Supporting Information

Polarity-triggered high-contrast fluorescent imaging of lipid droplets via a naphthalimide fluorophore

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Materials and Instrumentals

^{1}H NMR spectra were obtained from a 600 MHz Bruker Advance II. Mass spectrometry analysis was carried out using Aglient 7250, USA and JEOL-JMS-T100LP AccuTOF, Japan. Absorption spectra were determined using Shimadzu UV-3600 spectrophotometer. Fluorescence spectra were carried out at room temperature by Edinburgh FS5 fluorescence spectrometer. Theoretical calculations were performed using the Gaussian 09 program packages, and the structures were optimized at the B3LYP/6-31g(d) level. All chemical reagents were purchased at the highest commercial quality and used without further purification unless otherwise stated, and distilled water was used after purification by a water ultra-purification system.

Synthesis of NA-LD

Under nitrogen atmosphere, a mixture of 1[1] (381 mg, 1 mmol), 1-ethynyl-4-methoxybenzene (198 mg, 1.5 mmol), $Pd(PPh_3)_2Cl_2$ (70 mg, 0.1 mmol), CuI (19 mg, 0.1 mmol), and Et_3N (10 mg, 0.1 mmol) in toluene (30 mL) was reflux under stirring overnight, after cooling to room temperature, and the reaction mixture was poured into water and extracted with dichloromethane. The organic layer was dried with anhydrous Na_2SO_4 and concentrated. The residue was purified by column chromatography eluting with petroleum ether and dichloromethane (4/1, v/v) to afford a light-yellow solid (263 mg, 61%). ^{1}H NMR (600 MHz, $CDCl_3$) δ/ppm 8.75 (d, *J*=12 Hz, 1H), 8.64 (d, *J*=12 Hz, 1H), 8.55 (d, 2H), 7.93 (d, *J*=12 Hz, 1H), 7.84 (t, *J*=6 Hz, 1H), 7.73-7.66 (m, 1H), 7.65-7.58 (m, 3H), 7.52-7.45 (m, 1H), 6.98 (d, *J*=12 Hz, 2H), 4.61 (t, *J*=6 Hz, 2H), 3.90 (s, 3H), 3.26 (t, *J*=6 Hz, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ/ppm 163.90, 163.63, 160.58, 159.00, 136.35, 133.54, 132.49, 132.08, 131.56, 130.49, 130.37, 128.56, 128.16, 127.28, 123.23, 122.93, 121.67, 121.46, 114.34, 99.58, 85.38, 55.42, 40.18, 36.42. HRMS: m/z 433.1561 $[M+H]^+$, calcd. for $C_{28}H_{20}N_2O_3$: 432.1474.

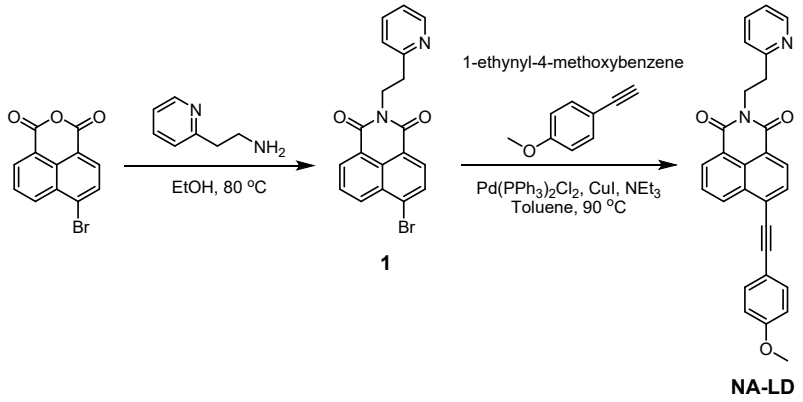


Figure S1. The synthetic route of probe **NA-LD**.

General Analytical Procedure

The stock solution of **NA-LD** was prepared in DMSO and stored at 4 °C before use. The solutions of K^+, Cu^{2+}, Mg^{2+}, Ca^{2+}, Zn^{2+} were prepared from their chloride salts. Sodium hypochlorite (ClO^-) was prepared by diluting 14.5% aqueous solution. Hydrogen peroxide (H_2O_2) was prepared by diluting the commercially available 30% aqueous solution. Peroxynitrite anion ($ONOO^-$) was prepared by mixing the HCl and H_2O_2 into a solution of sodium nitrite ($NaNO_2$) and sodium hydroxide (NaOH) at 0 °C. Glucose, cysteine (Cys) and glutathione (GSH) were dissolved in ultrapure water.

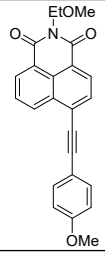
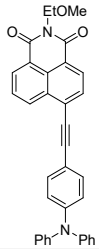
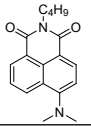
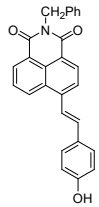
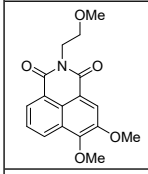
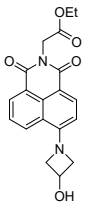
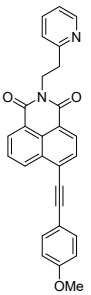
Cytotoxicity Cells

The in vitro cytotoxicity of **NA-LD** was assessed using the standard CCK-8 assay. 4T1 cells were inoculated in 96-well plates at 5×10^3 cells/well with DMEM in a humidified incubator at 37 °C with 5% carbon dioxide. After 24h, each well was treated with various concentrations of **NA-LD** (0, 2, 5, 10, 20 μM) in fresh medium. After 24 h, the cells were treated with CCK-8 reagent in fresh medium, and the absorbance of CCK-8 was determined by a Bio-Rad 680 ELIASA at 450 nm and all the measurements were completed for three times. Cell viability was calculated using the following formula: Cell viability ratio (%) = $(OD_{Sample} - OD_{PBS})/(OD_{Blank} - OD_{PBS}) \times 100\%$.

Fluorescence Imaging of Lipid Droplets in Living Cells

4T1 cells (human breast cancer cells), HepG2 cells (human liver cancer cells), 3T3 cells (embryonic mouse fibroblast cells), and HUVEC cells (human umbilical vein endothelial cells) were used for experiment. (1) 4T1 cells were treated with **NA-LD** (10 μM) and cocultured with Lyso-Tracker Red (2 μM), Mito Tracker Red (2 μM), ER Tracker Red (2 μM), HCS lipidTOX (2 μM), respectively, at 37 °C, then washed with PBS for three times, and conducted to fluorescence imaging. (2) 4T1 cells were treated with different concentrations of **NA-LD** (2.5, 5, 10, 20 μM) for 30 min at 37 °C, then washed with PBS for three times, and conducted to fluorescence image. (3) 4T1 cells were treated with oleic acid (oleic acid was used to stimulate cells to produce more LDs) for 0, 2, 4, 6 h respectively, the group of 0 h was set as the control group untreated oleic acid, after that the oleic acid was washed with PBS, then stained with **NA-LD** (10 μM) for 30 min, and conducted to fluorescence image after washing with PBS for three times. (4) 4T1, HepG2, 3T3 and HUVEC cells were incubated with **NA-LD** (10 μM) for 10 min at 37 °C, then conducted to fluorescence image after washing with PBS for three times. Fluorescence imaging was used with a ZEISS LSM900 confocal fluorescence microscope with proper excitation wavelength.

Table S1. Comparison of reported LD probe

Probe	$\lambda_{abs}/\lambda_{em}$ (nm)	Targeting specificity (LDs)	Photostability	Cytotoxicity	pH tolerance	Ref.
	382/470 (1,4-dioxane)	good	poor	low	high	[2]
	441/565 (1,4-dioxane)	good	NA	low	low	[3]
	404/500 (Toluene)	good	moderate	high	NA	[4]
	405/520 (1,4-dioxane)	poor	NA	low	high	[5]
	392/450 (Toluene)	good	NA	low	NA	[6]
	433/510 (1,4-dioxane)	good	moderate	low	NA	[7]
	382/470 (1,4-dioxane)	good	good	low	high	This work

NA: not available.

Calculation of Δ*f* values

The polarity value of the solvent is calculated by the following equation:

$$\Delta f = (\varepsilon - 1)/(2\varepsilon + 1) - (n^2 - 1)/(2n^2 + 1)$$

where ε denotes the dielectric constant and n denotes the refractive index.

Characteristics of NA-LD

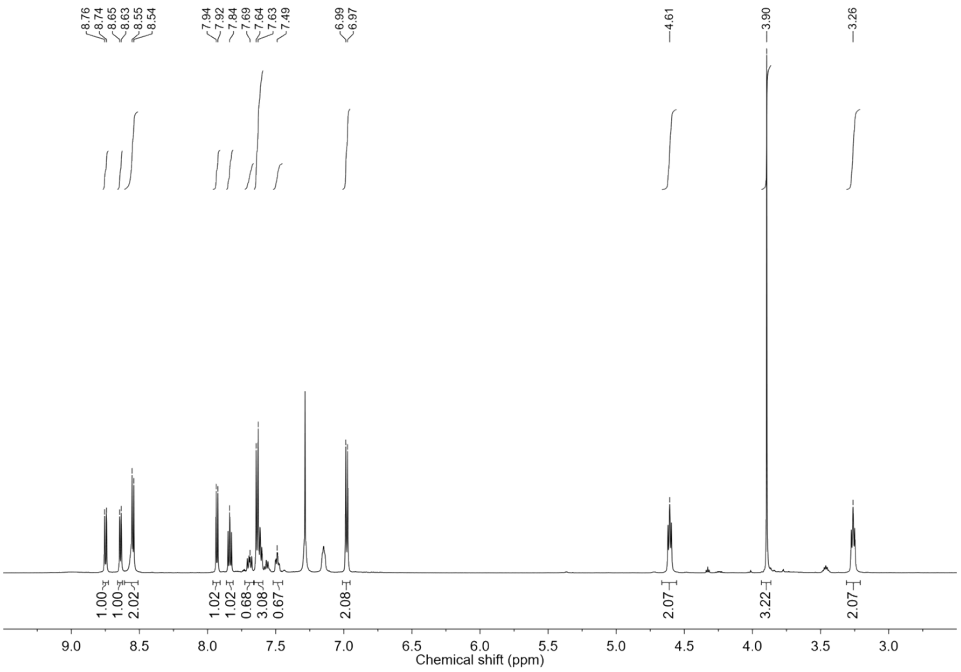


Figure S2. ^{1}H NMR spectrum of **NA-LD**.

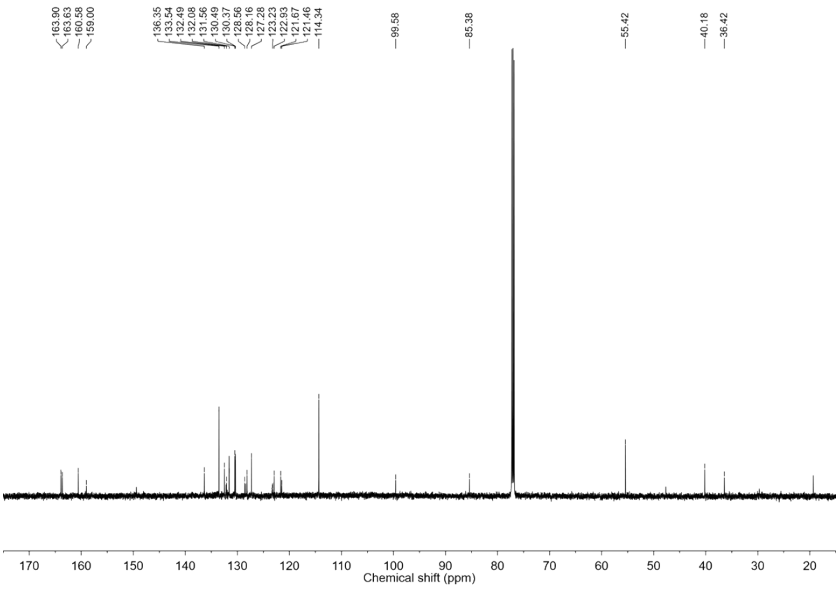


Figure S3. ^{13}C NMR spectrum of **NA-LD**.

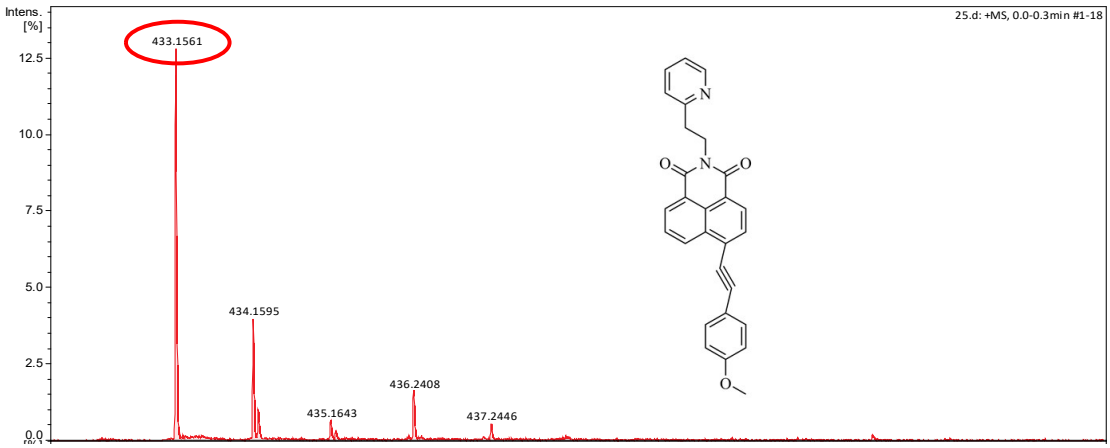


Figure S4. HR-MS spectrum of **NA-LD**.

Table S2. The properties of NA-LD in different solvents

Solvent	$\lambda_{abs,max}$ (nm)	$\lambda_{em,max}$ (nm)	Stokes shift (nm)	ε ($L \cdot moL^{-1} \cdot cm^{-1}$)	Δf
1,4-Dioxane	382	470	88	21300	0.0205
Ethyl acetate	387	494	107	22800	0.1998
THF	390	498	108	22300	0.2052
DMF	394	545	151	21000	0.2763
EtOH	396	549	153	20200	0.2897
MeOH	395	565	170	22100	0.3079
H_2O	369	573	204	7360	0.3212

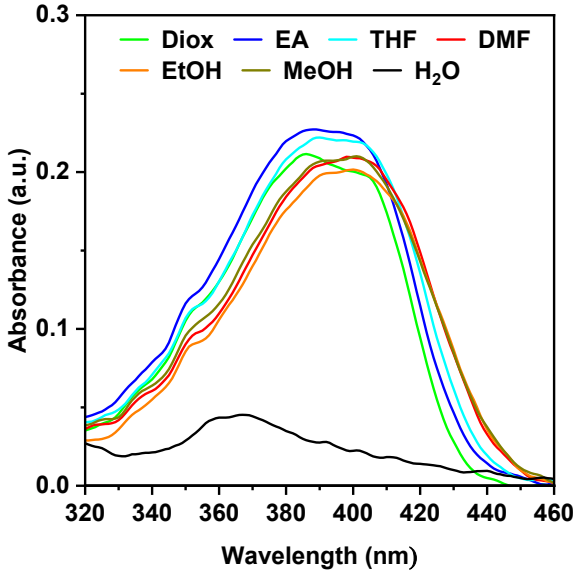


Figure S5. Absorption spectra of **NA-LD** (10 μM) in different polarity solvents.

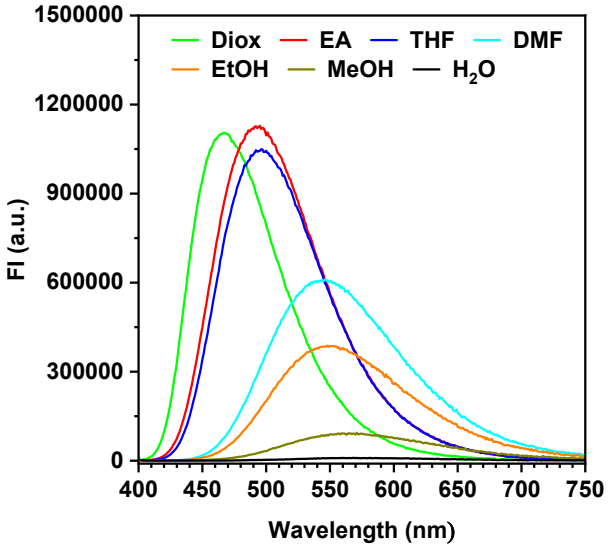


Figure S6. Fluorescence spectra of **NA-LD** (10 μM) in different polarity solvents.

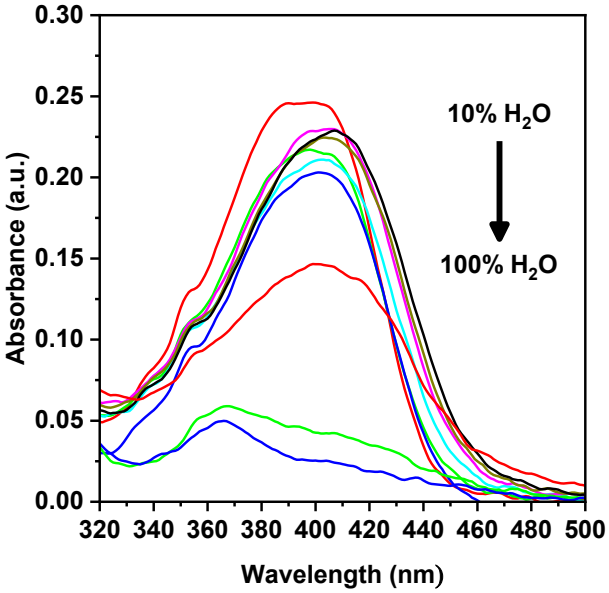


Figure S7. Absorption spectra of **NA-LD** (10 μM) in the H_2O/Diox mixture with H_2O fractions ranging from 10% to 100%.

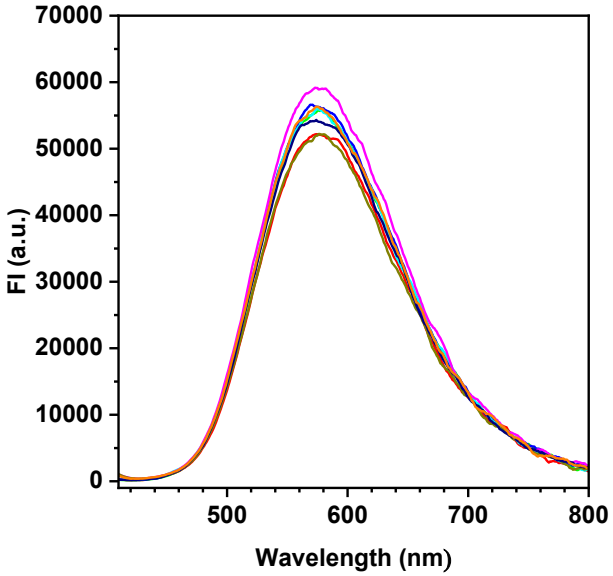


Figure S8. Fluorescence spectra of **NA-LD** (10 μM) in phosphate buffer (20 mM) with pH values ranging from 4 to 10.

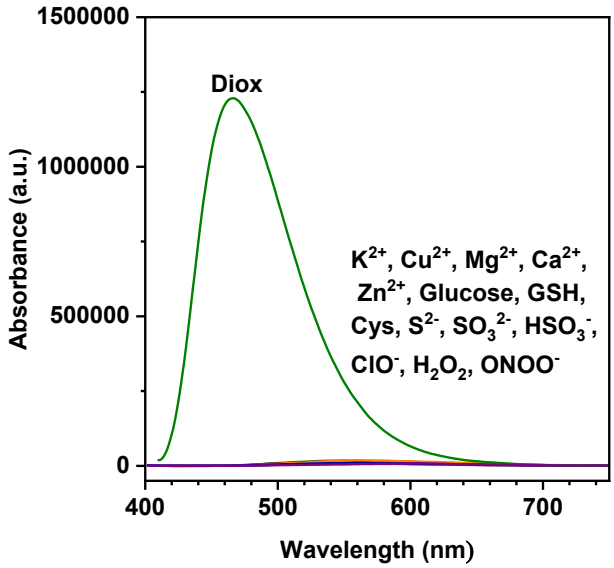


Figure S9. Fluorescence spectra of **NA-LD** (10 μM) to various substances: K^+ (150 mM), Cu^{2+} (100 mM), Mg^{2+} (2 mM), Ca^{2+} (2 mM), Zn^{2+} (100 mM), Glucose (10 mM), GSH (1 mM), Cys (100 mM), S^{2-} (100 mM), SO_3^{2-} (100 mM), HSO_3^- (100 mM), OCl^- (100 mM), H_2O_2 (100 mM), $ONOO^-$ (100 mM), Diox.

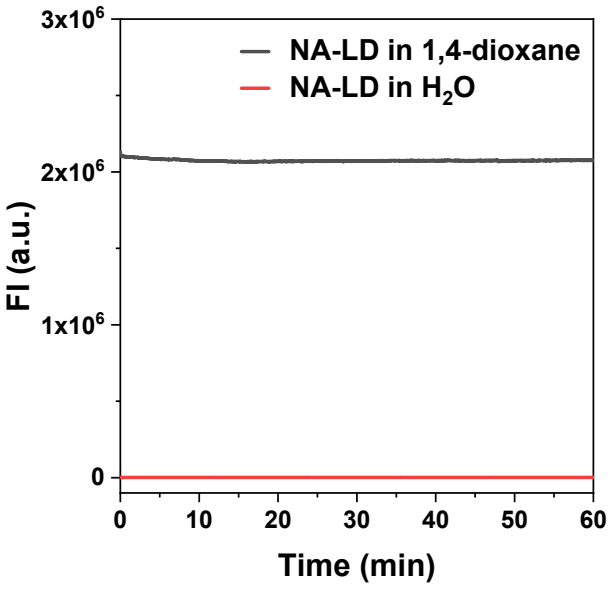


Figure S10. The time-dependent fluorescence of NA-LD in 1,4-dioxane and H_2O respectively.

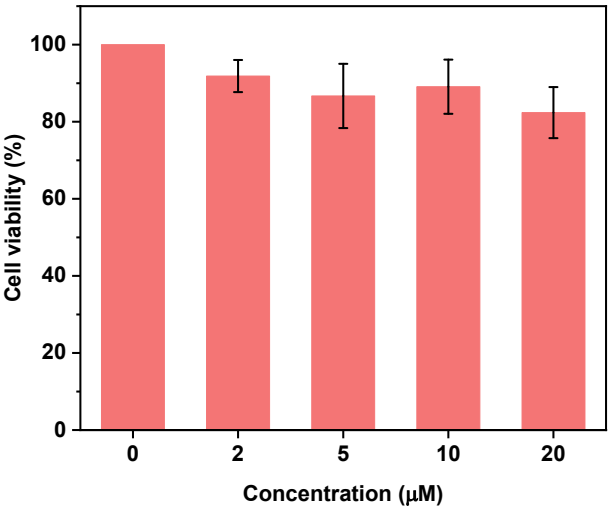


Figure S11. The viability of 4T1 cells treated with different concentrations of **NA-LD** (0, 2, 5, 10, 20 μM).

References

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[7] X. Li, J. Bian, M. Fu, Y. Zhang, H. Liu, B. Gao, Photostable fluorescent probes based on multifunctional group substituted naphthalimide dyes for imaging of lipid droplets in live cells, *Anal. Methods* 2022, 14, 1279-1284.