Supporting information for

POSS - Assisted Fluorescent Probe for Rapid Detection of HClO in Mitochondria with Large Emission Wavelength in Dual Channels

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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.

Determination of the detection limit

The detection limit was determined by fluorescence titration. It was observed that the emission intensity at 510 nm and 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.

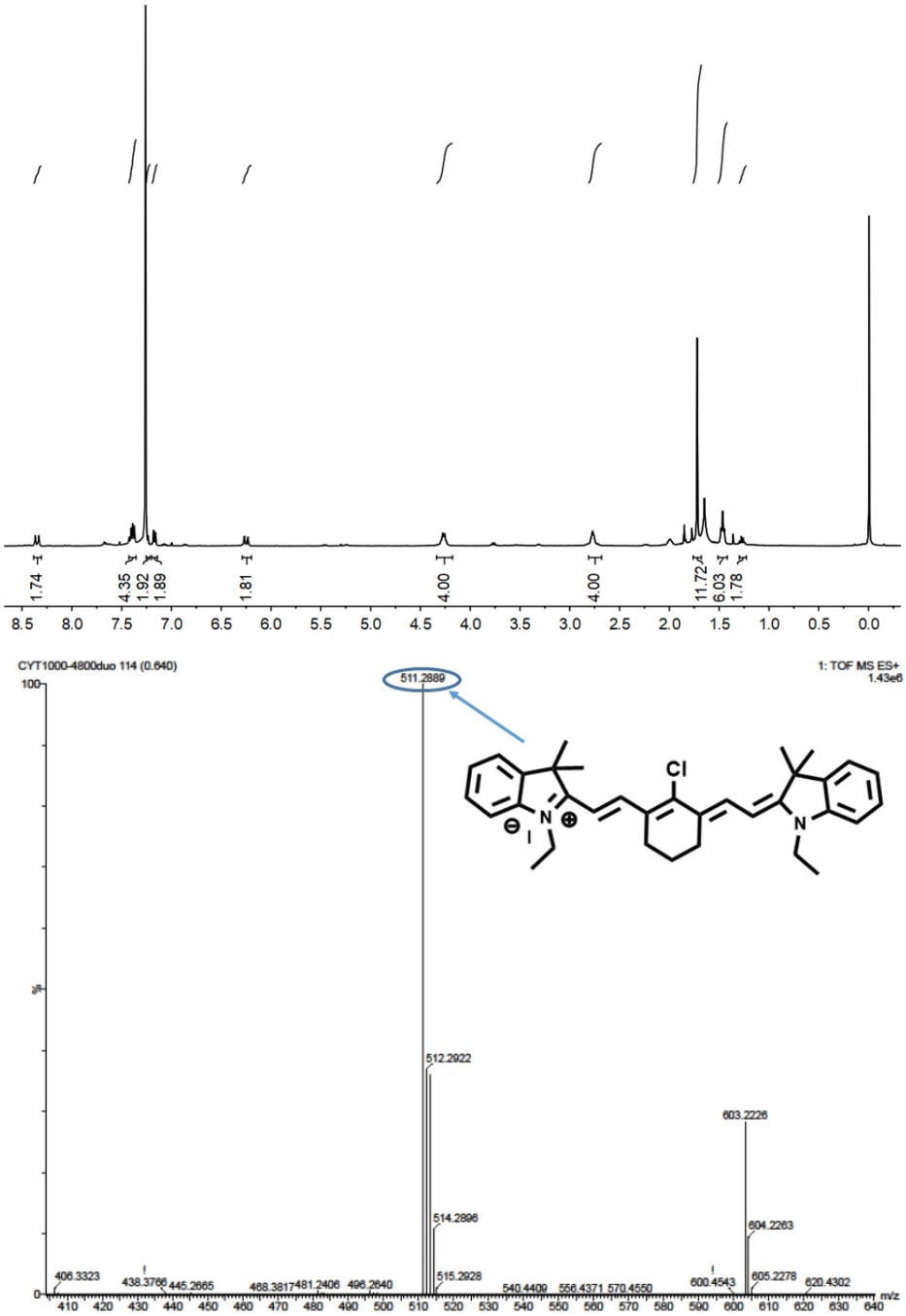
Synthesis of D1

Ethanethiol (0.3 g, 4.8 mmol), 4-Bromo-1, 8-naphthalic anhydride (1 g, 3.6 mmol), DMF (40 mL), appropriate amount of NaOH and K_2CO_3 were added to the

reaction flask, refluxed for 1 h at 45 ℃, ice water cooled, filtered 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 D5

OA-POSS (0.5 g, 0.6 mmol), D1(0.5856 g, 2.4 mmol), and ethanol (25 mL) were added to the reaction flask, heated to reflux at 80 °C for 36 h, filtered with suction, and spun dry.

Synthesis of D7

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 °C for 36 h, filtered with suction, and spun dry.

Synthesis of D6

D7 (0.5 g, 0.17 mmol), D1 (0.17 g, 0.69 mmol), and ethanol (25 mL) were added to the reaction flask, heated to reflux at 80 °C for 36 h, filtered with suction, and spun dry. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.64 (dd, J = 7.9, 4.3 Hz, 4H), 8.50 (d, J = 8.0 Hz, 2H), 8.36 (d, J = 14.4 Hz, 2H), 7.84 - 7.77 (m, 2H), 7.64 (d, J = 25.8 Hz, 2H), 7.57 (d, J = 7.8 Hz, 2H), 7.44 - 7.35 (m, 4H), 7.17 (d, J = 7.8 Hz, 2H), 6.25 (d, J = 13.6 Hz, 2H), 4.25 (s, 4H), 3.24 (q, J = 7.3 Hz, 4H), 2.77 (s, 4H), 2.02 (d, J = 14.0 Hz, 2H), 1.73 (s, 8H), 1.64 (d, J = 13.3 Hz, 12H), 1.52 (t, J = 7.4 Hz, 6H), 1.47 (s, 6H), 1.25 (s, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.89 (s), 160.58 (s), 150.58 (s), 148.19 (s), 144.47 (s), 141.47 (d, J = 61.2 Hz), 135.81 - 132.39 (m), 131.67 - 127.99 (m), 126.99 (s), 125.35 (s), 122.31 (s), 119.23 (s), 114.38 (s), 110.75 (s), 101.06 (s), 49.36 (s), 40.09 (s), 29.51 - 25.11 (m), 20.71 (s), 12.93 (d, J = 87.8 Hz).

Table S1. Molecular weights of **D6**

Sanple	Mn g/mol	Mw g/mol	PDI (Mw/Mn)
D6	3370	3876	1.15

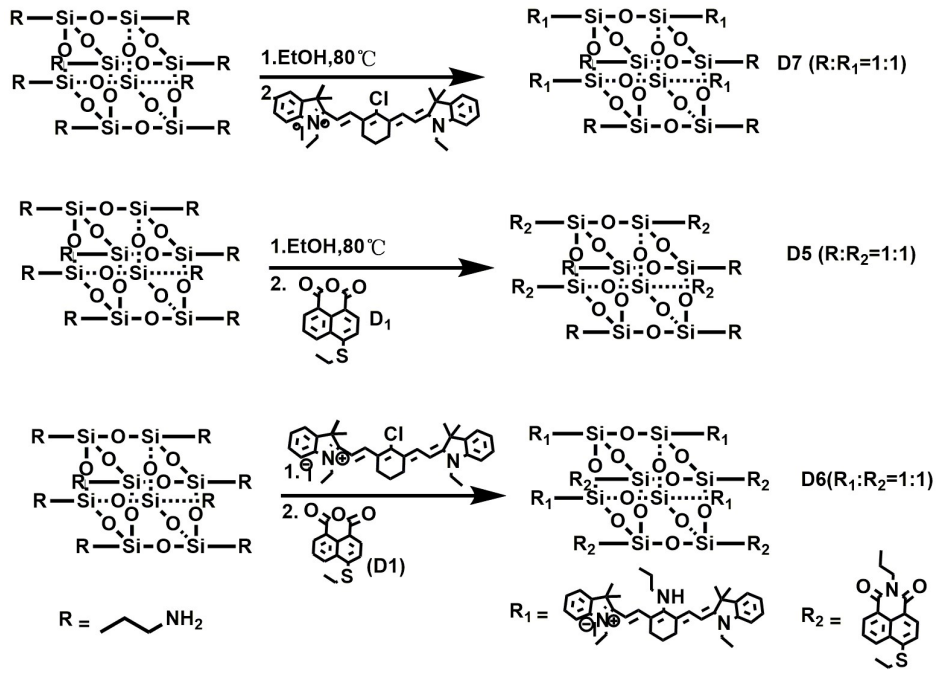


Fig. S1. Synthesis procedures of D5, **D6** and D5.

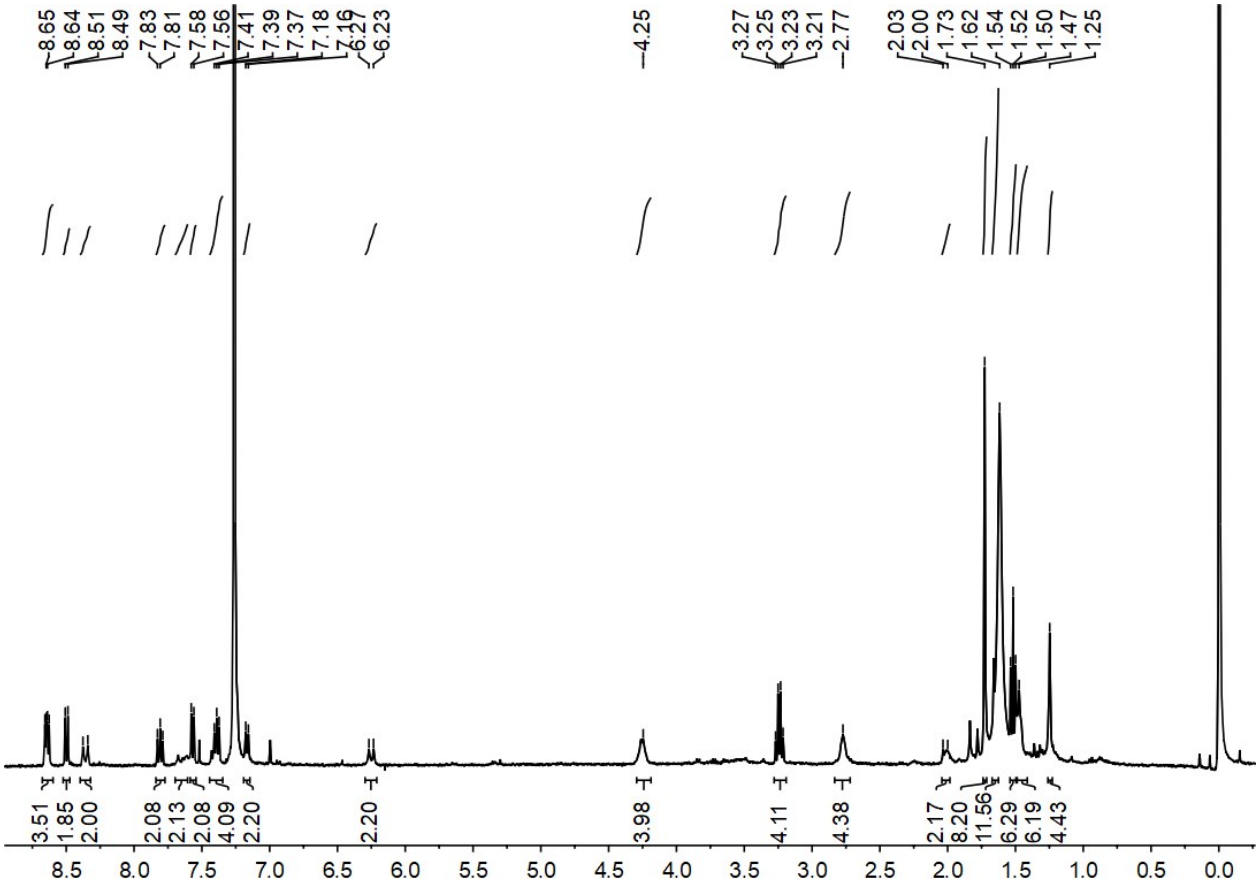


Fig. S2. ^{1}H NMR spectrums of **D6** ($CDCl_3$-d_1).

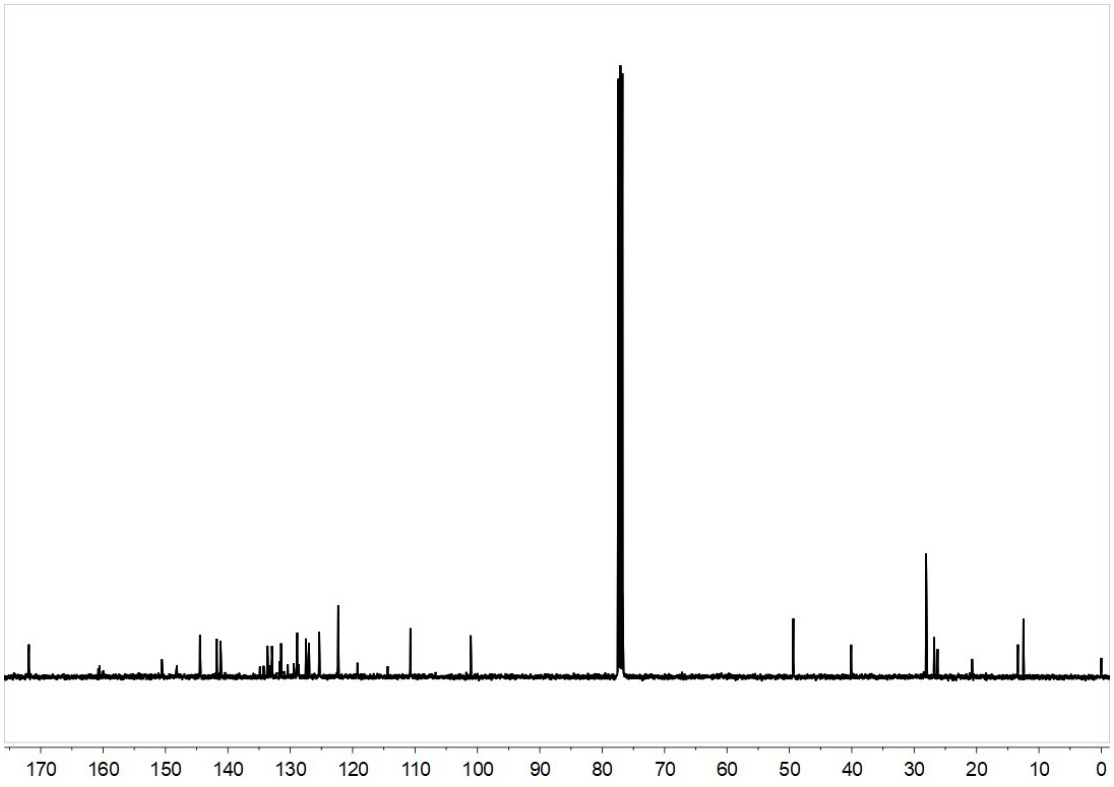


Fig. S3. ^{13}C NMR spectrums of **D6** ($CDCl_3$-d_1).

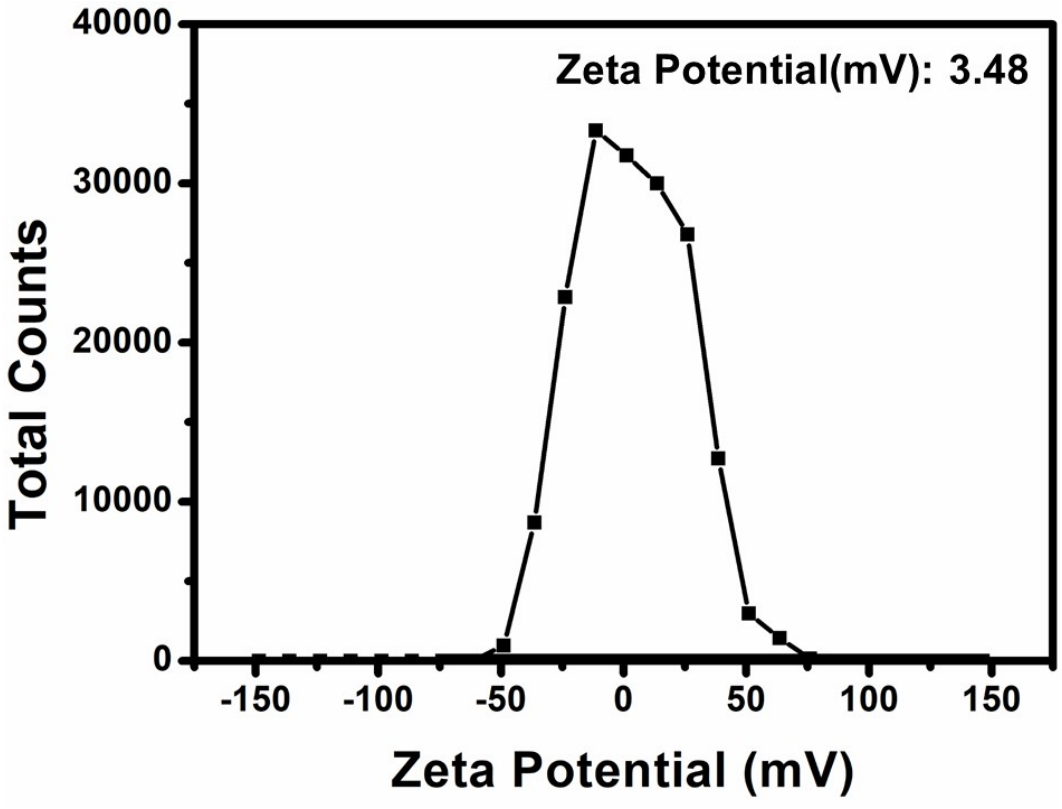


Fig. S4. Zeta potential of **D6**.

Table S2. Comparison of fluorescent probes for HClO in mitochondria

LOD/μM	Response time	applicability	λ_{em}/nm	Number of channels	Ref.
0.52	dozens of seconds	Imaging of exogenous and endogenous HClO	529	1	55
0.58	-	Imaging of exogenous HClO	546	1	56
0.62±0.09	30 seconds	Imaging of exogenous HClO	789	1	57
0.59	a few seconds	Imaging of exogenous HClO	585	2	58
0.15	15 seconds	Imaging of exogenous and endogenous HClO	820	2	This work

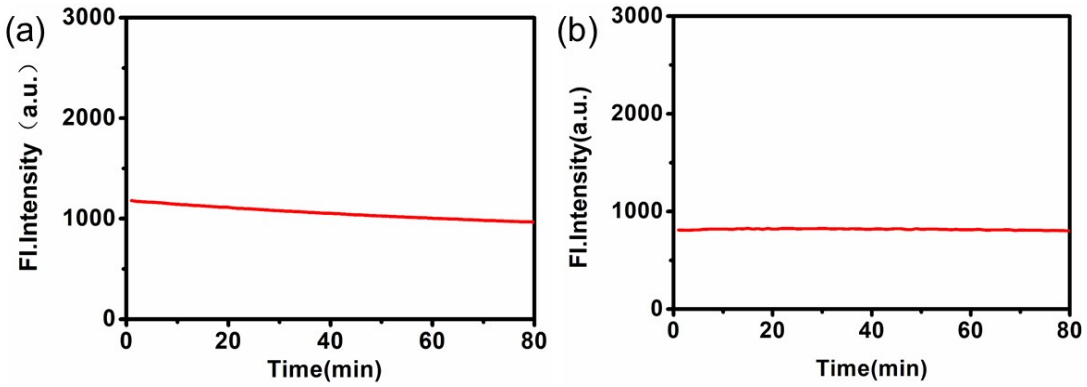


Fig. S5. (a) The photostability of **D6**, λ_{ex}= 420 nm; (b) The photostability of **D6**, λ_{ex}= 750 nm.

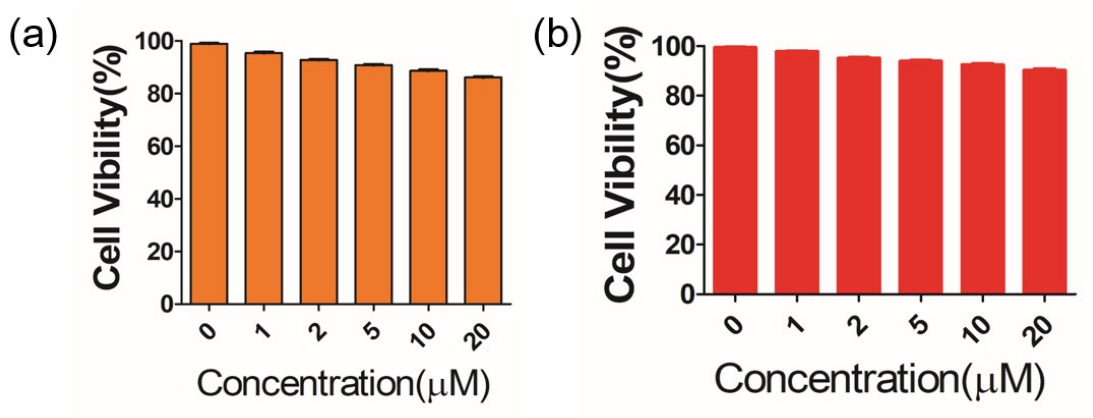


Fig. S6. Cytotoxicity assay of **D6** and **D7** at different concentrations (1: 0 μM; 2: 1 μM; 3: 5 μM; 4: 10 μM; 5: 20 μM) for HepG2 cells. Error bars represent the standard deviation (± S.D.) with n=3. Significant difference ($P < 0.01$) are analyzed with two-sided Students's *t*-test.

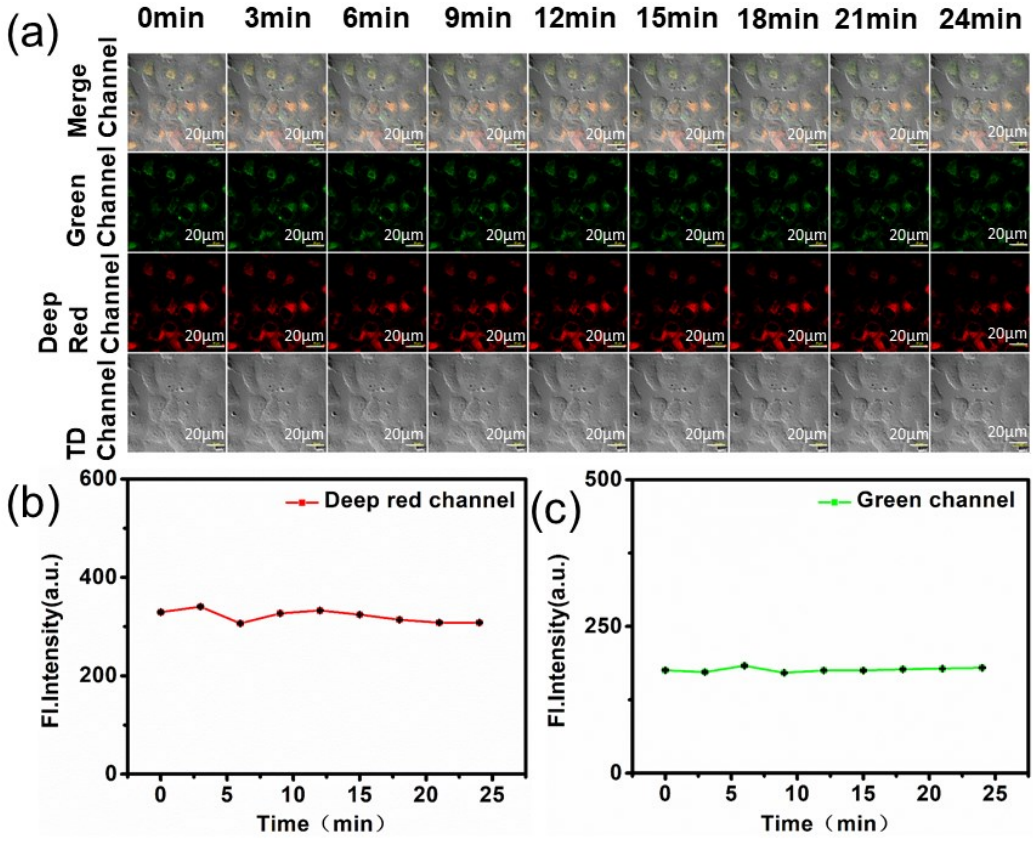


Fig. S7 (a) Photostability of the probe in HepG2 cells; (b) Changes in fluorescence intensity of deep red channel; (c) Changes in fluorescence intensity of green channel. Scale bar = 20 μm. Error bars represent the standard deviation (± S.D.) with n=3. Significant difference ($P < 0.01$) are analyzed with two-sided Students's *t*-test.