

Supplementary Information for

A lipid droplet-targeted fluorescent probe for fluorescence imaging of cell and zebrafish viscosity

Hua Zheng, Guofang Li, Langdi Zhang, Minguang Fan, Weiyang Lin*

Institute of Optical Materials and Chemical Biology, Guangxi Key Laboratory of Electrochemical Energy Materials, School of Chemistry and Chemical Engineering, Guangxi University, Nanning, Guangxi 530004, P. R. China

Email: weiyanglin2013@163.com

*Correspondence to: Weiyang Lin, Institute of Optical Materials and Chemical Biology, Guangxi Key Laboratory of Electrochemical Energy Materials, School of Chemistry and Chemical Engineering, Guangxi University, Nanning, Guangxi 530004, P. R. China

Email: weiyanglin2013@163.com.

Table of Contents

Experimental.....	S3
1. Materials and Instrumentations.....	S3
2. Steps of synthesis.....	S3
2.1 Synthesis routine of the probe CA-LD	S3
2.2 Synthesis of the compound 2.....	S3
2.3 Synthesis of the probe CA-LD	S3
3. Optical studies and analysis.....	S4
4. Culture and preparation of HeLa cells.....	S4
5. Cytotoxicity assay.....	S4
6. Co-location experimental imaging.....	S4
7. Fluorescence imaging of distinguishing normal cells and cancer cells.....	S5
8. Lipid droplet accumulation experiment.....	S5
9. Confocal imaging of intracellular viscosity.....	S5
10. Confocal imaging of zebrafish viscosity.....	S5
Table S1.....	S5
Fig. S1.....	S7
Fig. S2.....	S7
Fig. S3.....	S8
Fig. S4.....	S8
Fig. S5.....	S9
Fig. S6.....	S9
Fig. S7.....	S10
Fig. S8.....	S10
Fig. S9.....	S11
Fig. S10.....	S12
Fig. S11.....	S12
Fig. S12.....	S13
Fig. S13.....	S13
Fig. S14.....	S14
Fig. S15.....	S14

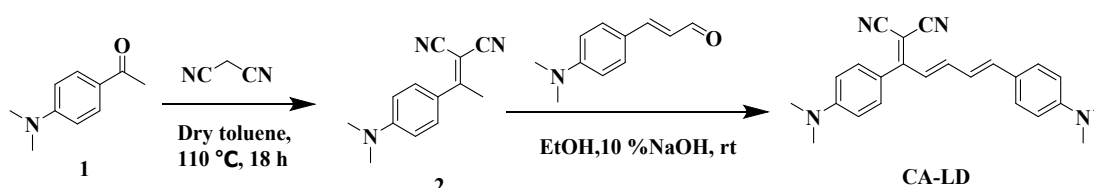
Experimental

1. Materials and Instrumentations

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. All experiments used ultra-pure water. Solvents were purified by standard methods prior. Ultra-pure water (18.2 MΩ cm) is used by ULPURE. TLC analysis was carried out on silica gel plates, and column chromatography was conducted over silica gel (mesh 200-300); both of them were purchased from Qingdao Ocean Chemicals. ¹H and ¹³C NMR spectra were measured on a Bruker Avance III HD 600 MHz NMR spectrometer (United States of America). High-resolution mass spectrometric (HRMS) analyses were measured on Brooke Solan X 70 FT-MS, Agilent 6540T. UV-vis absorption spectra were obtained on a Shimadzu UV-2700 spectrophotometer, and fluorescence spectra were measured on a HITACHI F4700 fluorescence spectrophotometer. The fluorescence imaging of cells was performed with a Leica TCS SP8 CARS confocal microscope.

2. Steps of synthesis

2.1 Synthesis routine of the probe CA-LD.



2.2 Synthesis of the compound 2

4-(dimethylamino) acetophenone (815 mg, 5 mmol), malononitrile (990 mg, 15 mmol) was dissolved in a round-bottomed flask containing 30 mL of toluene, and then ammonium acetate (770 mg, 10 mmol) and acetic acid (330 mg, 5.5 mmol) were added to the system and returned at 110 °C for 18 h. After the reaction was completed, the reaction system was spin dried, methanol was added, and a yellow solid was precipitated. compound 2 were filtered and dried to obtain 1.5 g with a yield of 94.8%. ¹H NMR (600 MHz, CDCl₃) δ 7.73 – 7.65 (m, 2H), 6.74 – 6.69 (m, 2H), 3.11 (s, 6H), 2.59 (s, 3H). ¹³C NMR (151 MHz, CDCl₃) δ 172.18, 153.25, 130.34, 122.06, 115.24, 114.84, 111.13, 76.55, 40.00, 22.95. ESI: m/z: calcd for [C₁₃H₁₄N₃] 212.1182, found: 212.1189.

2.3 Synthesis of the probe CA-LD

Compound 2 (211 mg, 1 mmol) was dissolved in a round-bottomed flask containing 3 mL of anhydrous ethanol, followed by 4-(dimethylamino) cinnamaldehyde (175 mg, 1 mmol) and 3 mL 10% NaOH solution, and reacted at room temperature for 3 h. After TLC monitoring of the reaction, it was poured into water and extracted with ethyl acetate. The product was obtained by vacuum distillation and purified by silica gel column (petroleum ether: ethyl acetate=7:1) to obtain 50 mg of red solid product with a yield of 13.6%. ¹H NMR (600 MHz, CDCl₃) δ 7.54 – 7.40 (m, 1H), 7.36 (dt, J = 16.9, 6.2 Hz, 4H), 7.01 – 6.96 (m, 1H), 6.88 (dd, J = 9.0, 4.4 Hz, 1H), 6.83 – 6.79

(m, 1H), 6.78 – 6.76 (m, 2H), 6.69 (d, J = 8.8 Hz, 2H), 3.09 (s, 6H), 3.05 (s, 6H). ¹³C NMR (151 MHz, CDCl₃) δ 171.02, 152.39, 151.50, 150.19, 144.25, 131.34, 129.46, 125.81, 123.81, 123.00, 120.47, 115.88, 115.03, 112.01, 111.30, 74.35, 40.13. ESI: m/z: calcd for [C₂₄H₂₄N₄] 368.2001, found: 368.2003.

3. Optical studies and analysis

A stock solution (1 mM) of the probe **CA-LD** was initially prepared in dimethyl sulfoxide (DMSO). All spectrometric probes were used at a concentration of 10 μM. The adjunction of 20 μL of stock solution was added to 2.0 mL of different solvent systems to obtain the probe **CA-LD** diluent. The solutions of various interfering substances (cations, anions, amino acids and active small molecules) were prepared with twice-distilled water. The providing solutions were mixed well before texting the spectra. Unless otherwise specified, the required fluorescence spectral measurement is generally an excitation wavelength of 467 nm, an excitation slit width of 5.0 nm, and an emission slit width of 5.0 nm.

4. Culture and preparation of HeLa cells

HeLa cells were cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO₂ and 95% air at 37 °C. Before the experiments, the HeLa cells in 35-mm glass-bottomed dishes were cultured to a density of 2×10⁵ cells per dish. Incubate the cells for 24 h. Cells will attach to the glass surface during this time.

5. Cytotoxicity assay

HeLa cells were seeded into 96-well plates, and 0, 1, 2, 5, 10, 20, and 50 μM (final concentration) of the probe **CA-LD** were added respectively. Subsequently, the cells were cultured at 37 °C in an atmosphere of CO₂ (5%) and air (95%) for 24 h. Next, MTT (10 μL, 5 mg/mL) was injected into every well and incubated for 4 h. Then, violet formazan was dissolved with DMSO (100 μL). The absorbance of the solution was measured at 467 nm by way of a microplate reader. The cell viability was determined by assuming 100% cell viability for cells without the probe **CA-LD**.

$$\text{The cell viability (\%)} = (\text{OD}_{\text{sample}} - \text{OD}_{\text{blank}}) / (\text{OD}_{\text{control}} - \text{OD}_{\text{blank}}) \times 100\%.$$

6. Co-location experimental imaging

The cells were incubated with the probe **CA-LD** (10 μM) for 10 min in an incubator of 95% air and 5% CO₂ at 37 °C. Then HeLa cells were incubated with BODIPY 493/503 (100 nM) (a commercial dye targeting LD) for 20 min. Afterward, the medium was removed and the cells were rinsed three times with PBS (10 mM, pH 7.4) for confocal imaging. The LD localization ability of the probe **CA-LD** was analyzed by the Pearson coefficient. laser confocal imaging. λ_{ex} = 488 nm, λ_{em} = 560-660 nm.

7. Fluorescence imaging of distinguishing normal cells and cancer cells

Normal cells (7702) and cancer cells (4T1, HepG 2, HeLa,) were respectively incubated with 10 μM probe for 20 min, and then the medium was removed and the cells were rinsed three times with PBS (10 mM, pH 7.4) for confocal imaging.

8. Lipid droplet accumulation experiment

HeLa cells were incubated with oleic acid (0 μM , 100 μM) for 30 min, and then incubated with the probe **CA-LD** for 20 min. Afterward, the medium was removed and the cells were rinsed three times with PBS (10 mM, pH 7.4) for confocal imaging. $\lambda_{\text{ex}} = 488 \text{ nm}$, $\lambda_{\text{em}} = 560\text{-}660 \text{ nm}$.

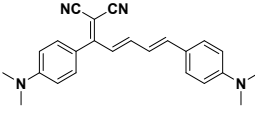
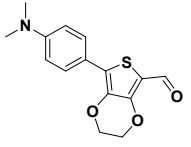
9. Confocal imaging of intracellular viscosity

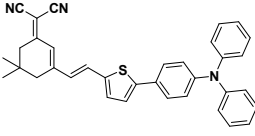
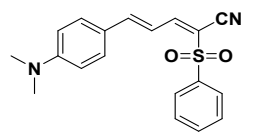
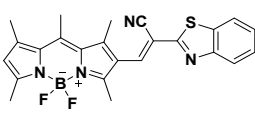
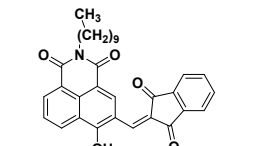
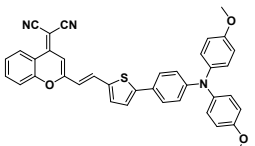
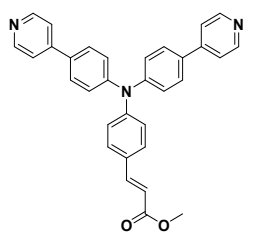
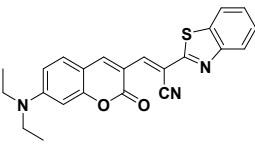
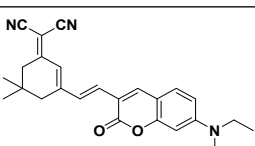
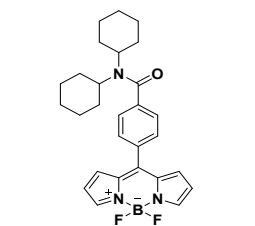
For cellular viscosity change, the HeLa cells were respectively incubated with rapamycin (10 μM), monensin (10 μM), nystatin (10 μM), LPS (10 μM) for 30 min and then incubated with the probe **CA-LD** for 20 min. Afterward, the medium was removed and the cells were rinsed three times with PBS (10 mM, pH 7.4) for confocal imaging. $\lambda_{\text{ex}} = 488 \text{ nm}$, $\lambda_{\text{em}} = 560\text{-}660 \text{ nm}$.

10. Confocal imaging of zebrafish viscosity

3-day-old zebra fish were transferred into a 30 mm glass culture dishes using a disposable sterilized dropper. Zebrafish were divided into multiple groups and incubated with rapamycin (10 μM), monensin (10 μM), nystatin (10 μM), LPS (10 μM) for 30 min and then incubated with the probe **CA-LD** for 20 min. Afterward, the medium was removed and the zebrafish were rinsed three times with PBS (10 mM, pH 7.4) for confocal imaging. $\lambda_{\text{ex}} = 488 \text{ nm}$, $\lambda_{\text{em}} = 560\text{-}660 \text{ nm}$.

Table S1 The latest research progress of viscosity probes

Probe structure	$\lambda_{\text{ex}}/\lambda_{\text{em}}$ (nm)	Targeted organelles	Imaging	References
	560/660	LDs	Living cells and zebrafish	This work
	530/600	LDs	Living cells and zebrafish	Dyes Pigm., 2024, 225, 112088

	500/540	LDs	Living cells and zebrafish	ACS Biomater. Sci. Eng., 2023, 9, 3c00409
	570/630	LDs	Living cells	Luminescence, 2024, 39, e4749
	415/480	LDs	Living cells	Talanta, 2024, 277, 126362
	500/550	LDs	Living cells and zebrafish	Anal. Chim. Acta, 2024, 1299, 342422
	520/800	LDs	Living cells	Anal. Chim. Acta, 2024, 1312, 342748
	560/620	LDs	Living cells	Sens. Actuators B-Chem., 2024, 414, 135942
	570/620	LDs	Living cells	Dyes Pigm., 2023, 220, 111656
	660/700	LDs	Living cells	J. PHOTOCH. PHOTOBIO. A., 2022, 425, 113656
	505/550	LDs	Living cells	J Mater Chem B., 2024, 12, 3022-3030

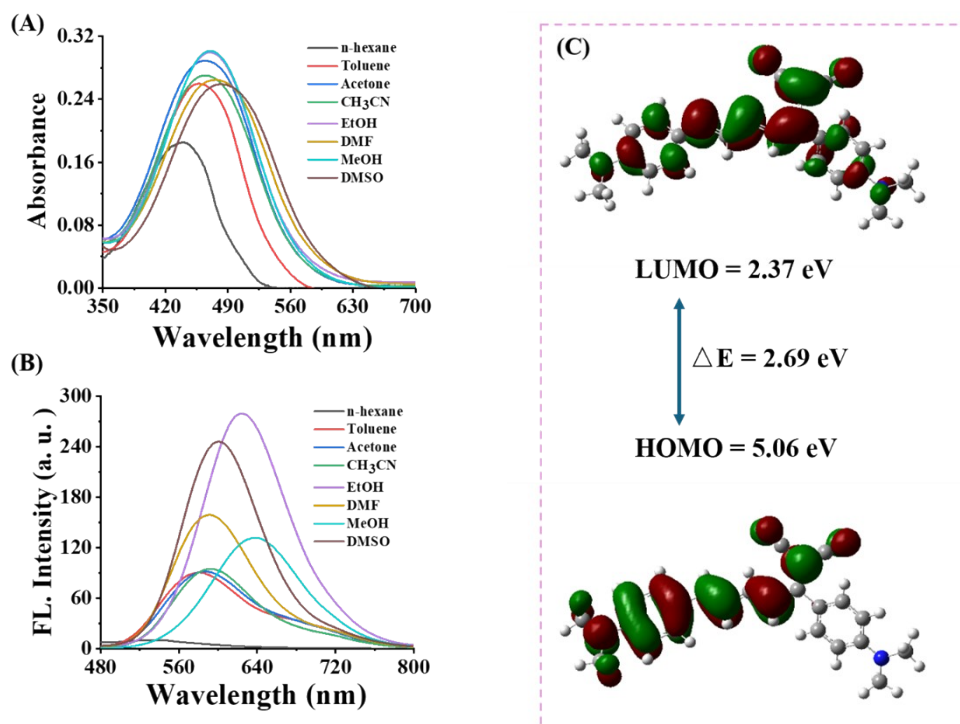


Fig. S1. (A) Absorption spectra of the probes **CA-LD** in various solvents (n-hexane, Toluene, Acetone, CH₃CN, EtOH, DMF, MeOH, DMSO). (B) Fluorescence spectra of the probe **CA-LD** in various solvents (n-hexane, Toluene, Acetone, CH₃CN, EtOH, DMF, MeOH, DMSO). (C) Spatial electron distribution B3LYP/6-31G (d, p) levels of HOMO and LUMO of probes.

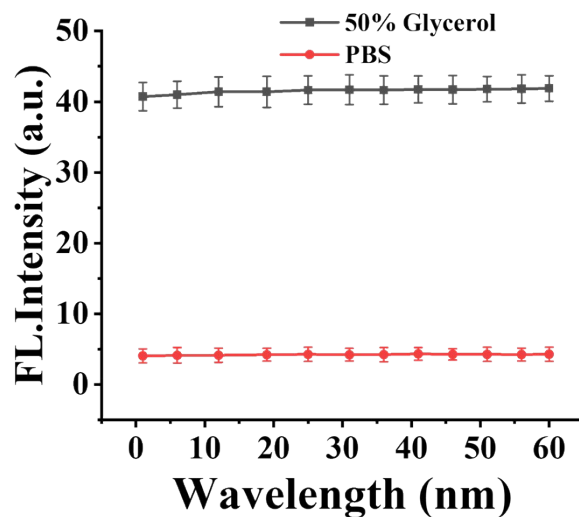


Fig. S2. The photostability of the probe **CA-LD** (10 μ M) with continuous laser scanning during 60 min in PBS buffer solutions and 50% glycerol, and the emission peak was collected at the time interval of 1 min, $\lambda_{\text{ex}} = 488$ nm, $\lambda_{\text{em}} = 560$ -660 nm.

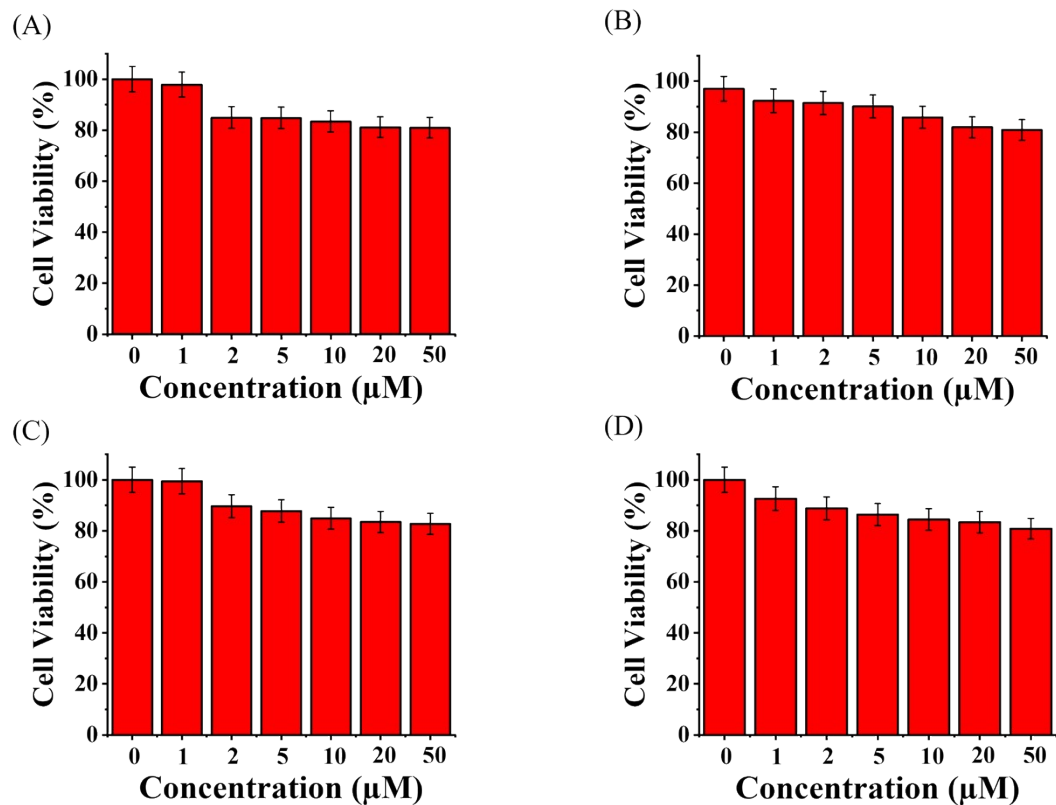


Fig. S3. Cytotoxicity assays of the probe **CA-LD** at different concentrations for (A) 7702, (B) 4T1, (C) HepG 2, (D) HeLa cells with MTT.

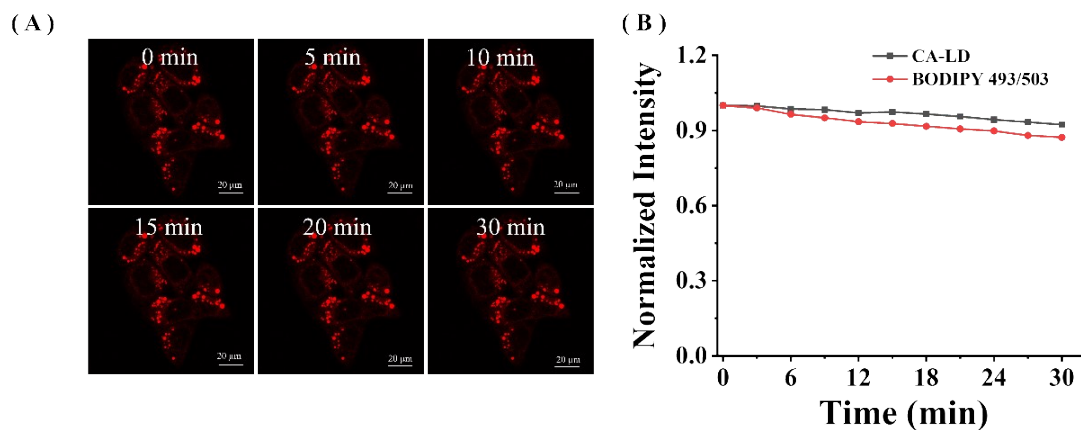


Fig. S4. (A) Photostability of the probe **CA-LD** in living HeLa cells. HeLa cells were incubated with 10 μM the probe **CA-LD** for 20 min, and then the medium was removed and the cells were rinsed three times with PBS (10 mM, pH 7.4) for confocal imaging; (B) the mean fluorescence intensity at different times of (A). Irradiation time: 60 s per scan. $\lambda_{\text{ex}} = 488 \text{ nm}$, $\lambda_{\text{em}} = 560\text{-}660 \text{ nm}$, Scale bars: 20 μm .

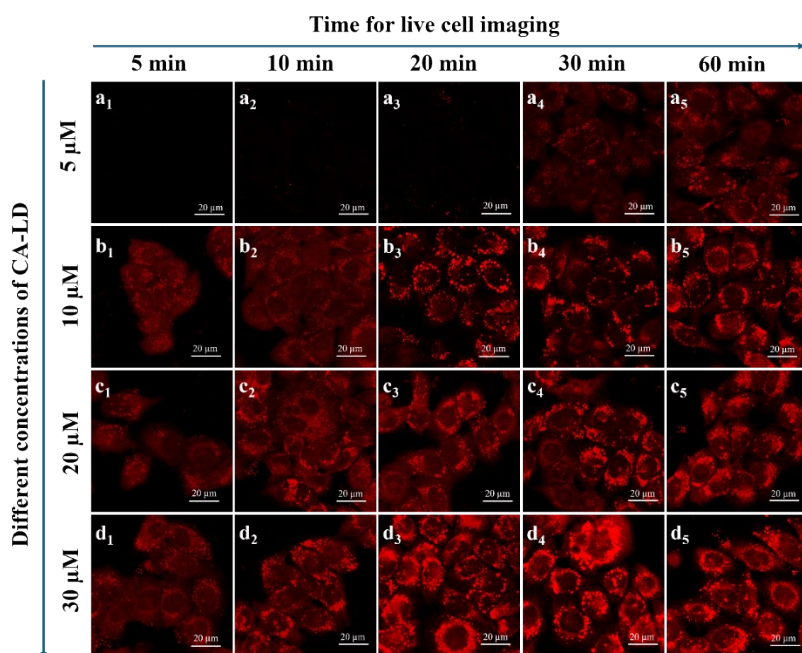


Fig. S5. Imaging of HeLa cells with different concentrations of CA-LD and incubation for 5-60 min. (a₁-a₅) Fluorescence imaging of HeLa cells incubated with 5 μM CA-LD for different times. (b₁-b₅) Fluorescence imaging of HeLa cells incubated with 10 μM CA-LD for different times. (c₁-c₅) Fluorescence imaging of HeLa cells incubated with 20 μM CA-LD for different times. (d₁-d₅) Fluorescence imaging of HeLa cells incubated with 30 μM CA-LD for different times. $\lambda_{ex} = 488$ nm, $\lambda_{em} = 560-660$ nm, Scale bars: 20 μm.

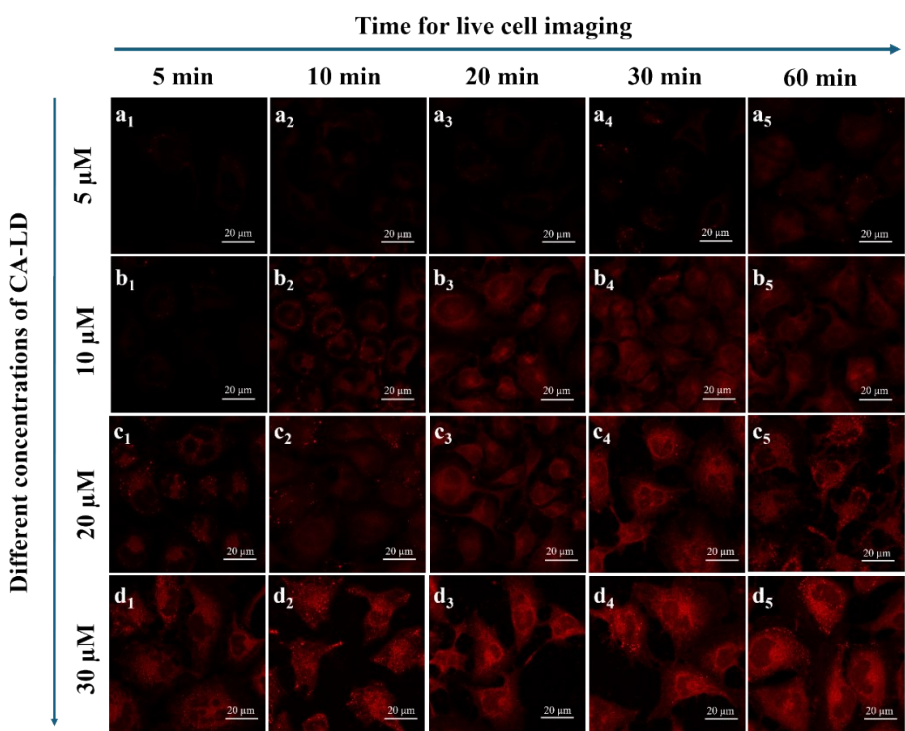


Fig. S6. Imaging of 7702 cells with different concentrations of CA-LD and incubation for 5-60 min. (a₁-a₅) Fluorescence imaging of 7702 cells incubated with 5 μM CA-LD for different times. (b₁-b₅) Fluorescence imaging of 7702 cells incubated with 10 μM CA-LD for different times. (c₁-c₅)

Fluorescence imaging of 7702 cells incubated with 20 μM CA-LD for different times. (d₁-d₅) Fluorescence imaging of 7702 cells incubated with 30 μM CA-LD for different times. $\lambda_{\text{ex}} = 488$ nm, $\lambda_{\text{em}} = 560\text{-}660$ nm, Scale bars: 20 μm .

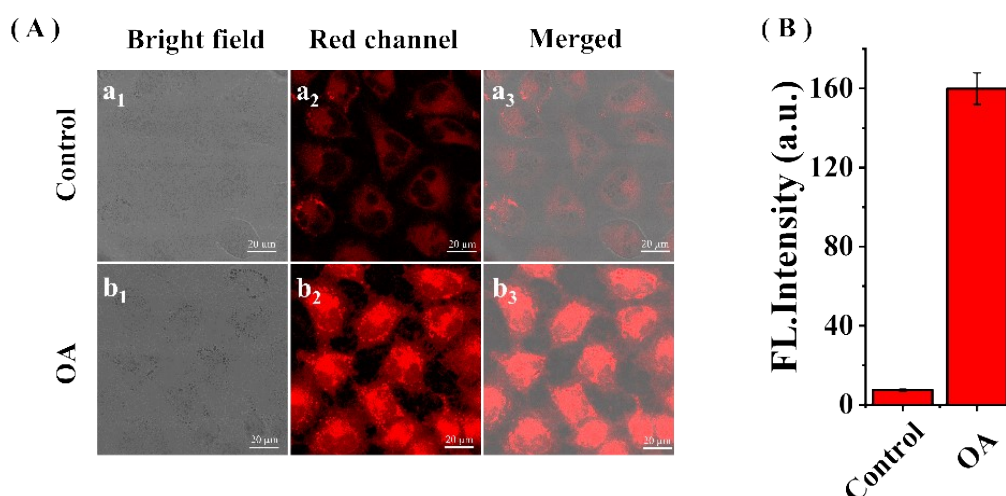


Fig. S7. LDs accumulation experiment in HeLa cells. (a₁-a₃) Only the probe CA-LD incubates with the cells; (b₁-b₃) the cells was incubated with the oleic acid (100 μM), and then incubated with the probe CA-LD for 20 min. Afterward, the medium was removed and the cells were rinsed three times with PBS (10 mM, pH 7.4) for confocal imaging. $\lambda_{\text{ex}} = 488$ nm, $\lambda_{\text{em}} = 560\text{-}660$ nm, Scale bars: 20 μm .

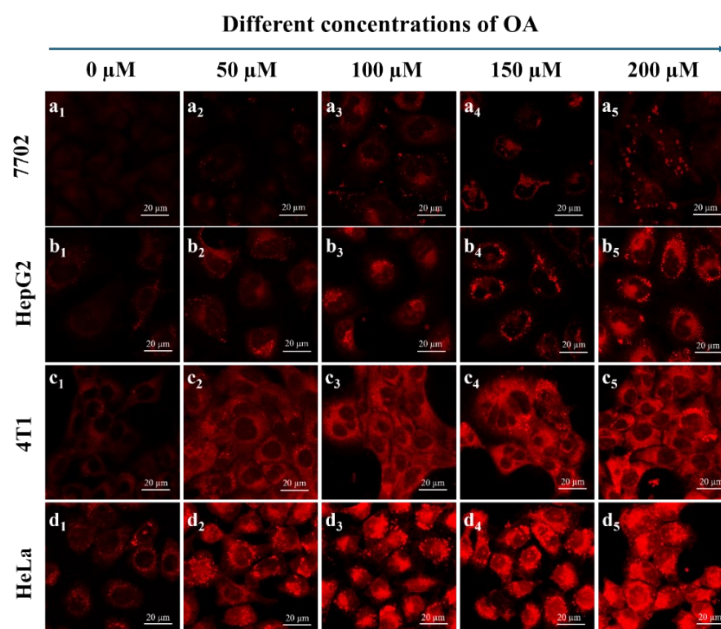


Fig. S8. Live cells imaging. incubate different cells with different concentrations of oleic acid for 30 min, then add CA-LD and continue incubation for 20 min. (a₁-a₅) Fluorescence imaging of 7702 cells incubated with different concentrations of OA (0, 50, 100, 150, 200 μM). (b₁-b₅) Fluorescence imaging of HepG2 cells incubated with different concentrations of OA (0, 50, 100, 150, 200 μM). (c₁-c₅) Fluorescence imaging of 4T1 cells incubated with different concentrations of OA (0, 50, 100,

150, 200 μM). (d₁-d₅) Fluorescence imaging of HeLa cells incubated with different concentrations of OA (0, 50, 100, 150, 200 μM). $\lambda_{\text{ex}} = 488 \text{ nm}$, $\lambda_{\text{em}} = 560\text{-}660 \text{ nm}$, Scale bars: 20 μm

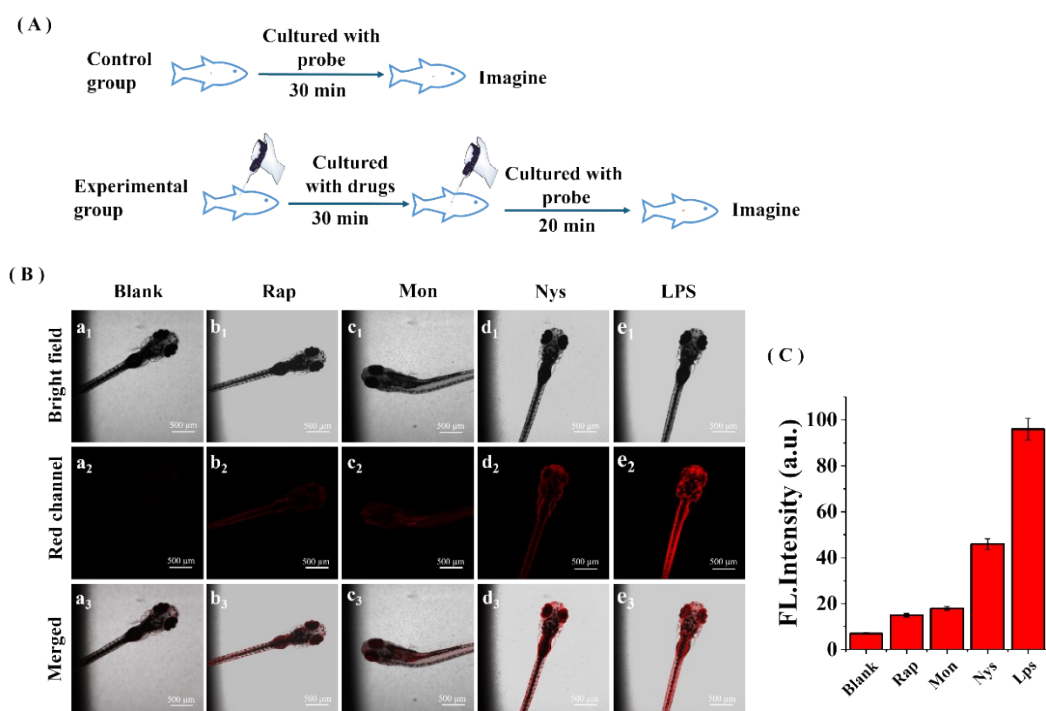


Fig. S9. (A) Imaging illustrations of the zebrafish viscosity experiment. (B) Confocal fluorescence images of the probe **CA-LD** (10 μM) in zebrafish: (a₁-a₃) zebrafish were incubated with the probe **CA-LD** only for 20 min; (b₁-b₃) zebrafish were treated with rapamycin (20 μM) for 30 min prior to incubation with the probe **CA-LD** for 20 min; (c₁-c₃) zebrafish were treated with monensin (20 μM) for 30 min prior to incubation with the probe **CA-LD** for 20 min; (d₁-d₃) zebrafish were treated with nystatin (20 μM) for 30 min prior to incubation with the probe **CA-LD** for 20 min; (e₁-e₃) zebrafish were treated with LPS (20 μM) for 30 min prior to incubation with the probe **CA-LD** for 20 min; (C) Average fluorescence intensity of zebrafish under the aforementioned conditions. $\lambda_{\text{ex}} = 488 \text{ nm}$, $\lambda_{\text{em}} = 560\text{-}660 \text{ nm}$, Scale bars: 500 μm

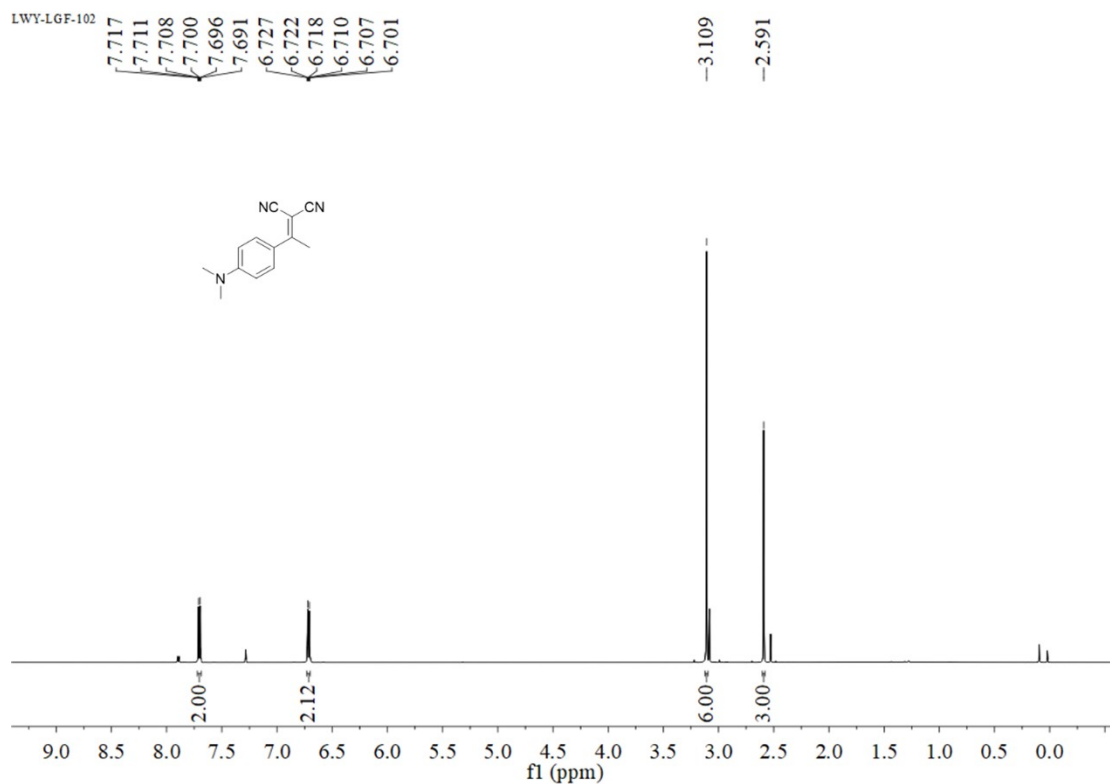


Fig. S10. The ^1H NMR spectrum of the compound **2** in CDCl_3 .

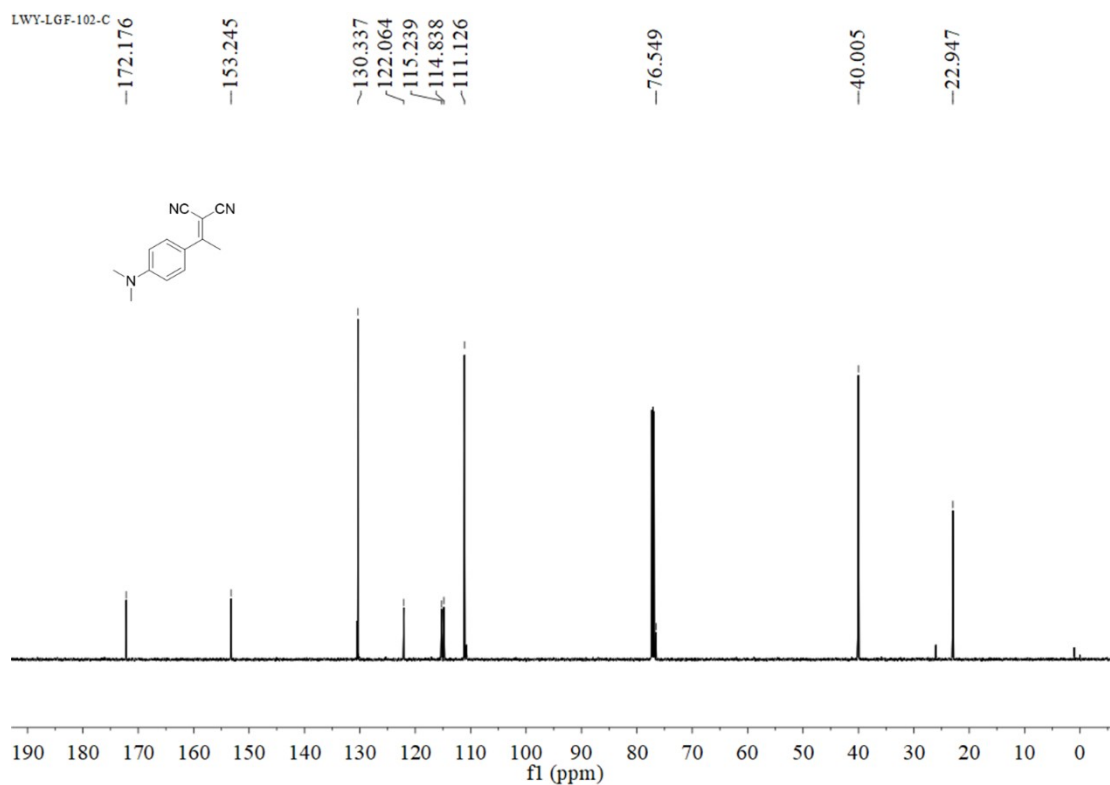


Fig. S11. The ^{13}C NMR spectrum of the compound **2** in CDCl_3

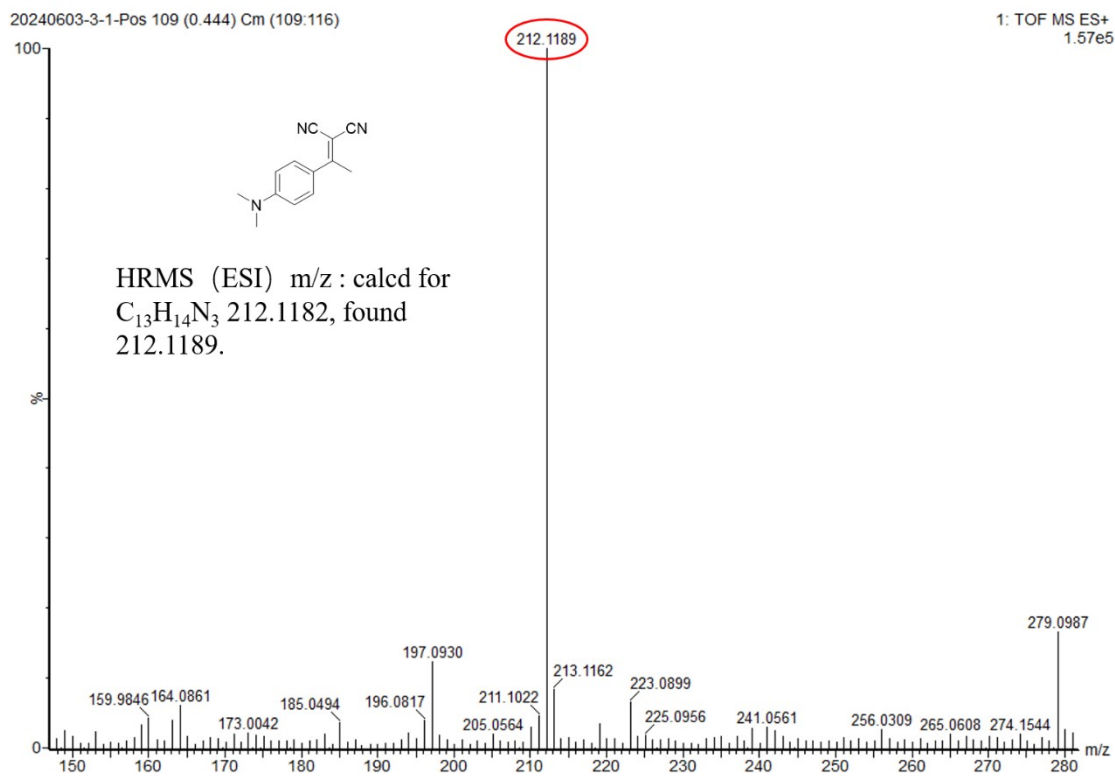


Fig. S12. The HRMS spectrum of the compound 2.

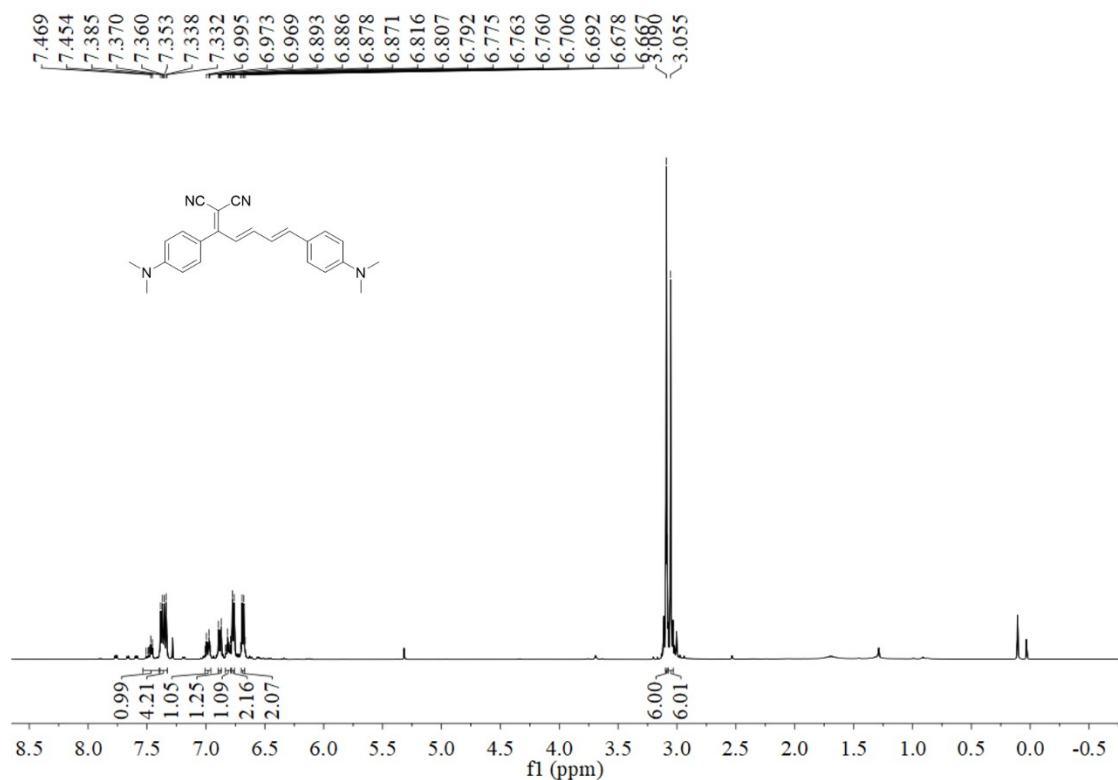


Fig. S13. The ¹H NMR spectrum of the probe CA-LD in CDCl₃.

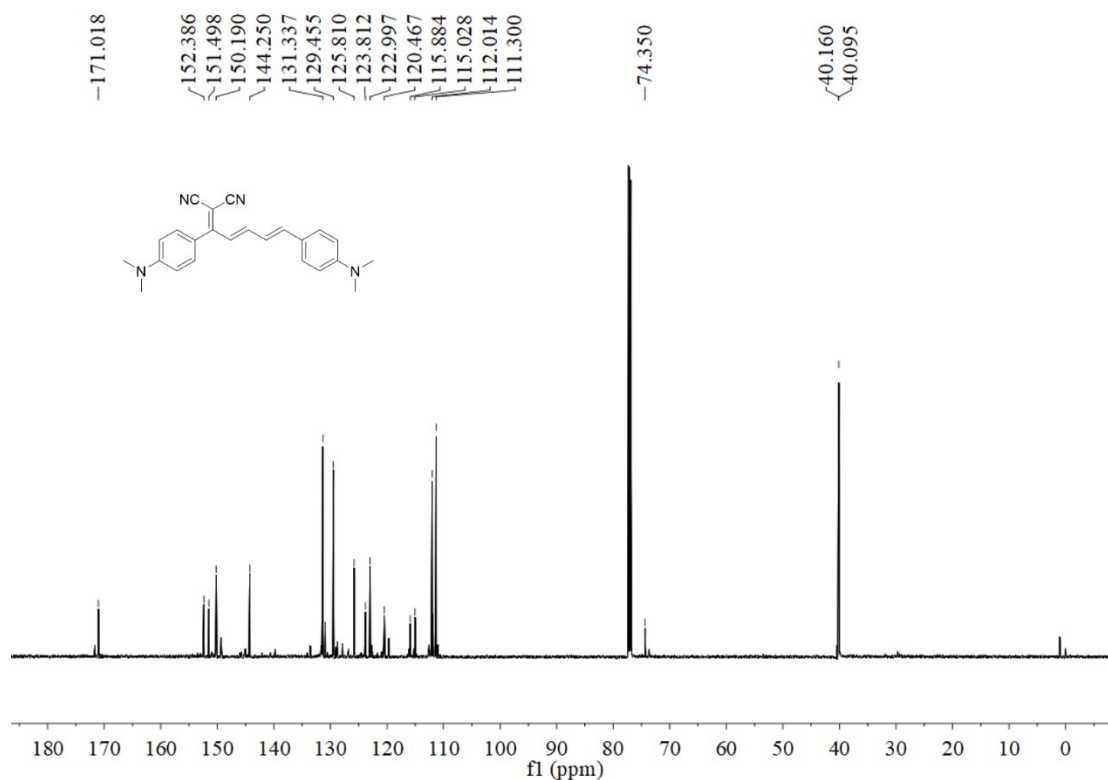


Fig. S14. The ¹³C NMR spectrum of the probe CA-LD in CDCl₃

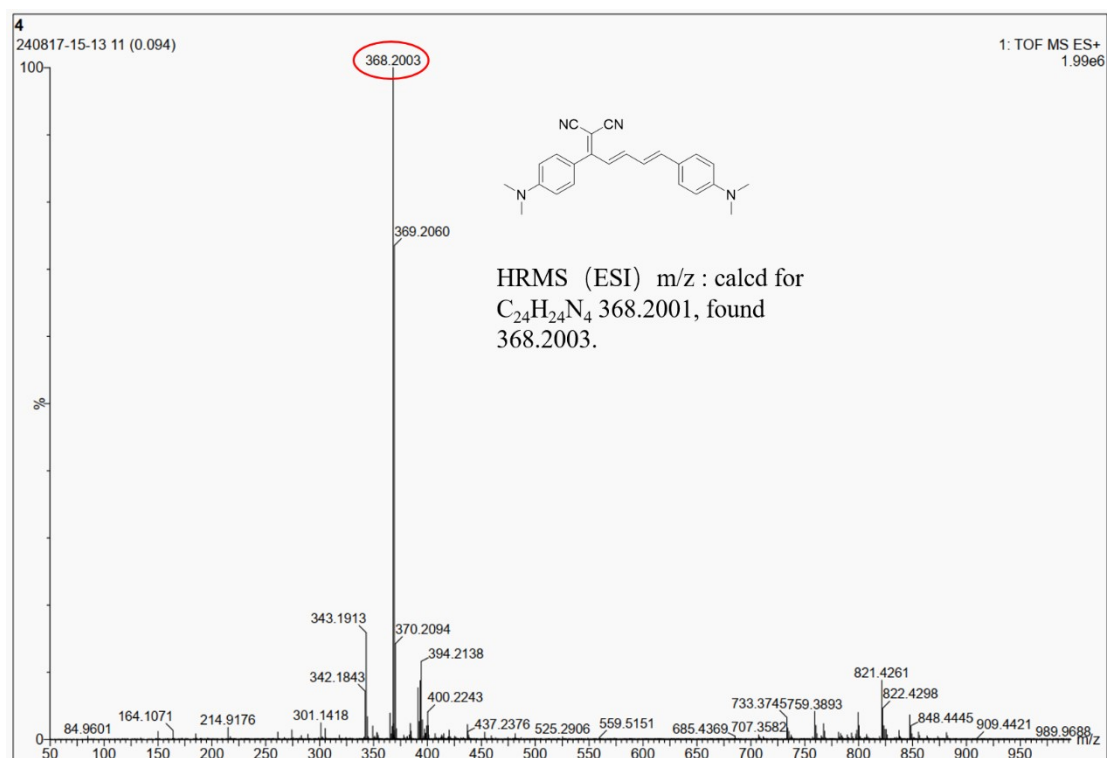


Fig. S15. The HRMS spectrum of the probe CA-LD.