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Monitoring mitochondrial pH with a hemicyanine-based ratiometric fluorescent probe

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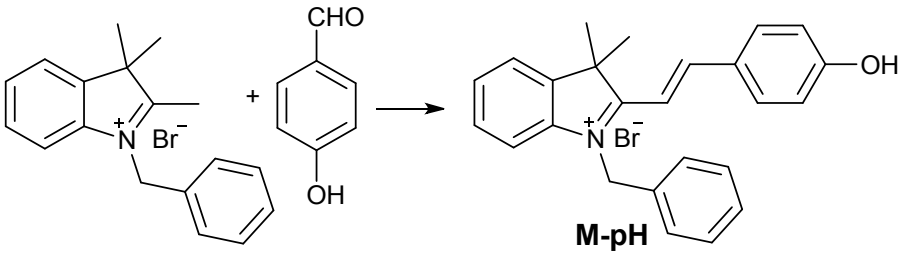
Reagents and instruments

All reagents and solvents were purchased from commercial sources and used without further purification. The solutions of metal ions were prepared from chloride or nitrate salts dissolved in deionized water. The organelle dye Mito-Tracker Deep Red was purchased from Invitrogen (USA). 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma. Silica gel (200-300 mesh) used for flash column chromatography was purchased from Qingdao Haiyang Chemical Co., Ltd. ^{1}H NMR and ^{13}C NMR spectra were determined by 400 MHz and 100 MHz using Bruker NMR spectrometers. The mass spectra were obtained by Bruker maxis ultra-high resolution-TOF MS system. The fluorescence spectra measurements were performed using F-380 fluorescence spectrometer. UV-vis absorption spectra were made with a UV-2450. All pH measurements were carried out on PHSJ-4A pH meter. Confocal fluorescence imaging in cells was performed with Leica TCS SP5 Confocal Laser Scanning Microscope. The laser power of confocal imaging is 15 mW for 476 and 633 nm laser. The hepatoma carcinoma cells (SMMC-7721 and HepG2) were purchased from Cell Bank of the Chinese Academy of Sciences (Shanghai, China).

Synthesis of probe M-pH

The facile synthesis of M-pH is shown in Scheme S1. 1-benzyl-2,3,3-trimethylindolenium bromide was synthesized in the previous work.[1] The

probe M-pH as a red solid was obtained by one step condensation of 1-benzyl-2,3,3-trimethylindolenium bromide and 4-hydroxybenzaldehyde. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.736 (s, 6H), 5.094 (s, 2H), 6.196 (d, J=13.6 Hz, 1H), 6.488 (d, J=9.2 Hz, 2H), 6.917 (d, J=8.0 Hz, 1H), 7.143 (t, J=7.6 Hz, 1H), 7.227 (d, J=7.2 Hz, 2H), 7.280 (d, J=7.2 Hz, 2H), 7.328-7.381 (m, 5H), 7.610 (d, J=13.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 28.49, 48.23, 52.62, 95.88, 109.16, 122.14, 122.38, 123.67, 126.09, 128.30, 128.54, 129.40, 134.28, 139.74, 143.06, 145.98, 169.15. MS m/z calcd. for $C_{25}H_{24}NO^+$ [M^+]: 354.1858, found 354.1861.



Scheme S1 The facile synthesis of M-pH

Sample preparation

The probe M-pH was dissolved in dimethyl sulfoxide (DMSO) to afford the stock solution (1.0×10^{-3} M). Test samples were prepared by displacing 20 μL of the stock solution and an appropriate aliquot of each testing species solution into a tube and the solution was diluted to 2.0 mL in a mixture of PBS buffer and DMSO (90/10, v/v). The resulting solution was shaken well and measured immediately.

Cell culture and imaging

SMMC-7721 and HepG2 cells were cultured in Dulbecco's modified

Eagle's medium (DMEM, Invitrogen) supplemented with 10 % fetal bovine serum (Invitrogen), 1.0 % penicillin and 1.0 % streptomycin. The cells were seeded in confocal culture dishes and then incubated for 24 h at 37 °C under a humidified atmosphere containing 5.0 % CO_2. For colocalization cell imaging experiments, the cells were seeded onto glass plates for 24 h for adherence, then washed by PBS and incubated with the probe M-pH and Mito-Tracker Deep Red before fluorescence imaging. For mitochondrial stress experiments, the SMMC-7721 cells were incubated with M-pH for 30 min, then washed with PBS for three times, and sodium selenite was added. The fluorescence images were collected at different times. To test the cytotoxicity of M-pH, the cell viability of HepG2 cells was determined by MTT assay. Fluorescence imaging in cells with M-pH were performed on Leica TCS SP5 Confocal Laser Scanning microscope.

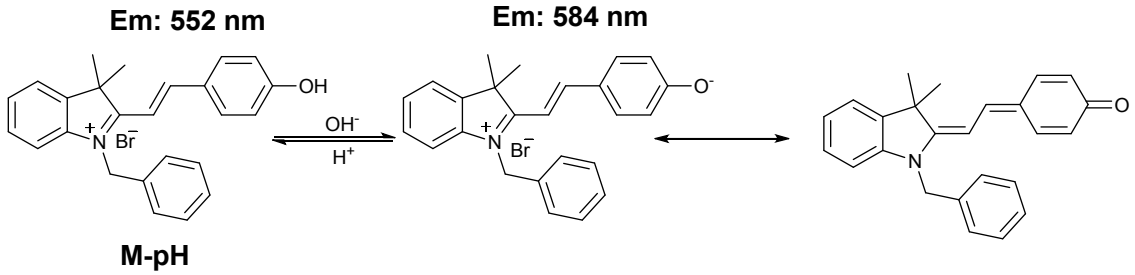


Figure S1 The sensing mechanism of M-pH to pH

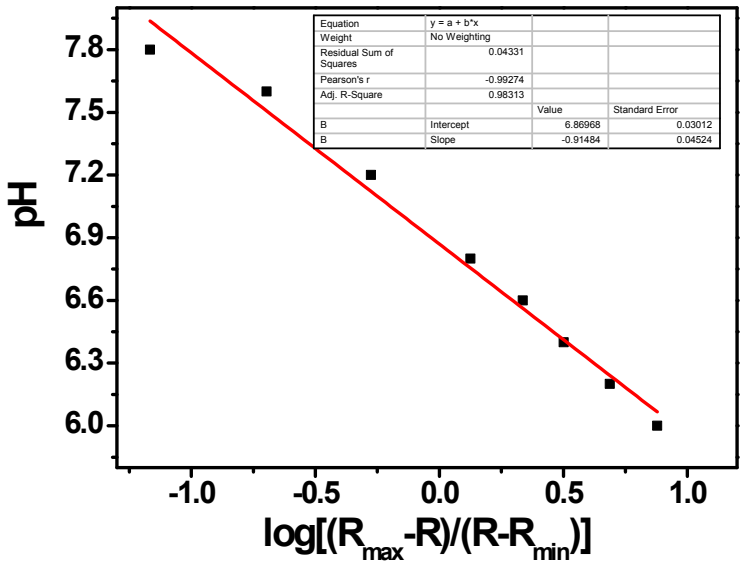


Figure S2 Plot of pH *vs* log[(R_{max}-R)(R-R_{min})], where R is the fluorescence intensity ratios between 584 and 552 nm of M-pH upon excitation at 480 nm. The y-intercept is the *pKa* value (6.87) of M-pH.

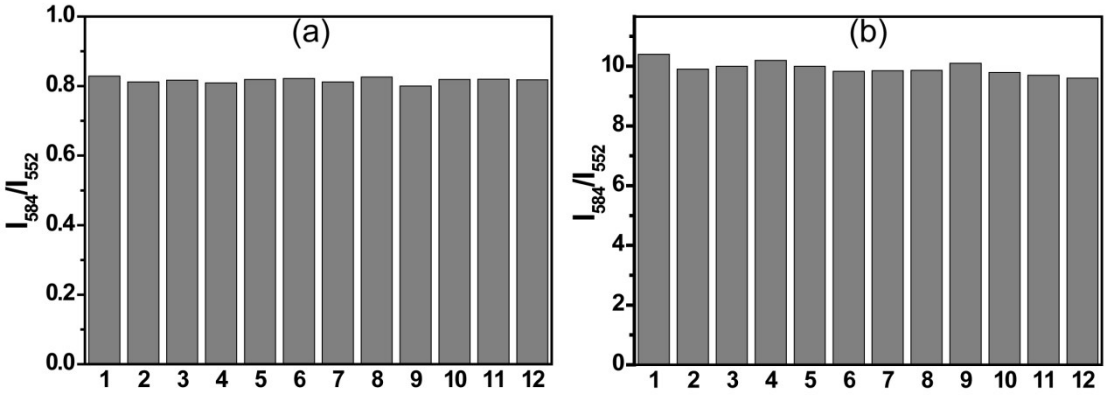


Figure S3 The fluorescence intensity ratio values I_{584}/I_{552} of M-pH (10 μM) with or without various ROS and RSS at pH 4.0 (a) and 9.0 (b). 1: blank; 2: Na_2SO_3, 1.0 mM; 3: Na_2S, 1.0 mM; 4: Na_2SO_4, 1.0 mM; 5: GSH, 5.0 mM; 6: Cys, 5.0 mM; 7: H_2O_2, 1.0 mM; 8: $ONOO^-$, 100 μM; 9: HClO, 100 μM; 10: $O_2^{\cdot-}$, 100 μM; 11: ·OH, 100 μM; 12: $^1O_2$, 100 μM. Ex=480 nm.

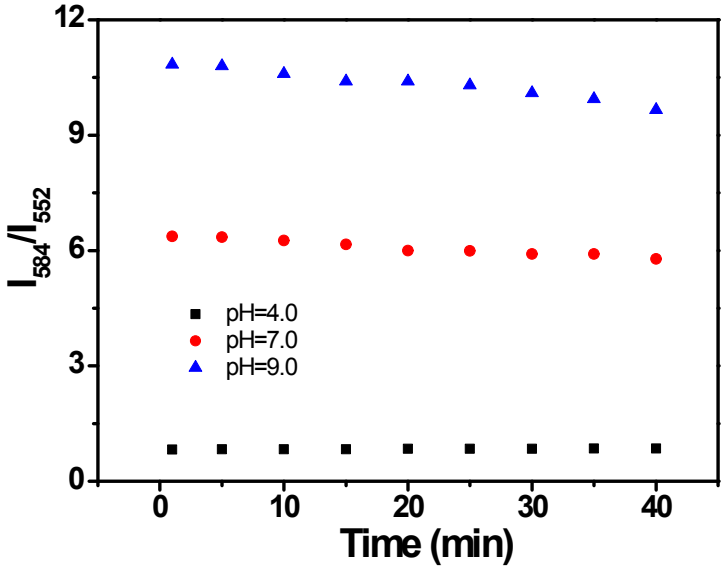


Figure S4 The stability of M-pH at different time. Under different pH values (4.0, 7.0, 9.0), the fluorescence spectra of M-pH were detected and the fluorescence intensity ratios I_{584}/I_{552} were record every 5 minutes. Ex=480 nm.

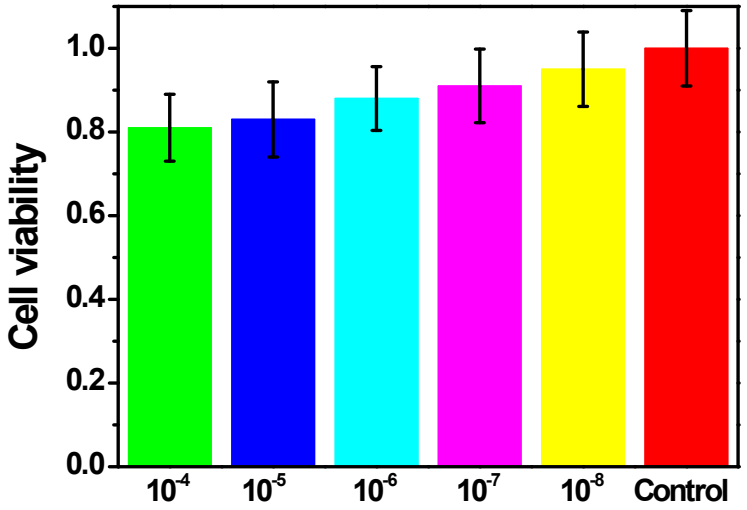


Figure S5 The cell viability of HepG2 cells incubated with different concentrations of M-pH. The cells were seeded at about $5x10^4$ cells/well on a 96-well plate. The cells were treated with media containing M-pH (10^{-4}-10^{-8} M) for 10 h, and MTT assay was then performed. The data are based on the average and show the standard deviation (n=5). The IC50 value was calculated to be 0.12 mM.

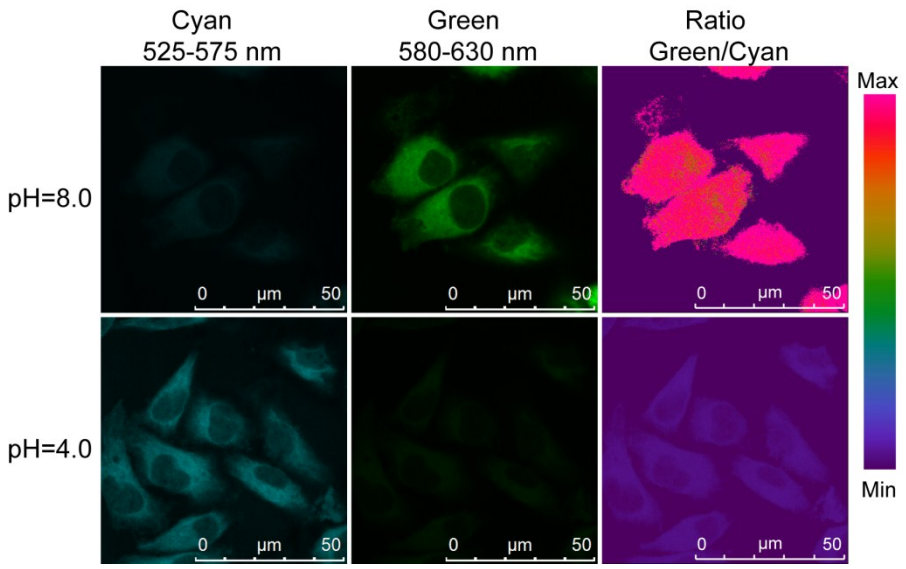


Figure S6 The ratiometric fluorescence imaging of different mitochondrial pH in SMMC-7721 cells with M-pH (10 μM). The first column was cyan channel collected 525-575 nm. The second column was green channel collected 580-630 nm. The third column was ratiometric fluorescence images between green and cyan channel. Ex=476 nm. Scale bar: 50 μm.

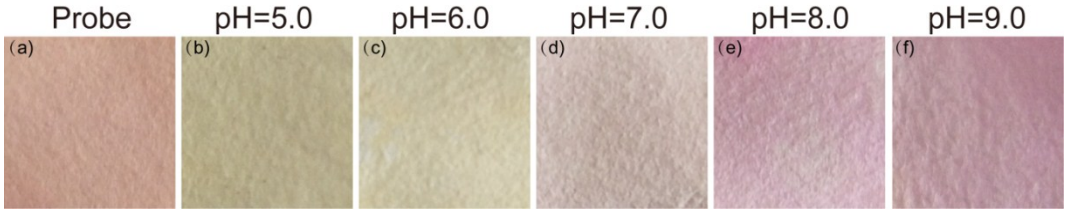


Figure S7 The color changes of M-pH (100 μM) coated test strips after exposure to different pH solutions. (a) The test strip coated with M-pH alone. (b-f) The test strips coated with M-pH at corresponding pH solutions.

Reference

1. D. Oushiki, H. Kojima, T. Terai, M. Arita, K. Hanaoka, Y. Urano and T. Nagano, *J. Am. Chem. Soc.*, 2010, **132**, 2795-2801.