Electronic Supplementary Material (ESI) for Analyst

Supporting Information

A Two-Photon "Turn-on" Fluorescent Probe Based on Carbon Nanodots for Imaging and Selective Biosensing of Hydrogen Sulfide in Live Cells and Tissues

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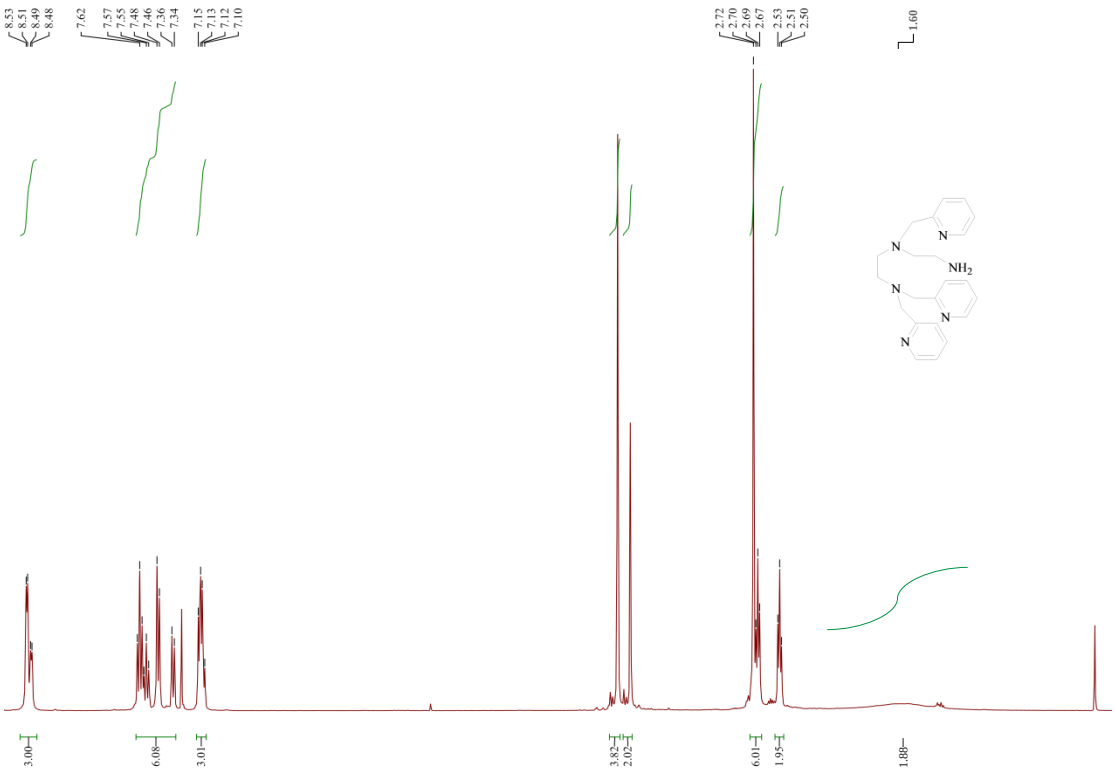


Fig. S1. ^{1}H NMR spectra (400 MHz) of AE-TPEA in $CDCl_3$.

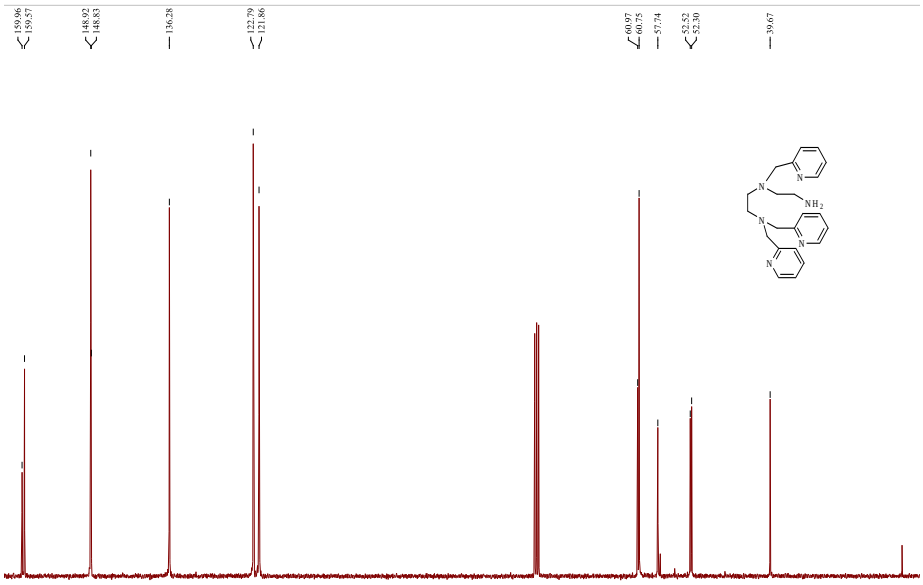


Fig. S2. ^{13}C NMR spectrum (100 MHz) of AE-TPEA in $CDCl_3$.

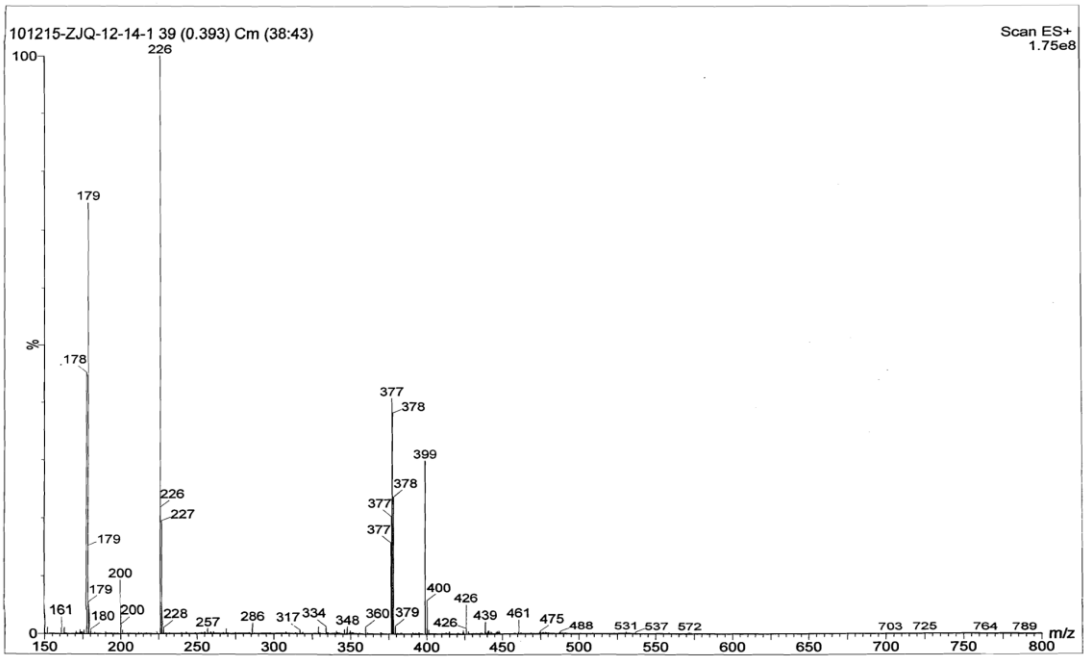


Fig. S3. MS spectra of AE-TPEA.

Elemental Composition Report

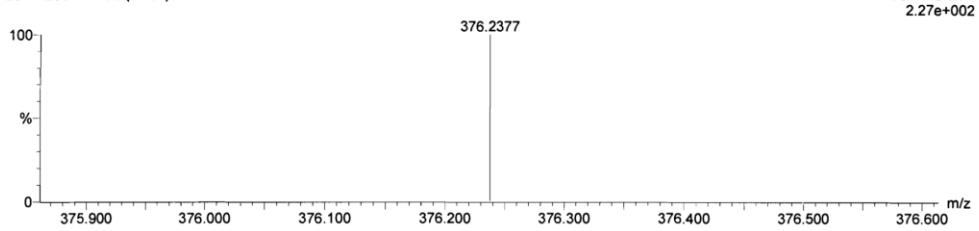
Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 35.0
Element prediction: Off

Monoisotopic Mass, Odd and Even Electron Ions
11 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:
C: 0-25 H: 0-40 N: 0-6

AE TPEA
20111206009 156 (5.184)

GCT Premier CAB073

06-Dec-201114:14:15
TOF MS EI+
2.27e+002



Minimum:		5.0	10.0	-1.5		
Maximum:				35.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
376.2377	376.2375	0.2	0.5	12.0	5546128.5	C22 H28 N6

Fig. S4. Elemental composition obtained by HR-MS for AE-TPEA.

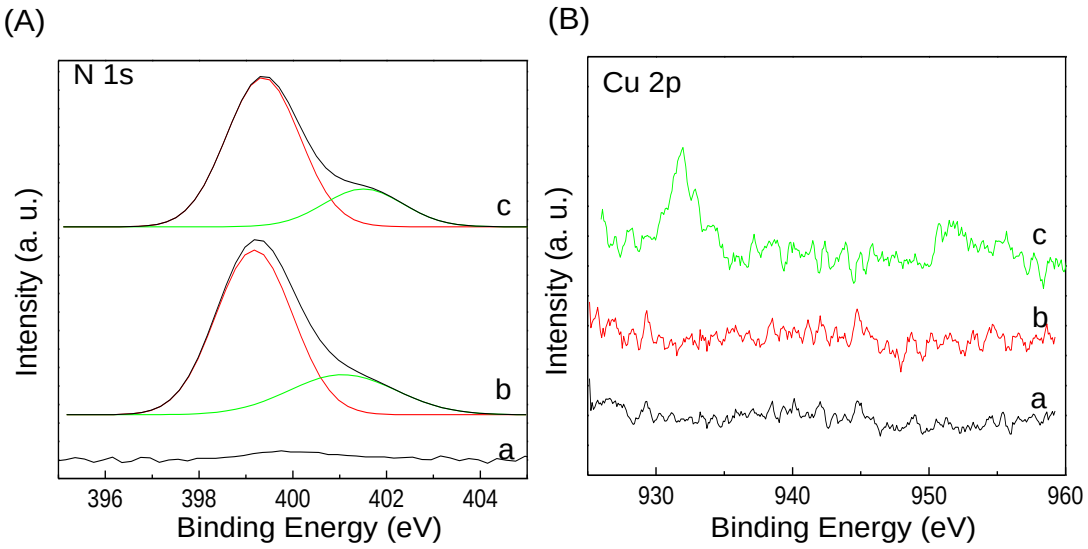


Fig. S5. X-ray photoelectron spectra for (A) N 1s and (B) Cu 2p obtained at (a) C-Dots, (b) C-Dot-TPEA, and (c) C-Dot-TPEA-Cu^{2+} probes.

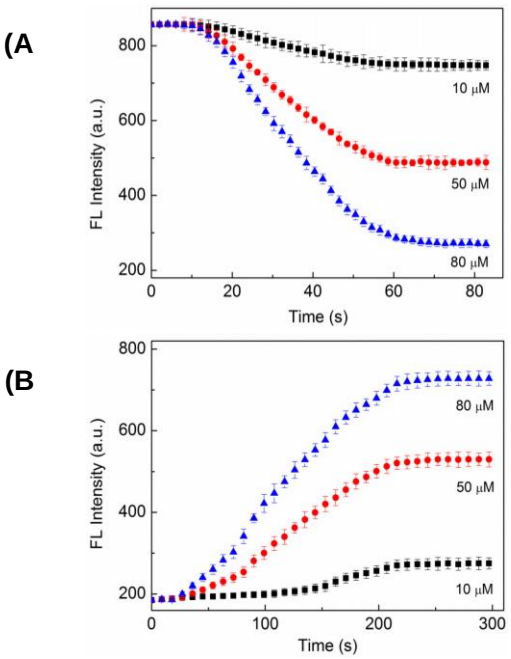


Fig. S6. (A) Time dependence of the fluorescence intensity of C-Dot-TPEA on the concentrations of Cu^{2+}; (B) Time dependence of the fluorescence intensity of C-Dot-TPEA-Cu^{2+} on the concentrations of H_2S.

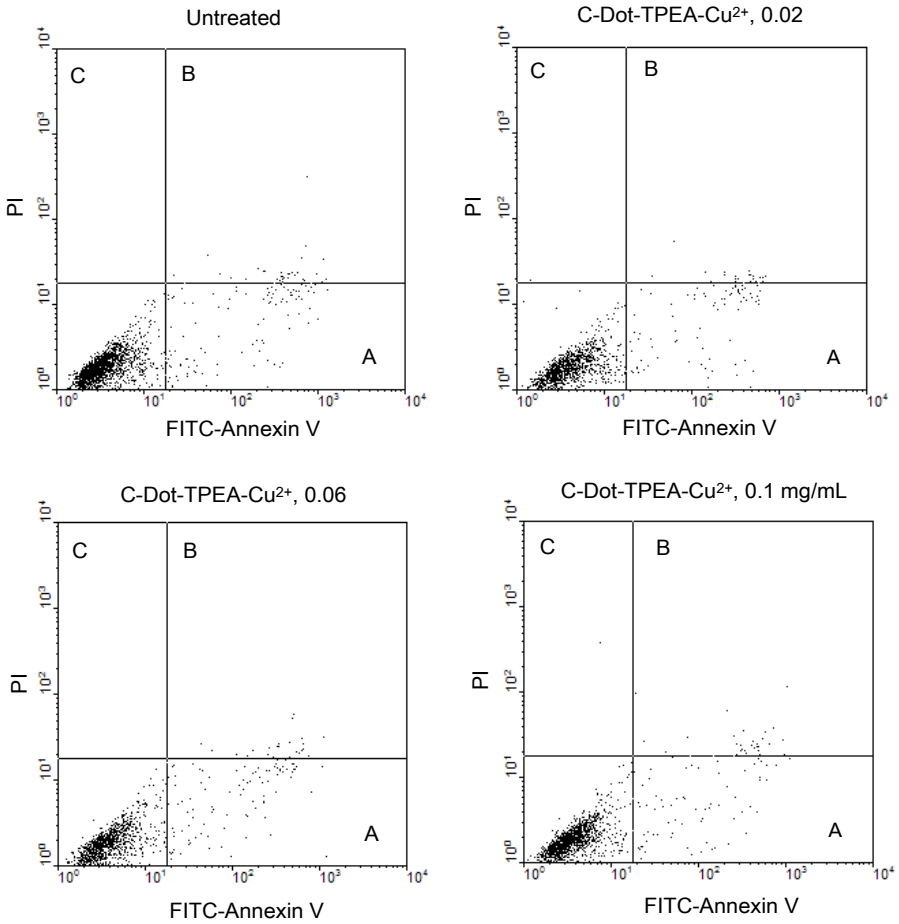


Fig. S7. Apoptosis assay of Hela cells after C-Dot-TPEA-Cu^{2+} treatment. HeLa cells were incubated with C-Dot-TPEA-Cu^{2+} at concentrations of 0.02, 0.06, and 0.1 mg mL^{-1} for 24 h. Cells were stained by FITC-annexin V and Propidium iodide (PI) to label the apoptosis cells and necrotic cells respectively for flow cytometry (FACS) measurement. A, B and C represent the regions of early apoptotic cells, late apoptotic cells and dead cells, respectively.

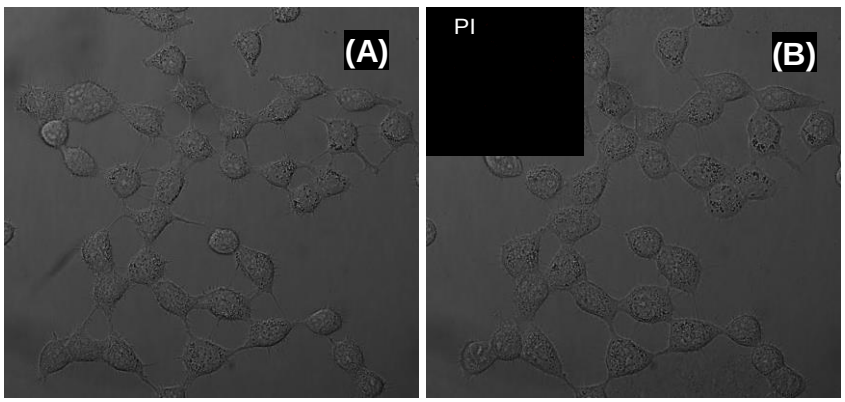


Fig. S8. The confocal bright-field images of living HeLa cells loaded with C-Dot-TPEA-Cu^{2+} (A) before and (B) after irradiated by the femtosecond laser at 800 nm (~5 mW average power in the focal plane) for 1 h in advance. Inset: propidium iodide (PI) staining is included to indicate the necrotic cells after two-photon excitation.

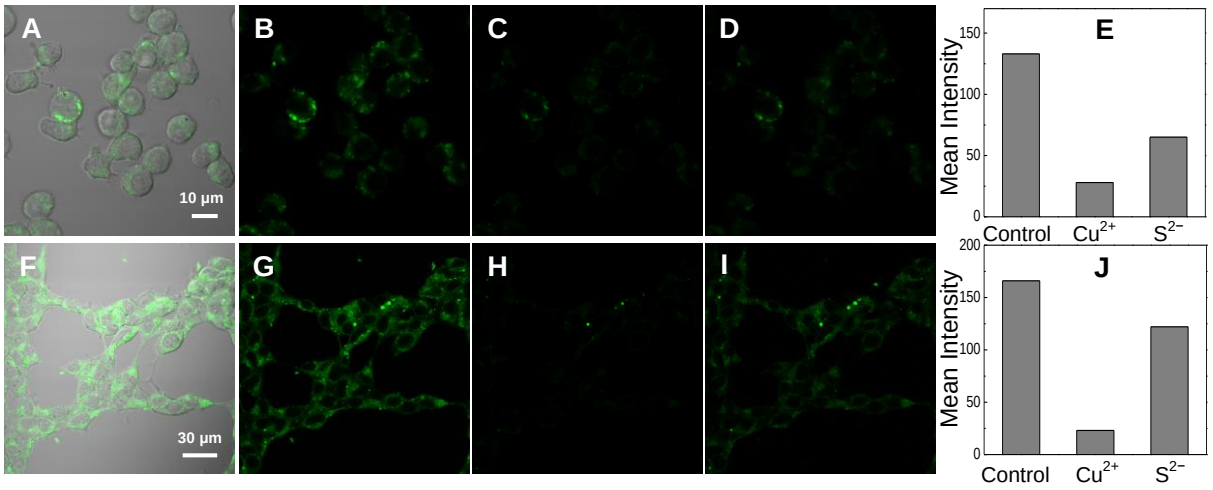


Fig. S9. Two-photon fluorescence microscopy images of live (A–D) RAW264.7 cells and (F–I) HEK 293T cells. (A) The overlay of fluorescence and bright-field images and (B) the fluorescence image of RAW264.7 cells after incubation with 0.04 mg mL^{-1} C-Dot-TPEA for 1 h; (C) RAW264.7 cells stained with 0.04 mg mL^{-1} C-Dot-TPEA for 1 h and then treated with100 μM $CuCl_2$ for 1 h at 37 ℃; (D) C-Dot-TPEA loaded-RAW264.7 cells pretreated with 100 μM $CuCl_2$ for 1 h and then with 100 μM Na_2S for 0.5 h; (E) The mean fluorescence intensity of RAW264.7 cells before and after the treatment; (F) The overlay of fluorescence and bright-field images and (G) the fluorescence image of HEK 293T cells after incubation with 0.04 mg mL^{-1} C-Dot-TPEA for 1 h; (H) HEK 293T cells stained with 0.04 mg mL^{-1} C-Dot-TPEA for 1 h and then treated with100 μM $CuCl_2$ for 1 h at 37 ℃; (I) C-Dot-TPEA loaded-HEK 293T cells pretreated with 100 μM $CuCl_2$ for 1 h and then with 100 μM Na_2S for 0.5 h; (J) The mean fluorescence intensity of HEK 293T cells before and after the treatment.

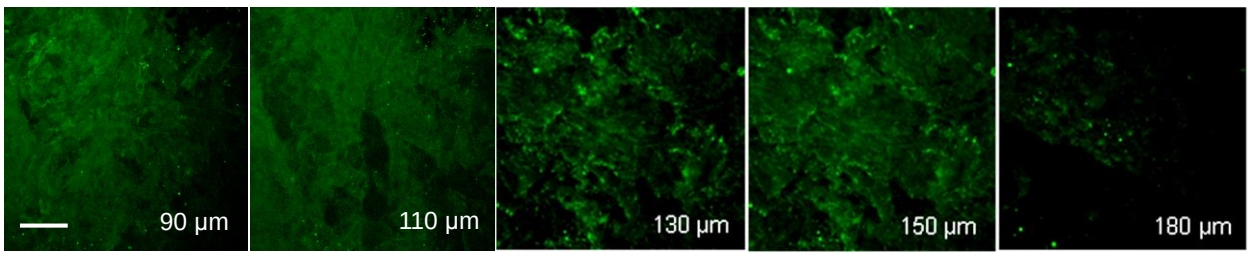


Fig. S10. Two-photon images of the lung cancer tissue slice treated with 0.2 mg mL^{-1} C-Dot-TPEA. Tissue was obtained from A549 cells. The images shown are 5 representative images obtained along the z-direction in the range of 90-180 μm from replicate experiments. Images were acquired using 800 nm excitation and fluorescent emission window: 450 nm-700 nm. Scale bar: 50 μm.