Supporting Information

A membrane-anchored fluorescent probe for the detection of pH in living cells and NAFLD

Mengqi Yan, Xuechen Li*, Jiarui Liu, Xinyue Li, Shining Wu, Mingyang Zhou, Yuezhi Cui

School of Chemistry and Chemical Engineering, Qilu University of Technology (Shandong Academy of Sciences), Daxue Road 3501, Changqing District, Jinan 250353, P. R. China.

E-mail: lxcred@126.com

Table of Content

1. Apparatus and Method

1.1 Apparatus. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance II spectrometer at 400 or 100 MHz (TMS as the internal standard). The UV absorption and fluorescent spectrum of probes were obtained on a Hitachi U-2500 spectrophotometer and a Hitachi F-2710 spectrofluorimeter equipped with a 450 W Xe lamp, respectively. Confocal fluorescent images were obtained from Leica TCS SP8 X.

1.2 Reagents. The Lyso-Tracker Red and Mito-Tracker Deep Red FM were purchased from ThermoFisher. The potassium hydrogen phthalate buffer and sodium tetraborate buffer was purchased from shnaghai INESA Scientific Instruments Co., Ltd. The perfluoro octanoic acid (PFOA) and lipopolysaccharides (LPS) was purchased from Shanghai Macklin Biochemical Co., Ltd.

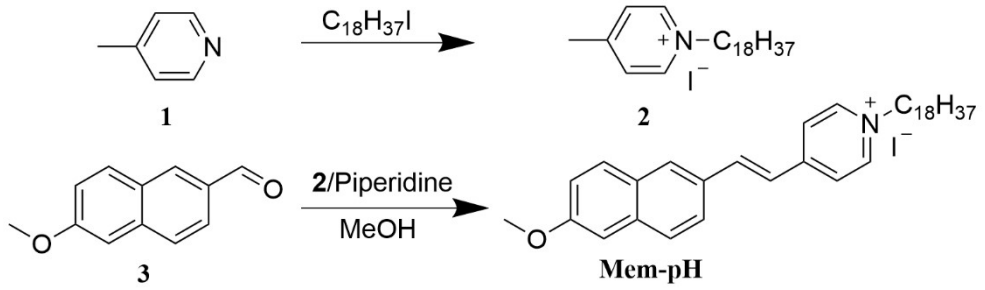
1.3 Cell Culture. All cells were grown in a 5% CO_2 incubator at 37 °C. The HeLa, SiHa, MIHA and A549 cells were cultured in the f12k medium including 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. The RAW cells were cultured in the RPMI1640 medium including 10% FBS and 1% penicillin/streptomycin.

1.4 Cytotoxicity of Mem-pH. The study of the cytotoxicity of Mem-pH on the cells was performed by methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay. SiHa, HeLa, A549, RAW and MIHA cells were seeded into 96-well plates (ca. 1×10^4 cells/ well) and adhered for 24 h. Mem-pH (200 μL/well) diluted in culture medium at corresponding concentration was added into the wells as the treatment group, and DMSO (200 μL/well) diluted in culture medium with final concentration of 0.2% as the negative control group, respectively. The cells in the 96-well plate were added 2, 4, 6, 8, 10 μM Mem-pH for 24 h at 37℃ under 5% CO_2, then MTT (5 mg/mL in PBS) was added into each well. After 4 h of incubation, the culture medium in each well was removed and DMSO (200 μL) was added to dissolve the purple crystals. After incubating 30 min, the optical density readings at 492 nm were taken using a plate reader. Each individual cytotoxicity experiment was repeated for three times.

1.5 Preparation and staining of the GUVs.

1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC) was used to prepare GUVs to simulate cell membrane. GUVs were prepared following the mild hydration method. Firstly, the stock solutions with concentration of18 mg/mL of DOPC, were prepared with the mixing solvents of $CHCl_3$ and CH3OH (V/V, 2:1), while the stock solutions of 1,2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (DOPG) with concentration of 2 mg/mL were also prepared with the same solvents. Secondly, add 50ul of the stock solution of DOPC and DOPG to the flaskets and then fully shocked. Thirdly, the mixed solutions were carefully layed over the inner wall of the flaskets and the solvents were removed under the protection of N_2. Fourthly, flaskets were treated with vacuum conditions for the further removing of the solvents. Finally, the flaskets was filled with 0.1 M sucrose solutions and heated under 60 ℃ for about 24 h. The GUVs were formed as white cloudlike subjects suspending in the sucrose solutions. When 10 mL GUVs was firstly taken out staining the GUVs with the probes and mixed evenly before imaging experiments.

2. Synthetic details, NMR and HRMS spectra



Scheme S1. The synthesis route of Mem-pH.

1. Synthesis of compound 2: 4-methylpyridine (0.900 mL, 9.19 mmol) and 1-Iodooctadecane (3.42 g, 9.19 mmol) were added into a three-necked flask, stirred until the white solid precipitated. For 4-methyl-1-octadecylpyridin-1-ium iodide: 3.80 g, yield 86%. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.92 (d, J = 5.0 Hz, 2H), 7.99 (d, J = 6.6 Hz, 2H), 4.51 (t, J = 7.4 Hz, 2H), 2.61 (s, 3H), 1.87 (p, J = 7.5 Hz, 2H), 1.23 (s, 30H), 0.89 – 0.83 (m, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm): 184.09, 159.11, 153.05, 139.23, 138.64, 137.80, 135.19, 133.55, 131.95, 131.23, 130.50, 129.89, 128.89, 127.71, 127.67, 127.60, 127.19, 124.85, 123.60, 120.38, 114.09, 111.99, 110.01, 59.36, 54.24, 49.79, 26.29.

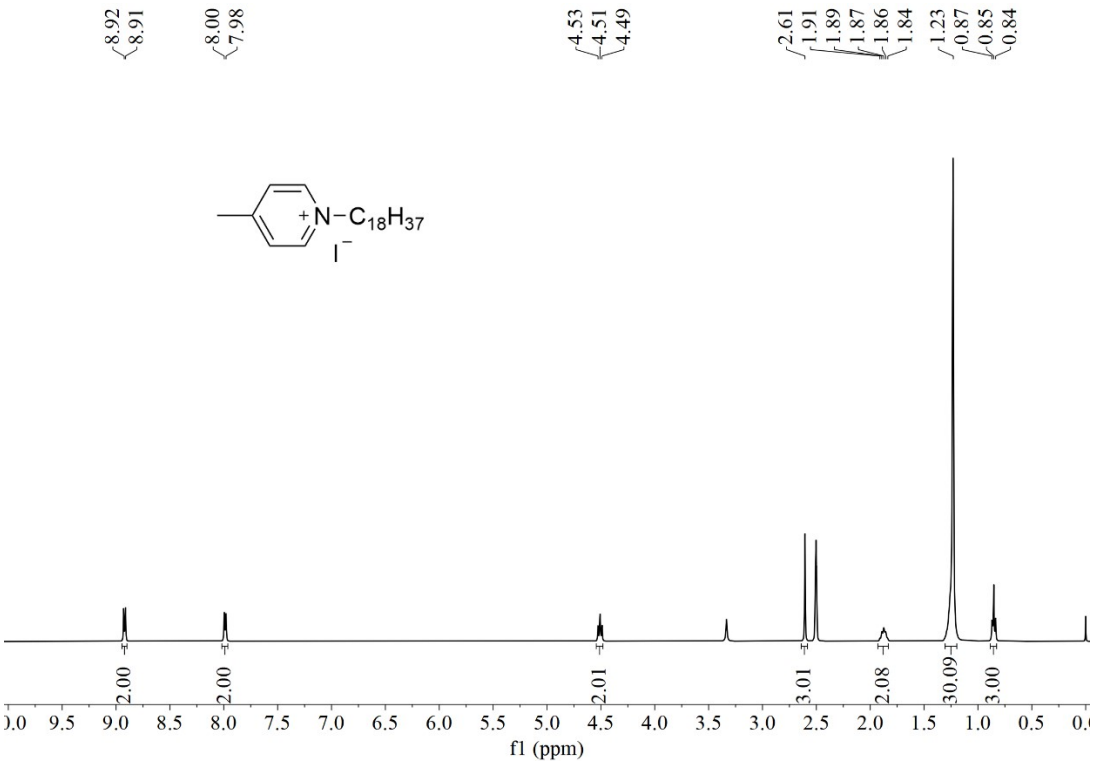


Fig. S1 The ^{1}H NMR spectrum of compound 2 in DMSO-d_6.

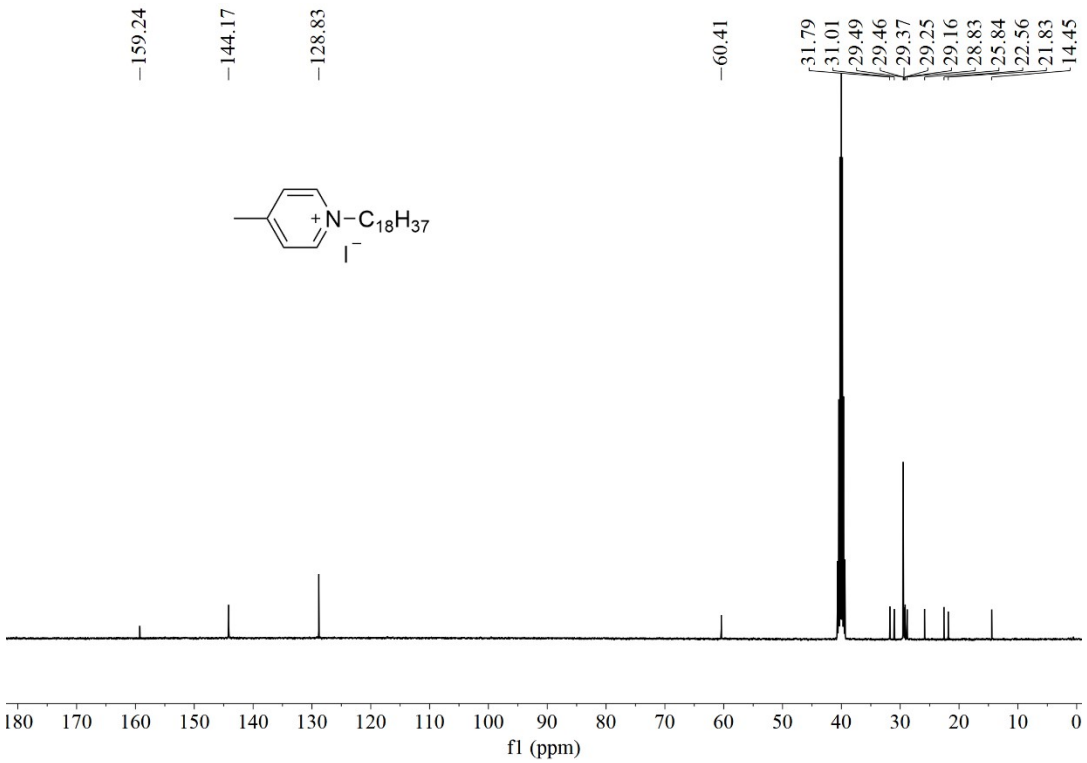


Fig. S2 The ^{13}C NMR spectrum of compound 2 in DMSO-d_6.

2. Synthesis of Mem-pH: The compound 2 (0.400g, 0.845mmol) and anhydrous methanol (25mL) were added into a three-necked flask, heated at 55 °C until they all were dissolved, and then added the compound 3 (0.198 g, 0.845 mmol) and piperidine (6 drops). On completion of the addition, raise the temperature and reflux until the reddish brown solid precipitated. For (E)-4-(2-(6-methoxynaphthalen-2-yl)vinyl)-1-octadecylpyridin-1-ium iodide, Mem-pH: 0.28 g, yield 51%.^1H NMR (DMSO-d_6., 300 MHz): δ (ppm): 8.83 (d, J=8 Hz, 2H), 8.13 (d, J=8 Hz, 2H), 8.04 (d, J=16 Hz, 2H), 7.79(s, 3H), 7.47 (m, 1H), 7.28 (d, 1H), 7.09(s, 1H), 4.37(d, J=16 Hz, 2H), 3.78(s, 2H), 1.79(s, 2H), 1.13 (m, 30H), 0.72 (d, J=12 Hz, 3H). ^{13}C NMR (101 MHz, DMSO-d_6): δ(ppm): 158.55, 152.92, 144.08, 141.11, 135.36, 130.47, 130.11, 129.68, 128.17,127.58, 124.14, 123.54, 122.32, 119.33, 106.30, 59.58, 55.31, 31.19, 30.38, 28.94, 28.89, 28.77, 28.65, 28.60, 28.26, 25.29, 21.99, 13.85. HRMS (m/z): **calcd for $C_{36}H_{52}INO$: 641.30; found: 514.41 $(M-I)^+$**.

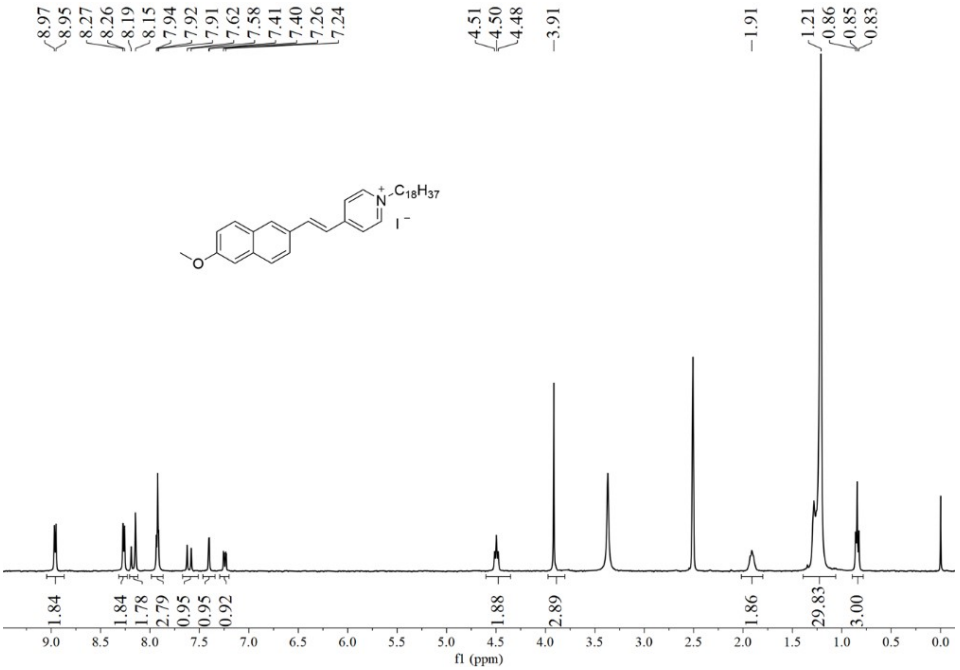


Fig. S3 The ^{1}H NMR spectrum of compound Mem-pH in DMSO-d_6

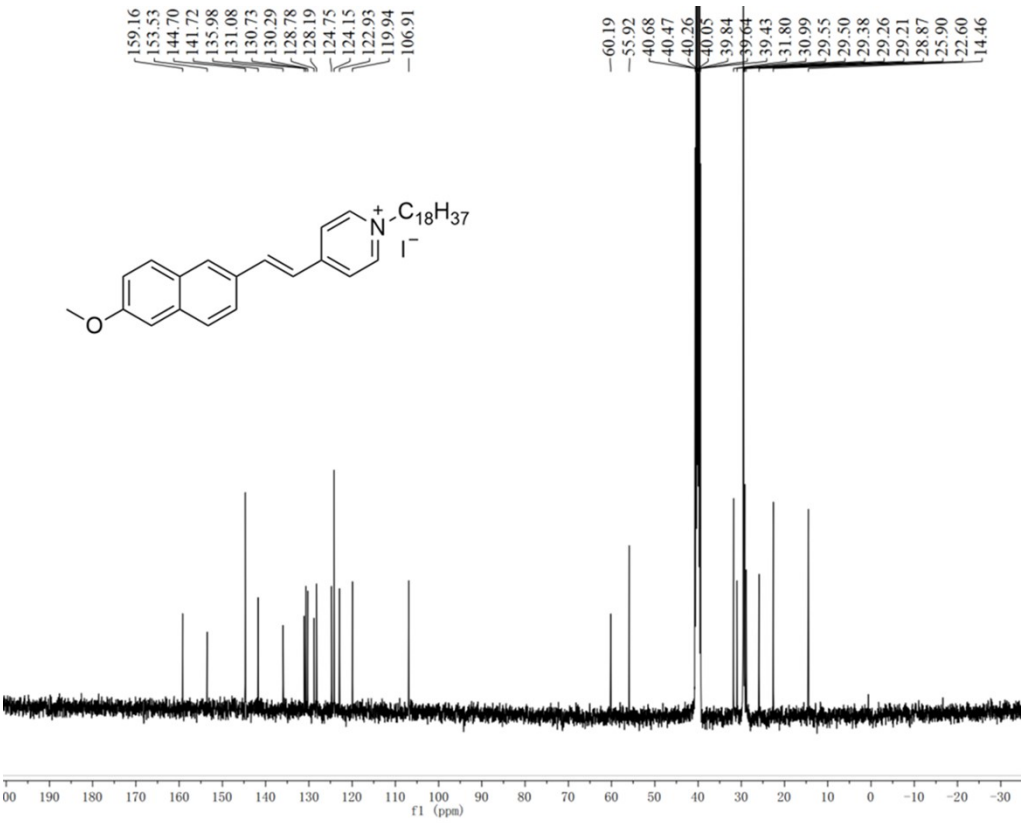


Fig. S4 The ^{13}C NMR spectrum of compound Mem-pH in DMSO-d_6.

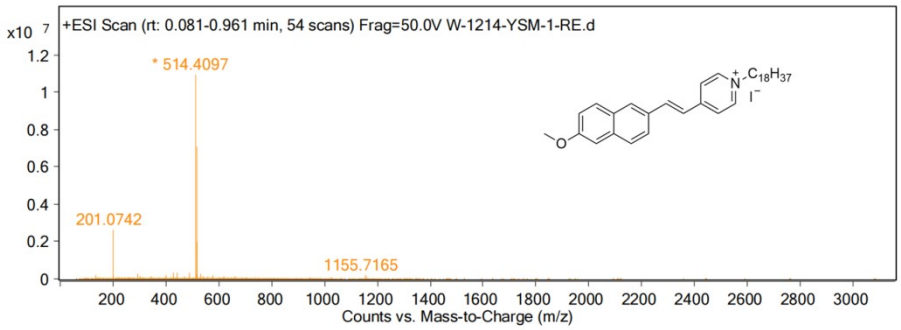


Fig. S5 HRMS spectrum of Mem-pH in methanol.

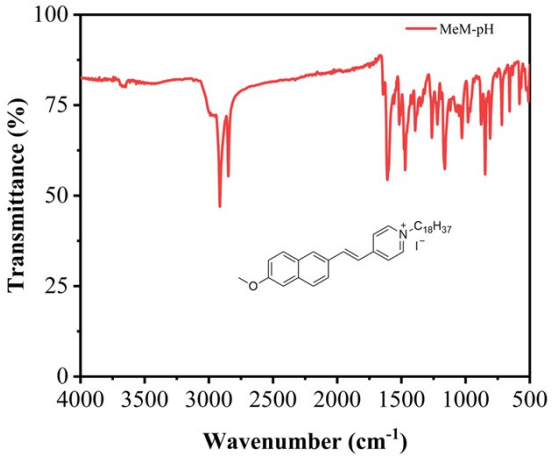


Fig. S6 IR spectrum of Mem-pH.

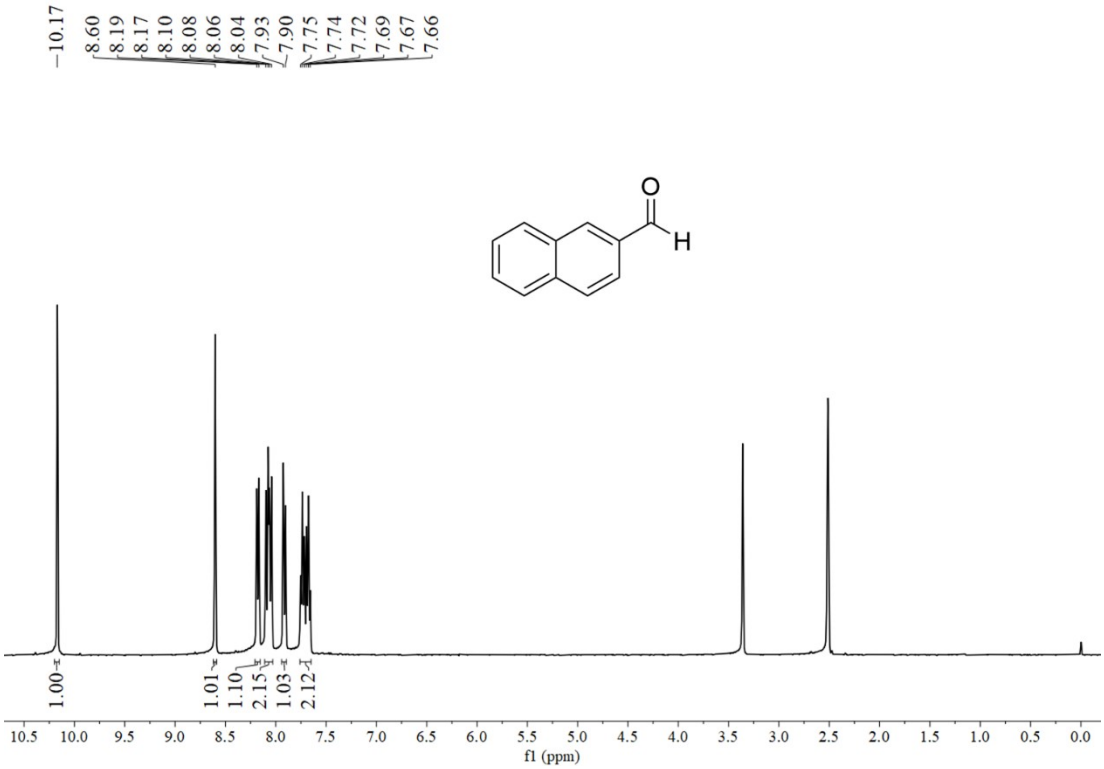


Fig. S7 The ^{1}H NMR spectrum of control molecule in DMSO-d_6.

3. Structural characterization

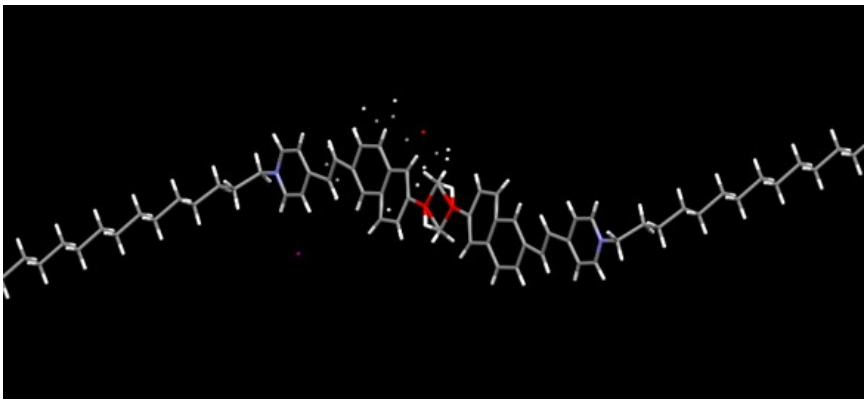


Fig. S8 Molecular configurations of Mem-pH under positive charged form in the single crystal cell with atoms labeled in color. C, gray; H, white; N, blue; O, red; I, purple.

Table S1 Crystal data and structure refinement for probe Mem-pH.

Identification code	c18_0m
Empirical formula	$C_{36}H_{52}INO$
Formula weight	641.68
Temperature/K	170.0
Crystal system	triclinic
Space group	P-1
a/Å	6.6992(12)
b/Å	7.6455(18)
c/Å	33.360(8)
α/°	84.805(7)
β/°	84.464(6)
γ/°	88.654(7)
Volume/Å^3	1693.5(6)
Z	2
ρ_{calc}g/cm^3	1.258
μ/mm^{-1}	0.972
F(000)	672.0
Crystal size/mm^3	0.12 × 0.05 × 0.04
Radiation	MoKα (λ = 0.71073)
2Θ range for data collection/°	4.926 to 60.182
Index ranges	-7 ≤ h ≤ 8, -9 ≤ k ≤ 9, -41 ≤ l ≤ 41
Reflections collected	19042
Independent reflections	6852 [R_{int} = 0.0658, R_{sigma} = 0.0912]
Data/restraints/parameters	6852/581/415
Goodness-of-fit on F^2	1.021
Final R indexes [I>=2σ (I)]	R_1 = 0.0873, wR_2 = 0.2022
Final R indexes [all data]	R_1 = 0.1615, wR_2 = 0.2448
Largest diff. peak/hole / e Å^{-3}	1.15/-1.13

4. Spectra data

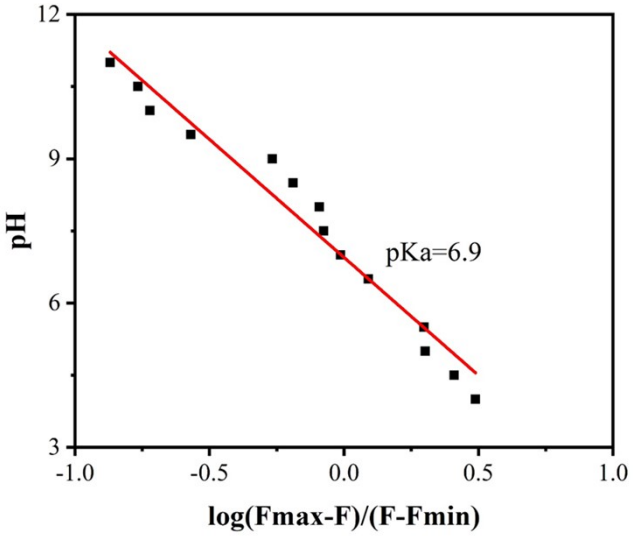


Fig. S9 Plot of pH vs log [(Imax - I) / (I - Imin)].

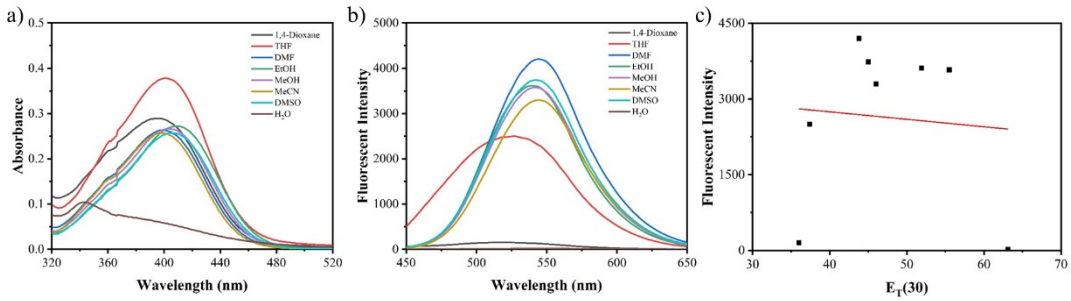


Fig. S10 Absorption and fluorescent spectra of probe Mem-pH (10 μM) with the change of polarity (a, b), (c) plot of Mem-pH's fluorescent intensity vs polarity.

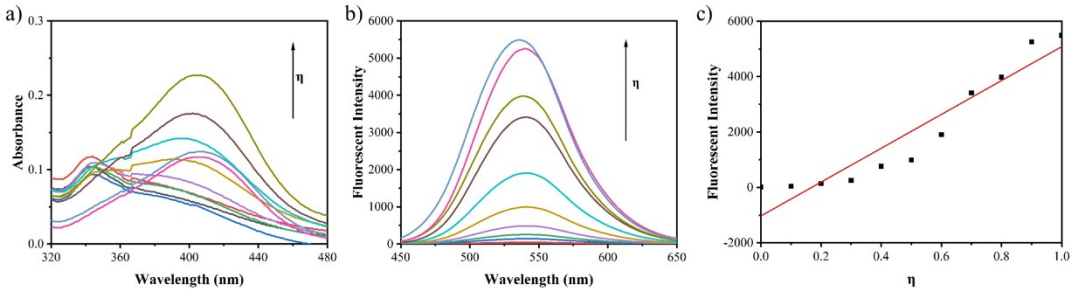


Fig. S11 Absorption and fluorescent spectra of probe Mem-pH (10 μM) with the change of viscosity (a, b), (c) plot of Mem-pH's fluorescent intensity vs viscosity.

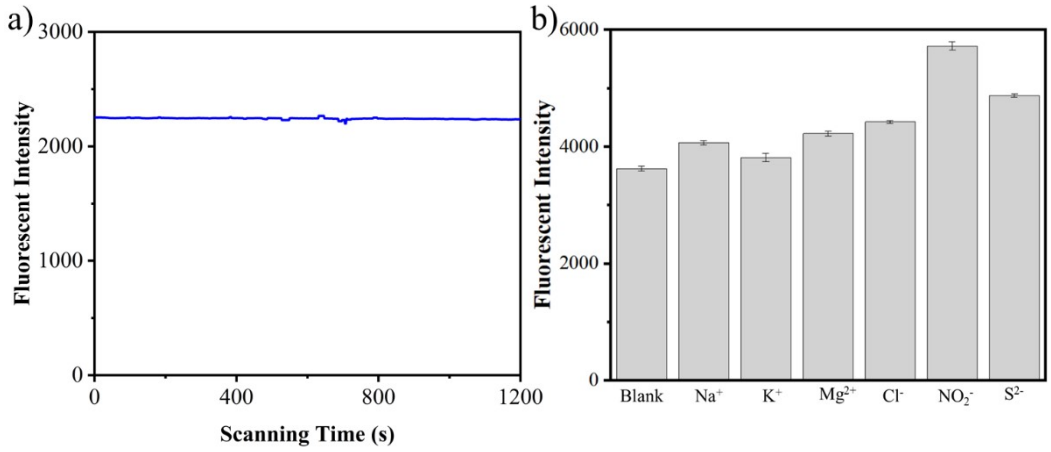


Fig. S12 (a) Light stability of the probe Mem-pH. (b) Comparison of maximum fluorescence intensity of different ions (C_{ion} =0.1mM) in aqueous solution.

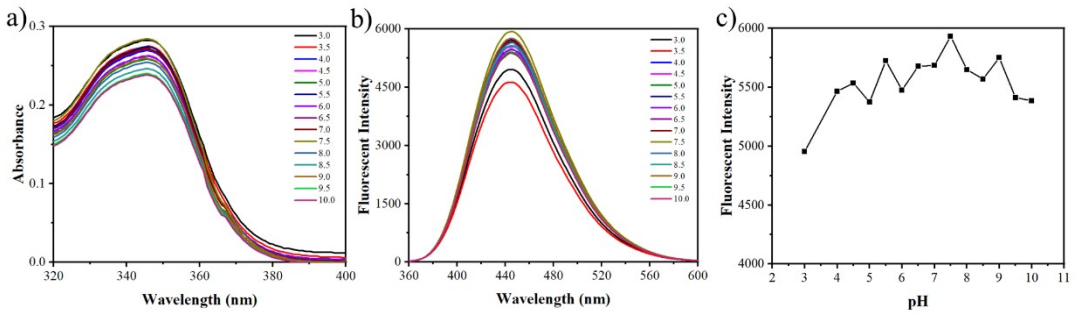


Fig. S13 Absorption and fluorescent spectra of control molecule (10 μM) with the change of pH (a, b), (c) plot of control molecule's fluorescent intensity vs pH.

5. Cell viability and imaging data

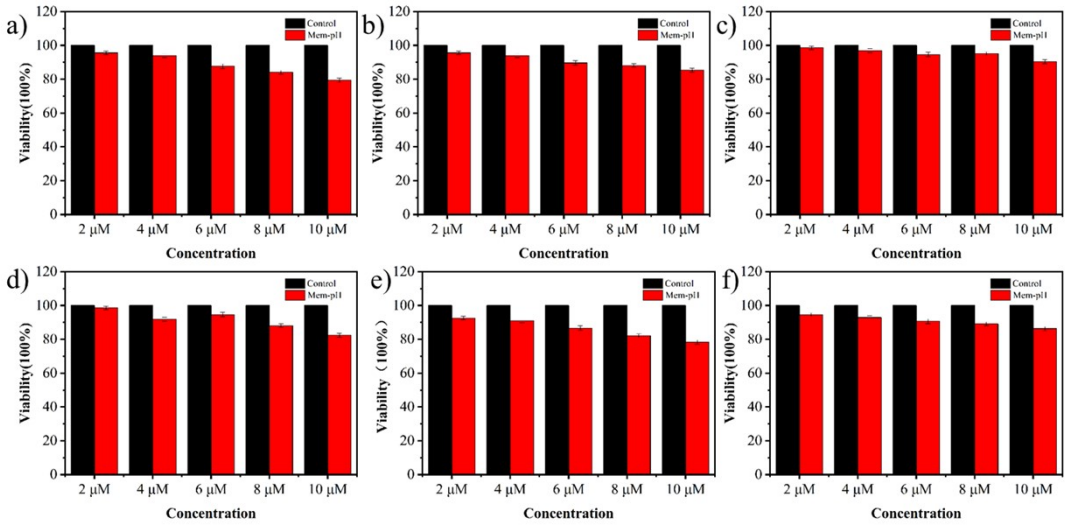


Fig. S14 MTT results of a) HeLa, b) A549, c) SiHa, d) RAW, e) MIHA, and f) RAW and HeLa cells viabilities after incubation with Mem-pH for 24 h at different incubation concentrations.

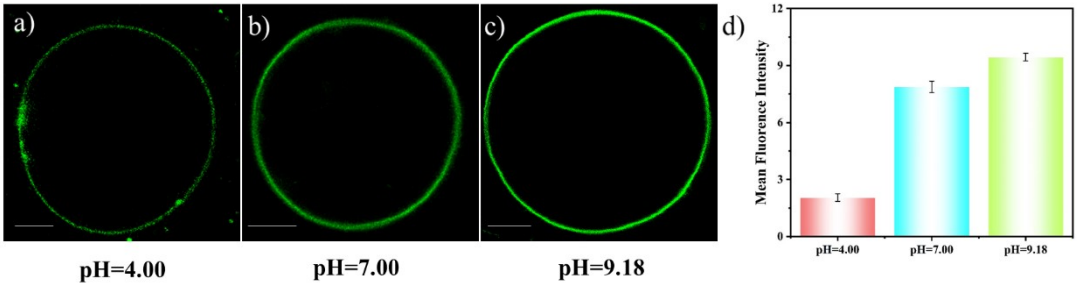


Fig. S15 Fluorescent images of GUVs with different pH stained with 10 mM Mem-pH (a-c). d) Mean fluorescent intensities were obtained in circular areas in a, b, c. Ex = 405 nm, Em = 550−620 nm, bar = 20 μm.

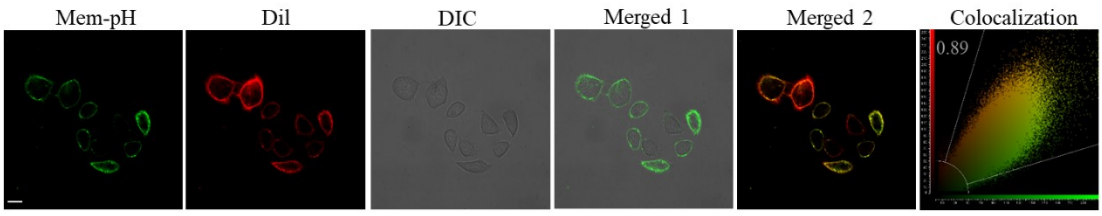


Fig. S16 Fluorescent， DIC and merged images of HeLa cells stained with Mem-pH (2 uM, 10 min) and Dil (0.2 uM, 10 min). Merged 1: the merged image of the Mem-pH and DIC. Merged 2: the merged image of the Mem-pH and Dil channel. Mem-pH: E_x=405 nm, E_m=550-620 nm; Dil: E_x = 532 nm, E_m = 550-580 nm. bar = 20 μm.

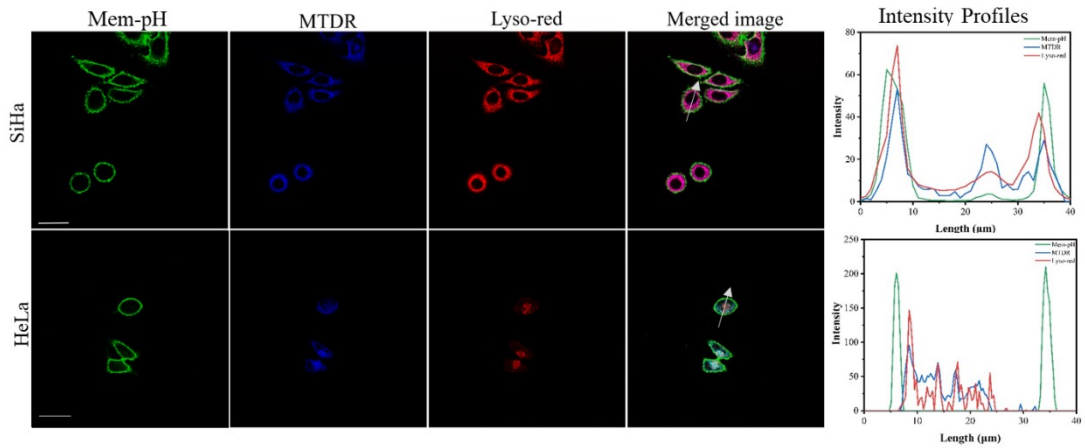


Fig. S17 Fluorescent, and merged images of SiHa and HeLa cells stained by Mem-pH (2 μM, 20 min), Lyso-red (0.5 μM, 10 min) and MTDR (0.5 μM, 10 min), Mem-pH: E_x = 405 nm, E_m = 550-620 nm; Lyso-red: E_x = 552 nm, E_m = 570-610 nm; bar = 20 μm; MTDR: E_x = 635 nm, E_m = 650-680 nm.

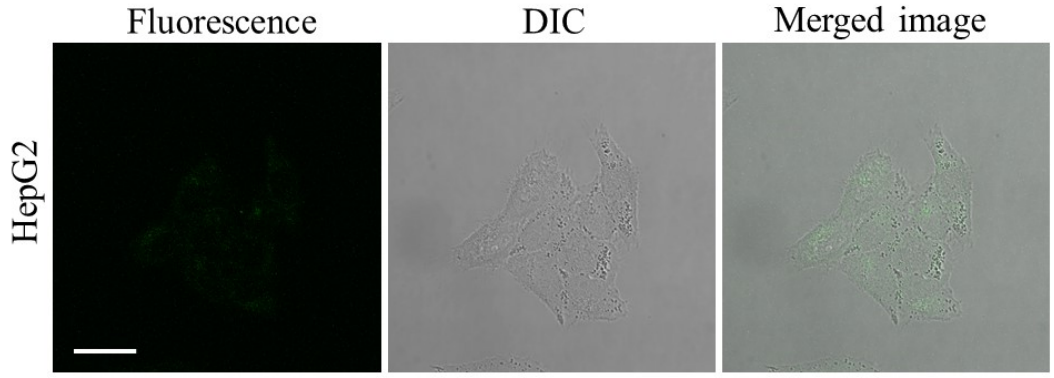


Fig. S18 Fluorescent, DIC and their merged images of HepG2 stained by control molecule (2 μM, 20 min).

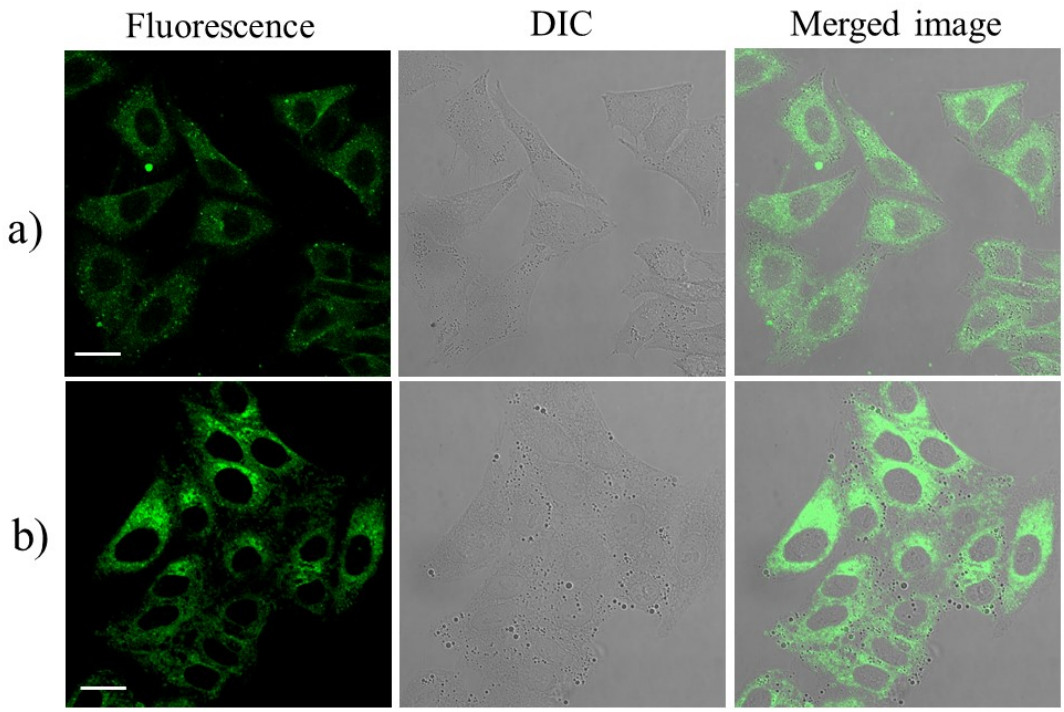


Fig.S19 Confocal fluorescent images of (a) β-CD solution-pretreated and (b) NaCl solution-pretreated HepG2 cells, both stained with Mem-pH (2 μM, 20 min). Ex = 405 nm, Em = 550-620 nm, bar = 20 μm.

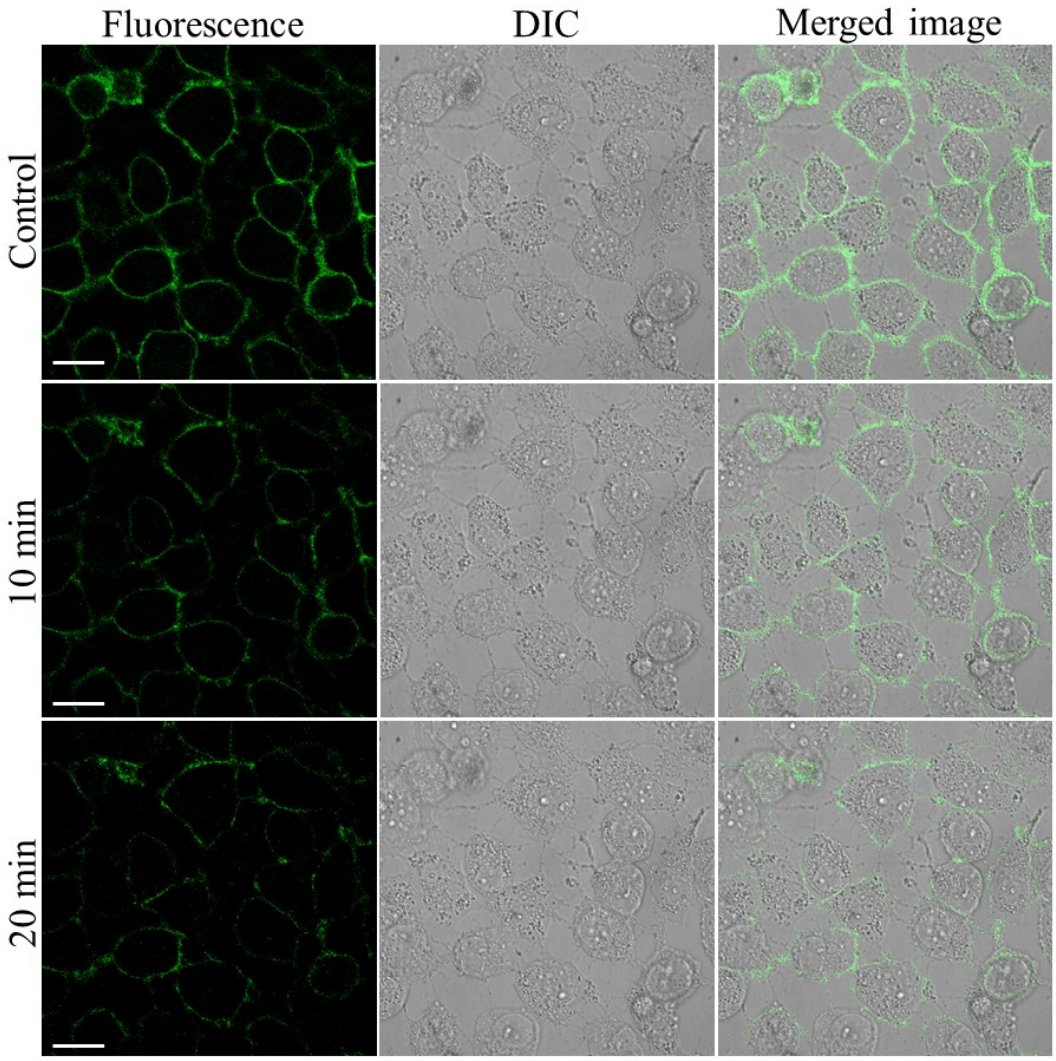


Fig. S20 Fluorescent, DIC and their merged images of HeLa stained by Mem-pH (2 μM, 20 min) cells under the normal status and after incubated with LPS for different time, E_x = 405 nm, E_m = 550-620 nm, bar = 20 μm.

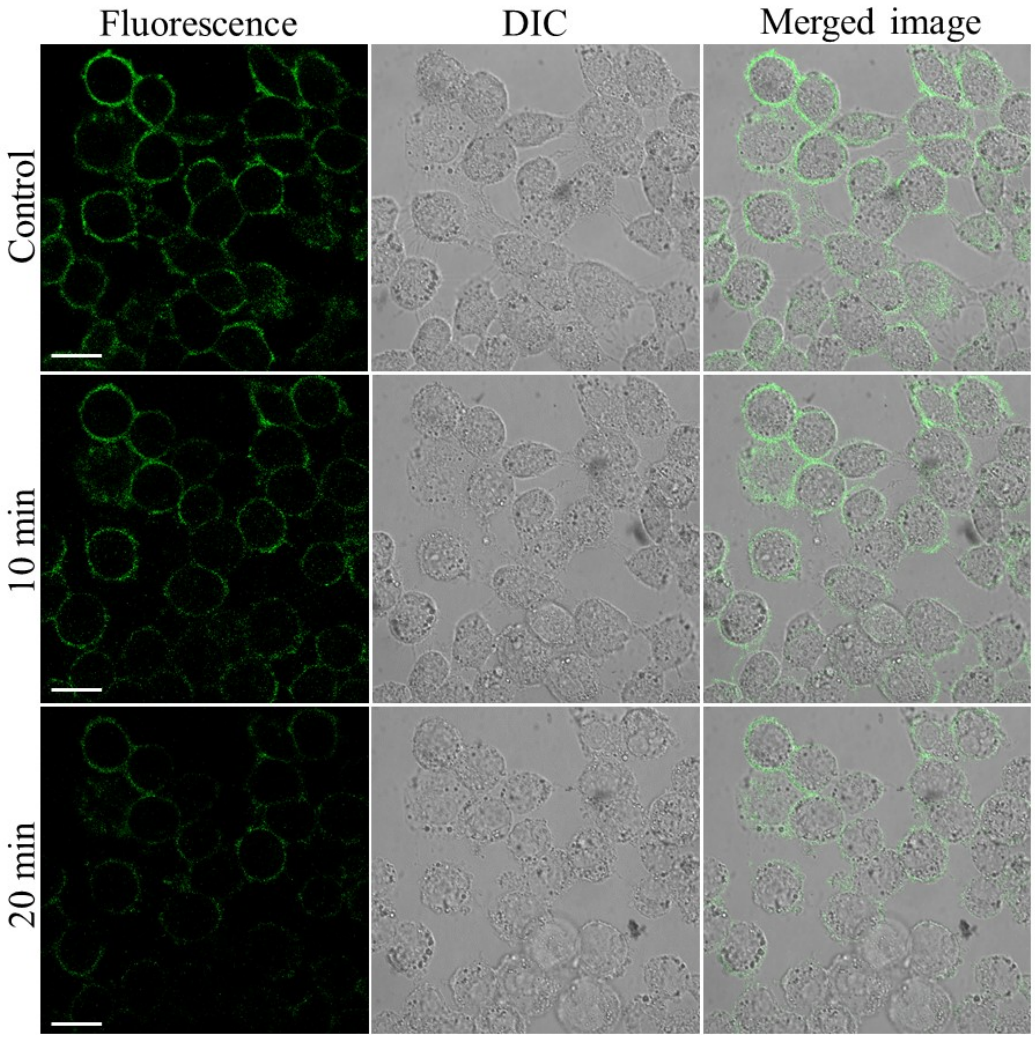


Fig. S21 Fluorescent, DIC and their merged images of HeLa stained by Mem-pH (2 μM, 20 min) cells under the normal status and after incubated with H_2O_2 for different time, E_x = 405 nm, E_m = 550-620 nm, bar = 20 μm.

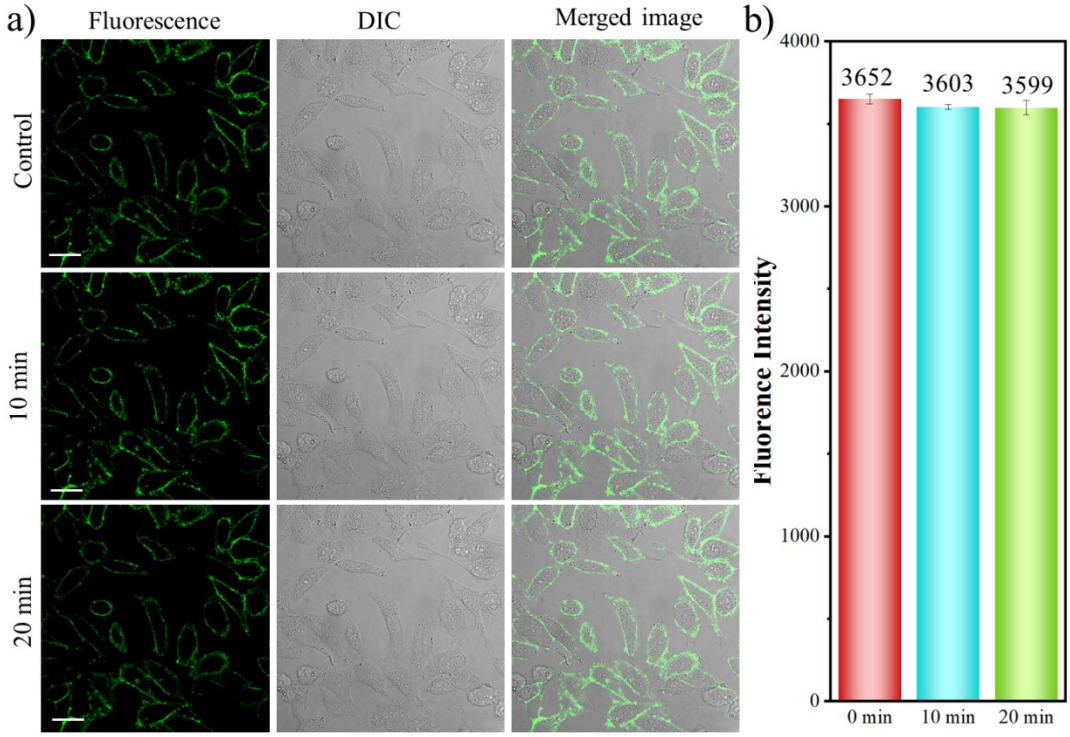


Fig. S22 Fluorescent, DIC and their merged images (a) and the fluorescent intensity change (b) of HepG2 stained by Mem-pH (2 μM, 20 min) cells under the normal status for different time, Ex = 405 nm, Em = 550-620 nm, bar = 20 μm.