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Visualizing the changes of cellular redox environment using a novel profluorescent rhodamine nitroxide probe

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Experimental Section

Materials and methods

4-hydroxy-2, 2, 6, 6-tetramethyl-piperidinooxy free radical (4-OH-TEMPO), Rhodamine B, 2DG, GSH, ascorbic acid, cysteine (Cys) and uric acid were purchased from Sigma-Aldrich. HepG2 and L02 cells were purchased from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences. All reagents used were of analytical grade or chromatographically pure.

ESI-MS studies were carried out with an Esquire 5000 spectrometer and NMR spectra were performed on a Varian INOVA 400 MHz spectrometer. The fluorescence spectra were recorded on a Perkin Elmer LS55 spectrometer while the fluorescence and bright field images were acquired with Olympus BX51.

Synthesis of the probe

Rhodamine B (0.27 mmol) was mixed with equimolar 4-OH-TEMPO in dichloromethane, and then 4-dimethylamiopryidine (DMAP, 0.027 mmol) and dicyclohexyl carbodiimide (DCC, 0.27 mmol) were added as catalyst and dehydrating agent. The mixture was stirred under an Ar atmosphere for 24 h at ambient. After removing the solvent under vacuum, the residue was purified by chromatography on neutral aluminum oxide column (eluent: CH_2Cl_2/CH_3OH 20/1, v/v). The probe was obtained in 31% yield and stored in a -20 °C freezer. ^1H NMR (400 MHz, $CDCl_3$, tetramethylsilane (TMS)): δ=6.7-8.2 (aryl H, 11 H), 3.4-3.7 (NCH_2; OCH, 9 H), 1.2-1.7 ppm (CH_3; CH_2, 24 H); MS (ESI-MS): m/z calcd: 597.36, found: 597.5 $[M]^+$.

HPLC analysis of the reducing product

The mixture of the probe and ascorbic acid was stirred for 2 h at ambient, and then subjected to the HPLC analysis with the conditions: eluent, CH_3OH (90%)/ammonium acetate (0.01 M, 10%) aqueous solution; flow rate, 1.0mL/min; injection volume, 20 μl, temperature, 25℃，analytical column，octadecyl (C18). The elution was monitored by FLD (E_x556 nm, E_m 590 nm.) and DAD (absorbance at 550 nm) detector.

Fluorescence imaging

HepG2 and L02 cells were cultured in MEM medium supplemented with 10% fetal calf serum and incubated at 37°C in 5% CO_2/air atmosphere. Cells were seeded into culture dishes with appropriate density and cultured for 12 h, then choose the well-grown cells and treated accordingly. The probe was added to all cell samples at a final concentration of 2 μM to 4 μM from a 2 mM stock solution. A fresh stock solution of 2DG (0.2 M) was prepared in H_2O and appropriate concentrations were added to the cell samples.

The cells were treated with 2DG for 4 h before the probe was added, and then incubated continue for 2 h. After all the treatment, the medium was removed from the cells and washed 3 times with 10% PBS. Fluorescence and bright field images were acquired with Olympus BX51 with 10× eye lens and 20× objective lens.

Results and discussion

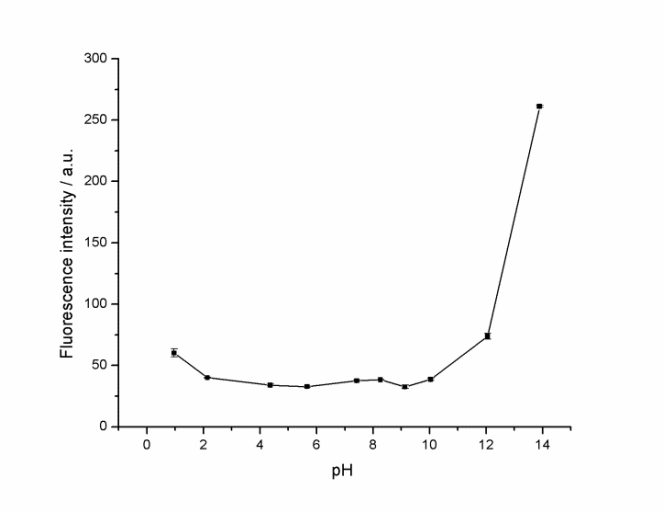

Fig.S1 The effect of pH (1～14) on the fluorescence intensity of the probe. The pH was controlled by HCl or NaOH and the final concentration of the probe was 2.5 μM.

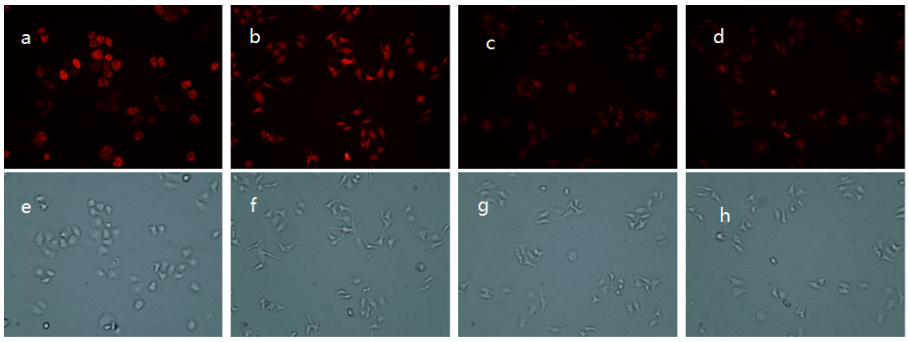

Fig.S2 Fluorescence and bright field images of HepG2 cells treated with 2DG. Fluorescence imaging of cells treated with 0 mM (a), 2 mM (b), 10 mM (c), 20 mM (d) 2DG for 4 h before incubated with 4 μM probe for another 2 h, e, f, g, h were the corresponding bright field images.

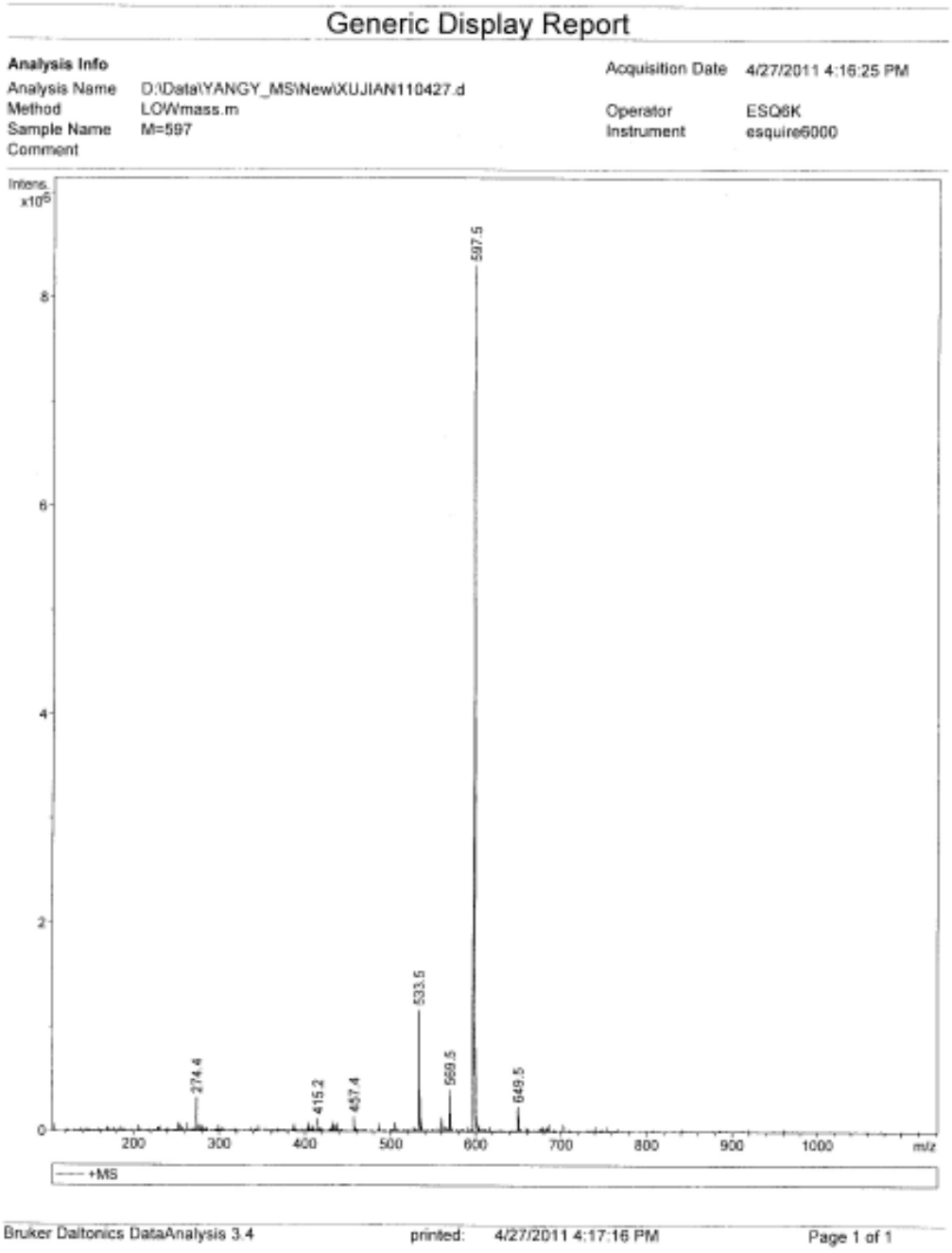

Fig.S3 HRMS spectra of the probe