Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting information for

Fluorescent Probe Based on POSS for facilitating visualization of HClO and NO in living cells and zebrafish

Guowei Ding,[a] Fengqing Gai,[a] Zhiming Gou,[a] and Yujing Zuo,[a]

School of Chemistry and Chemical Engineering, School of Materials Science and Engineering, University of Jinan, Jinan, Shandong 250022, P.R. China.

*Corresponding Author.

E-mail address: zuoyujing0888@163.com

Table of contents

Materials and instruments 1
Determination of the detection limit 1
Synthesis of OA-POSS 1
Synthesis and characterization of Cy7-Cl 2
Synthesis of Mito-Cy 3
Table S1. 3
Figure S1.. 4
Figure S2. 3
Figure S3. 4
Figure S4. 5
Figure S5. 5
Figure S6. 6
Figure S7. 6
Figure S8. 7

Materials and instruments

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. NMR spectra were recorded on an AVANCE III 400 MHz Digital NMR Spectrometer, using TMS as an internal standard; Electronic absorption spectra were obtained on a Shimadzu UV-2700 power spectrometer; Photoluminescent spectra were recorded with a HITACHI F4600 fluorescence spectrophotometer with a 1 cm standard quartz cell; The fluorescence imaging of cells was performed with a Nikon A1MP confocal microscope; The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter; TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from the Qingdao Ocean Chemicals.

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

Determination of the detection limit

The detection limit was determined by fluorescence titration. It was observed that the emission intensity at 812 nm was linear with the concentration of HClO.

$$\text{Detection limit} = 3\sigma / k$$

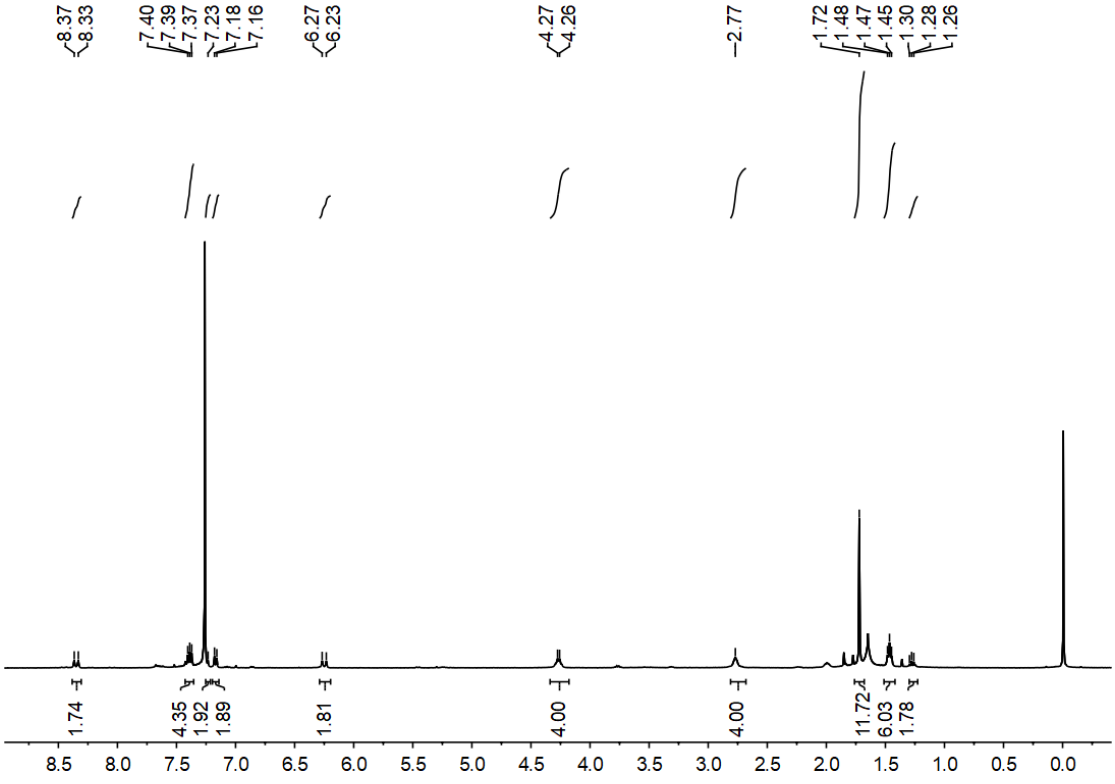
Where σ is the standard deviation of the blank sample and ‘k’ is the slope of the linear regression equation.

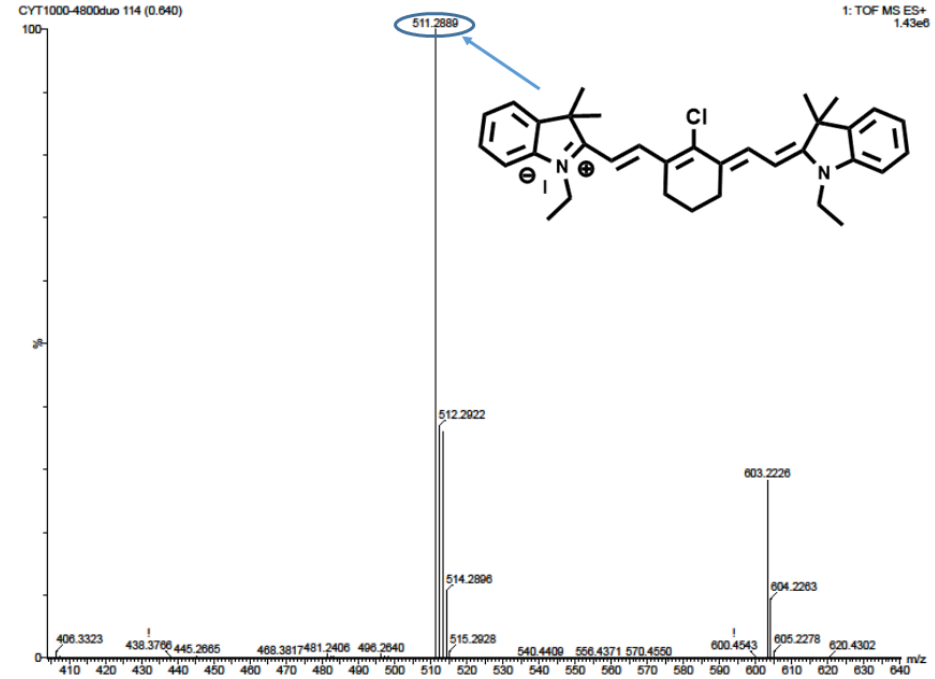
Synthesis of OA-POSS

Methanol (180 mL), (3-Aminopropyl) trimethoxysilane (20 mL, 0.115 mmol), , HCl (27 mL) were added to the reaction flask, stirred at room temperature for 7 days until white precipitate appeared, washed and dried.

Synthesis and characterization of Cy7-Cl

Synthesized of Cy7-Cl according to traditional methods. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (d, J = 13.8 Hz, 2H), 7.40 (dd, J = 13.8, 7.1 Hz, 4H), 7.23 (s, 2H), 7.17 (d, J = 7.9 Hz, 2H), 6.25 (d, J = 14.1 Hz, 2H), 4.27 (d, J = 6.8 Hz, 4H), 2.77 (s, 4H), 1.70 (d, J = 16.7 Hz, 12H), 1.47 (t, J = 6.8 Hz, 6H), 1.30 - 1.23 (m, 2H).





Synthesis of Mito-Cy

OA-POSS (0.5 g, 0.6 mmol), Cy7-Cl (1.25 g, 2.4 mmol), and ethanol (25 mL) were added to the reaction flask, heated to reflux at 80 ℃ for 36 h, filtered with suction, and spun dry. ^{1}H NMR (400 MHz, DMSO) δ 8.25 (d, J = 14.1 Hz, 2H), 7.63 (d, J = 7.3 Hz, 2H), 7.48 - 7.39 (m, 4H), 7.28 (t, J = 7.0 Hz, 2H), 6.32 (d, J = 14.2 Hz, 2H), 4.25 (d, J = 7.2 Hz, 4H), 3.34 (s, 4H), 2.71 (s, 4H), 1.84 (s, 2H), 1.66 (s, 12H), 1.29 (t, J = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.95, 150.56, 144.47, 141.78, 141.18, 128.88, 127.46, 125.35, 122.29, 110.75, 101.04, 49.36, 40.02, 28.06, 26.73, 20.72, 12.45.

Table S1. Molecular weights of **Mito-Cy**

Sanple	Mn g/mol	Mw g/mol	PDI (Mw/Mn)
Mito-Cy	3384	4332	1.28

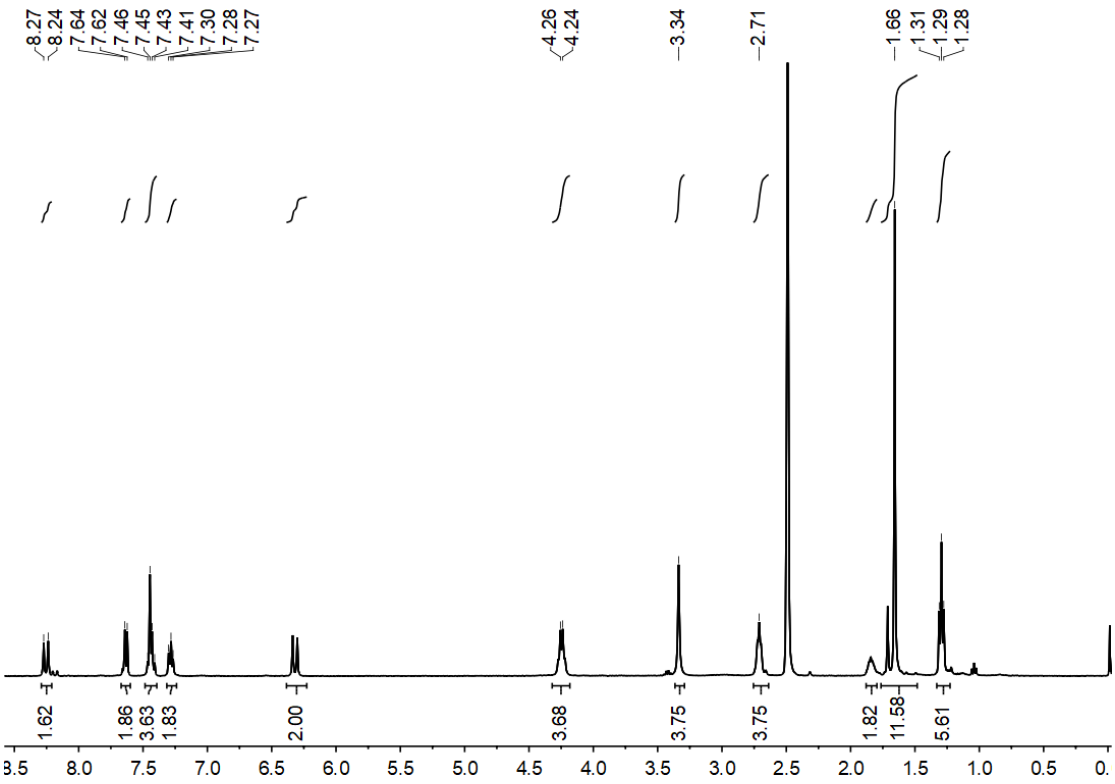


Figure S1. ^{1}H NMR spectrums of **Mito-Cy** (DMSO-d_6).

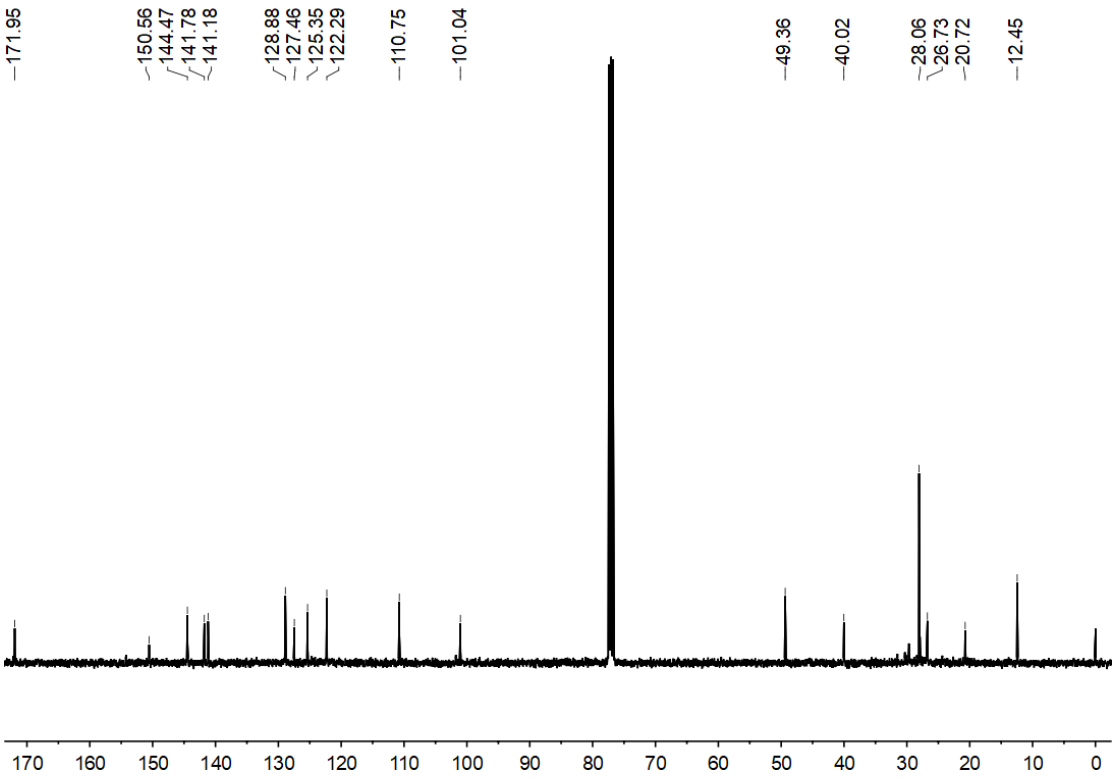


Figure S2. ^{13}C NMR spectrums of **Mito-Cy** ($CDCl_3$-d_1).

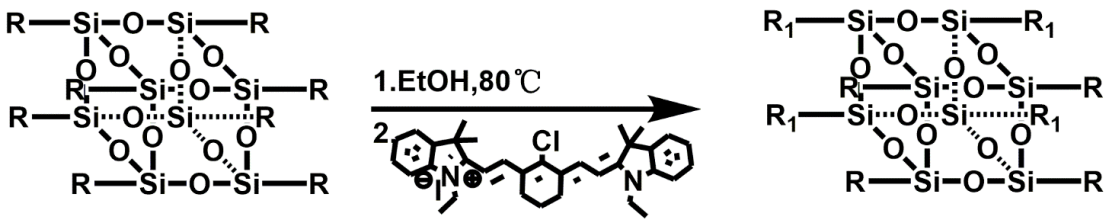


Figure S3. Synthesis procedures of **Mito-Cy**.

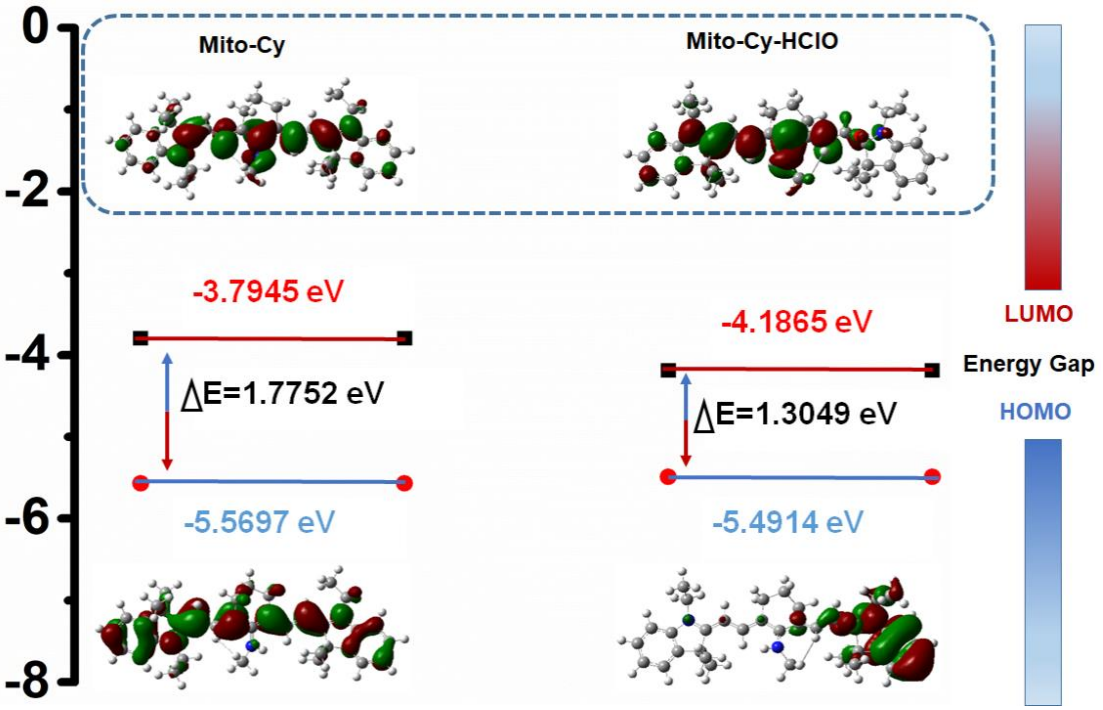


Figure S4. The spatial electron distributions of HOMOs and LUMOs of **Mito-Cy** and **Mito-Cy-HClO** in optimized state.

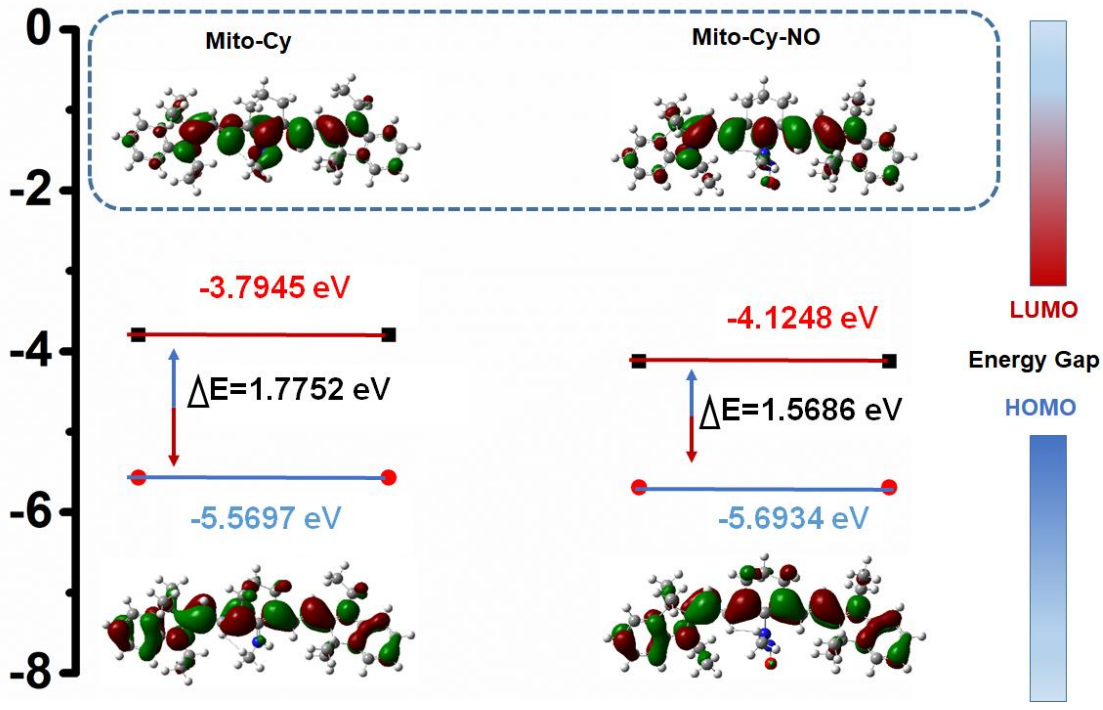


Figure S5. The spatial electron distributions of HOMOs and LUMOs of **Mito-Cy** and **Mito-Cy-NO** in optimized state.

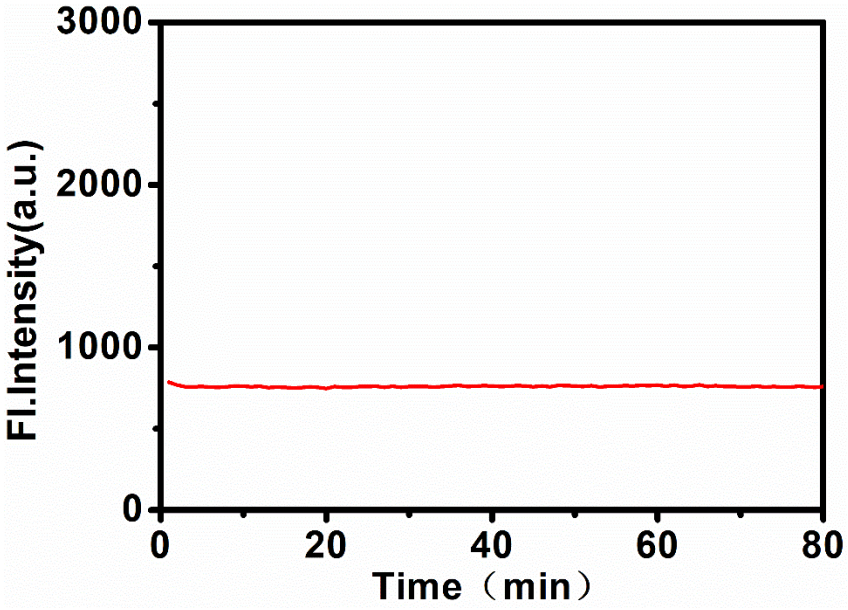


Figure S6. (a) The photostability of **Mito-Cy**, λ_{ex}= 750 nm.

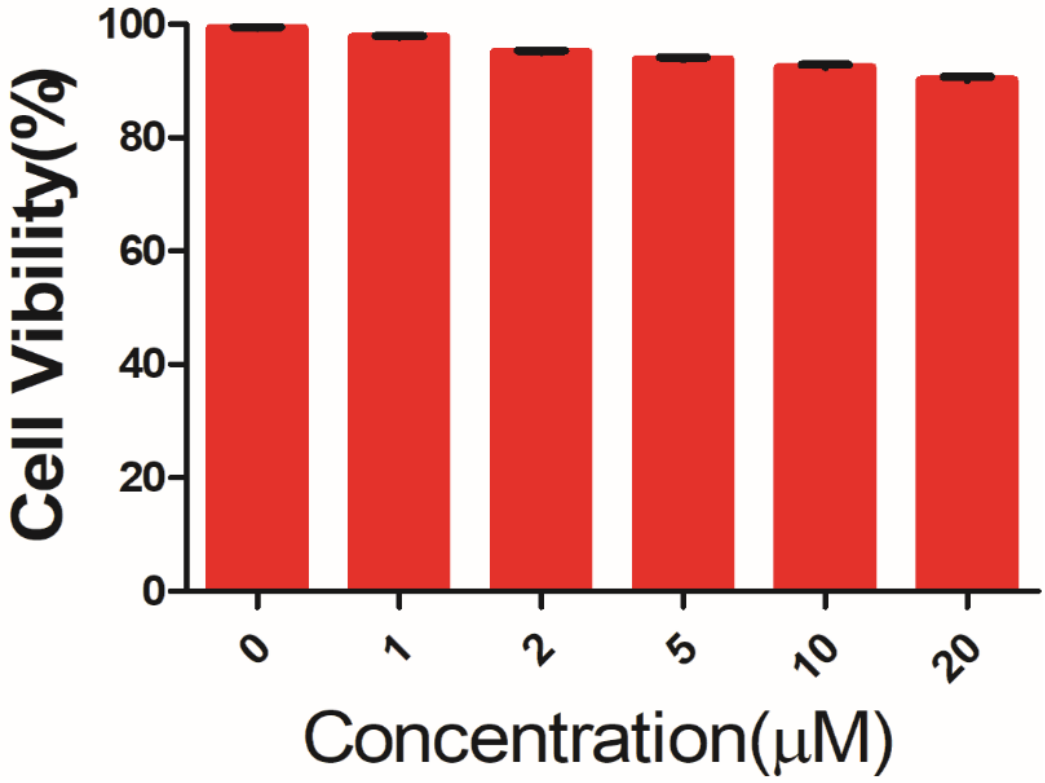


Figure S7. Cytotoxicity assay of **Mito-Cy** at different concentrations (1: 0 μM; 2: 1 μM; 3: 5 μM; 4: 10 μM; 5: 20 μM) for HeLa cells. Error bars represent the standard deviation (± S.D.) with n=3. Significant difference ($P< 0.01$) are analyzed with two-sided Students's *t*-test.

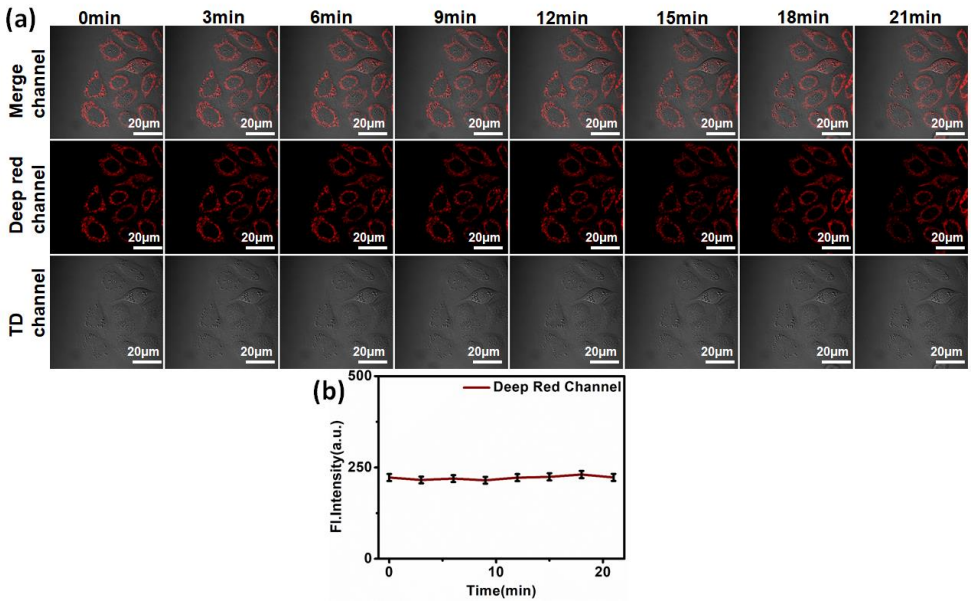


Figure S8. (a) Photostability of the probe in HeLa cells; (b) Changes in fluorescence intensity of deep red channel; Scale bar = 20 μm. Error bars represent the standard deviation (± S.D.) with n=3. Significant difference ($P < 0.05$) are analyzed with two-sided Students's *t*-test.