Electronic Supplementary Information

An ICT-switched fluorescent Probe for visualizing lipid and HClO in lipid droplets during ferroptosis

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Table of contents:

1. General Information

1.1 Materials

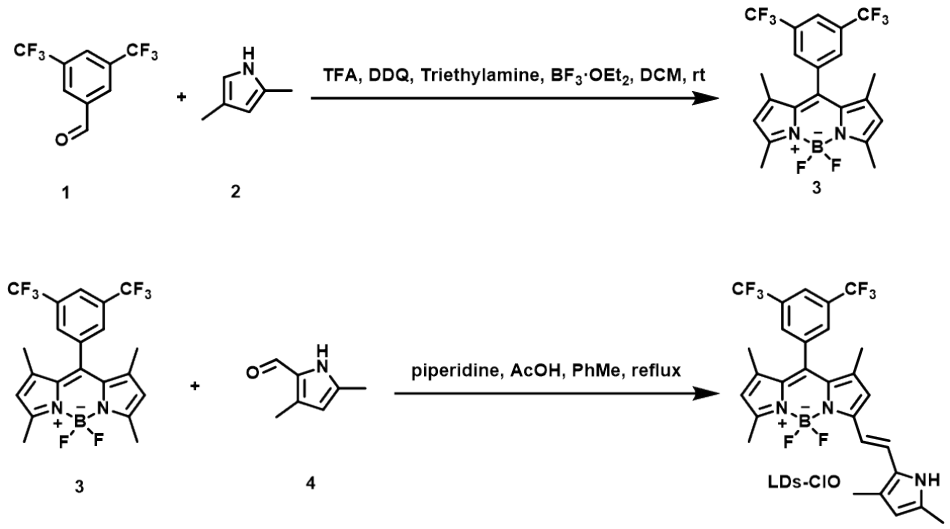
The chemicals needed for the experiment were purchased from Aladdin Reagent (Shanghai) Co., Ltd. and Shanghai Anaiji Chemical Co., Ltd., and the reagents needed were purchased from Shanghai Chemical Industry. All chemicals and solvents used were of analytical grade and no further purification was required. Distilled water was used after passing through a water ultra-purification system. PBS buffer solution was obtained by mixing of 0.05 mol/L Na_2HPO_4 water solution and 0.05 mol/L KH_2PO_4 water solution with the volume ratio 4:1.

1.2 Instruments

Reaction processes were monitored on thin layer chromatography (TLC). TLC analysis was performed using precoated silica plates. Fluorescence spectra were carried out a HITACHI F-7000 spectrophotometer. UV-visible spectra were recorded with a HITACHI U-3900 spectrophotometer. Shanghai Huamei Experiment Instrument Plants (China) provided a PO-120 quartz cuvette (10 mm). NMR spectra were recorded on a JBruker AVANCE-600MHz spectrometer and chemical shifts were referenced relative to tetramethylsilane. Coupling constants (J values) are reported in hertz. HR-MS was measured with a Thermo Scientific Q Exactive. Cell imaging experiments of living cells were carried out by Zeiss LSM880 Airborne confocal laser scanning microscopy.

2. Experimental Section

2.1 **Scheme S1**. Synthesis route of probe **LDs-ClO**.



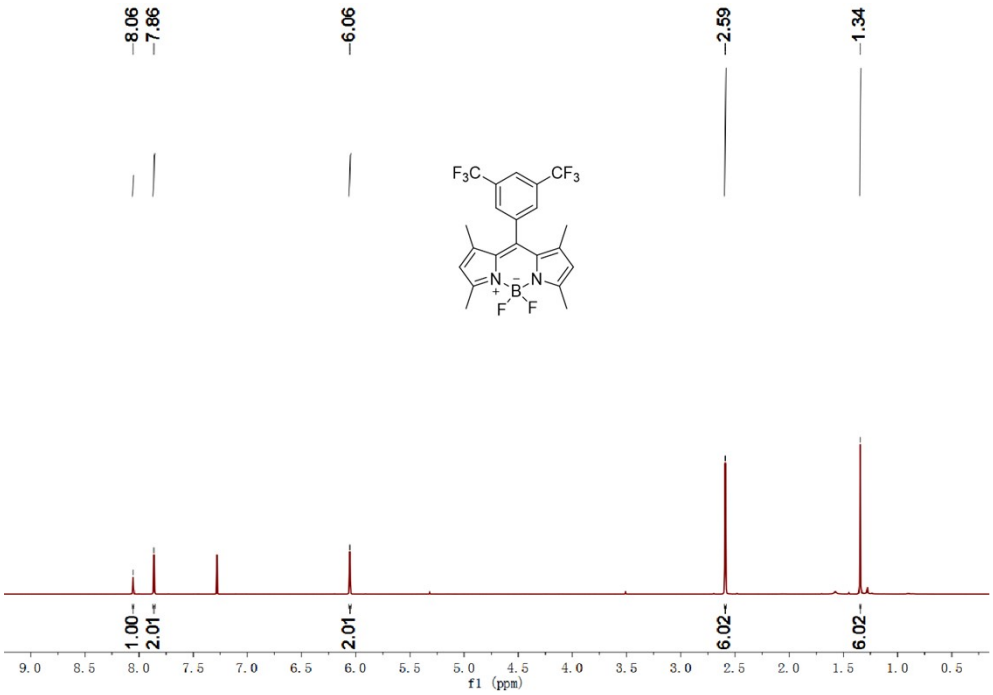
2.2 Synthesis and characterization.

Compound **3**[1].Compound 1 (1.05 mL, 10 mmol) and compound 2 (2.1 mL, 20 mmol) were dissolved in 125 mL DCM, added 2 drops of TFA into the above mixture system. The reaction system was stirred at

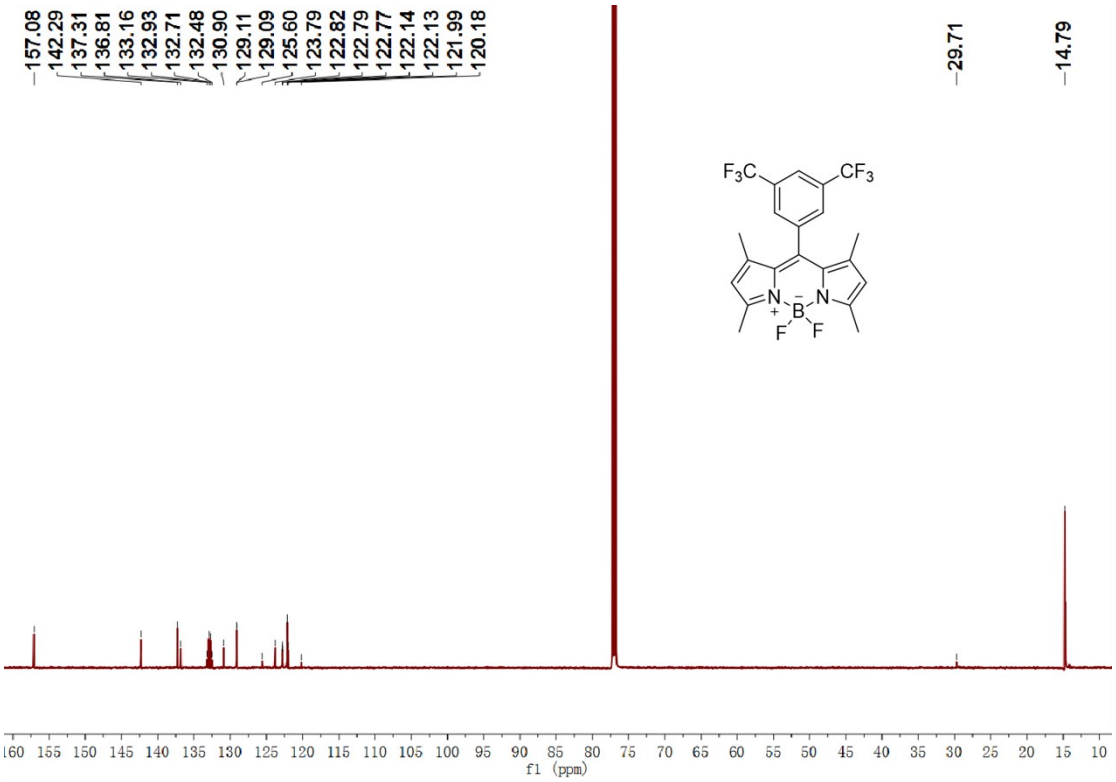
30 ℃ for 12 h under the protection of N_2. Then, DDQ (2.724 g, 12 mmol) was added in N_2 atmosphere and continue stirring for 1.5 h. Later, added excessive $BF3{\cdot}OEt_2$ (40 mL) and triethylamine (30 mL), and stired at room temperature for 2 h. The crude product was diluted with proper amount of DCM and washed with proper amount of water, dried with anhydrous sodium sulfate. Later, distilled the solvent under reduced pressure, the crude product was purified with silica-gel column chromatography (PE/DCM, 5:1, v/v), affording the probe as the orange solid (1.61 mg, yield 35%).^{1}H-NMR (600 MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.86 (s, 2H), 6.06 (s, 2H), 2.59 (s, 6H), 1.34 (s, 6H). ^{13}C-NMR (151 MHz, $CDCl_3$) δ 157.08, 142.29, 137.31, 136.81, 133.16, 132.93, 132.71, 132.48, 130.90, 129.11, 129.09, 125.60, 123.79, 122.82, 122.79, 122.77, 122.14, 122.13, 121.99, 120.18, 29.71, 14.79.

LDs-ClO. Compound 3 (460 mg, 1 mmol) was dissolved in toluene (50 mL), then 3,5-Dimethyl-1H-pyrrole-2-carbaldehyde (160 mg, 1.3 mmol) and 2 drops of piperidine were added to the above mixture. The reaction system was heated to 80 ℃ under the protection of N_2, and then 2 drops of acetic acid were added. After then, the reaction mixture was stirred at 110 ℃ for 8 h. Later, distilled the solvent under reduced pressure, the crude product was purified with silica-gel column chromatography (PE/DCM, 5:1, v/v), affording the probe as the purple-black solid. ^{1}H-NMR and ^{13}C-NMR spectra and HR-MS of the probe are shown in Figure S3-5. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.44 (s, 1H), 8.04 (s, 1H), 7.88 (s, 2H), 7.17 (d, J = 16.1 Hz, 1H), 6.96 (d, J = 16.1 Hz, 1H), 6.64 (s, 1H), 6.00 (s, 1H), 5.89 - 5.83 (m, 2H), 2.60 (s, 3H), 2.30 (s, 3H), 2.19 (s, 3H), 1.36 (d, J = 31.3 Hz, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 156.66, 152.04, 142.18, 138.31, 137.87, 134.63, 133.09, 132.87, 132.65, 132.42, 132.40, 132.20, 130.14, 129.66, 129.64, 127.47, 126.75, 126.10, 123.89, 122.53, 122.08, 120.60, 118.67, 111.65, 108.71, 99.99, 15.21, 14.55, 14.52, 13.40, 11.27. HR-MS: m/z calcd. for the probe ($C_{28}H_{24}BF_8N_3$, [M]), 565.19355; found, 564.19202.

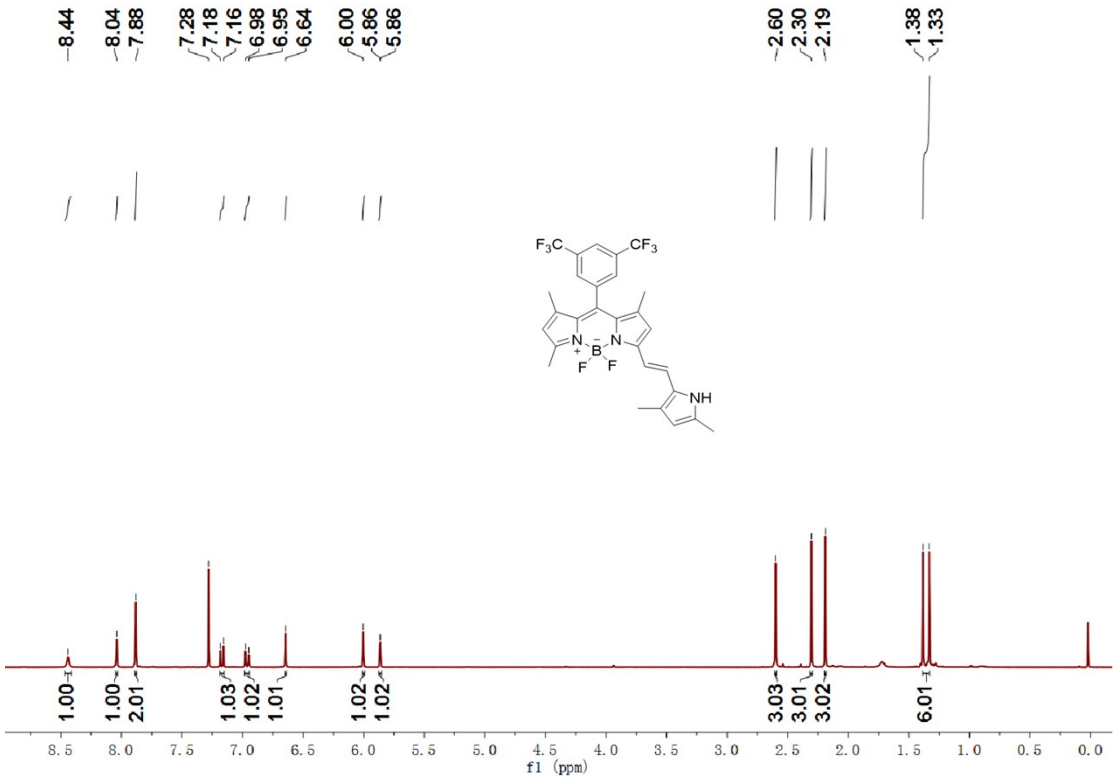
Fig. S1: Structure characterization.



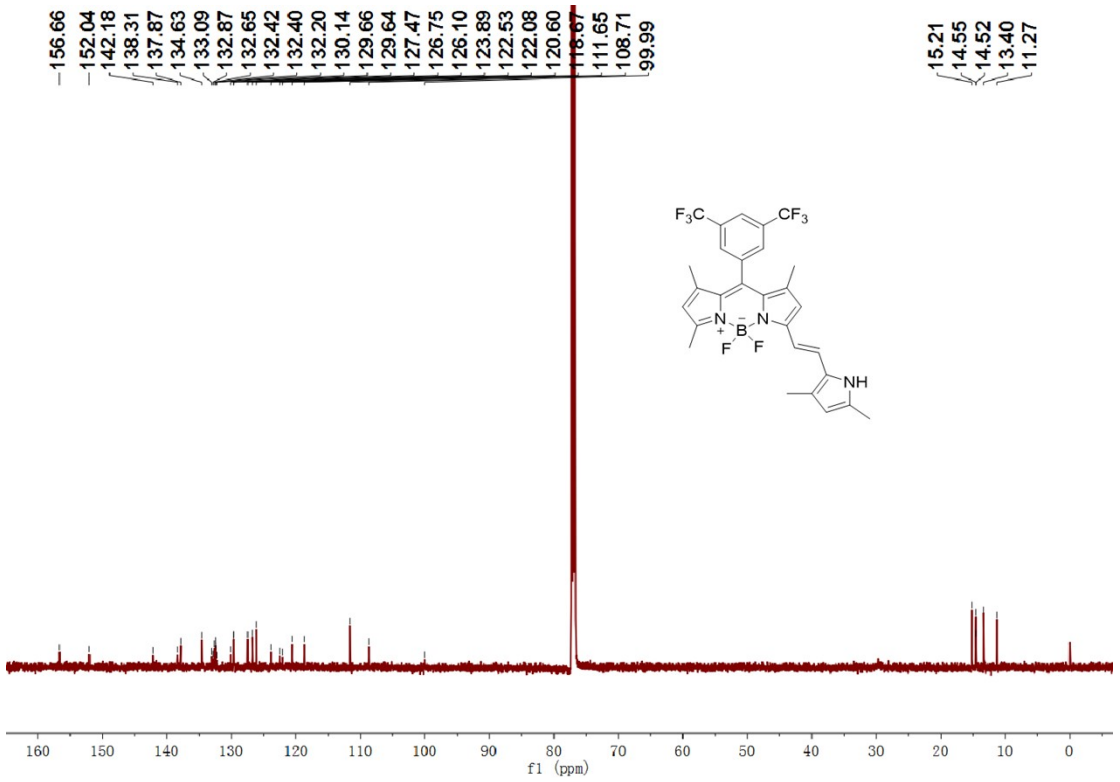
^{1}H-NMR spectrum of compound **3** in $CDCl_3$-*d*



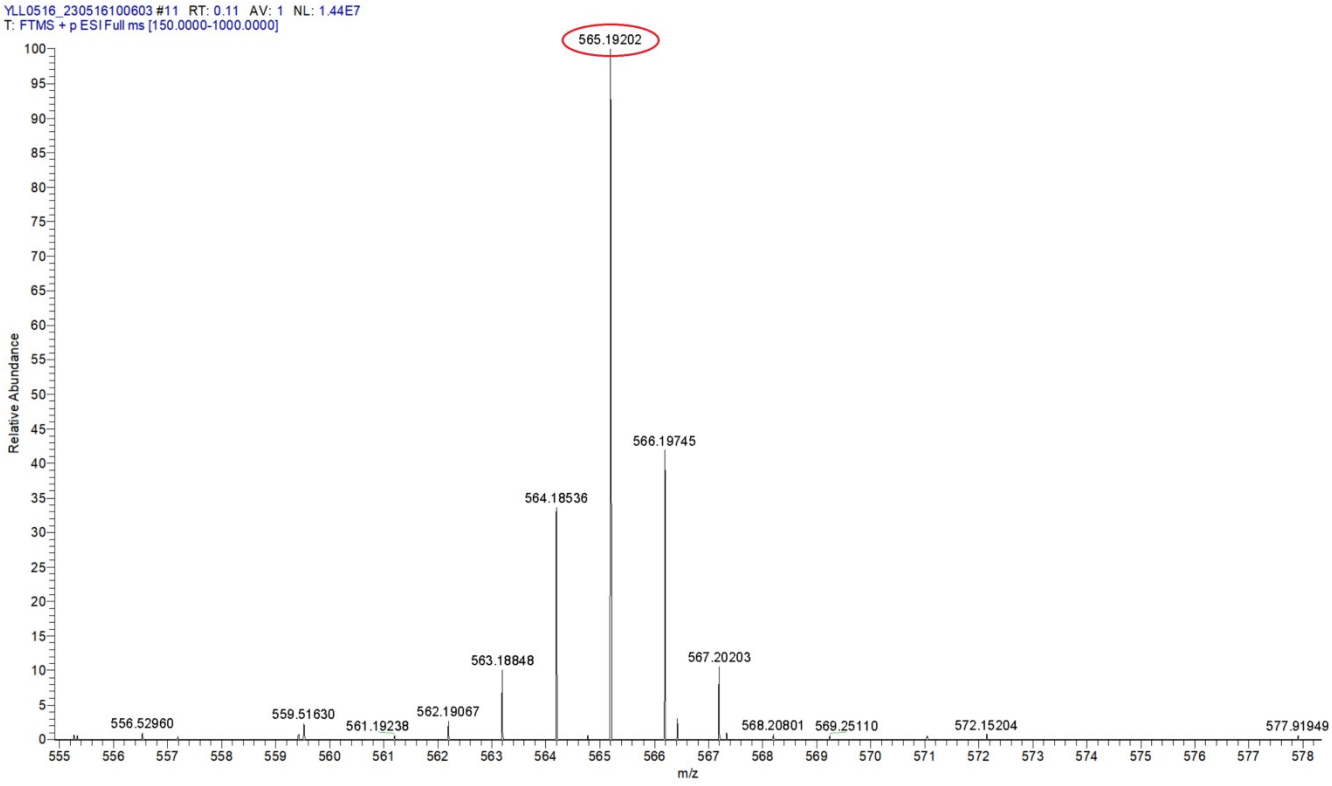
^{13}C-NMR spectrum of compound **3** in $CDCl_3$-*d*



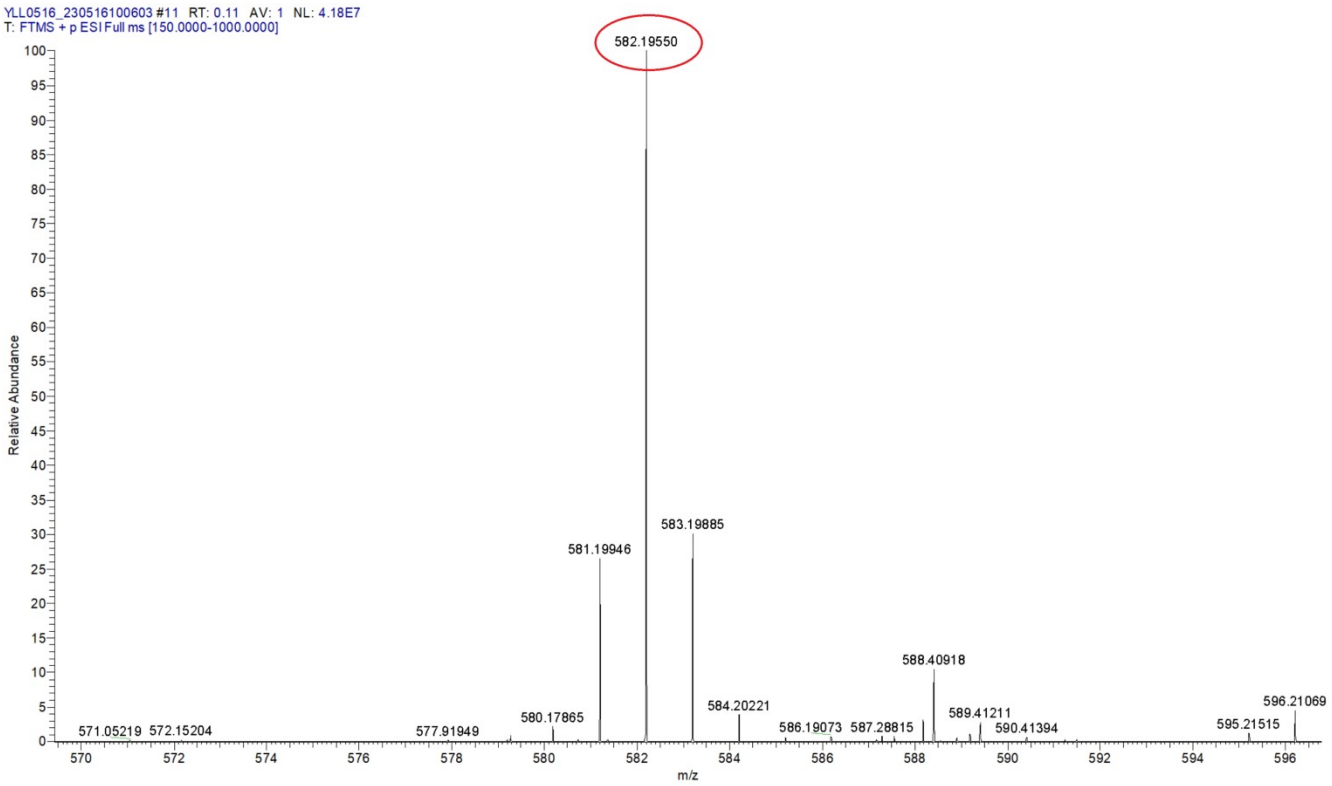
^{1}H NMR spectrum of **LDs-ClO** in $CDCl_3$-*d*.



^{13}C NMR spectrum of **LDs-ClO** in $CDCl_3$-*d*.



The HR-MS spectrum of **LDs-ClO:** *m/z*: [M] Calcd. for $C_{28}H_{24}BF_8N_3$, 565.19355; found, 565.19202;



The HR-MS spectrum of **LDs-ClO**+HClO: *m/z*: $[M+H]^+$ Calcd. for $C_{28}H_{25}BF_8N_3O$, 582.19629; found, 582.19550.

3. Spectrometric Studies

3.1 Photochemical properties of **LDs-ClO** measurement

The stock solution of LDs-ClO required for property testing was made by dissolving probe in DMSO. NaClO stock solution, other common interfering ions stock and some biological small molecules solutions (SO_3^{2-}, NO_3^{2-}, Cl^-, $S_2O_3^{2-}$, S^{2-}, OAc^-, I^-, Br^-, Na^+, Cu^{2+}, Ca^{2+}, K^+, NH_4^+, GSH, L-Cys, Hcy, Glycine, L-Glu, L-Lys, ClO_2^-) were prepared by dissolving solid in PBS buffer solution. All the PBS solutions used in the experiment were prepared by dissolving in deionized water. Fluorescence measurements were performed on LDs-ClO. Both the fluorescence spectrum and UV-Vis were obtained in DMF: PBS = 1: 1 (pH 7.4, v: v).

3.2 Preparation of ROS

$\cdot O_2^-$ stock solution (10 mM). Make a stock solution (10 mM) of $\cdot O_2^-$ by adding ClO^- stock solution into H_2O_2 stock (1:1, mol/mol), and stir the mixture solution for 10 min.
TBO· stock solution. TBO· (10 μM) was generated by mixing 3.0 μL of 10 mM TBHP stock solution (Reagent setup) with 3.0 μL of 10 mM Fe^{2+} stock solution (Reagent setup).
NO stock solution (10 mM). 2.98 mg of sodium nitroprusside dihydrate was dissolved in 1,000 μL of H_2O.
TBHP stock solution (10 mM). 1.2 μL of 70% (wt/vol) TBHP was added to 998.8 μL of H_2O.
H_2O_2 stock solution (100 mM, 10 mM, and 0.7 M). Make a stock solution (100 mM) of H_2O_2 by adding 11 μL of 30% (wt/vol) H_2O_2 to 989 μL of H_2O. 10 mM stock was prepared by diluting 10 μL of

100 mM H_2O_2 with 90 μL of H_2O. 0.7 M stock was prepared by diluting 154 μL of 30% (wt/vol) H_2O_2 in 1,846 μL of H_2O. H_2O_2 concentration is determined by measuring the absorbance at 240 nm (ε = 43.6 M^{-1} cm^{-1}).

HCl stock solution (0.6 M). 100 μL of 36–38% (wt/vol) HCl was diluted with 2,000 μL of H_2O. The HCl stock solution can be stored at 4°C for up to 1 week.

$NaNO_2$ stock solution (0.6 M). 124.2 mg of sodium nitrite was dissolved in 3,000 μL of H_2O. The $NaNO_2$ stock solution can be stored at 4°C for up to 1 d.

NaOH stock solution (1.5 M). 180.0 mg of sodium hydroxide was dissolved in 3,000 μL of H_2O. A 0.1 M stock solution was prepared by dissolving 12.0 mg of sodium hydroxide in 3,000 μL of H_2O. The NaOH stock solution can be stored at 4°C for up to 1 week.

$ONOO^-$ stock solution. Mix H_2O_2 (0.7 M, 1.5 mL), HCl (0.6 M, 1.5 mL), $NaNO_2$ solution (0.6 M, 3 mL), and NaOH solution (1.5 M, 3 mL) in an ice bath; the final concentration was determined by measuring the absorbance at 302 nm (ε = 1,670 M^{-1} cm^{-1}).

Fe^{2+} stock solution (10 mM). A stock solution of Fe^{2+} was prepared for radical generation by Fenton reaction. 10 mM stock was prepared by dissolving 1.99 mg of $FeCl_2$·4H2O in 1,000 μL of H_2O. The Fe^{2+} stock solution can be stored at 4°C for up to 1 d.

·OH stock solution. Hydroxyl radical (·OH) was generated by the Fenton reaction between $FeCl_2$ and H_2O_2, and the concentration of ·OH was determined by Fe^{2+}.

NaClO stock solution (10 mM). Add 100 μL of commercial NaClO (0.1 M) to 900 μL of H_2O. Determine the NaClO concentration by measuring the absorbance at 292 nm (ε = 350 M^{-1} cm^{-1}).

Critical: stock solutions of ROS, including NaOCl, should be freshly prepared before use.

3.3 Calculation of the limit of detection (LOD)

The limit of detection (LOD) is calculated according to the following equitation: DL = 3σ / K. Where σ is the standard deviation of 10 blank sample, and K is the slope of the calibration curve.

Fig. S2: Molecular structures and the ClogP values of Nile red, BODIPY (493/503), and our work.

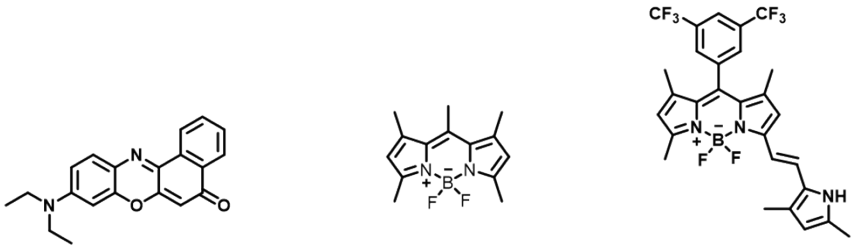


Fig. S3: Absorption spectrum changes of probe (3 μM) upon treatment with various concentrations of NaClO standard solution (0-130 μM).

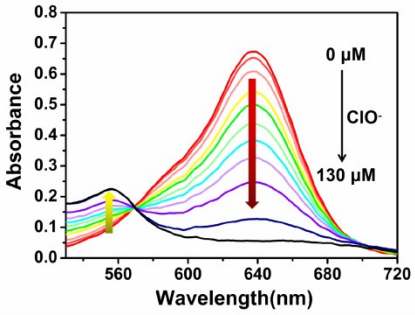


Fig. S4: Time-dependent fluorescence intensity of probe (10 μM) upon treatment with ClO- (NaClO is 0, 50, 100 μM, respectively). The reactions were performed in DMF: PBS = 1: 1.

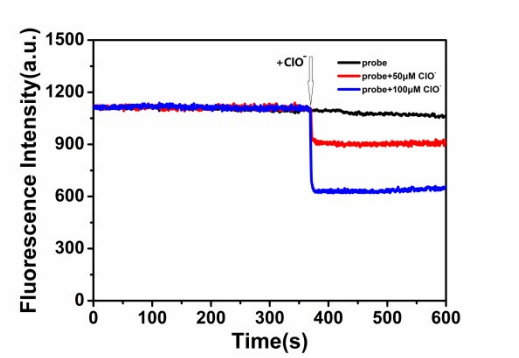
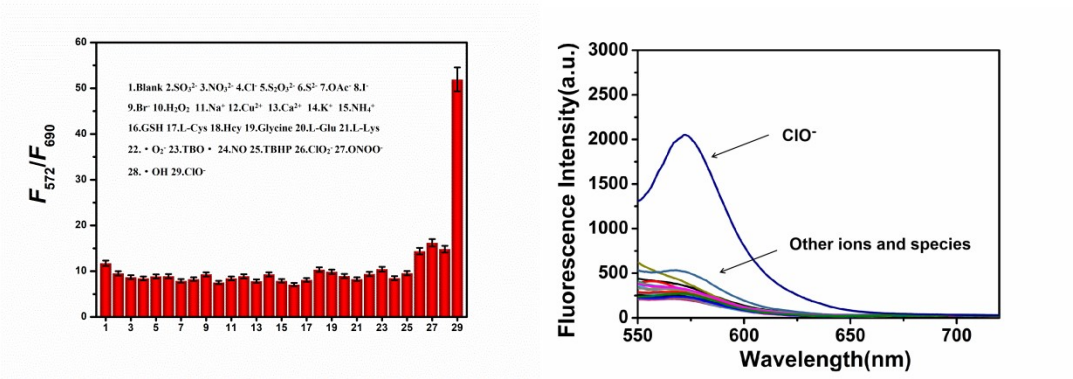


Fig. S5: Fluorescence intensity of probe (3 μM) upon treatment with ClO^- (50 μM) and other species (300 μM). The reactions were performed in DMF: PBS = 1: 1.



4. Cell culture and imaging

4.1 Cell culture

RAW 264.7 cells was cultured in Dulbecco's Modified Eagle Medium containing 10% Fetal Bovine Serum, and grown in an incubator at 37℃ for approximately 24 h.

4.2 The cytotoxicity test of **LDs-ClO**

After incubating RAW 264.7 cells in 96 well plates for 24 hours, LDs-ClO (0, 1, 2.5, 5, 7.5, 10, 20, 30

μM) were used respectively treat cells for 6 h and 12 h, and then test the cell survival rate using the CCK-8.

Fig. S6: Cytotoxicity data of **LDs-ClO** (0, 1, 2.5, 5, 7.5, 10, 20, 30 μM) in RAW 264.7 cells (a) 6 h (b) 12 h.

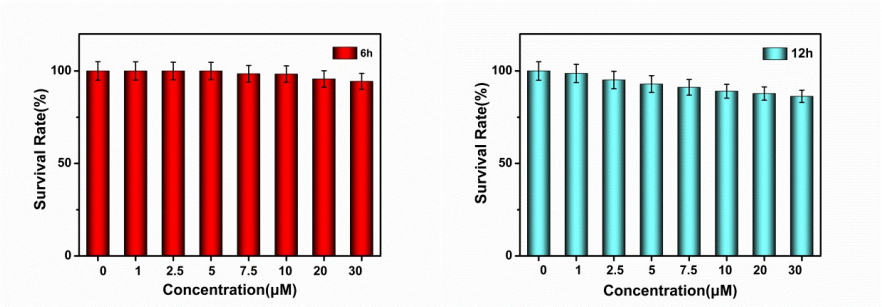


Fig. S7: Confocal images of Raw 264.7 cells induced by **LDs-ClO** (10 μM). Red channel: λ_{ex}=488 nm, λ_{em}=660-720 nm; Yellow channel: λ_{ex}=488 nm, λ_{em}=540-600 nm.

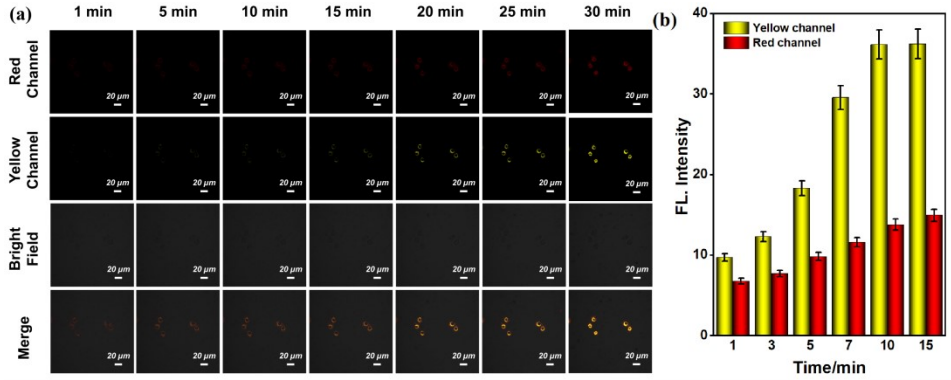


Fig. S8: Co-localization imaging of Raw 264.7 cells stained with (a) BODIPY 493/503: Green channel: λ_{ex}=488 nm, λ_{em}=470-530 nm; Red channel: λ_{ex}=488 nm, λ_{em}=660-720 nm. (b) Lyso-Tracker Red: Blue channel: λ_{ex}=561 nm, λ_{em}=560-620 nm; Red channel: λ_{ex}=488 nm, λ_{em}=660-720 nm; (c) Mito-Tracker Green: Green channel: λ_{ex}=488 nm, λ_{em}=490-540 nm; Red channel: λ_{ex}=488 nm, λ_{em}=660-720 nm.

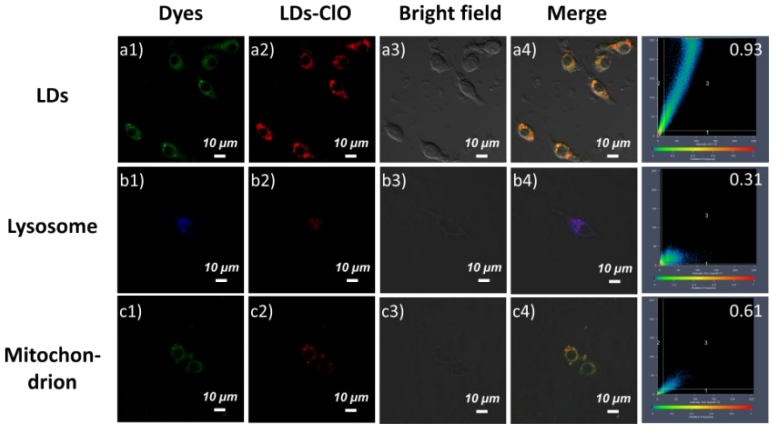


Fig. S9: Cell viability of RAW 264.7 cells incubated with different ferroptosis inducers and inhibitor for 12 h.

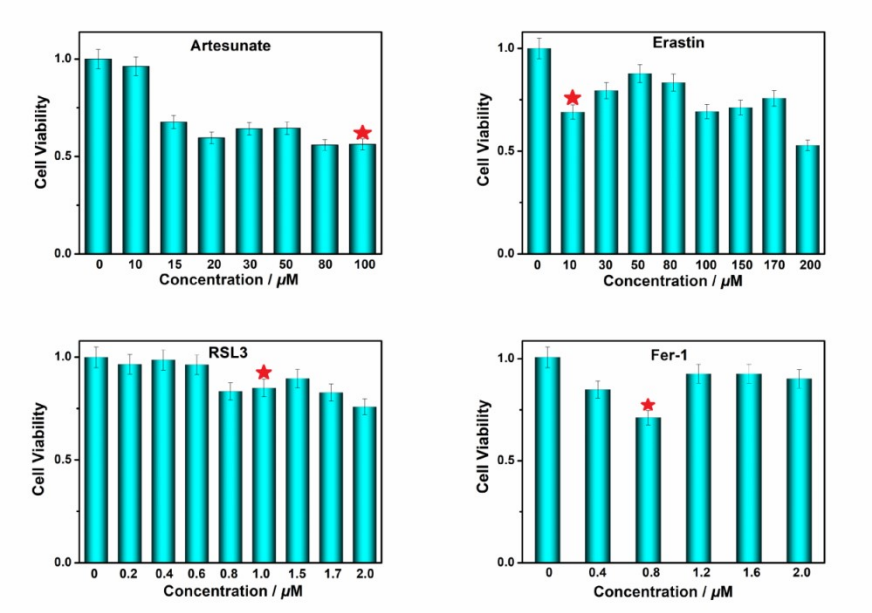


Fig. S10: The corresponding average fluorescence intensity curves for 1-30 min.

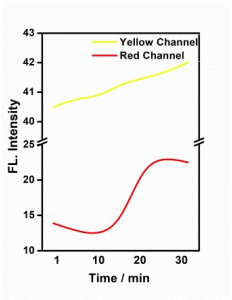


Fig. S11: The corresponding average fluorescence intensity curves for 1-30 min.

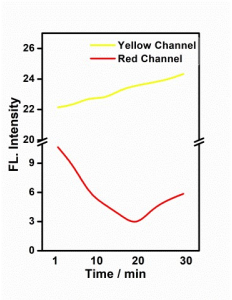


Fig. S12: The corresponding average fluorescence intensity curves for 1-30 min.

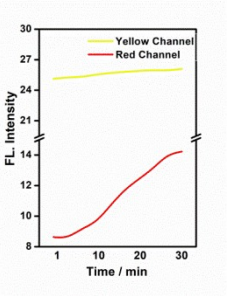
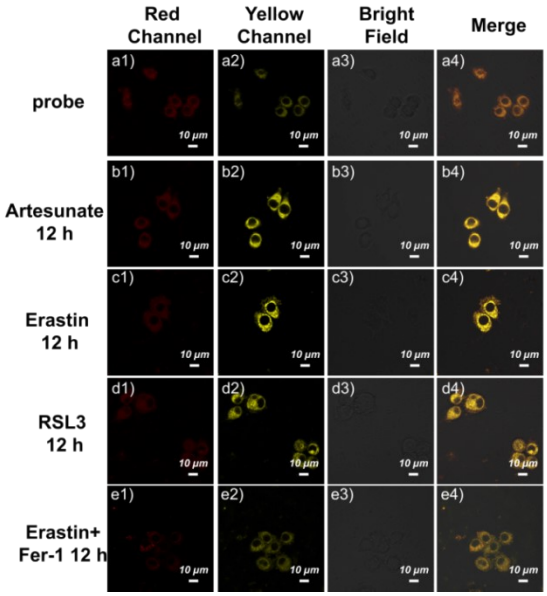


Fig. S13: Confocal microscopy fluorescence images of Raw 264.7 cells induced by (b) Artesunate (100 μM); (c) 1 μM Erastin (10 μM); (d) RSL3 (1 μM); (e) Erastin (10 μM) + Fer-1 (0.8 μM) 12h and then LDs-ClO (10 μM) for 10 min. λ_{ex} =488 nm, Red channel: λ_{em}=660-720 nm; Yellow channel: λ_{em} =540-600 nm.



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1. Z. Ye, M. Ji, K. Wu, J. Yang, A. A. Liu, W. Sun, D. Ding, D. Liu, *Angew. Chem. Int. Ed.* 2022, **61**, e202204518.