Supporting Information

A novel hydroxynaphthalimide-derived regenerative fluorescent probe for the detection of formaldehyde in cells and zebrafish

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1. Materials and instruments

All chemicals are obtained from commercial suppliers and can be used directly without further purification. Absorption spectra were determined by UV-3101PC spectrophotometer. Fluorescence spectra were determined by Horiba FluoroMax-4 spectrophotometer. High resolution mass spectra (HRMS) were obtained by LC-MS2010A instrument. ^{1}H and ^{13}C NMR data were obtained by Bruker AV-400 NMR spectrometer. Fluorescence imaging of formaldehyde in cells and zebrafish was performed under the Olympus FV1000-IX81 confocal fluorescence microscope.

2. Synthesis of compound 1 and compound 2.

4-Chloro-1,8-naphthalene anhydride (9.8 g, 42 mmol) and *N*-butylamine (6.1 g, 84 mmol) were dissolved in 100 mL anhydrous ethanol and refluxed under heating conditions for 48 h. After the reaction, compound **1** was obtained by filtration.

The compound **1** (2.0 g, 7 mmol) and *N*-hydroxylphthalimide (1.4 g, 8.4 mmol) were dissolved in 15 mL DMSO. Potassium carbonate (2.9 g, 21 mmol) was added to the reaction mixture. The reaction mixture was refluxed under heated conditions for 6 h. After the reaction was completed, potassium carbonate was precipitated by filtration. Subsequently, the solid was precipitated from the filtrate by adding concentrated hydrochloric acid and water, and compound **2** was obtained by filtration.

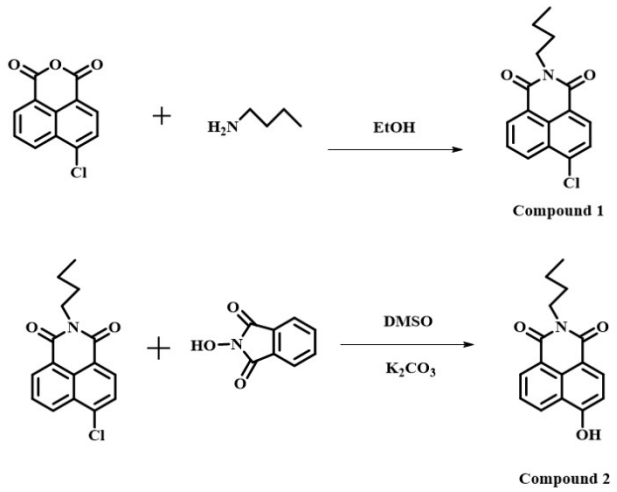


Fig. S1. Synthesis of compound **1** and compound **2**.

3. Determination of detection limit

The detection limit was calculated based on the fluorescence titration. Measured probe **NE-FA** 4 times using fluorescence emission spectroscopy, and the standard deviation of blank measurement was obtained. To obtain the slope, we plotted a standard curve based on the relationship between the fluorescence intensity value at 550 nm and the formaldehyde concentration. The detection limit was calculated with the following equation:

$$\text{Detection limit} = 3\sigma/k \qquad (1)$$

Where σ is the standard deviation of blank measurement, k is the slope between the fluorescence intensity versus the concentrations of formaldehyde.

4. Absorption spectra of probe NE-FA

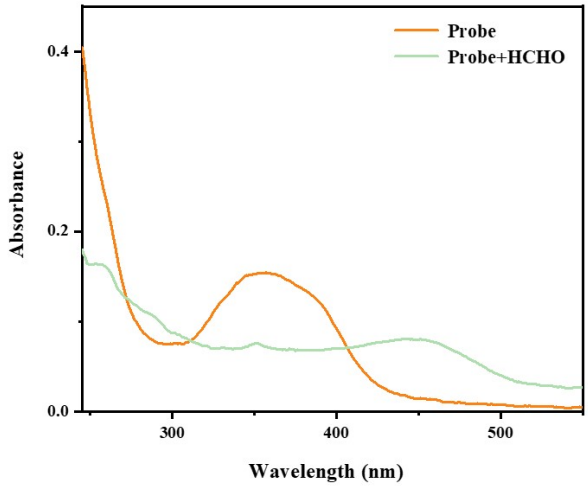


Fig. S2. Absorption responses of **NE-FA** (10 μM) toward formaldehyde (200 mM).

5. The effects of pH on NE-FA

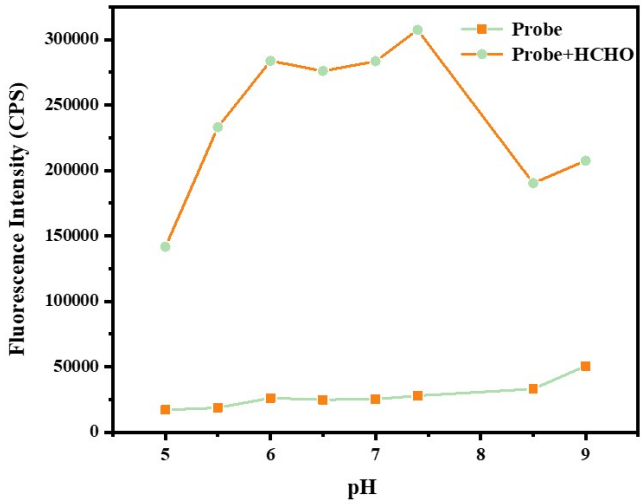


Fig S3. Identification effect of probe **NE-FA** (5 μM) on formaldehyde (50 mM) at different pH values.

6. ^{1}H NMR and ^{13}C NMR of probe NE-FA

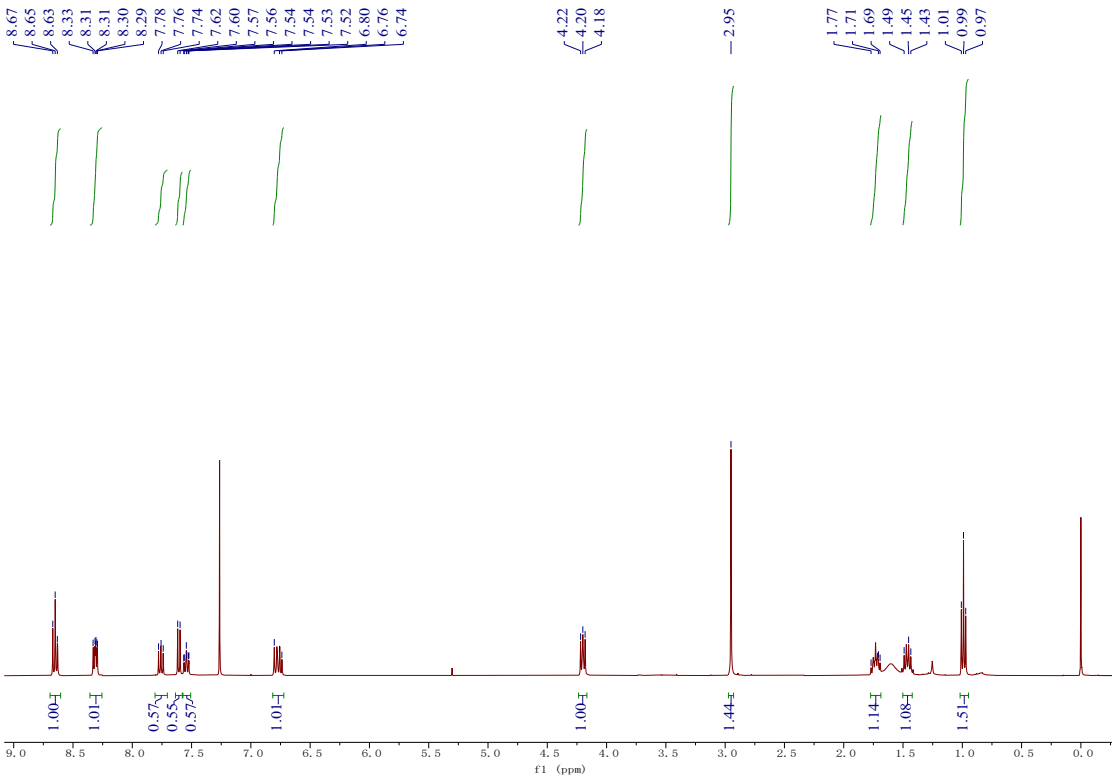


Fig. S4. The ^{1}H-NMR data of probe **NE-FA**.

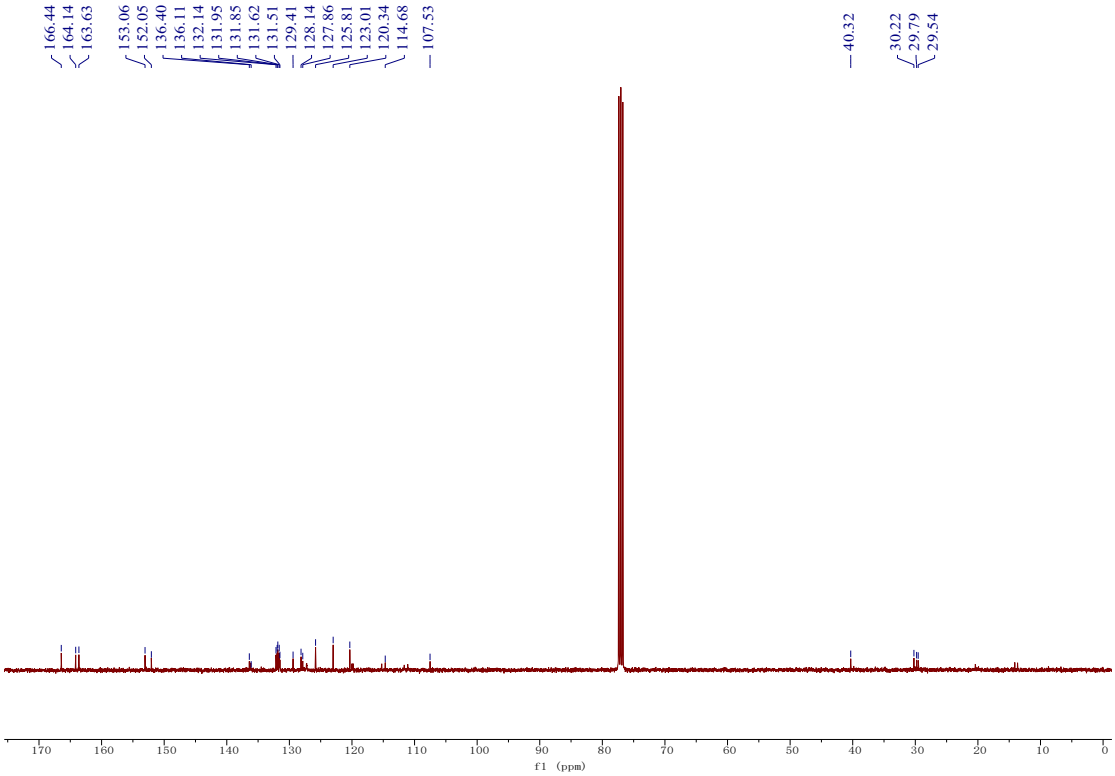


Fig. S5. The ^{13}C-NMR data of probe **NE-FA**.

7. ^{1}H NMR and ^{13}C NMR of compound 1 and 2

Compound **1**: ^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 0.98 (t, J = 6 Hz, 3H), 1.41-

1.48 (m, 2H), 1.69-1.74 (m, 2H), 4.17 (t, J = 4 Hz, 2H), 7.80-7.85 (m, 2H), 8.49 (d, J = 4 Hz, 1H), 8.58 (d, J = 8 Hz, 1H), 8.65 (d, J = 4 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm): 13.83, 20.37, 30.19, 40.37, 121.68, 123.17, 127.36, 127.83, 129.08, 129.31, 130.55, 131.08, 131.96, 138.95.163.49, 163.74.

Compound **2**: ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 0.91 (t, J = 6 Hz, 3H), 1.30-1.36 (m, 2H), 1.56-1.61 (m, 2H), 4.01 (t, J = 6 Hz, 2H), 7.14 (d, J = 4 Hz, 1H), 7.74 (t, J = 6 Hz, 1H), 8.33 (d, J = 8 Hz, 1H), 8.45 (d, J = 4 Hz, 1H), 8.51 (d, J = 4 Hz, 1H). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 14.18, 20.28, 30.22, 33.81, 110.42, 113.08, 122.27, 122.84, 126.04, 129.32, 129.63, 131.55, 133.98, 160.69, 163.45, 164.12.

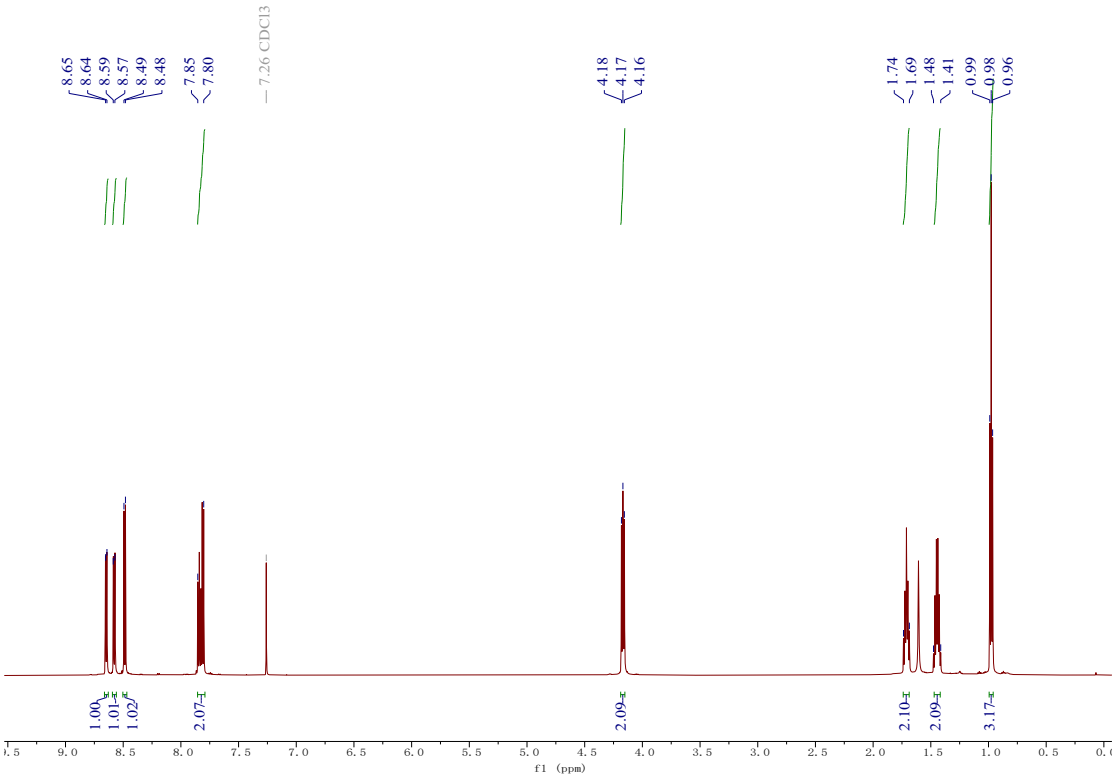


Fig. S6. The ^{1}H-NMR data of compound **1**.

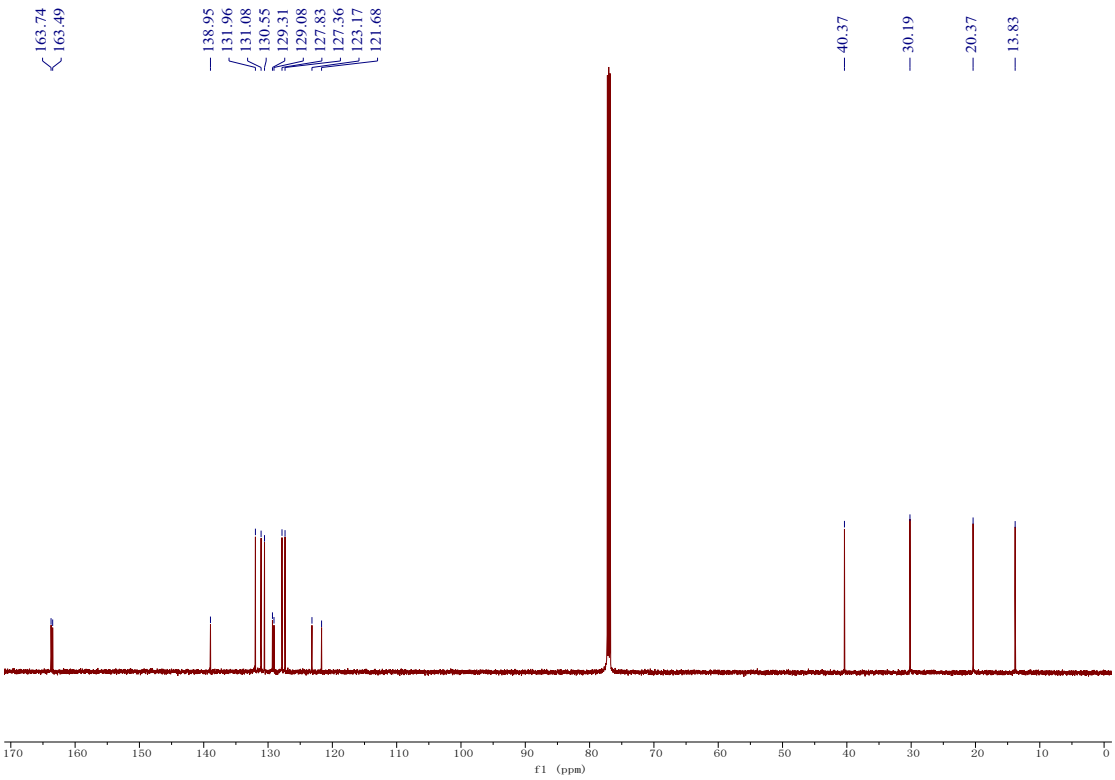


Fig. S7. The ^{13}C-NMR data of compound **1**.

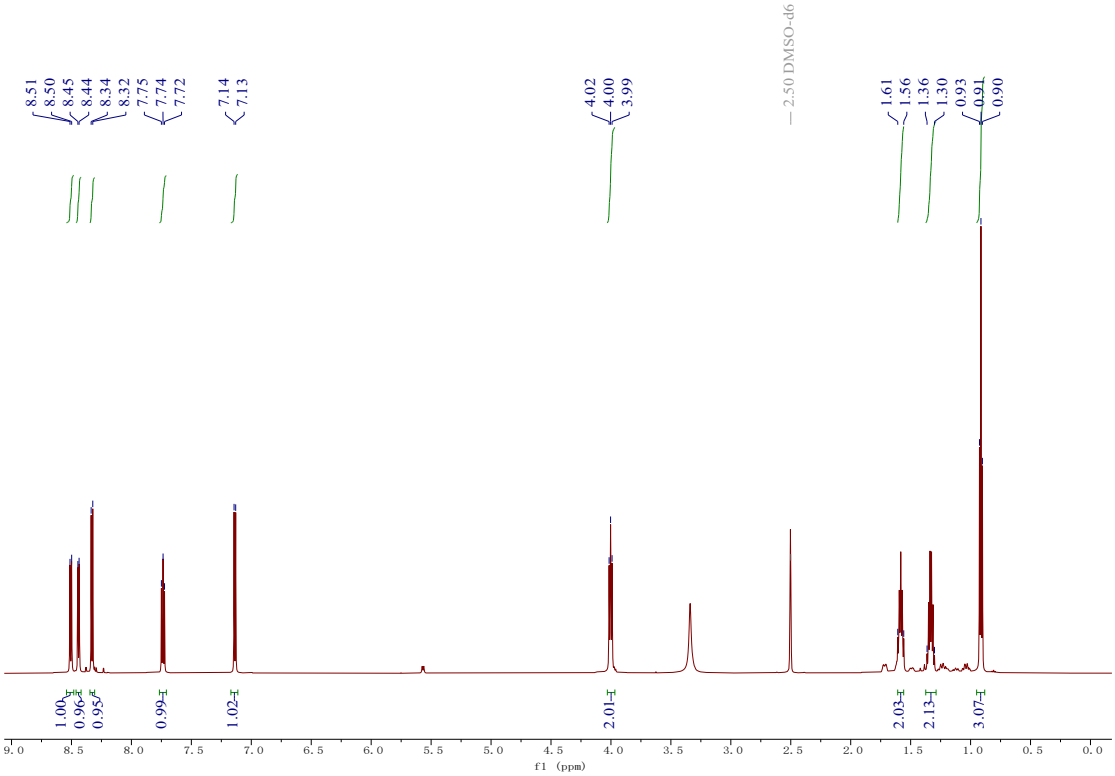


Fig. S8. The ^{1}H-NMR data of compound **2**.

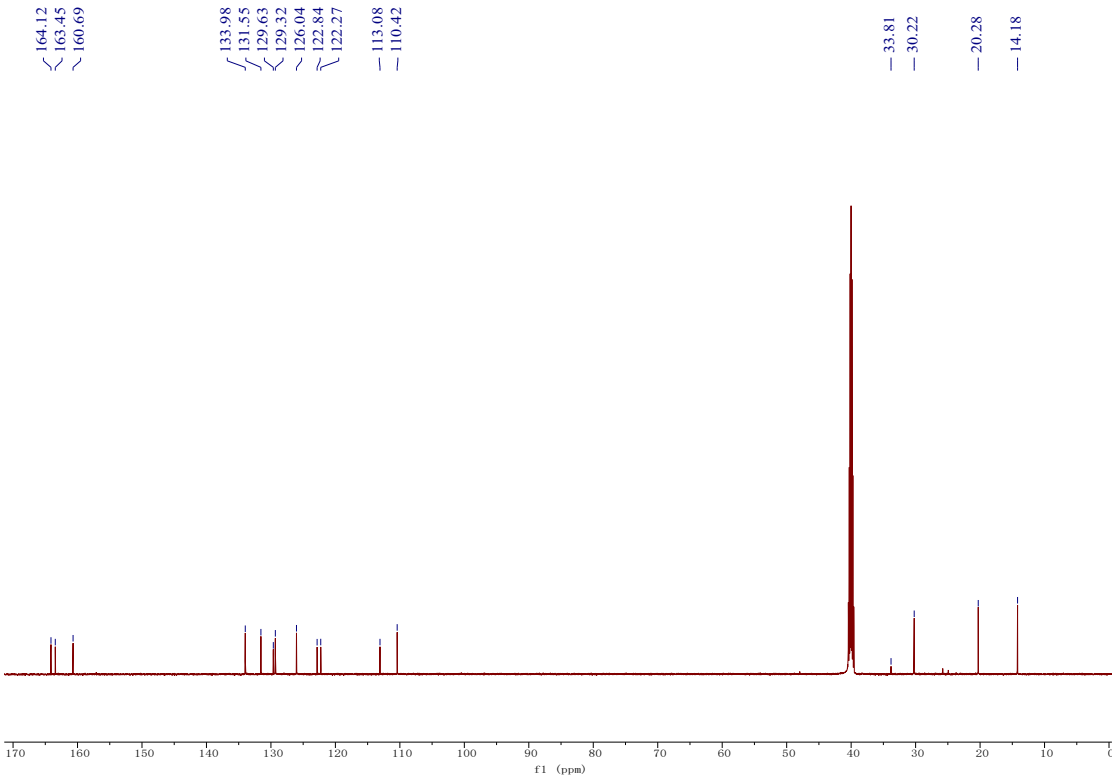


Fig. S9. The ^{13}C-NMR data of compound **2**.

8. Recognition mechanism of probe NE-FA for FA

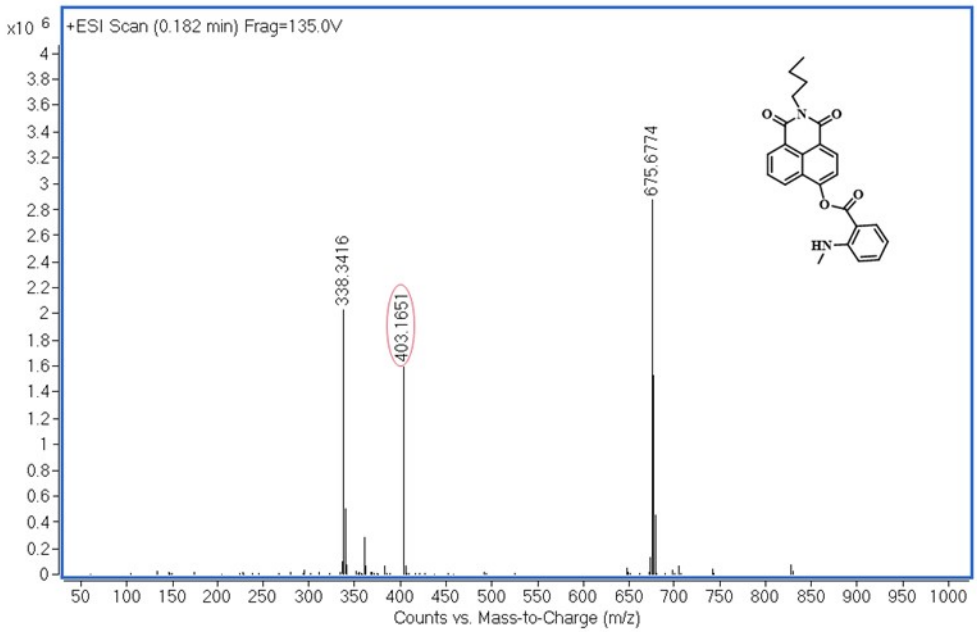


Fig. S10. HRMS of probe **NE-FA**.

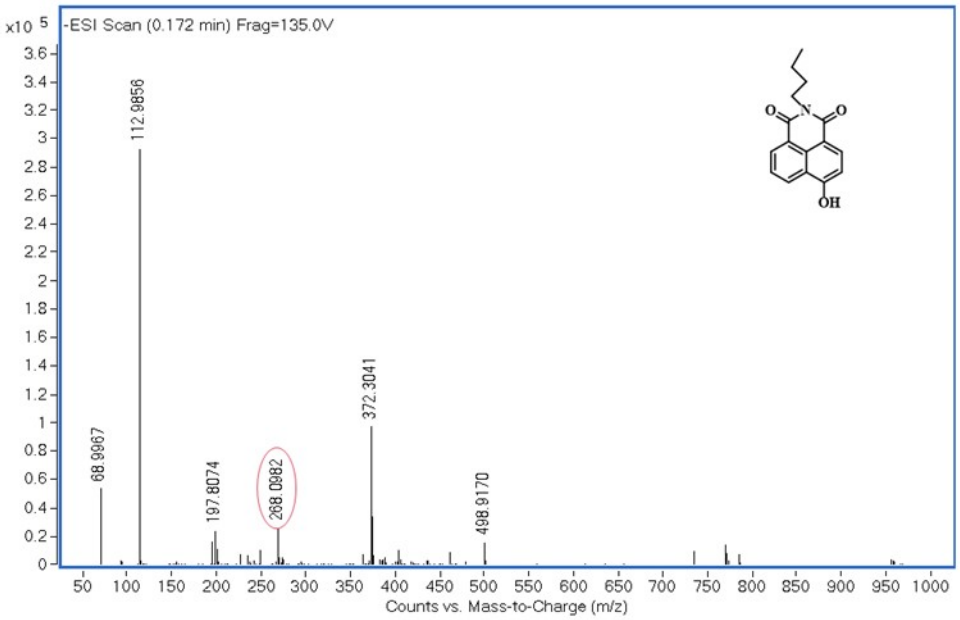


Fig. S11. HRMS of the reaction products of probe **NE-FA** and formaldehyde.

9. Fluorescence images in cells

The HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) and incubated under an atmosphere containing 5% CO_2 at 37 °C humidified air for 24 h. DMEM contains 10% fetal bovine serum and 1% penicillin-streptomycin.

The first group of cells was imaged directly as a control group. The second group of cells were incubated with probe (10 μM) only for 30 min and then imaged. The third group of cells were treated with $NaHSO_3$ (800 μM) for 1 h, then incubated with probe (10 μM) for 30 min, and the cells were imaged. The fourth group of cells were treated with formaldehyde (2 mM) for 1 h, and then incubated with probe (10 μM) for 30 min, and the cells were imaged. The fifth group of cells were treated with THFA (800 μM) for 1 h, then incubated with probe (10 μM) for 30 min, and the cells were imaged.

10. Fluorescence images in zebrafish

Healthy male and female zebrafish (AB stain) were maintained in different tanks with a 14 h light/10 h dark cycle at 28 °C. Then, sexually mature zebrafish were selected to induce spawning in tanks and the zebrafish eggs were obtained by giving light stimulation in the morning. After sterilizing and cleaning, the fertilized eggs were added to zebrafish embryo culture water (5 mM NaCl, 0.17 mM KCl, 0.4 mM $CaCl_2$, 0.16 mM $MgSO_4$) and cultured in illumination incubator at 28 °C.

The first group of zebrafish were incubated with probe (10 μM) only for 30 min and then imaged. The second group of zebrafish were treated with $NaHSO_3$ (800 μM) for 1 h, and then incubated with probe (10 μM) for 30 min. The third group of zebrafish were treated with formaldehyde (2 mM) for 1 h, and then incubated with probe (10 μM) for 30 min. The fourth group of zebrafish were treated with THFA (800 μM) for 1 h, then incubated with probe (10 μM) for 30 min, and the zebrafish were imaged.

11. Cytotoxicity assays

The cell viability of HeLa cells, treated with probe **NE-FA**, was assessed by a cell counting kit-8 (CCK-8, Dojindo Molecular Technologies, Tokyo, Japan). Briefly, HeLa, seeded at a density of 1×10^6 cells·mL^{-1} on a 96-well plate, were maintained at 37℃ in a 5% CO_2 / 95% air incubator for 12 h. Then the live HeLa cells were incubated with various concentrations (0, 5, 10, 20 μM) of probe **NE-FA** suspended in culture medium for 12 h. Subsequently, CCK-8 solution was added into each well for 2 h, and absorbance at 550 nm was measured.

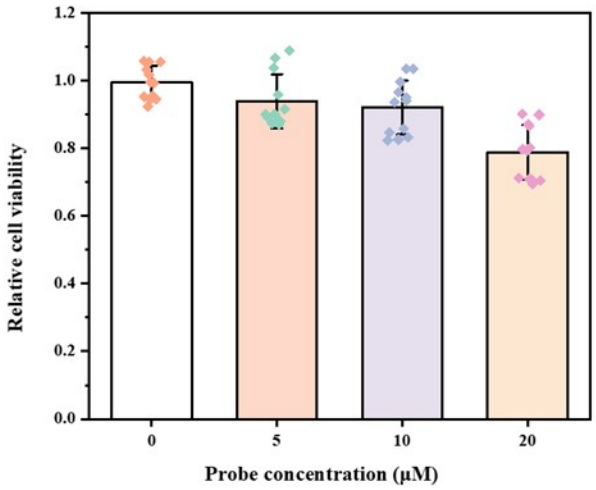
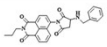
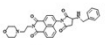


Fig. S12. Cytotoxicity assays of different concentrations probe **NE-FA** for HeLa cells.

12. Comparison of the properties of probes for FA

Probes	λ_{ex}, λ_{em}/ nm	Detection limit	Response time	Bio-imaging	Regeneration performance	Ref.
	359, 470	0.48 μM	10 min (pH=7.4) 2 h (pH=5.5)	Cell (HeLa)	✓	J. Am. Chem. Soc., 2018, 140, 16408−16412
	359, 470	0.145 μM	30 min	Cell (HeLa)	✓	J. Am. Chem. Soc., 2018, 140, 16408−16412
	368, 445	1.0 μM	2 h	Cell (HepG 2)	✓	J. Mater. Chem. B., 2023, 11, 4408-4415
	633, 650	10 μM	3 h	Cell (HEK293TN and NS1)	—	J. Am. Chem. Soc., 2015, 137: 10890-10893
	318, 359/451	59.6 μM	2 h	Cell (HeLa)	—	Chem. Commun., 2016, 52(21): 4029.
	405, 451	41.6 μM	3.3 h	Cell (HeLa); zebrafish; liver tissue	—	New J. Chem., 2019, 43(30): 11844.
	350, 460/525	20 nM	6 min	Cell (HeLa); AD brain slice	—	Chem. Commun., 2021, 57, 3496-3499
	363, 480	0.58 mM	1 h	—	—	Indones. J. Chem., 2019, 19 (4), 1074 - 1080
	385, 525	8.1 nM	10 min	Cell (A549); zebrafish	—	Anal. Chim. Acta., 2023, 1239, 340723
	480, 550	0.296 mM	60 min	Cell (HeLa); zebrafish	✓	This work