Supporting Information

A novel cell membrane-targeting fluorescent probe for imaging endogenous/exogenous formaldehyde in live cells and zebrafish

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1. Materials and instruments

Unless otherwise stated, chemical reagents were purchased from commercial vendor and were used as received. Absorption spectra were carried out using a UV-3101PC spectrophotometer. Fluorescence emission spectra were performed using a Horiba FluoroMax-4 spectrophotometer. The slit width was 2.0 nm for both excitation and emission. High resolution mass spectra (HRMS) were carried out using a LC-MS2010A instrument. Fluorescence imaging of formaldehyde in live cells and zebrafish were carried out on an Olympus FV1000-IX81 confocal fluorescence microscope.

2. Determination of the detection limit

The detection limit was calculated based on the fluorescence titration. The detection limit (LOD) was calculated as follows:

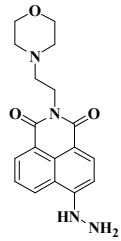
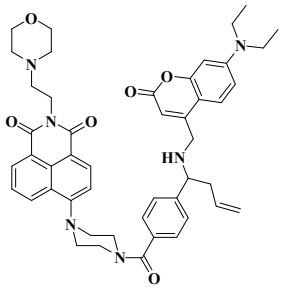
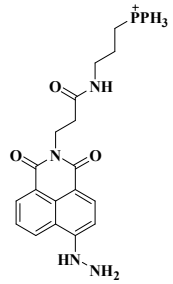
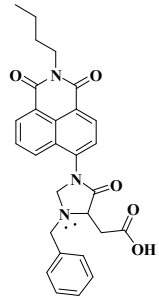
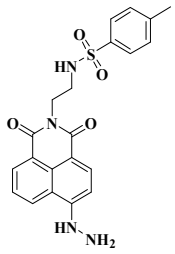
$$DL = 3\sigma/k,$$

$$\sigma = \sqrt{\frac{\sum(\bar{x} - x_i)^2}{n - 1}}$$

where σ is the standard deviation of the blank solution, $\bar{x}$ is the mean of the blank measures, x_i is the value of blank measures, n is the number of tested blank measures (n = 5), and k is the slope between the fluorescence intensities versus the concentrations of formaldehyde.

3. Additional table of comparison between reported FA probes and Mem-FA

Table S1. Comparison of organelle-targeted fluorescent probes for FA

Probe	λ_{em}	Detection limit	Response time	Imaging	references
	543 nm	5.02 μM	within 30 min	lysosome-targeted living cells	Anal. Chem. 88 (2016) 9359-9363
	506/566 nm	3 μM	---	lysosome-targeted living cells and rat's abdomen tissue	Chem. Commun. 53 (2017) 6520-6523
	539 nm	4.9 μM	40 min	mitochondrial - targeted living cells and liver slides	New J. Chem. 42 (2018) 8325-8329
	470 nm	---	---	lysosome-targeted living cells	Chem. Commun. 55 (2019) 7053-7056
	543 nm	---	within 50 min	endoplasmic reticulum-targeted living cells	Methods Appl. Fluoresc. 5 (2017) 024005

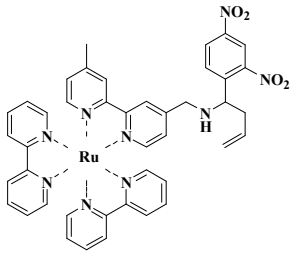
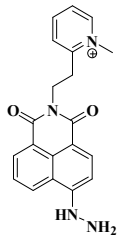
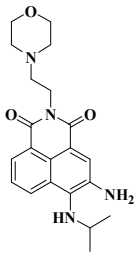
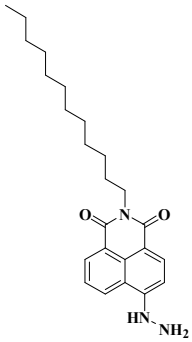
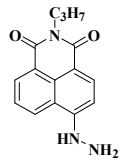
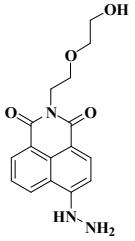
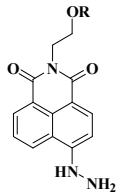
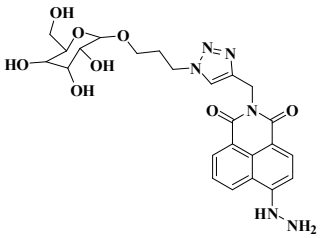
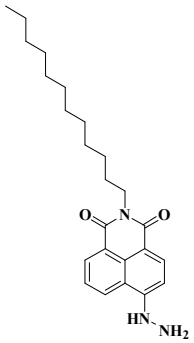
	644 nm	19.8 nM	120 min	lysosome-targeted living cells, mice and rat's organs	J. Am. Chem. Soc. 141 (2019) 8462-8472
	550 nm	12.4 μM	within 20 min	mitochondrial - targeted living cells	Analyst 144 (2019) 2297-2303
	444 nm	0.27 μM	60 min	lysosome-targeted living cells	Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 245 (2021) 118949
	520 nm	214 nM	30 min	membrane-targeted living cells and zebrafish	This work

Table S2. Comparison of naphthalimide-based fluorescent probes for FA

Probe	λ_{em}	Detection limit	Response time	Imaging	references
	543 nm	0.71 μM	within 30 min	endogenous FA in living cells and liver slides	Angew. Chem. Int. Ed. 55 (2016) 3356-3359
	553 nm	0.76 μM	within 90 min	FA in leather products	Dyes and Pigments 188 (2021) 109175

	541 nm	0.78 μM	8 min	exogenous and endogenous FA in living cells and tumour tissue slices	Chem. Commun. 52 (2016) 11247-11250
	541 nm	1.2 μM	within 80 min	exogenous and endogenous FA in living cells	Sensor Actuat. B-Chem. 332 (2021) 129494
	520 nm	214 nM	30 min	distinguishing exogenous and endogenous FA in living cells, FA produced by one-carbon cycle in living cells and zebrafish	This work

4. Determination of quantum yield

Fluorescence quantum yields of probe **Mem-FA** and reaction solution were determined in a mixture aqueous solution of cetyltrimethylammonium bromide (CTAB, 5 mM) and PBS (10 mM, pH 7.4, 10% DMSO) with optically matching solutions of fluorescein (Φ = 0.95 in 0.1 M NaOH solution)[1] as the standard and the quantum yield was calculated using the following equation:

$$\Phi_s = \Phi_r (A_r F_s / A_s F_r) (n_s^2 / n_r^2)^2$$

Where, s and r denote sample and reference, respectively. A is the absorbance. F is the relative integrated fluorescence intensity and n is the refractive index of the solvent.

Quantum yield of probe **Mem-FA**: Φ = 0.035

Quantum yield of reaction solution: Φ = 0.124

5. Cytotoxicity assays

The cell viability of HeLa cells, treated with probe **Mem-FA**, was assessed by a cell counting kit-8 (CCK-8; Dojindo Molecular Technologies, Tokyo, Japan). Briefly, HeLa cells, seeded at a density of 1×10^6 cells·mL^{-1} on a 96-well plate, were maintained at 37 °C in a 5% CO_2 / 95% air incubator for 12 h. then, the cells were treated with increasing amounts of **Mem-FA** (0, 5, 10, 20 and 30 μM) for 24 h. Subsequently, CCK-8 solution was added into each well for 2 h, and absorbance at 420 nm was measured.

6. Cell imaging experiments

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.

Firstly, control cells were imaged. Then, prior to imaging, HeLa cells were treated with probe **Mem-FA** (10 μM) for 30 min. The other groups of cells were incubated with **Mem-FA** (10 μM) for 30 min, washed with culture water, and then treated with FA (400 μM) for 30 min for imaging. Next, in order to confirm that probe **Mem-FA** can detect basal FA, another cells were incubated with $NaHSO_3$ (200 μM) for 30 min, washed with culture water, and then treated with probe **Mem-FA** (10 μM) for 30 min. The other groups of cells were pretreated with Tet (400 μM), and then treated with probe **Mem-FA** (10 μM) for 30 min. Finally, the fluorescence imaging of cells was carried out by confocal fluorescence microscope.

7. Behavioral analysis of zebrafish

All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Qilu University of Technology and approved by the Animal Ethics Committee of Qilu University of Technology.

Behavioral test was conducted to evaluate the safety of **Mem-FA**. The normal zebrafish larvae at 96 hpf were randomly divided into five well plates and exposed to various concentrations of **Mem-FA** (0, 5, 10, 20, 30 μM) dissolved in the bathing medium. The larvae were transferred and maintained in a 14 h light (~1000 lux): 10 h dark (LD) cycle at 28 °C with changing fresh **Mem-FA** daily. Then the zebrafish larvae were cleaned in bathing medium and placed in 48-well plates (one larva per well) at 144 hpf. After a 30 min acclimation period, the locomotor activity of each larva was monitored for 20 min in a silent room using an automated computerized video-tracking system (Viewpoint, Lyon, France), and the detailed track was recorded with Zebralab software (Viewpoint). The swimming duration and movement distance were analyzed. A total of twenty-four zebrafish larvae (n=24) were used for each group. Additionally, to reduce possible diurnal factors on level of locomotor activity, all behavioral tests were performed at zeitgeber time 6-8 (ZT6-8). Care and handling of zebrafish assuredly adhered to the Institutional Animal Care and Use Committees of Qilu University of Technology.

8. zebrafish imaging experiments

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.

Firstly, control 4-day-old zebrafish were imaged. Then, probe **Mem-FA** (10 μM) was used to incubate zebrafish for 30 min for imaging. The other groups of zebrafish were incubated with probe **Mem-FA** (10 μM) for 30 min, washed with culture water, and then treated with FA (400 μM) for 30 min for imaging. Next, in order to confirm that probe **Mem-FA** can detect basal FA, another group zebrafish were incubated with $NaHSO_3$ (200 μM) for 30 min, washed with culture water, and then treated with probe **Mem-FA** (10 μM) for 30 min. The other groups of zebrafish were pretreated with Tet (400 μM), and then treated with probe **Mem-FA** (10 μM) for 30 min. Finally, the fluorescence imaging of zebrafish was carried out by confocal fluorescence microscope.

9. colocalization experiments

In the membrane localization experiment chemical reagent for targeting membrane was obtained from commercial suppliers. DiD Perchlorate was used as a commercial membrane targeting dye (λ_{ex} = 633 nm; λ_{em} = 650-750 nm).

For distinguishing endogenous and exogenous FA, HeLa cells were divided into two groups. One group of cells was treated with probe **Mem-FA** (10 μM) for 10 min, and then treated with FA (400 μM) for 30 min. The other group was preincubated with Tet (400 μM) for 2 h, and then treated with probe **Mem-FA** (10 μM) for 30 min.

Finally, the fluorescence imaging of cells was carried out by confocal fluorescence microscope.

10. Preparation of reactive oxygen species (ROS) and reactive nitrogen species (RNS)

$^1O_2$

Singlet oxygen ($^1O_2$) was generated from HOCl and H_2O_2.

H_2O_2

The concentration of hydrogen peroxide (H_2O_2) was determined from the absorption at 240 nm (ε = 43.6 M^{-1} cm^{-1}).

NaOCl

The concentration of sodium hypochlorite (NaOCl) was determined from the absorbance at 292 nm (ε = 350 M^{-1} cm^{-1}).

$ONOO^-$

Simultaneously, 0.6 M KNO_2, 0.6 M HC1 and 0.7 M H_2O_2 was added to a 3 M NaOH solution at 0 ºC. The concentration of peroxynitrite was estimated by using the extinction co-efficient of 1670 cm^{-1} M^{-1} at 302 nm in 0.1 M sodium hydroxide aqueous solutions.

11. HRMS proof for detection mechanism of probe Mem-FA for FA

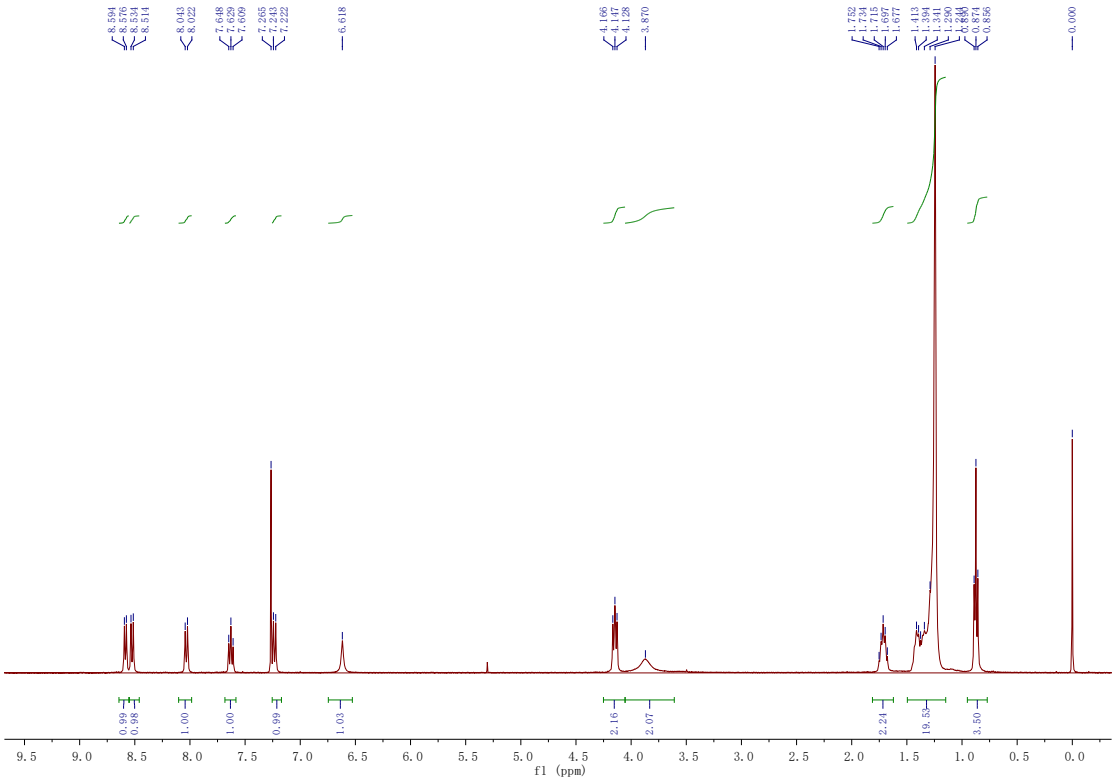


Fig. S1 ^{1}H-NMR data of probe **Mem-FA**

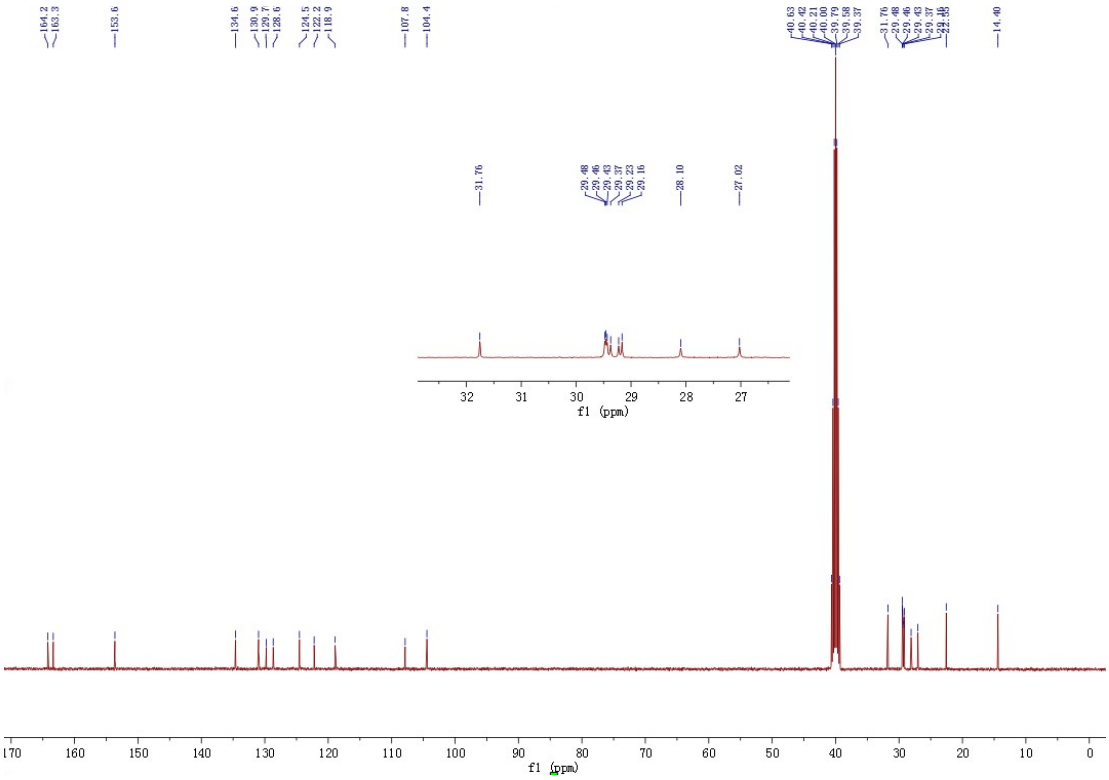


Fig. S2 ^{13}C-NMR data of probe **Mem-FA**

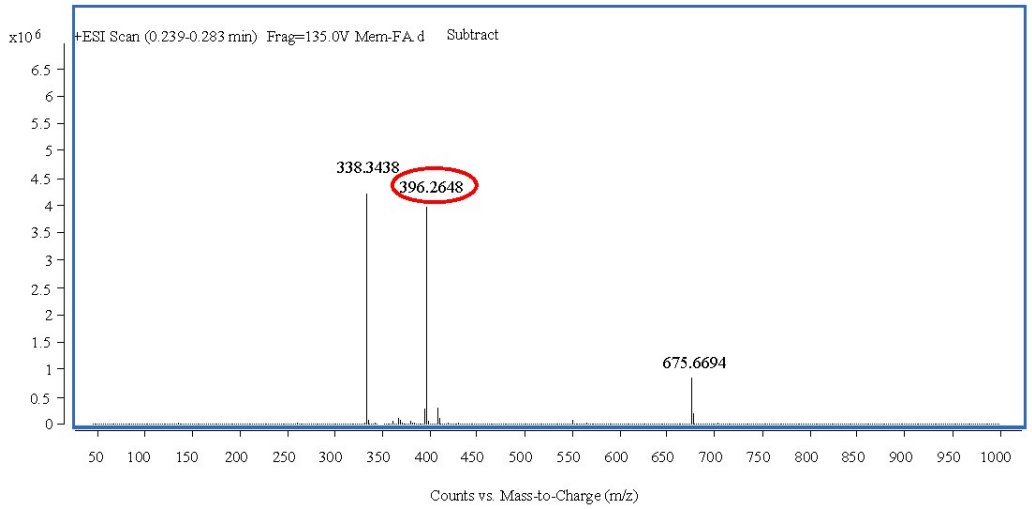


Fig. S3 HRMS data of probe **Mem-FA**

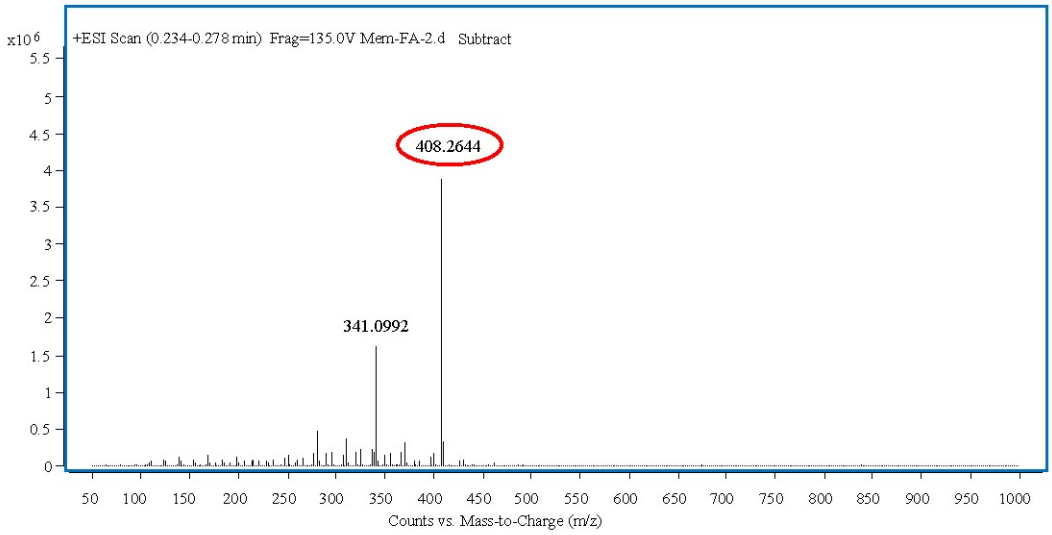


Fig. S4 HRMS data of the reaction products of probe **Mem-FA** with FA.