## **Supporting Information**

## Lysosome-Targeted Carbon Dots for Ratiometric Imaging of Formaldehyde in Living Cells

Haifang Liu,<sup>‡a</sup> Yuanqiang Sun,<sup>‡a</sup> Zhaohui Li,<sup>a\*</sup> Jie Yang,<sup>a</sup> Aaron Albert Aryee,<sup>a</sup>

Lingbo Qu,<sup>a\*</sup> Dan Du,<sup>b\*</sup> and Yuehe Lin<sup>b</sup>

 <sup>a</sup> Institute of Chemical Biology and Clinical Application at the First Affiliated Hospital, Henan Joint International Research Laboratory of Green Construction of Functional Molecules and Their Bioanalytical Applications, College of Chemistry and Molecular Engineering, Zhengzhou University, Zhengzhou 450001, P.R. China;
 <sup>b</sup> School of Mechanical and Materials Engineering, Washington State University, Pullman, Washington 99164, United States.

\**Corresponding authors. Email: <u>zhaohui.li@zzu.edu.cn</u>, <u>qulingbo@zzu.edu.cn</u>, <u>annie.du@wsu.edu</u>.* 

## **Table of contents**

Figure S3. Fluorescence ratio response of CDs ( $50 \mu g/mL$ ) to various species (1 mM), including FA (1), amino acids (2. Lys, 3. Arg, 4. Tyr, 5. Trp, 6. Thr, 7. Ser, 8. Leu, 9. Ile, 10. His, 11. Pro, 12. Met, 13. Gln, 14. Glu, 15. Asn, 16. Asp, 17. Phe, 18. Val, 19. Ala, 20. Gly), cations (21. Zn<sup>2+</sup>, 22. Fe<sup>3+</sup>, 23. Fe<sup>2+</sup>, 24. Cu<sup>2+</sup>, 25. K<sup>+</sup>, 26. Ca<sup>2+</sup>, 27. Na<sup>+</sup>, 28. Mg<sup>2+</sup>), PBS (29.10 mM pH=7.4 PBS), proteins (30. HSA: human serum albumin, 31. bovine serum albumin, 32. albumin from chicken egg white), serum (33. fetal bovine serum), culture medium (34. high glucose Dulbecco's modified Eagle medium).-----S6 Figure S4. The fluorescence ratio values of CDs with or without FA at different pH values.----S7 Figure S5. FTIR spectra of CDs and the product after reaction with FA.-----S8 Figure S6. Cell viability of HeLa cells after incubated with a series concentrations of CDs (the viability of the cells without CDs is defined as 100%). The results are the mean SD of six separate measurements.-----S9 Figure S7. Fluorescence images of HeLa cells after being incubated with CDs (10-30 µg/mL) at 37 °C for 10 min. The images were collected in bright field (A1-C1), 450-550 nm (A2-C2) and 550-650 nm (A3-C3) with the excitation wavelength at 405 nm. Scale bar: 20 μm.-----S10 Figure S8. Fluorescence images of HeLa cells which incubated with CDs in the presence of NaHSO<sub>3</sub> or FA. (A) HeLa cells with a treatment of 200 µM NaHSO<sub>3</sub> for 30 min before incubated with CDs (30 µg/mL, 10 min). (B) HeLa cells were incubated with CDs (30 µg/mL) for 10 min. (C) HeLa cells with a treatment of 200  $\mu$ M FA for 20 min before incubated with CDs (30  $\mu$ g/mL, 10 min). (D) HeLa cells with a treatment of 400  $\mu$ M FA for 20 min before incubated with the CDs (30  $\mu$ g/mL,

10 min). The images were collected in bright field (A1-D1), 450–550 nm (green channel) (A2-D2) and 550–650 nm (orange red channel) (A3-D3) with the excitation wavelength at 405 nm. Scale bar: 20 μm.-----S11 **Figure S9**. The hydrodynamic diameter distribution of CDs (A) and the product after reaction with FA (B).-----S12 **Figure S10**. The zeta potential of CDs and the product after reaction with FA.----S13 **Figure S11**. High-resolution XPS spectra of C1s (A), O1s (B) and F1s (C) peaks of CDs. (A) The peaks at 283.9 eV for C-F, 284.5 eV for C-C/C-H, 285.3 eV for C-N, 286.3 eV for C-O. (B) The peaks at 531.8 eV for C=O, 532.8 eV for C-O. (C) The peaks at 686.1 eV for C-F (between sp<sup>2</sup> C and F), 687.7 eV for C-F (between sp<sup>3</sup> C and F).-----S14 **Figure S12**. The photograph of CDs under 405 nm light irradiation.----S15 **Table S1**. Comparison of the reaction time of the developed method for the determination of FA with published methods.-----S16

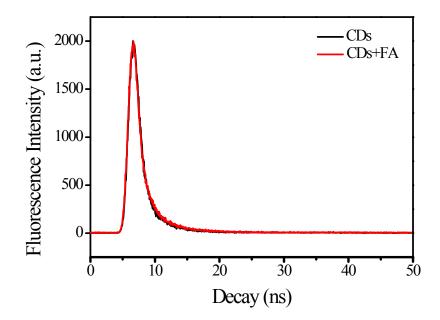
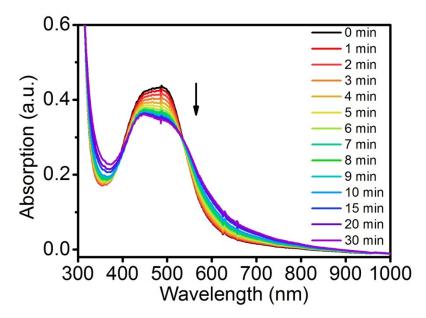
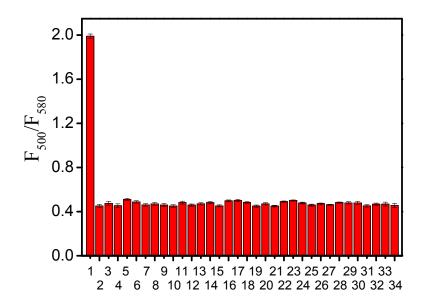


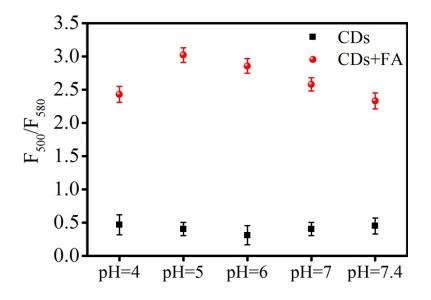
Figure S1. Fluorescence lifetime decay of CDs in the absence and presence of FA.



**Figure S2**. UV-vis spectra of the time dependent manner on CDs sensing for FA (the concentration of FA used is 1 mM).



**Figure S3**. Fluorescence ratio response of CDs (50  $\mu$ g/mL) to various species (1 mM), including FA (1), amino acids (2. Lys, 3. Arg, 4. Tyr, 5. Trp, 6. Thr, 7. Ser, 8. Leu, 9. Ile, 10. His, 11. Pro, 12. Met, 13. Gln, 14. Glu, 15. Asn, 16. Asp, 17. Phe, 18. Val, 19. Ala, 20. Gly), cations (21. Zn<sup>2+</sup>, 22. Fe<sup>3+</sup>, 23. Fe<sup>2+</sup>, 24. Cu<sup>2+</sup>, 25. K<sup>+</sup>, 26. Ca<sup>2+</sup>, 27. Na<sup>+</sup>, 28. Mg<sup>2+</sup>), PBS (29.10 mM pH=7.4 PBS), proteins (30. HSA: human serum albumin, 31. bovine serum albumin, 32. albumin from chicken egg white), serum (33. fetal bovine serum), culture medium (34. high glucose Dulbecco's modified Eagle medium).



**Figure S4**. The fluorescence ratio values of CDs with or without FA at different pH values.

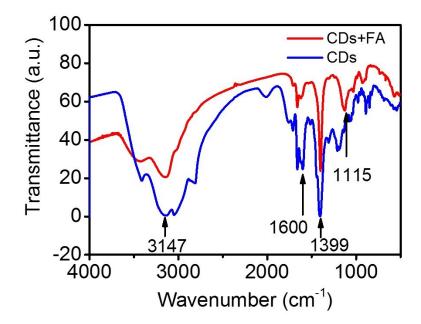
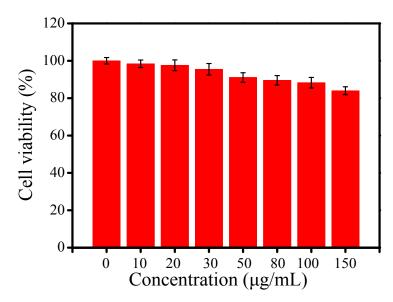
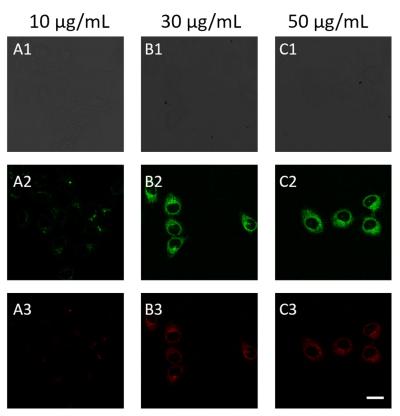


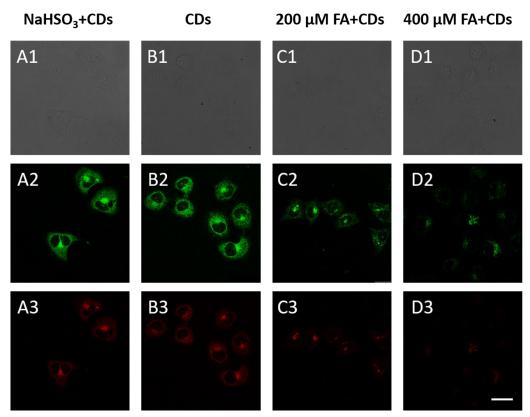
Figure S5. FTIR spectra of CDs and the product after reaction with FA.



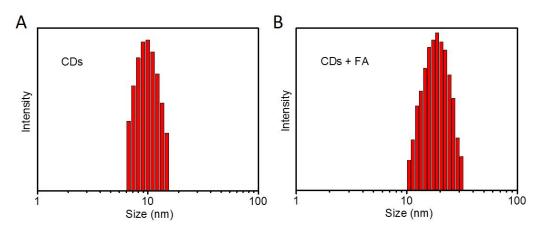
**Figure S6**. Cell viability of HeLa cells after incubated with a series concentrations of CDs (the viability of the cells without CDs is defined as 100%). The results are the mean SD of six separate measurements.



**Figure S7**. Fluorescence images of HeLa cells after being incubated with CDs (10-30  $\mu$ g/mL) at 37 °C for 10 min. The images were collected in bright field (A1-C1), 450–550 nm (A2-C2) and 550–650 nm (A3-C3) with the excitation wavelength at 405 nm. Scale bar: 20  $\mu$ m.



**Figure S8**. Fluorescence images of HeLa cells which incubated with CDs in the presence of NaHSO<sub>3</sub> or FA. (A) HeLa cells with a treatment of 200  $\mu$ M NaHSO<sub>3</sub> for 30 min before incubated with CDs (30  $\mu$ g/mL, 10 min). (B) HeLa cells were incubated with CDs (30  $\mu$ g/mL) for 10 min. (C) HeLa cells with a treatment of 200  $\mu$ M FA for 20 min before incubated with CDs (30  $\mu$ g/mL, 10 min). (D) HeLa cells with a treatment of 400  $\mu$ M FA for 20 min before incubated with CDs (30  $\mu$ g/mL, 10 min). (D) HeLa cells with a treatment of 400  $\mu$ M FA for 20 min before incubated with the CDs (30  $\mu$ g/mL, 10 min). (D) HeLa cells with a treatment of 400  $\mu$ M FA for 20 min before incubated with CDs (30  $\mu$ g/mL, 10 min). (D) HeLa cells with a treatment of 400  $\mu$ M FA for 20 min before incubated with the CDs (30  $\mu$ g/mL, 10 min). The images were collected in bright field (A1-D1), 450–550 nm (green channel) (A2-D2) and 550–650 nm (orange red channel) (A3-D3) with the excitation wavelength at 405 nm. Scale bar: 20  $\mu$ m.



**Figure S9.** The hydrodynamic diameter distribution of CDs (A) and the product after reaction with FA (B).

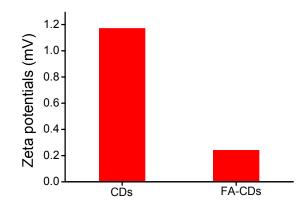
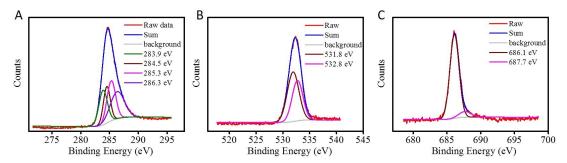


Figure S10. The zeta potential of CDs and the product after reaction with FA.



**Figure S11.** High-resolution XPS spectra of C1s (A), O1s (B) and F1s (C) peaks of CDs. (A) The peaks at 283.9 eV for C-F, 284.5 eV for C-C/C-H, 285.3 eV for C-N, 286.3 eV for C-O. (B) The peaks at 531.8 eV for C=O, 532.8 eV for C-O. (C) The peaks at 686.1 eV for C-F (between sp<sup>2</sup> C and F), 687.7 eV for C-F (between sp<sup>3</sup> C and F).



Figure S12. The photograph of CDs under 405 nm light irradiation.

Fluorescent Probe	Reaction time	Reference
HOOC NH SI FAP-1	2 h	S1
N N HN. <sub>NH2</sub> Na-FA-Lyso	30 min	S2
NH2 Na-FA	30 min	\$3
$\bigcup_{NH_2\\\mathsf$	2 h	S4
$\begin{array}{c} \begin{array}{c} & & \\ $	8 min	85
HO H H H H H H H H H H H H H H H H H H	1 h	S6
$O_2N$ V V V V V V V V	3 h	S7
HO HO RFFP	3 h	S8
FAP	2 h	S9
HO NH <sub>2</sub> HO NH <sub>2</sub> RFAP-J	2 h	S10

**Table S1.** Comparison of the reaction time of the developed method for the determination of FA with published methods.

NH <sub>2</sub>	1 h	S11
MeO CFAP540		
NH <sub>2</sub>	2.5 h	S12
MQAP		
	3 min	S13
$ \begin{array}{c}  & \\  & \\  & \\  & \\  & \\  & \\  & \\  & $	2 h	S14
CDs	5 min	This work

## **Reference:**

- S1 T. F. Brewer and C. J. Chang, J. Am. Chem. Soc., 2015, 137, 10886-10889.
- S2 Y. Tang, X. Kong, Z. R. Liu, A. Xu and W. Lin, Anal. Chem., 2016, 88, 9359-9363.
- S3 Y. Tang, X. Kong, A. Xu, B. Dong and W. Lin, Angew. Chem. Int. Ed., 2016, 55, 3356-3359.
- S4 K. J. Bruemmer, R. R. Walvoord, T. F. Brewer, G. Burgos-Barragan, N. Wit, L. B. Pontel, K. J. Patel and C. J. Chang, J. Am. Chem. Soc., 2017, 139, 5338-5350.
- S5 Y. H. Lee, Y. Tang, P. Verwilst, W. Lin and J. S. Kim, *Chem. Commun.*, 2016, 52, 11247-11250.
- S6 W. Liu, C. Truillet, R. R. Flavell, T. F. Brewer, M. J. Evans, D. M. Wilson and C. J. Chang, *Chem. Sci.*, 2016, 7, 5503-5507.
- S7 A. Roth, H. Li, C. Anorma and J. Chan, J. Am. Chem. Soc., 2015, 137, 10890-10893.
- S8 L. He, X. Yang, Y. Liu, X. Kong and W. Lin, Chem. Commun., 2016, 52, 4029-4032.
- S9 Z. Li, Y. Xu, H. Zhu and Y. Qian, Chem. Sci., 2017, 8, 5616-5621.
- S10 T. F. Brewer, G. Burgos-Barragan, N. Wit, K. J. Patel and C. J. Chang, *Chem. Sci.*, 2017, 8, 4073-4081.
- S11 K. J. Bruemmer, O. Green, T. A. Su, D. Shabat and C. J. Chang, *Angew. Chem. Int. Ed.*, 2018, 130, 7630-7634.
- S12 H. Yang, G. Fang, M. Guo, P. Ning, Y. Feng, H. Yu and X. Meng, Sens. Actuators. B, 2018, 270, 318-326.
- S13 F. Wu, Y. Zhang, L. Huang, D. Xu and H. Wang, Anal. Methods, 2017, 9, 5472-5477.
- S14 X. Song, X. Han, F. Yu, J. Zhang, L. Chen and C. Lv, Analyst, 2018, 143, 429-439.