Supplementary information

One Step Synthesis of PY-NBD to Distinguish Cys/Hcy and GSH in Aqueous Solution and Living Cells by Dual Channels

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CONTENTS

1. Experimental reagents and instruments

All other chemicals were obtained from commercial suppliers and used without further purification. Silica gel (200-300 mesh, Qingdao Haiyang Chemical Co.) was used for column chromatography. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Advance at 400MHz or at 100 MHz, δ values are in parts per million relatives to TMS in DMSO-d_6. Mass spectra (MS) were measured with Bruker Apex IV FTMS using electrospray ionization (ESI). Absorption spectra were recorded on a Purkinje TU-1901 spectrophotometer. Fluorescence measurements were taken on a Hitachi F-7000 fluorescence spectrometer with a 10mm quartz cuvette. pH measurements were carried out with a pH acidometer (Mettler Toledo FE-30). Fluorescence imaging was observed under an Olympus IX81 confocal fluorescence microscope.

2. Characterization data

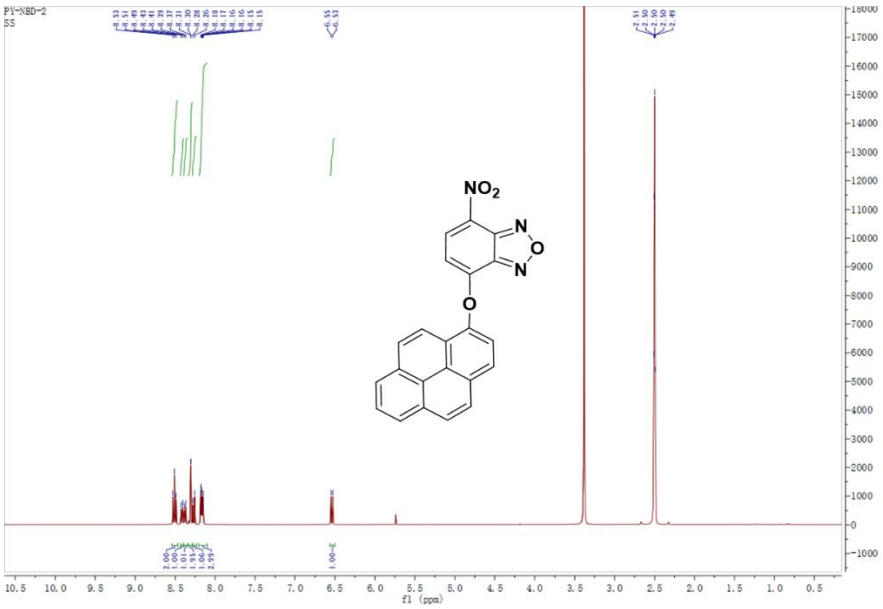

Fig. S1. ^{1}H NMR (400 MHz, DMSO-d_6) spectra of probe **PY-NBD**.

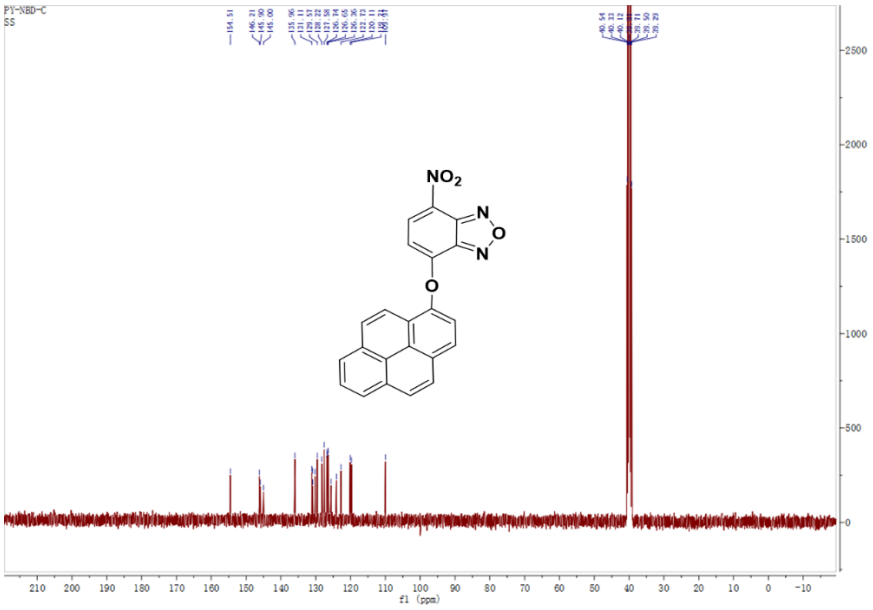

Fig. S2. ^{13}C NMR (101 MHz, DMSO-d_6) spectra of probe **PY-NBD**.

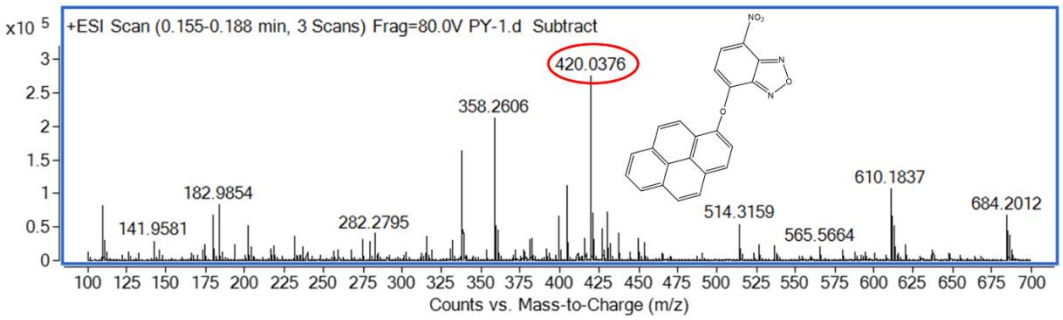

Fig. S3. ESI-MS of probe **PY-NBD**.

3. Time-dependent fluorescence changes of PY-NBD

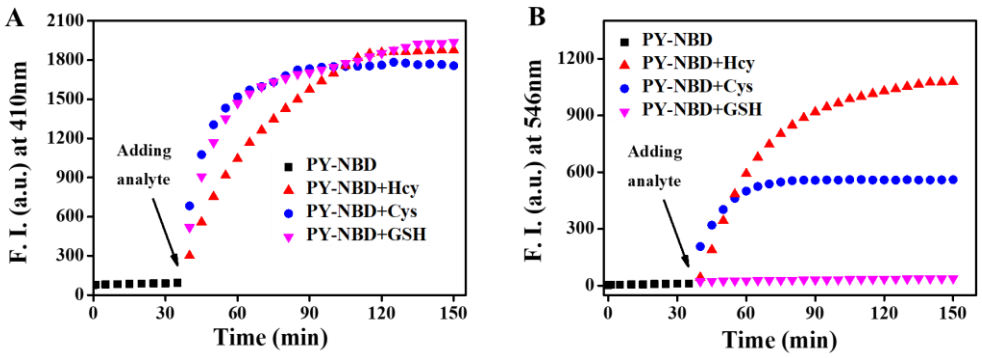

Fig. S4. The stability of **PY-NBD** (5 μM) in PBS (10 mM, pH 7.4, 50% CH_3CN, V/V) system and the fluorescence spectrum of response time to 50 μM Hcy/Cys/GSH. (A) At 410nm, λex=345 nm. Slit: 2.5 nm/2.5 nm; (B) At 546nm, λex=473 nm. Slit: 5.0 nm/5.0 nm.

4. The capabilities of PY-NBD for detecting Hcy/Cys and GSH at different pH

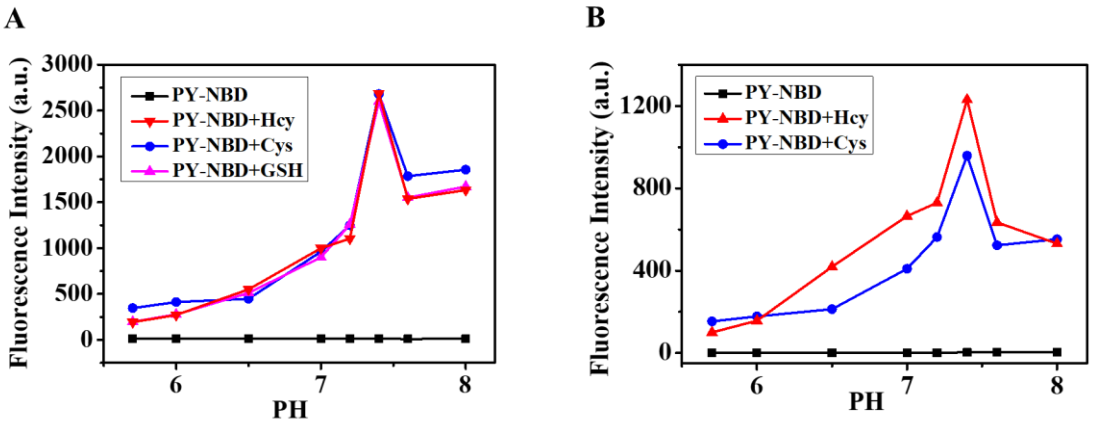

Fig. S5. Fluorescence intensity change graph of **PY-NBD** (5 μM) and Hcy/Cys/GSH (50 μM) at different pH. (A) At 410nm, λex=345 nm. Slit: 2.5 nm/2.5 nm; (B) At 546nm, λex=473 nm. Slit: 5.0 nm/5.0 nm.

5. ESI-MS of PY-NBD after upon addition Cys, Hcy, and GSH

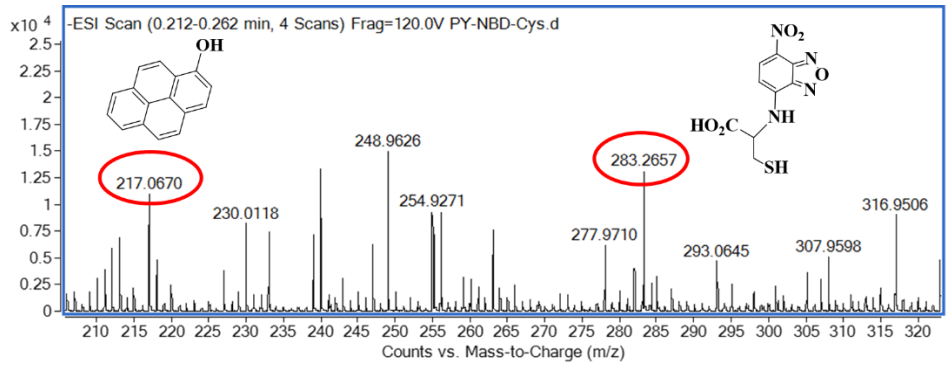

Fig. S6. ESI-MS of **PY-NBD** after upon addition Cys.

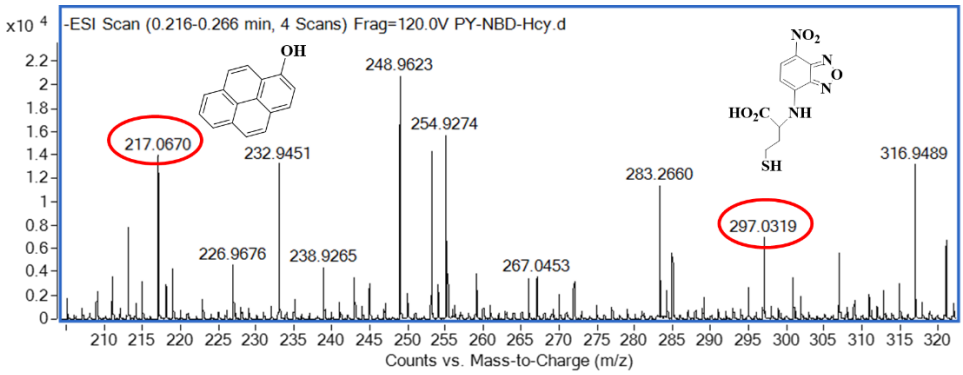

Fig. S7. ESI-MS of **PY-NBD** after upon addition Hcy

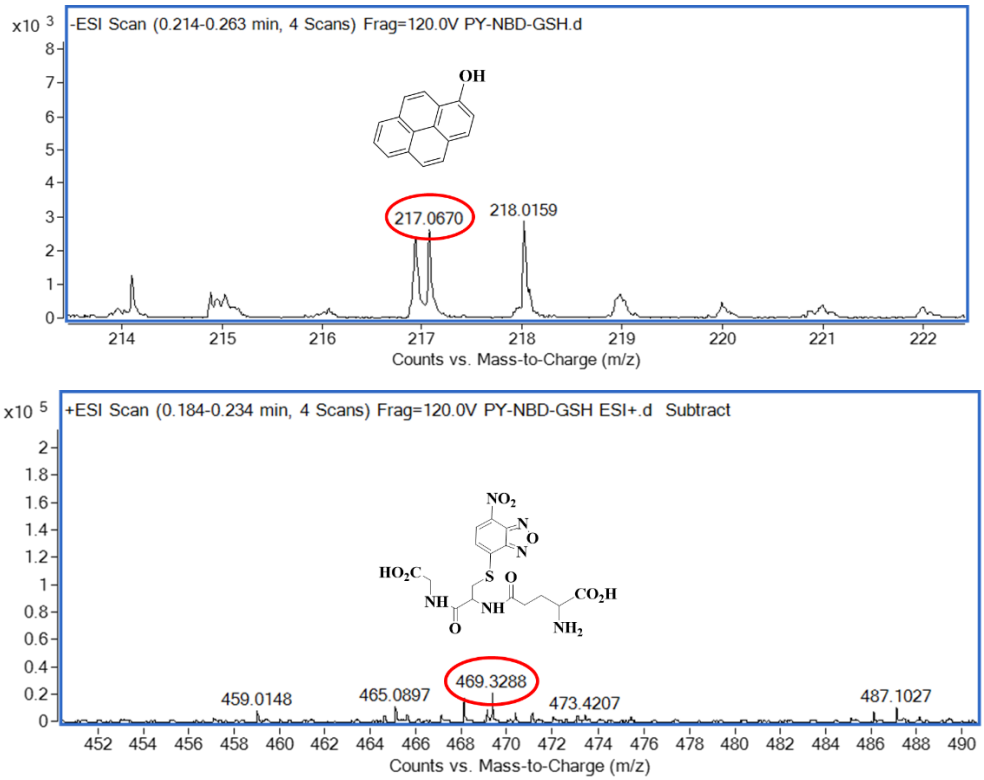

Fig. S8. ESI-MS of **PY-NBD** after upon addition GSH

6. Cytotoxicity assay

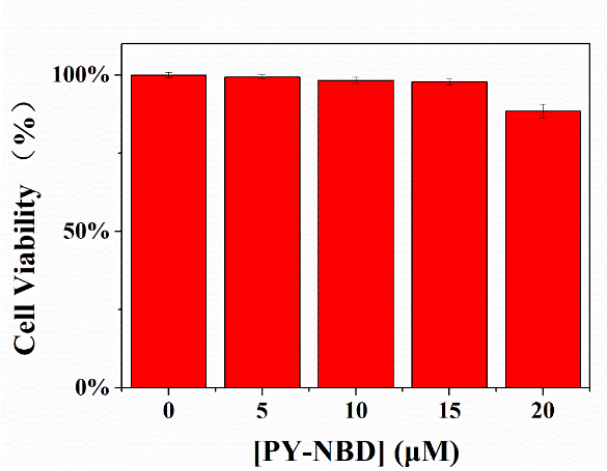

Fig. S9. Cell viability of HeLa cells treated with different concentrations of **PY-NBD**