DMEM medium were added (100 μL/well) and clutured for furthe 24 h at 37°C. Finally, MTT (5 mg mL-1, 20 μL/well) was added and incubated for another 4 h. After removing the culture medium, DMSO (150 μL/well) was added and shaken at 37°C for 10 min. A microplate reader (Bio Tek Epoch) was used to measure the absorbance of each sample at 490 nm. The data represent the mean ± standard deviation of four independent experiments. The cell viability was expressed as the below formula:

where ODcontrol was acquired before addition of Orn-CDs and ODtreated was acquired in the presence of Orn-CDs.

FitureS5 MTT.tif

**Fig. S11** Viability of the prepared Orn-CDs against A549 cells.

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