Supplementary Material

Dual-color reversible fluorescent carbon dots designed for dynamic monitoring of Cellular superoxide anion radicals

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Section 1. Materials and Apparatus

Caffeic acid (CA) was purchased from Yuanye (Shanghai, China), 4,4′,4″,4‴-(Porphine-5,10,15,20-tetrayl) tetrakis (benzoic acid) (TCPP) was purchased from Macklin (Shanghai, China), Superoxide ($O^{\bullet-}_2$) was delivered from KO_2 in a DMSO solution. H_2O_2 and ClO^- were obtained by direct dilution of aqueous solutions of NaClO and H_2O_2, respectively. The hydroxyl radical (•OH) was generated through the reaction of 1 mM Fe^{2+} with 200 μM H_2O_2 [1].

All fluorescence spectra were measured with a Horiba Jobin Yvon Fluoromax-4 spectrofluorometer (France). Fourier-transform infrared (FT-IR) spectra were carried out using an IR Affinity-1S spectrometer (Shimadzu, Japan). Fluorescent images were observed with a confocal laser scanning microscope (STELLARIS, Leica, Germany) and processed using the LAS X imaging software.

Section 2. CDs selectivity and disruptive concentration of various active species

60 μM $O^{\bullet-}_2$, 100 μM Na^+, 100 μM K^+, 100 μM Zn^{2+}, 100 μM Ca^{2+}, 100 μM ATP, 100 μM AMP, 100 μM Glu, 100 μM Cys, 100 μM GSH, 100 μM AA, 100 μM Glu,E, 100 μM ASP, 100 μM Trp, 100 μM Ala, 100 μM Mg^{2+}, 50 μM Cu^{2+}, 50 μM Fe^{3+}, 50 μM H_2O_2, 50 μM •OH, 20 μM ClO^-,20 μM $ONOO^-$,100 μM TRY,20 μM NO.

Section 3. Cytotoxicity assay

LO2 cells were seeded at a density of 3 × 10^5 cells/well in 96-well plates and cultured in DMEM complete medium for 24 h at 37 °C in a sterile incubator with 5% CO_2. Afterward, 100 μL of fresh medium containing different concentrations of CDs was added to each well, and the culture was continued for 24 h. Then, 20 μL of a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (5 mg/mL) was added to each well and incubated for an additional 4 h. Afterward, the supernatant was removed, and 200 μL of DMSO was added to each well. The relative cell viability was calculated by measuring the absorbance at 492 nm of the cells in each well using a microplate reader [2].

Section 4. Figures

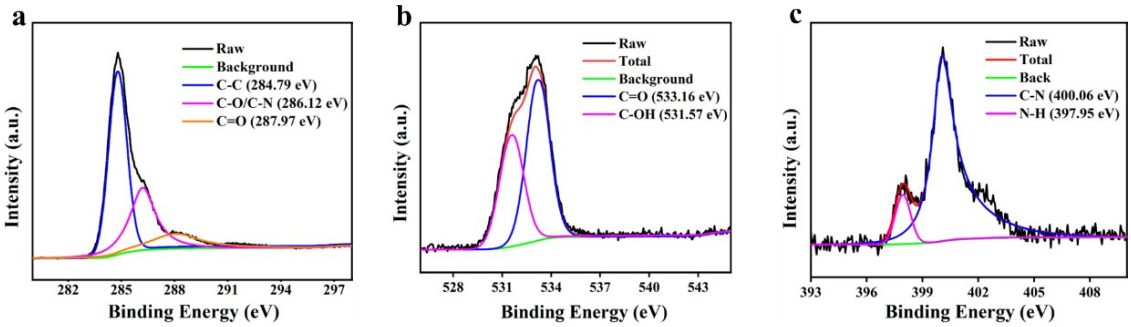

Fig. S1 XPS analysis of (a) C 1s, (b) O 1s, (c) N 1s.

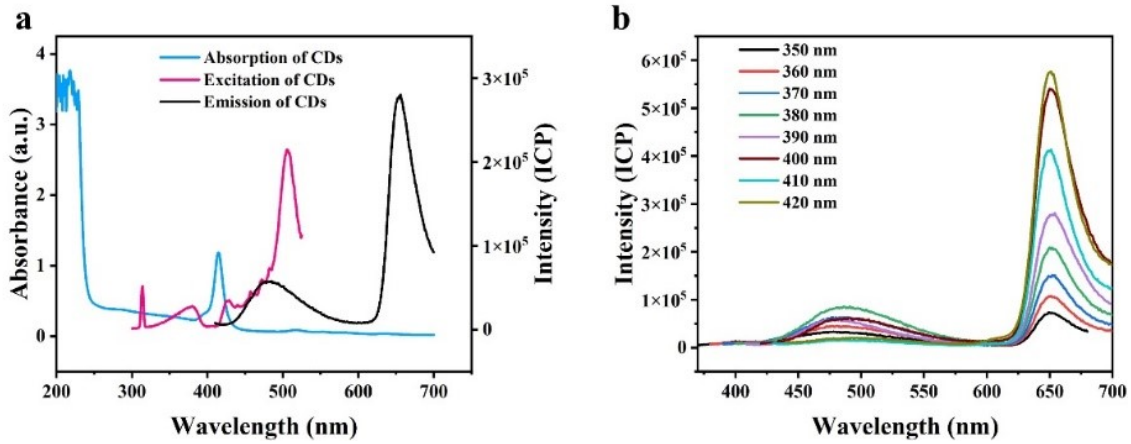

Fig.S2 (a) UV/vis absorption spectrum of CDs, emission and excitation spectra of CDs. (b) the fluorescence spectra of CDs at different excitation wavelengths.

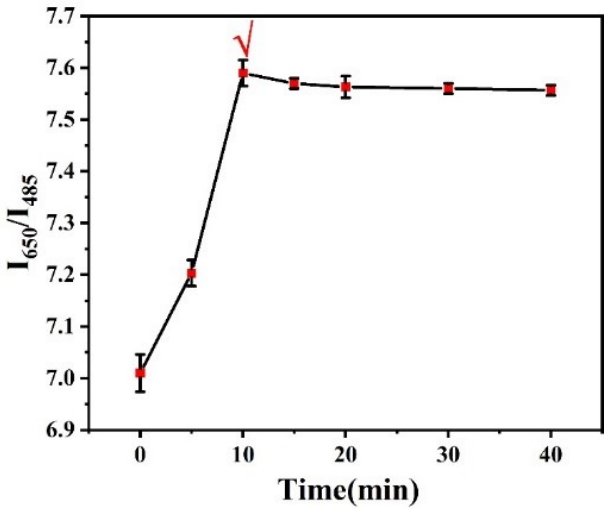

Fig. S3 Incubation time on the sensing system.

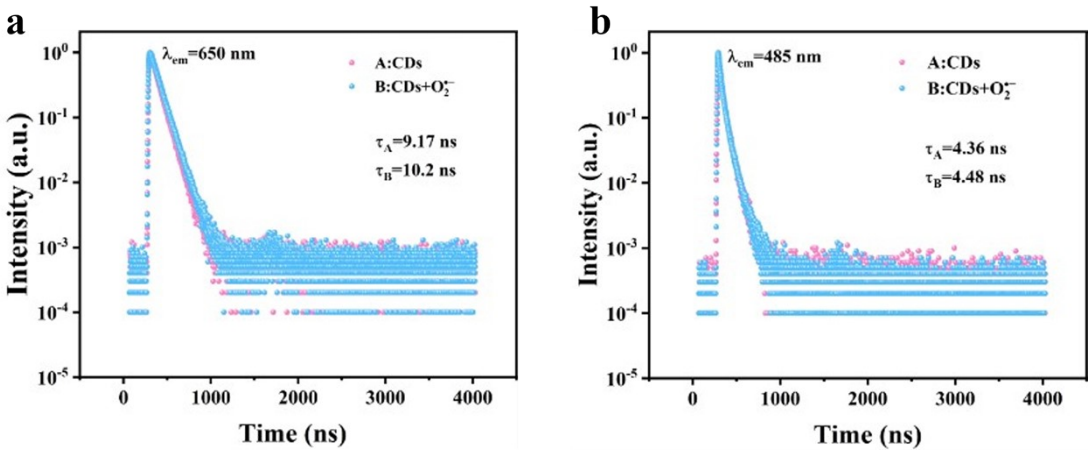

Fig. S4 Fluorescence decay lifetime of CDs in the absence and presence of $O^{\bullet}_{2}^{-}$ (a) λ_{em} = 485 nm and (b) λ_{em} = 650 nm.

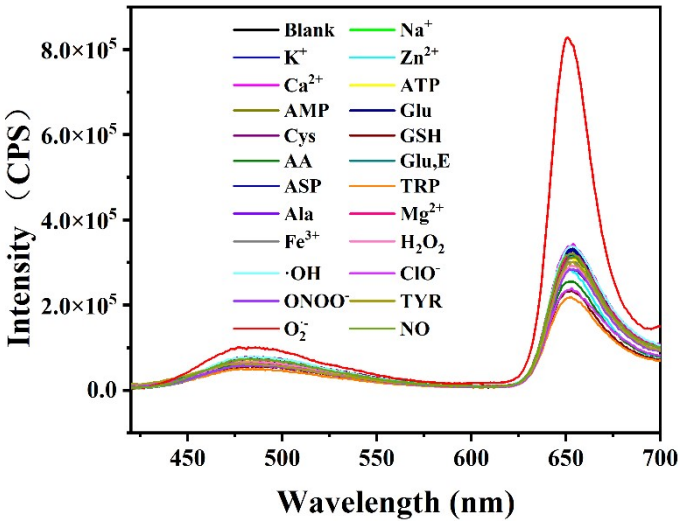

Fig. S5 Fluorescence profiles of various interfering substances after reaction with CDs

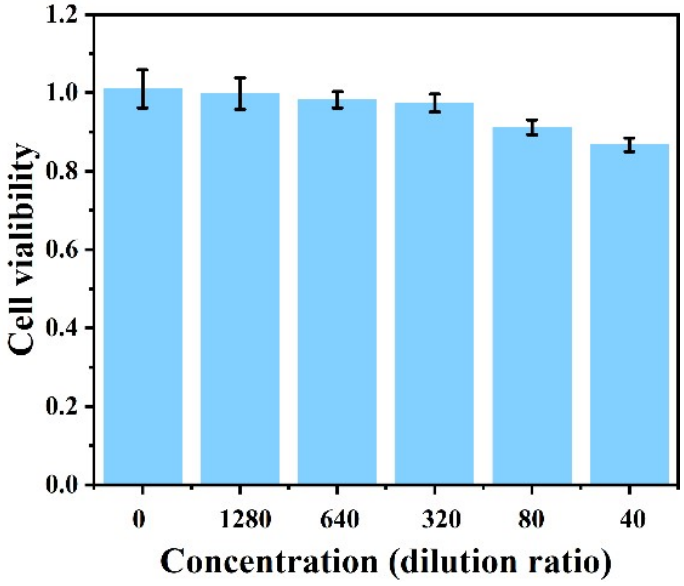

Fig. S6 Cellular activity after co-incubation with CDs at different dilution levels

Section 4 References

1. Y. Zhou, P. Li, N. Fan, X. Wang, X. Liu, L. Wu, W. Zhang, W. Zhang, C. Ma and B. Tang, *Chemical Communications*, 2019, 55, 6767-6770.
2. Y. Zhou, E. O. Hileman, W. Plunkett, M. J. Keating and P. Huang, *Blood*, 2003, 101, 4098-4104.