Electronic supplementary information (ESI)

A novel fluorescent probe with red emission and a large Stokes shift for selective imaging of endogenous cysteine in living cells

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Contents

Materials and instruments

All the solvents and reagents were obtained commercially. UV–vis absorption spectra were recorded on a Schimadzu 160A spectrophotometer. Fluorescence spectra were recorded on a Hitachi F-4500 spectrometer. The pH measurements were made with a Sartorius basic pH-meter PB-10. HPLC analyses were performed on Shimadzu LC-20A high performance liquid chromatography. Fluorescence imagings were carried out by a confocal laser scanning microscope (CLSM, Zeiss LSM 880, Jena, Germany). ^{1}H NMR spectra were recorded on Bruker Ascend 400 MHz spectrometers, and ^{13}C NMR spectra were recorded on 100 MHz spectrometers. Mass spectra (MS) were recorded on an Ion Spec 4.7 T FTMS instrument.

Synthesis of NT-OH

6-hydroxy-2-naphthaldehyde (0.095 g, 0.55 mmol), TCF (0.10 g, 0.50 mmol) and ammonium acetate (0.05 g, 0.60 mmol) were mixed in a Schlenk tube which was fully filled with argon. Then 1 mL of THF and 0.3 mL of ethanol were injected to the mixture by syringe. The solution was stirred at room temperature overnight. The solvent was concentrated under vacuum and **NT-OH** was obtained by silica column chromatography with dichloromethane/methanol (30/1, v/v) as eluent. Yield: 0.035 g, 20%. ^{1}H NMR (400 MHz, DMSO) δ [ppm]: 10.36 (s, 1H), 8.32 (s, 1H), 8.10 (d, J = 16 Hz, 1H), 7.98 (d, J = 12 Hz, 1H), 7.91 (d, J = 8 Hz, 1H), 7.80 (d, J = 8Hz, 1H), 7.27 (d, J = 16 Hz, 1H), 7.19-7.15 (m, 2H), 1.82 (s, 6H).

Preparation of solutions

Stock solution of **ANT** (1 mM) were prepared in analytical grade DMSO. Other analytes including Cys, Hcy, GSH, H_2O_2, Vc, NaClO, t-BuOOH, Gly (glycine), Glu (glutamic acid), His (histidine), Pro (proline), and Lys (lysine) were dissolved in deionized water to afford 10 mM aqueous solution.

Measurements of absorption and fluorescence spectra

The test solution was prepared by adding 30 μL of stock solution of probes and an appropriate volume of each analytical solution in a 3 mL volumetric flask. The hybrid solutions were diluted to the corresponding concentration with the mixed solution of HEPES buffer (20 mM, pH 7.4)/ethanol (v/v, 1/1). The final concentration of probe was 10 μM. All the measurements were conducted after incubation with analytes for 120 min.

Cytotoxicity evaluation

The cell viability of **ANT** on MCF-7 cells was examined by MTT assay. Briefly, cells were seeded in 96-well microplates at a density of 5×10^{3} cells/well in 100 μL of complete DMEM media and incubated at 37 ºC in an atmosphere containing 5% CO_2 and 95% air. After the cells reached about 80% confluence, the cells were incubated with 0, 2.5, 5, 10, 20 and 50 μM of **ANT** for 24 h. After that, 20 mL of MTT solution (5 mg mL^{-1}) in PBS was added to each well and further cultured for another 4 h at 37 ºC. Then the DMEM solution was removed and 150 mL of DMSO was added to dissolve the formed purple crystals derived from MTT. The plates were then analyzed with a microplate reader (Tecan M200 PRO, Austria) at the absorbance wavelength of 570 nm.

Cell culture and cell imaging

MCF-7 cells were obtained from Xiangya Central Experiment Laboratory (Hunan Province, China). MCF-7 cells were maintained under the standard culture conditions (atmosphere of 5% CO_2 and 95% air at 37°C) in RPMI 1640 medium, supplemented with 10% FBS (fetal calf serum) and 100 IU/mL penicillin−streptomycin.

Before confocal microscopy imaging of cells with probes, MCF-7 cells in the exponential phase were plated on 35 mm glass-bottom culture dishes for 2 days to reach around 80% confluency, respectively. Cell culture was maintained at 37 ºC under an atmosphere of 5% CO_2

and 95% air for desired time. Culture medium was changed every two days to keep the exponential growth of the cells. On the day of treatment, the cells were incubated with the appropriate concentrations of probe for different time at 37 ºC and washed with 1 mL of PBS for three times at room temperature, then they were added to 1 mL of PBS culture medium and observed under confocal microscopy (Zeiss LSM 880) with a 63× oil-immersion objective.

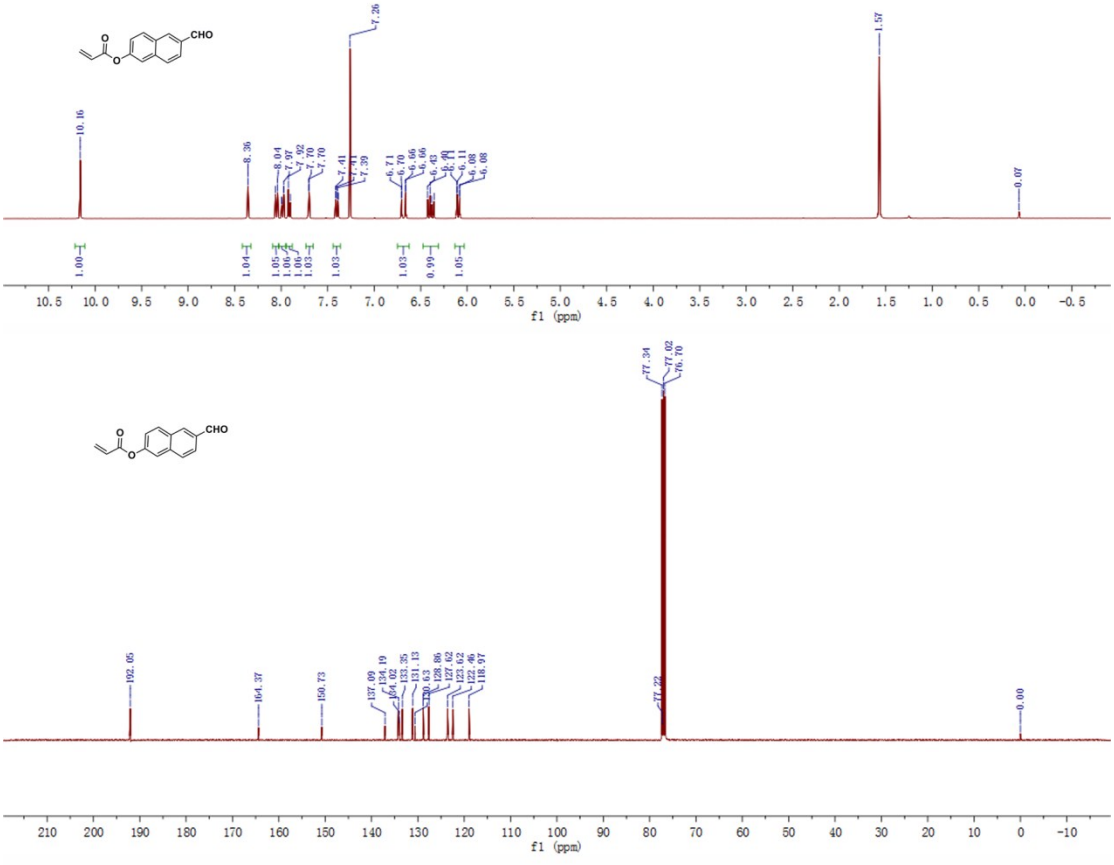

Figure S1 The ^{1}H NMR and ^{13}C NMR spectra of **AN-CHO**.

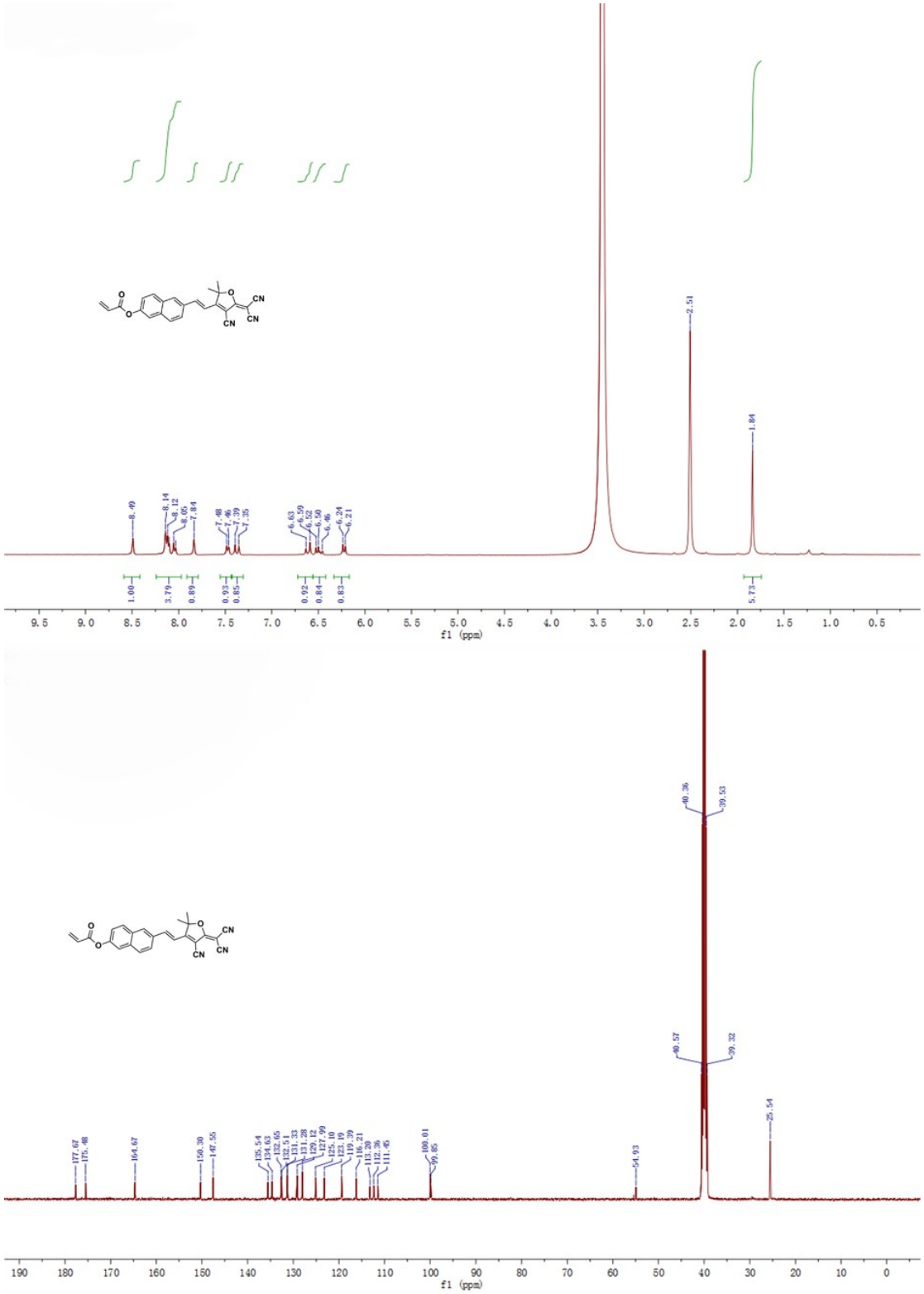

Figure S2 The ^{1}H NMR and ^{13}C NMR spectra of **ANT**.

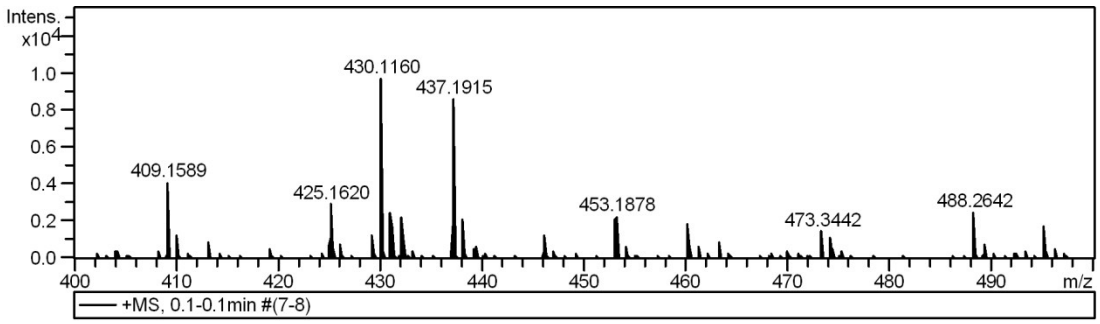

Figure S3 The HRMS of **ANT**. [**ANT** + Na]$^+$ calculated: 430.1168; found: 430.1160.

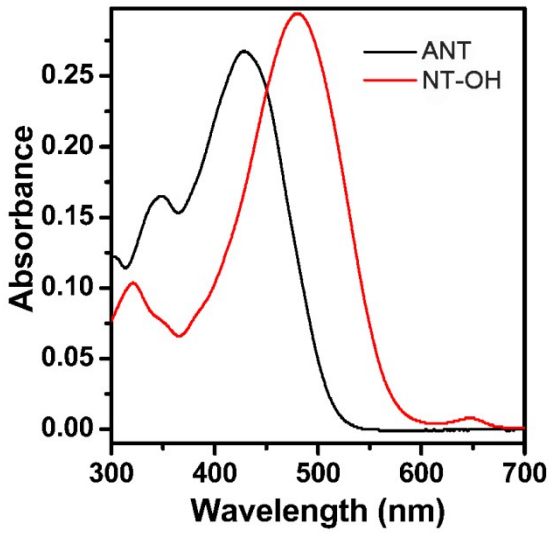

Figure S4 The UV-vis spectra of **ANT** and **NT-OH** in ethanol/HEPES buffer (1/1, v/v, pH 7.4).

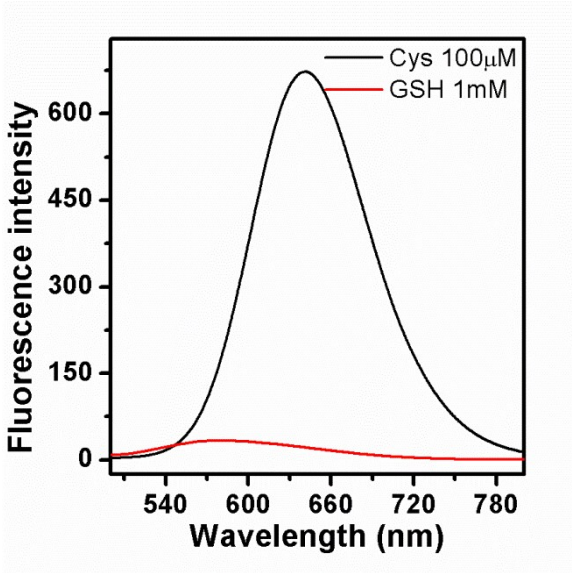

Figure S5 The fluorescence spectra of **ANT** (10 μM) with addition of Cys (100 μM) and GSH (1 mM) in ethanol/HEPES buffer (1/1, v/v, pH 7.4).

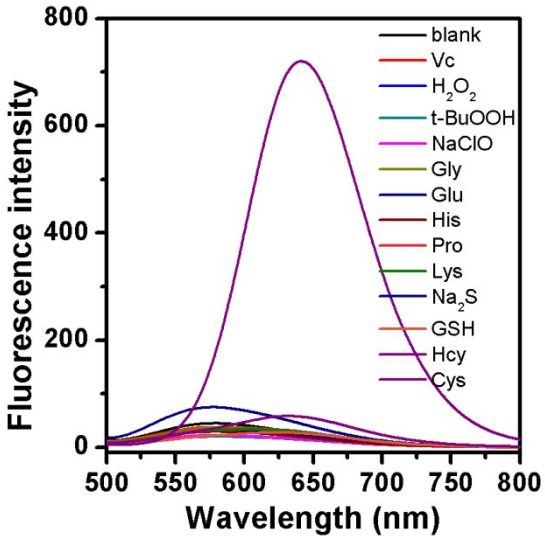

Figure S6 The fluorescence spectra of **ANT** (10 μM) with different bioanalytes (100 μM) in ethanol/HEPES buffer (1/1, v/v, pH 7.4).

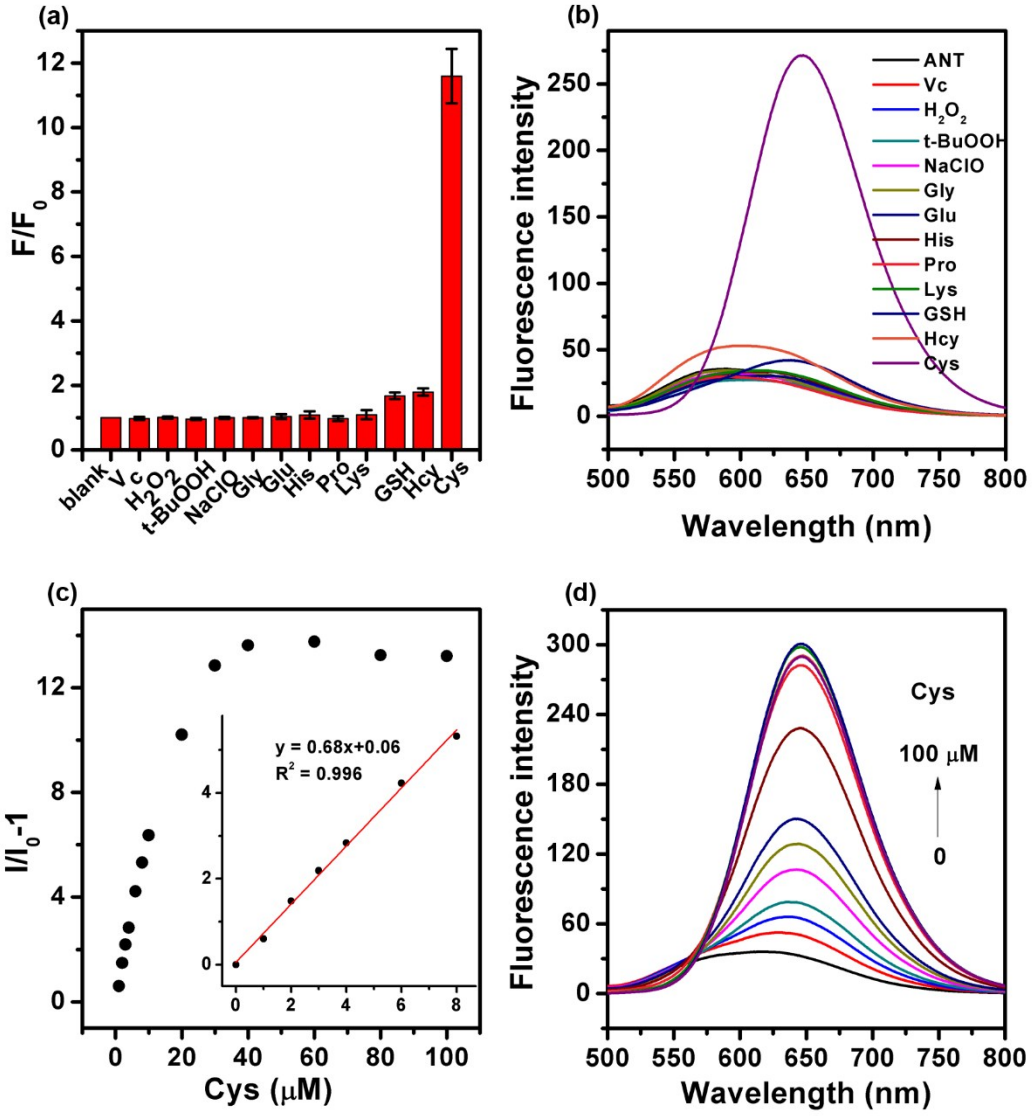

Figure S7 (a) The fluorescence response and (b) the fluorescence intensity changes of **ANT** (10 μM) to different analytes (100 μM), (c) the changing curve of fluorescence intensity during the titration of Cys and (d) the fluorescence spectra of **ANT** (10 μM) with titration of various concentration of Cys. All in ethanol/HEPES buffer (pH 7.4, v/v = 1/2), λ_{ex} = 480 nm, λ_{em} = 645 nm.

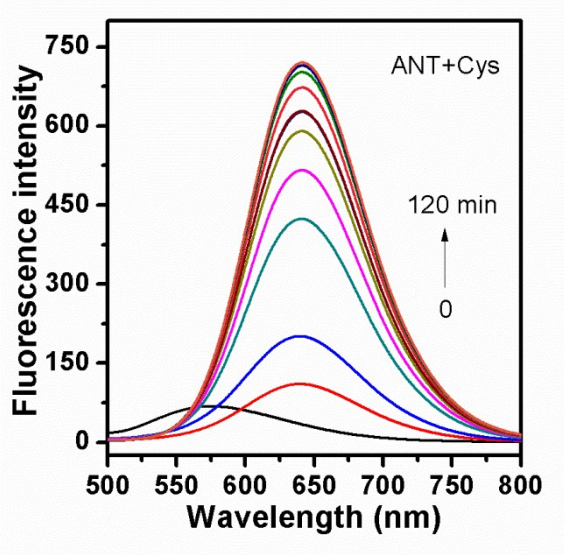

Figure S8 The time-dependent fluorescence spectra of **ANT** (10 μM) upon addition of Cys (100 μM) in ethanol/HEPES buffer (1/1, v/v, pH 7.4).

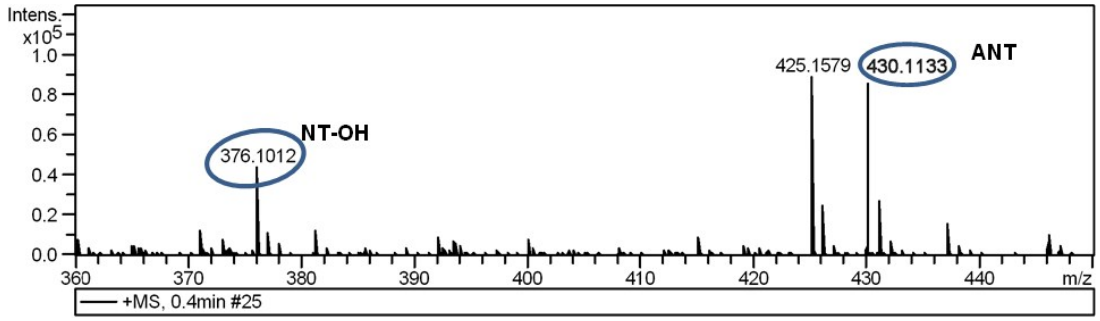

Figure S9 The HRMS of **ANT** with addition of Cys in ethanol/HEPES buffer (1/4, pH 7.4). [**NT-OH** + Na]$^+$ calculated: 376.1062; found: 376.1012. [**ANT** + Na]$^+$ calculated: 430.1168; found: 430.1133.

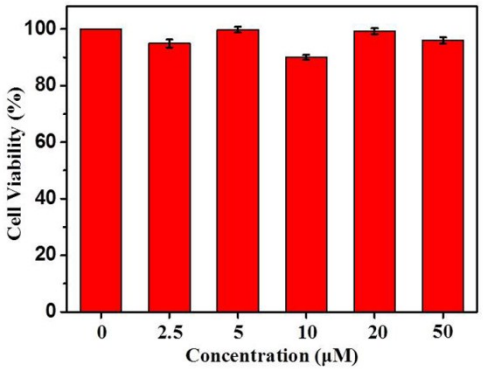

Figure S10 The cell viability incubated with different concentration of **ANT** for 24 h.

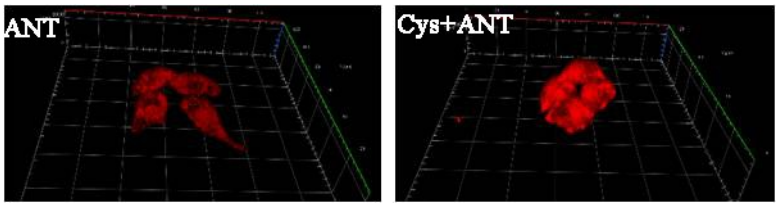

Figure S11 The 3D confocal laser microscope image of MCF-7 cells incubated with **ANT** (10 μM) for 40 min, and pretreated with Cys (100 μM) for 60 min then incubated with **ANT** (10 μM) for 40 min. Excitation at 488 nm. Emission collected at 600-700 nm.