Supporting Information

A Selective Near-Infrared Fluorescent Probe for Singlet Oxygen in Living Cells

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1. Apparatus and Materials

Apparatus. ^{1}H NMR spectra were taken on a Bruker 300 MHz spectrometer and chemical shifts were collected in $CDCl_3$ in parts per million referenced to an internal standard [$(CH_3)_4Si$ = 0.00 ppm] under argon. Mass spectra were measured with an Agilent 6520 Q-TOF (US). Fluorescence spectra were obtained with a Cary Eclipse fluorescence spectrophotometer (Varian, Inc., USA) with a 1.0 cm quartz cells at the slits of 10/10 nm. Infrared spectrum was measured with Bruker infrared spectrometer. Absorbance was measured in a TRITURUS microplate reader in the MTT assay.

Materials. 2-[4-Chloro-7-(1-ethyl-3,3-dimethyl(indolin-2-ylidene)]-3,5-(propane-1,3 -diyl)-1,3,5-heptatrien-1-yl)-1-ethyl-3,3-dimethyl-3H-indolium (Cy.7.Cl) was synthesized in our laboratory. Histidine (His), Phorbol myristate acetate (PMA), and methyl thiazolyl tetrazolium (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, MTT) were purchased from Sigma-Aldrich. MitoTracker Green FM was purchased from Shanghai DoBio Biotech. Co., LTD. All other chemicals were from commercial sources and of analytical reagent grade, unless indicated otherwise. Mouse macrophages (RAW264.7)

were purchased from the Committee on type Culture Collection of Chinese Academy of Sciences. Sartorius ultrapure water (18.2 MΩ cm) was used throughout the analytical experiments.

2. Synthesis and Characterization of His-Cy

2-[4-Histidine-7-(1-ethyl-3,3-dimethyl(indolin-2-ylidene)]-3,5-(propane-1,3-diyl)-1,3,5-heptatrien-1-yl)-1-ethyl-3,3-dimethyl-3H-indolium (His-Cy). The newly prepared cyanine 330 mg (0.516 mmol) was dissolved in 10 mL of anhydrous DMF in a 50 mL round bottom flask under argon. And histidine 160 mg (1.03 mmol) dissolved in 5 mL of water was added. The mixture was stirred at 80 °C for 24 h. Then it was cooled to room temperature and added to a large number of ether with violent stirring. The obtained blue solid was filtered and dried under vacuum, and the solid was purified on silica gel chromatography eluted with ethyl acetate/methanol (4:1 v/v).

^{1}H-NMR ($CDCl_3$, 300MHz): 0.95 (t, 6H, J = 6 Hz), 1.66 (s, 12H), 1.86 (m, 2H), 2.52 (m, 4H), 3.56 (m, 2H), 3.95 (m, 1H), 4.32 (m, 4H), 5.65-5.70 (d, 2H, J = 15 Hz), 6.91 (d, 2H, J = 9 Hz), 7.09 (m, 2H), 7.31 (m, 4H), 7.49-7.54 (d, 2H, J = 15 Hz), 7.54 (s, 1H), 7.72 (d, 1H, J = 3 Hz) ppm. ^{13}C-NMR ($CDCl_3$, 300MHz): 172, 154, 151, 145, 142, 141, 129, 127, 125, 122, 111, 102, 101, 49, 40, 30, 28, 27, 21, 12 ppm. IR (KBr): 3414, 1728, 1637, 1552, 1384 cm^{-1}. ESI-MS: m/z Calcd 630.38 [M-I^-], found 675.67 [M-I^- + formic acid]$^+$. MS solvents: acetonitrile, ultrapure water and formic acid.

3. Mass Spectrometry of Oxidized His-Cy

2-{4-[2-amino-3-(2,5-dihydro-5-hydroxy-2-oxo-1H-imidazol-5-yl)propanoicacid]-7-(1-ethyl-3,3-dimethyl(indolin-2-ylidene)}-3,5-(propane-1,3-diyl)-1,3,5-heptatrien-1-yl)-1-ethyl-3,3-dimethyl-3H-indolium (Oxidized His-Cy). $^{1}O_2$ generated by $NaClO/H_2O_2$ was added to a solution of purified His-Cy 50 mg (0.066 mM) in acetonitrile (2 mL), under 37 °C water bath. The end of the reaction was determined when the fluorescence intensity at 794 nm reached a maximum. Then the solvent was removed under reduced pressure to afford the desired product. ESI-MS: m/z Calcd 662.37 [M-I^-], found 662.44 [M-I^-]$^+$. MS solvents: acetonitrile, ultrapure water and formic acid.

4. Cell Culture and Confocal Imaging

Cell Culture. Mouse macrophages (RAW264.7) were maintained in continuous culture in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen, Carlsbad, CA) supplemented with 10% Fetal Bovine Serum (FBS, Invitrogen), and penicillin /streptomycin (50 g·mL^{-1}, Invitrogen) at 37 °C in a 5% CO_2/95% air incubator MCO-15AC (Sanyo, Japan).

Confocal Imaging. Cells were passed and plated on 18 mm glass coverslips in 1.5 mL DMEM one day before imaging. A Leica TCS SP5 laser scanning microscopy system containing a 40× air-immersion objective lens or a 63× oil-immersion objective lens (HCX Apo) was used for the probe experiments. Confocal images of macrophage fluorescence were collected using a 633 nm excitation light from a Helium/Neon laser, and emission was collected from 700 to 800 nm using a detection system.

5. Optimization of pH value, buffer concentration and probe concentration

Effect of pH value and buffer capacity: Effect of pH and the buffer capacity on the fluorescence intensity of the reaction system was discussed. The fluorescence intensity changes could reach the maximum and be relative stable when the pH was in the range 7.30-7.50 (Figure S1a) and 20 mM Tris-HCl buffer solution was chosen in subsequent experiments (Figure S1b).

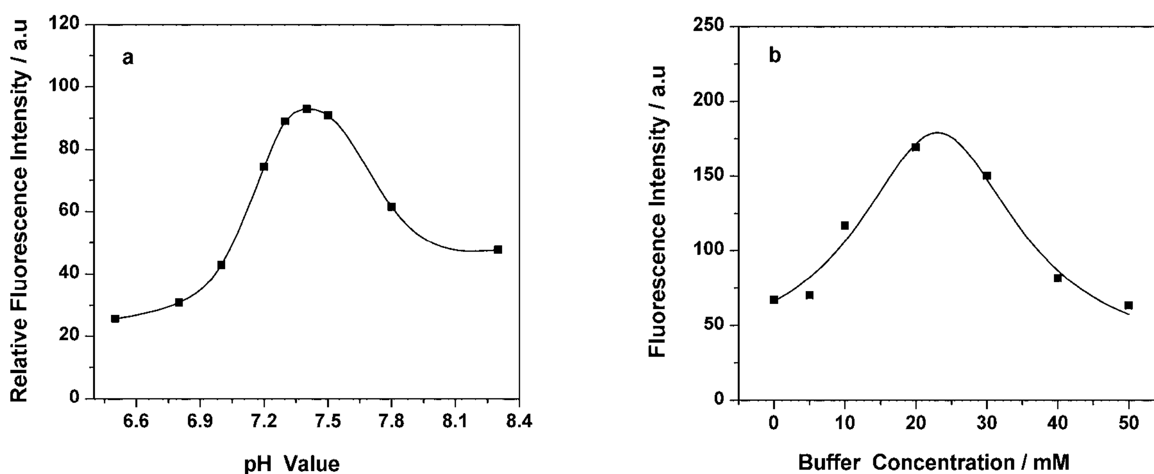

Figure S1. (a) The effect of pH values. (b) The effect of different concentrations of Tris-HCl buffer solution on the fluorescence of the system. (His-Cy concentration: 3.0 μM; $^1O_2$: 1.0 μM)

Effect of probe concentration: Whether the $^1O_2$ could be completely captured or not was determined by the probe concentration. Figure S2 showed that, when the probe concentration was 3.0 μM, the His-Cy showed the best response to 1.0 μM $^1O_2$.

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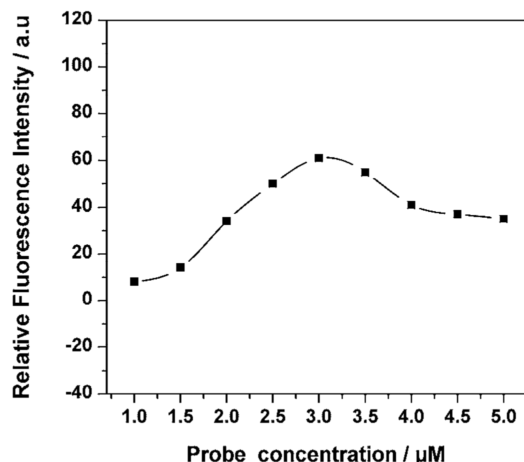


Figure S2. The relationship between the fluorescence intensities and probe concentrations (1.0 μM $^1O_2$ in 20 mM Tris-HCl buffer, pH 7.40 at 37°C)

6. Studies on interference from metal ions on determination of $^1O_2$ with His-Cy

In consideration that amines could bind many metal ions in solution, the interference from metal ions on determination of $^1O_2$ with His-Cy was studied. A an additional test was performed in order to determine whether metal ions such as K^+, Na^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Cd^{2+}, Co^{2+}, Hg^{2+}, Ni^{2+}, Cu^{2+}, Fe^{3+} and $Fe^{2+}$are potential interferences. An error of <5.0% in the relative fluorescence intensity was considered tolerable in Figure. S3.

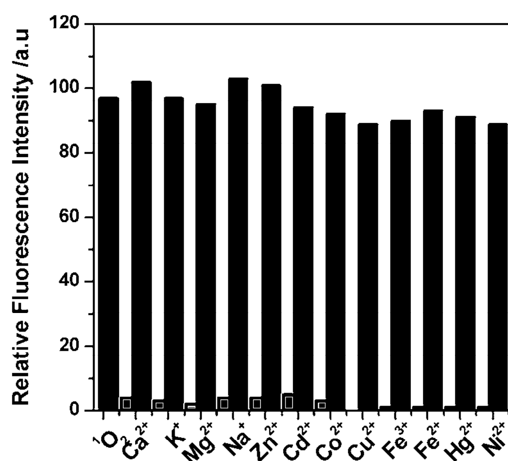


Figure S3. The relative fluorescence responses of 3.0 μM His-Cy to diverse metal ions (1.0 mM for Ca^{2+}, K^+, Mg^{2+}, Na^+; 0.20 mM for Zn^{2+}; 0.050 mM for Cd^{2+}, Co^{2+}, Hg^{2+}, Ni^{2+}; 1.0 μM for Cu^{2+}, Fe^{3+}, Fe^{2+}). Gray bars represent the addition of one of metal ions to the probe solution in 20 mM Tris-HCl buffer, pH 7.4. Blank bars represent the addition of $^1O_2$ (1.0 μM) or $^1O_2$ (1.0 μM) plus one of metal ions to the probe solution in 20 mM Tris-HCl buffer, pH 7.4. All data were obtained after incubation with the appropriate metal ions or $^1O_2$ at 37°C for 10 min. Collected emission was integrated between 780 and 850 nm (λex = 754 nm).

7. Tests of the cytotoxicity and photostability of His-Cy

To investigate BzSe-Cy cytotoxicity, MTT assay was carried out when the probe existed in macrophages. RAW 264.7 cells (5×10^4 cell mL^{-1}) were dispersed within replicate 96-well microtiter plates to a total volume of 100 μL $well^{-1}$. Plates were maintained at 37 °C in a 5% CO2/95% air incubator for 12 h. The probe was diluted to different concentrations of solution with medium and added to each well after the original medium had been removed. Macrophages were incubated upon probe concentrations from 1.0 μM to 400.0 μM for 4 h. And then 150 μL MTT (0.5 mg mL^{-1}, PBS) was added to each well. After 4 h, the remaining MTT solution was removed, and 100 μL of DMSO was added to each well to dissolve the formazan crystals. Absorbance was measured at 490 nm in a TRITURUS microplate reader in Figure. S4. In addition, photobleaching tests were carried out by means of time-sequential scanning of RAW 264.7 cells incubated with 10 μM His-Cy for 20 min after PMA stimulation for 30 min, as shown in Figure. S5 and Figure. S6.

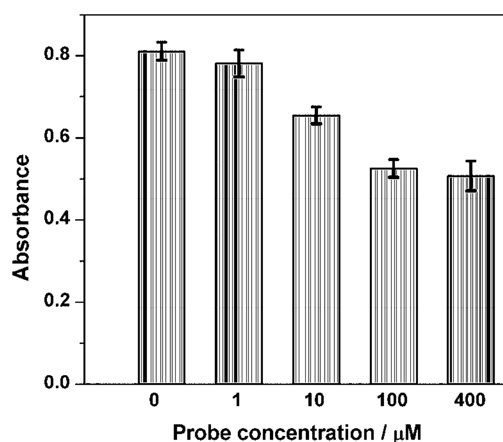


Figure S4 Cell viability estimated by MTT assay. RAW 264.7 macrophages were incubated with 0-400 μM His-Cy at 37 °C for 4 h.

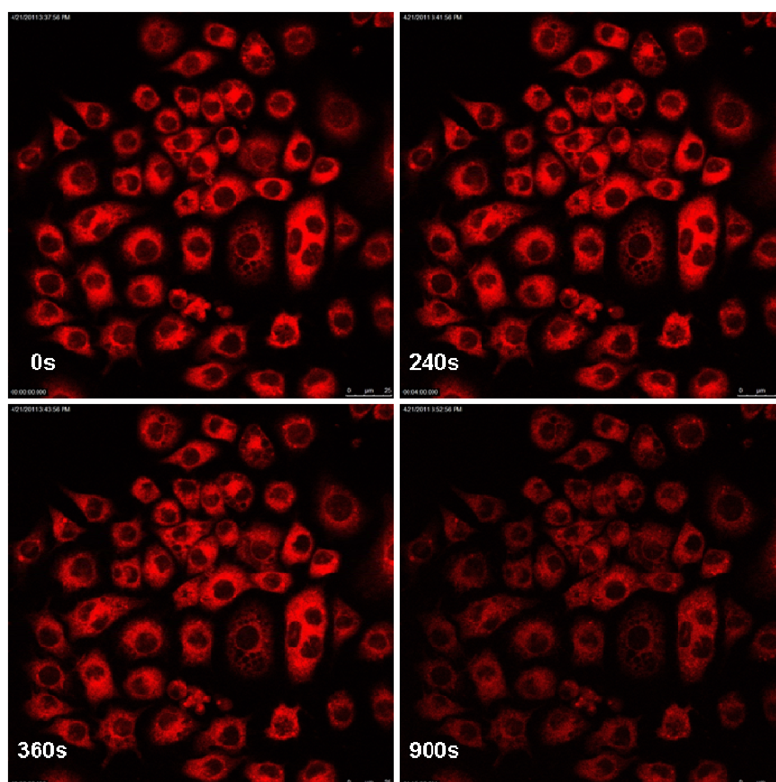


Figure S5 Test of photostability of oxidized His-Cy. Confocal fluorescence images (0s, 240s, 360s and 900s) were achieved by means of time-sequential scanning of RAW 264.7 cells. Cells were incubated with 10 μM His-Cy for 20 min after PMA stimulation for 30 min.

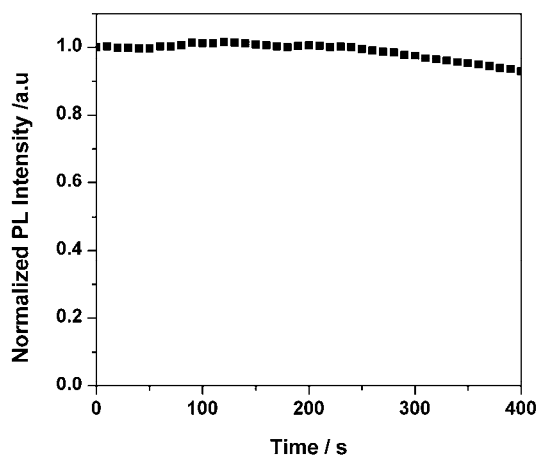
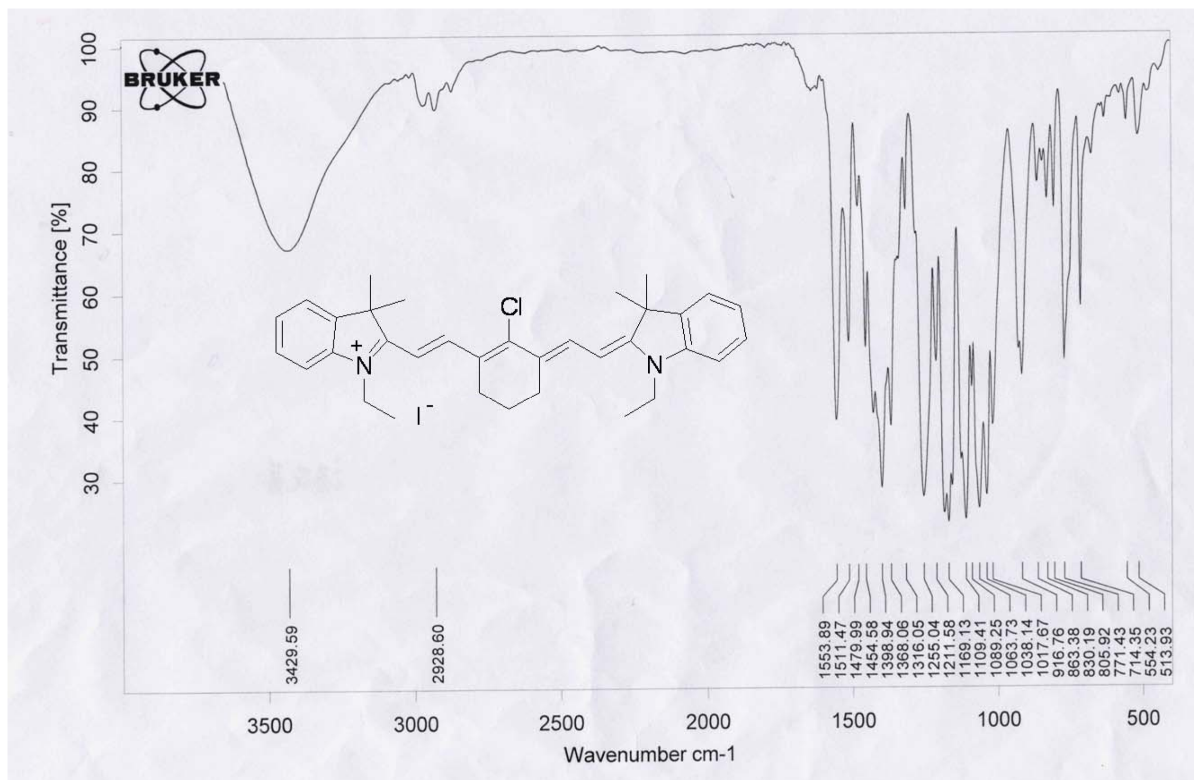
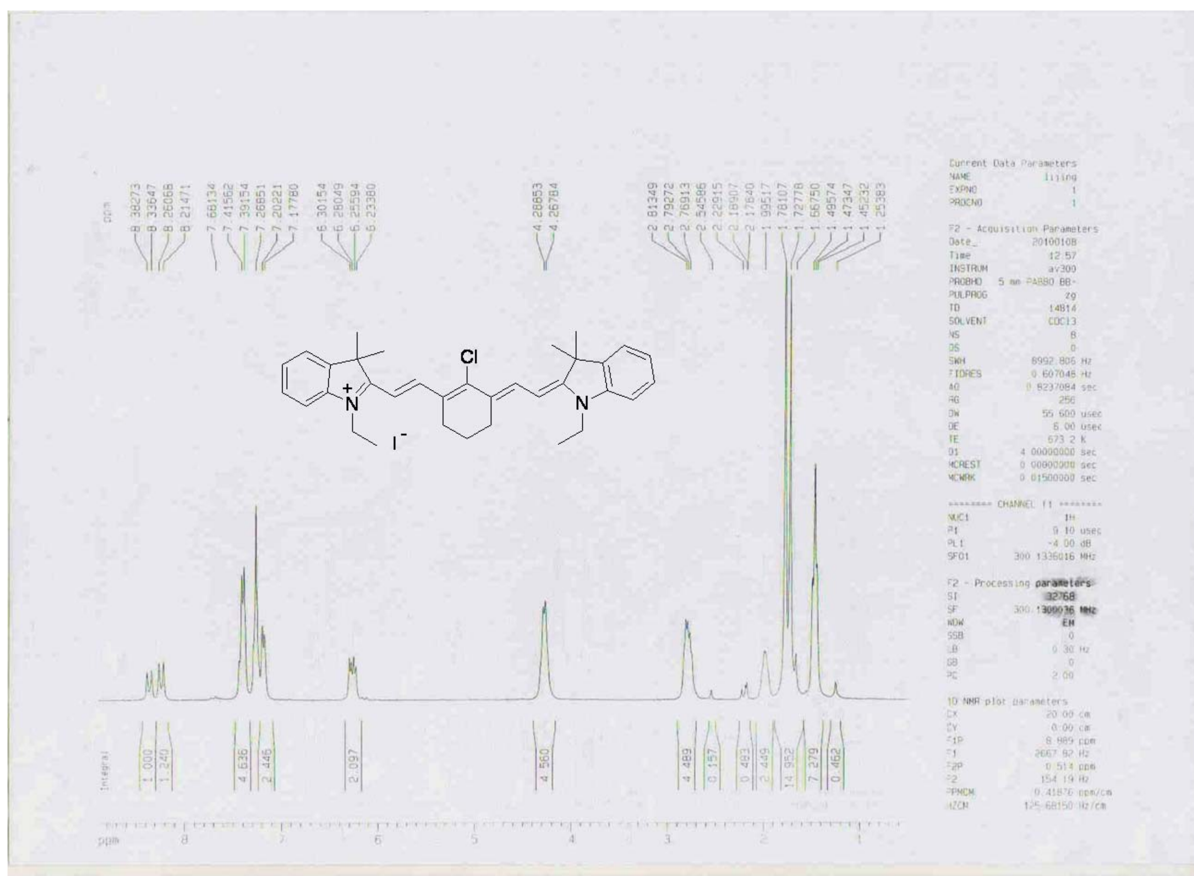
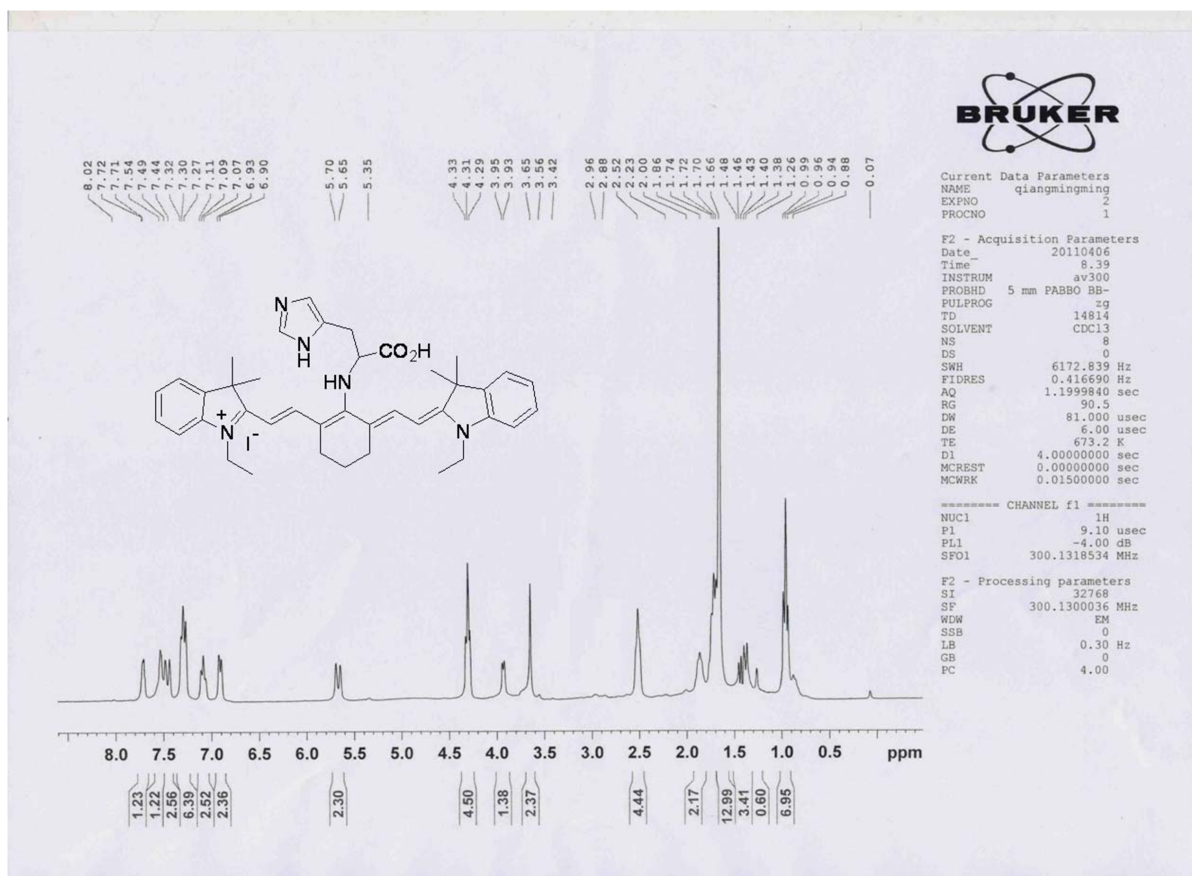


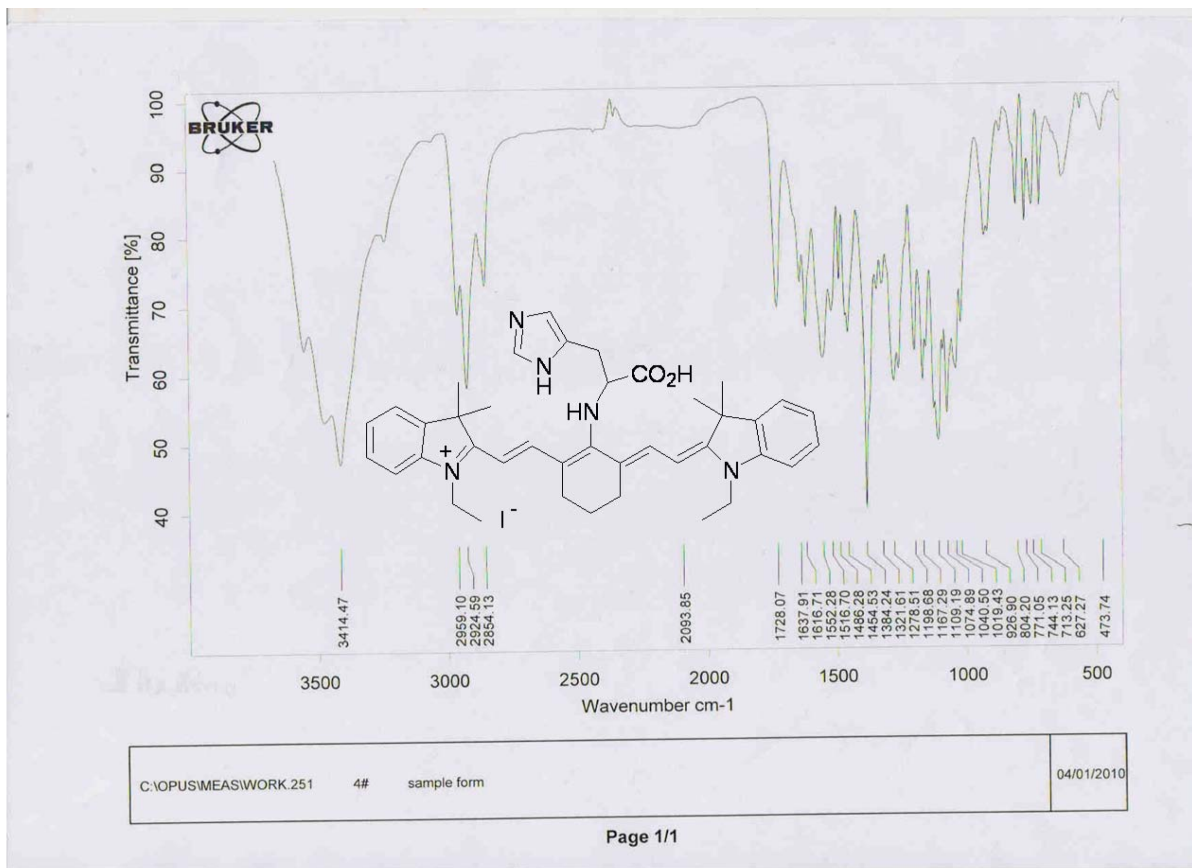
Figure S6 Photoluminescence (PL) intensity of oxidized His-Cy by means of time-sequential scanning of RAW 264.7 cells incubated with 10 μM His-Cy for 20 min after PMA stimulation for 30 min.

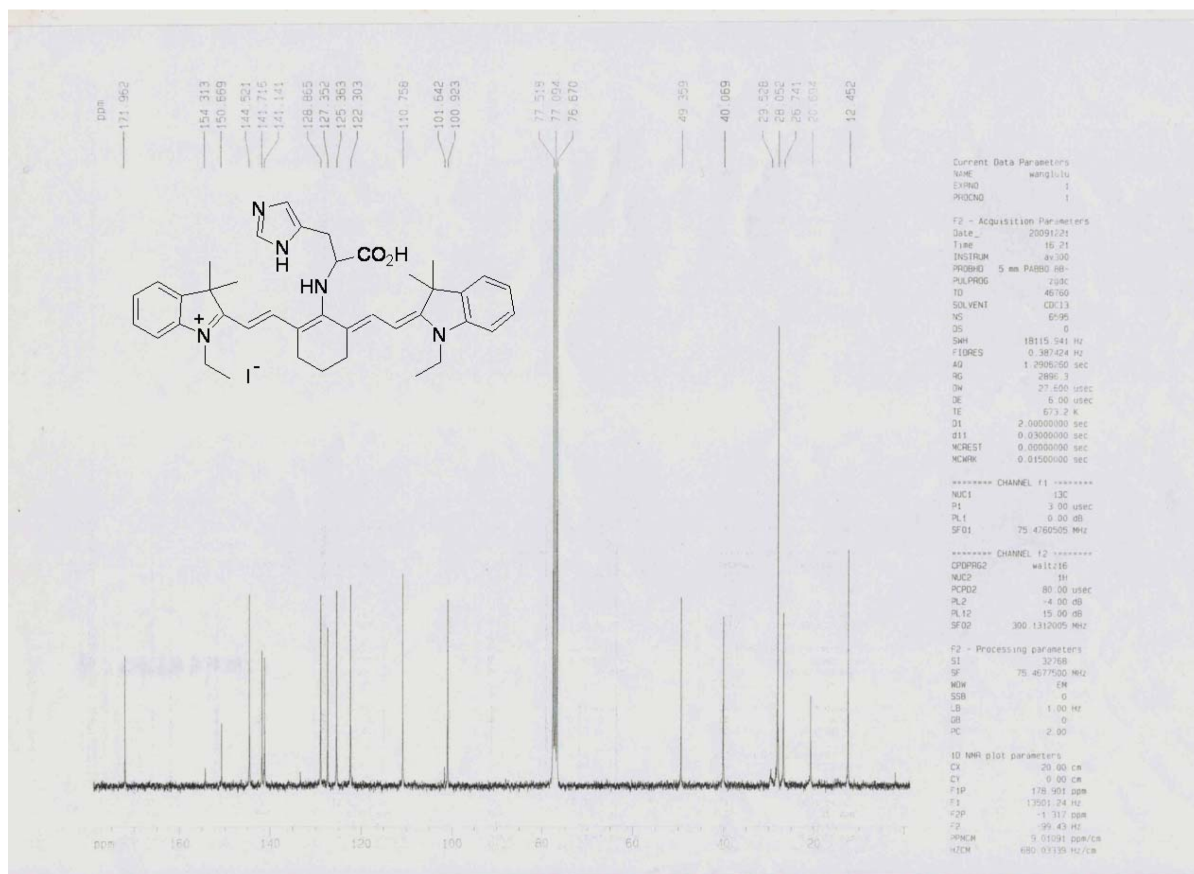
8. ^{1}H-NMR, ^{13}C-NMR, IR and MS spectra of Cy.7.Cl, His-Cy and Oxidized His-Cy

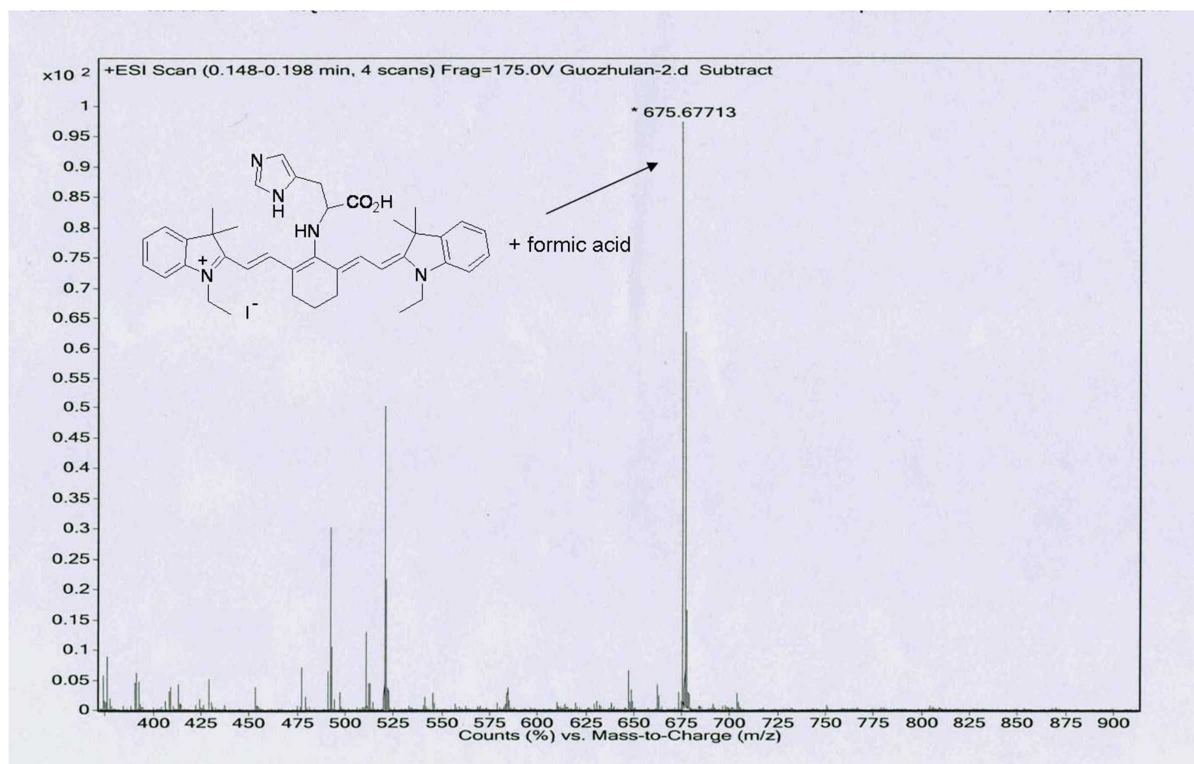








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Counts (%) vs. Mass-to-Charge (m/z)

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