Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

Dual-Targeted Activatable Photosensitizer with Aggregation-Induced Emission (AIE) Characteristics for Image-Guided Photodynamic Cancer Cell Ablation

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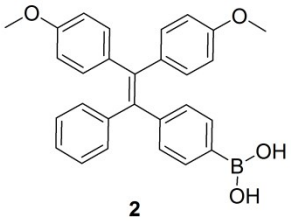
General Information. All chemicals were purchased from Alfa Aesar or Sigma-Aldrich unless otherwise specified and were used as received. Alkyne-functionalized cyclic RGD (Arg-Gly-Asp) was ordered from GL Biochem Ltd (Shanghai). Propidium iodide (PI), trypsin-EDTA, fetal bovine serum (FBS) and Hoechst 33342 were purchased from Life Technologies.

Characterization. NMR spectra were performed on a Bruker ARX 400 NMR spectrometer. Particle size was studied by laser light scattering (LLS, Brookhaven Instruments Co., United States). High-resolution TEM images were obtained using a JEOL JEM-2010 transmission electron microscope operating at 200 kV accelerating voltage. UV-vis absorption spectra were taken on a Shimadzu Model UV-1700 spectrometer. Photoluminescence (PL) spectra were studied on a Perkin-Elmer LS 55 spectrofluorometer.

Synthesis of compound 2.



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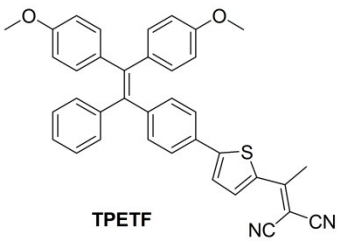
To a dry THF solution (40 mL) of compound **1** (4.0 g, 8.4 mmol) at -78 ^{o}C was added *n*-butyllithium (8.0 mL, 12.5 mmol) in hexane. The mixture was stirred at -78 ^{o}C for 2 h, and then trimethyl borate (1.89 mL, 16.4 mmol) was added. The reaction mixture was allowed to warm to room temperature slowly. Then HCl (3 M, 20 mL) solution was added and the mixture was stirred for another 3 h. The mixture was extracted with dichloromethane and organic layer was washed by brine, dried over anhydrous sodium sulfate and evaporated to dryness. The residue was subjected to flash column chromatography with dichloromethane and acetone (10:1 by volume) as eluention buffer to afford compound **2** as white power(1.1 g), which was used directly for the next step without further purification.

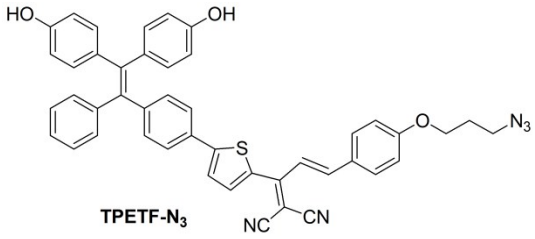
Synthesis of TPETF.



TPETF

4-aceyle-5-bromothiophene (246.0 mg, 1.2 mmol) and compound **2** (500.0 mg, 0.96 mmol) were dissolved in THF (8.0 mL), then 2 M aqueous K_2CO_3 solution (1.0 mL) and Aliquat 336 were added. The mixture was stirred for 40 min under an argon atmosphere at room temperature. Then the $Pd(PPh_3)_4$ catalyst was added and the reaction mixture was stirred at 75 ^oC for 16 h. After cooling to room temperature, the mixture was extracted with ethyl acetate (50 mL × 3). The combined organic phase was concentrated and added a solution of malononitrile (165.0 mg, 2.50 mmol) and ammonium acetate (192.2 mg, 2.50 mmol) in the mixture of dichloromethane (10 mL) and methanol (2 mL). Then silica gel (1.2 g) was added to the above mixture. Then the solvent was removed under reduced pressure. The resulting mixture was heated at 100 °C for 4 h. The mixture was cooled down and subsequently separated with chromatography (hexane/ethyl acetate = 20/1) to give the desired product as red solid (390.0 mg, 72.3%). ^1H NMR (400 MHz, $CDCl_3$): δ 7.92 (d, J = 4.2 Hz, 1H), 7.36 – 7.31 (m, 2H), 7.28 (d, J = 4.3 Hz, 1H), 7.07 – 6.94 (m, 7H), 6.91 – 6.84 (m, 4H), 6.61 – 6.55 (m, 4H), 3.69 – 3.66 (m, 6H), 2.60 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 23.153, 29.701, 55.108, 55.137, 113.063, 113.276, 113.990, 114.486, 124.582, 125.708, 126.428, 127.902, 129.745, 131.409, 132.294, 132.600, 132.637, 135.196, 135.952, 136.393, 138.069, 141.514, 143.767, 146.460, 154.082, 158.326, 158.465, 161.548. ESI-MS, m/z: $[M+H]^+$ calcd 564.7, found 565.2.

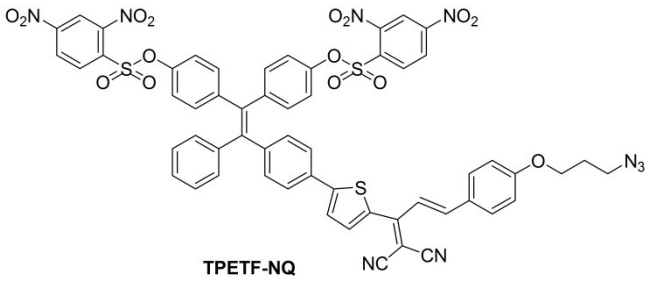
Synthesis of TPETF-N_3.



TPETF-N$_3$

TPETF (200.0 mg, 0.360 mmol), 4-(3-azidopropoxy)benzaldehyde (96.0 mg, 0.468 mmol) and piperidine (3.4 mg, 0.04 mmol) were stirred in 2-propanol(1.0 mL) at 70 ℃ for 24 h. The reaction was cooled to room temperature, and the solution was filtered to obtain a red solid. Recrystallization from hot ethanol afforded the pure **TPETF-N$_3$** as red solid (75.8 mg, 28.1%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.64 (d, *J* = 8.1 Hz, 2H), 7.44 – 7.38 (m, 3H), 7.34 (dd, *J* = 8.0, 2.4 Hz, 4H), 7.06 (d, *J* = 7.7 Hz, 4H), 6.95 – 6.76 (m, 8H), 6.58 (t, *J* = 8.7 Hz, 4H), 4.02 (t, *J* = 5.9 Hz, 2H), 3.67 (s, 6H), 3.45 (t, *J* = 6.5 Hz,2H), 2.03 – 1.93 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ14.198, 21.040, 28.661, 29.360, 31.438, 48.087, 50.888, 53.411, 55.102, 60.393, 64.841, 113.049, 113.248, 114.062, 115.223, 122.330, 124.016, 125.443, 126.362, 127.449, 130.212, 130.719, 131.413, 132.218, 132.264, 132.596, 132.632, 134.327, 136.042, 138.206, 141.199, 143.856, 145.711, 147.919, 151.913, 158.269, 158.388, 161.882. EI-MS, m/z: $[M]^+$ calcd 751.7, found 751.5.

Synthesis of TPETF-NQ.



TPETF-NQ

To the solution of **TPETF-N$_3$** (30.0 mg, 0.04 mmol) in dichloromethane (3 mL) was added boron tribromide (0.02 mL, 0.2 mmol) at ice-water bath. Then the reaction was stirred at room temperature for 2 h. The reaction was quenched by addition of water (5 mL) and dichloromethane (10 mL) at ice-water bath. Then the organic layer was taken, washed with

water (30 mL × 3), brine (30 mL) and dried over $MgSO_4$. The mixture was filtered and the resulting filtrate was concentrated to give a red residue. This residue was then dissolved in in dry dichloromethane (20 mL), followed by addition of triethylamine and stirred in an ice−water bath for 15 min. After 2,4-dinitrobenzenesulfonyl chloride (32.0 mg, 0.12 mmol) was added, the solution was further stirred for 3 h at room temperature. The solvent was then evaporated, and the crude product was purified by silica gel column, using hexane/ethyl acetate as the eluent. **TPETF-NQ** was obtained as a red solid (20.4 mg, 43 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.50 (s, 1H), 8.34 (s, 2H), 8.24 (d, J = 8.4 Hz, 1H), 7.54 (d, J = 4.0 Hz, 1H), 7.41 (d, J = 8.6 Hz, 2H), 7.37 – 7.22 (m, 5H), 7.01 (dt, J_1 = 13.1 Hz, J_2 = 7.6 Hz, 7H), 6.94 – 6.85 (m, 4H), 6.77 (d, J = 8.7 Hz, 2H), 6.59 (dd, J_1 = 14.5 Hz, J_2 = 8.9 Hz, 4H), 3.98 (s, 2H), 3.68 (d, J = 6.4 Hz, 4H), 3.40 (s, 2H). ESI-MS, m/z: $[M+H]^+$ calcd 1183.1, found 1183.3.

Synthesis of the probe TPETF-NQ-cRGD. TPETF-NQ (10.0 mg, 8.4 μmol) and alkyne-functionalized cRGD (9.5 mg, 16.8 μmol) were dissolved in mixture solvent of DMSO and H_2O (v/v = 10/1). Then $CuSO_4$ (2.7 mg, 16.8 μmol) and sodium ascorbate (3.3 mg, 16.8 μmol) were sequentially added and stirred at room temperature for 24 h. The final product was obtained after purification by preparative HPLC and freeze dried to yield the probe TPETF-NQ-cRGD as red powders in 45% yield (8.7 mg). HPLC (λ = 214 nm): purity 96%; Mass: *m*/*z* $[M+H]^+$ calc. 1754.755, found 1754.426.

ROS quantum yield measurements. The ROS generated from the PSs upon white light irradiation (400–800 nm) was studied by using 9,10-anthracenediyl-bis(methylene) dimalonic acid (ABDA) as an indicator. The absorbance decrease of ABDA at 400 nm was recorded for different duration of light irradiation to obtain the decay rate of the photosensitizing process. Using Rose Bengal (RB) as a reference, the ROS quantum yield of TPETF-N_3 (Φ_{PS}) was calculated according to the following formula:

$$\Phi_{PS} = \Phi_{RB}\frac{K_{PS} * A_{RB}}{K_{RB} * A_{PS}}$$

Where K_{PS} and K_{RB} are the decomposition rate constants of ABDA by TPETF-N_3 and RB. A_{PS} and A_{RB} represent the light absorbed by TPETF-N_3 and RB, which are determined by integration of the absorption bands in the wavelength range of 400–800 nm. Φ_{RB} is the ROS quantum yield of RB, which is 0.75 in water.

Cell culture. Normal cell lines of murine fibroblast NIH 3T3 cells and human embryonic kidney 293T cells, human breast cancer cell lines of MDA-MB-231 and MCF-7, human malignant glioma U87-MG cancer cells were provided by American Type Culture Collection (ATCC) and cultured in DMEM medium containing penicillin (100 U mL^{-1}), heat-inactivated FBS (Invitrogen, 10%) and streptomycin (100 μg mL^{-1}). The cells were maintained in a humidified incubator at 37 °C with 5% CO_2.

Confocal imaging. The cells were cultured in 8-well chambers and precultured overnight. Then the medium was replaced with fresh one and incubated with the probe (10 μM). In some experiments, the cells were pretreated with DMEM medium containning buthionine sulphoximine (BSO, 50 μM) or free cRGD (50 μM) before the probe incubation. The nuclei of the cells were living stained with Hoechst 33342 (E_x: 405 nm; E_m: 430-470 nm). The intracellular ROS generation was studied using 2′,7′-dichlorodihydrofluorescein diacetate (DCF-DA, E_x: 488 nm; E_m: 505-525 nm) as a cell permeable indicator. The cell apoptosis was studied by using FITC-tagged Annexin V (Life Technologies, E_x: 488 nm; E_m: 505-525 nm). For the AIEgen detection, the excitation wavelength was 453 nm and the emission was collected above 560 nm. The cells were imaged by confocal laser scanning microscope (CLSM, Zeiss LSM 410, Jena, Germany). The images were analyzed by Image J 1.43 × program (http://rsbweb.nih.gov/ij/).

Flow cytometry study. The cells in 24-well plate (Costar, IL, USA) were incubated with the probe (10 μM) for different time durations or different treatments. Then the cells were digested with trypsin and washed with PBS before flow cytometric studies using Cyan-LX (DakoCytomation). The mean fluorescence was determined by counting 10,000 events.

Cytotoxicity studies. The metabolic activity of the cells was assessed by 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays. After incubation of the cells in DMEM medium overnight, the medium was removed, washed with PBS and the cells were incubated with the probe for 6 h, then the medium was replaced with the fresh one and exposed with white light irradiation for different time durations. The cells were further incubated for 24 h and washed with 1× PBS before the addition of 100 μL of MTT solution (0.5 mg mL^{-1}) into each well. After 3 h incubation, the MTT solution was removed and DMSO (100 μL) was added into each well. The absorbance of MTT at 570 nm was studied by microplate reader (Genios Tecan). The cells without any treatment were used as control.

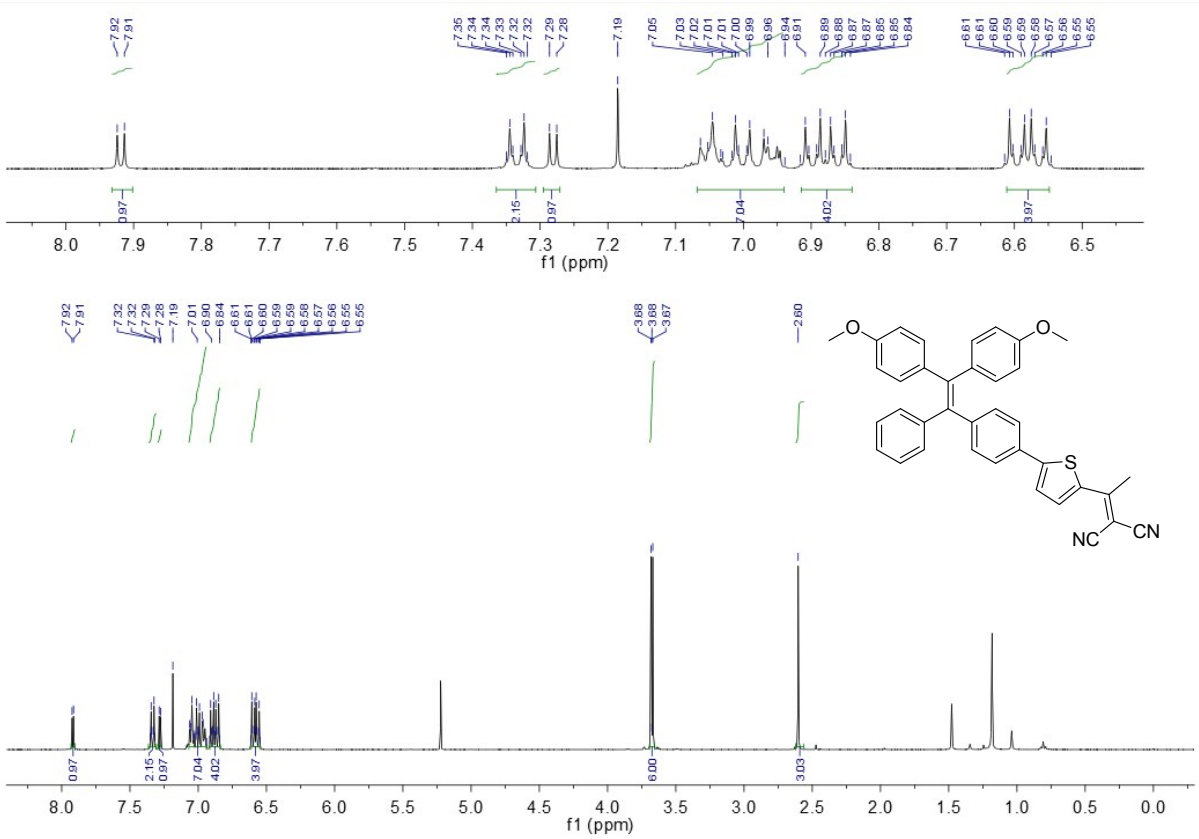


Figure S1. ^{1}H NMR spectrum of **TPETF** in $CDCl_3$.

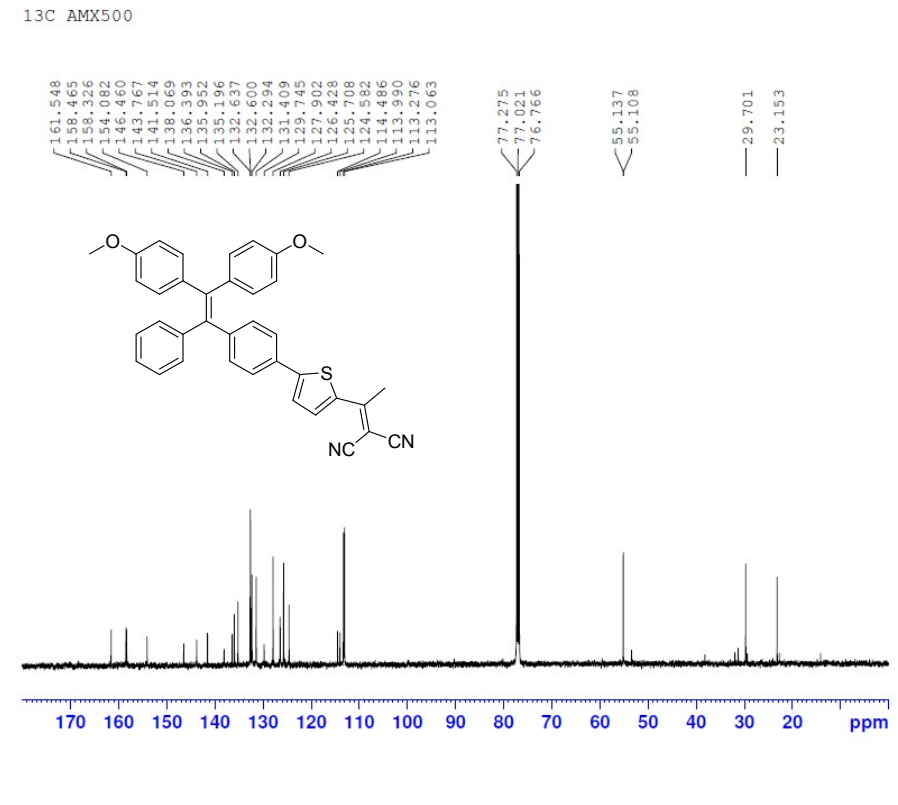



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Figure S2. ^{13}C NMR spectrum of **TPETF** in $CDCl_3$.

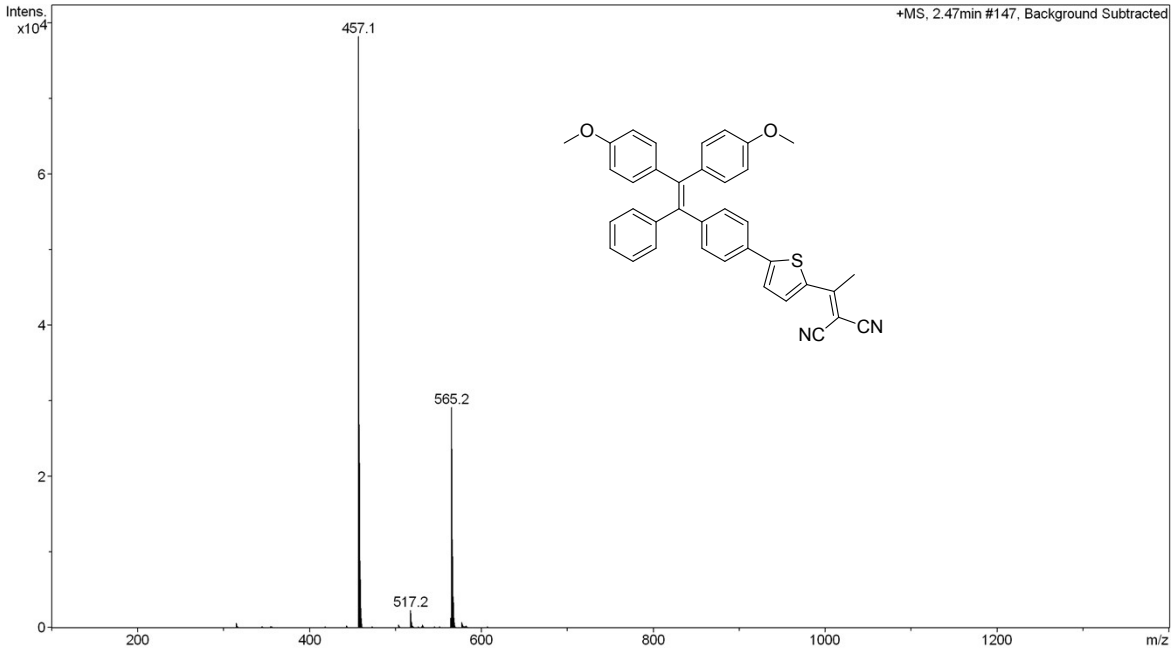

Figure S3. Mass spectrum of **TPETF**.

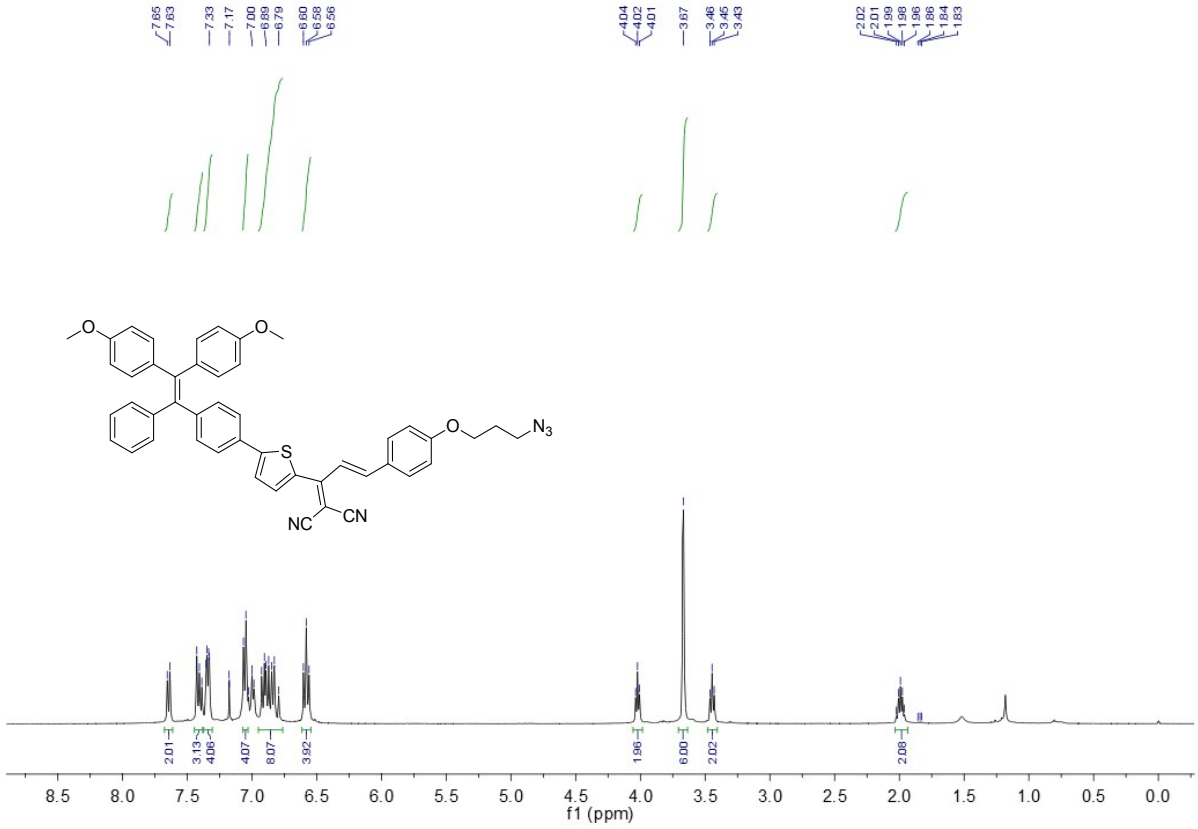

Figure S4. ^{1}H NMR spectrum of **TPETF-N_3** in $CDCl_3$.

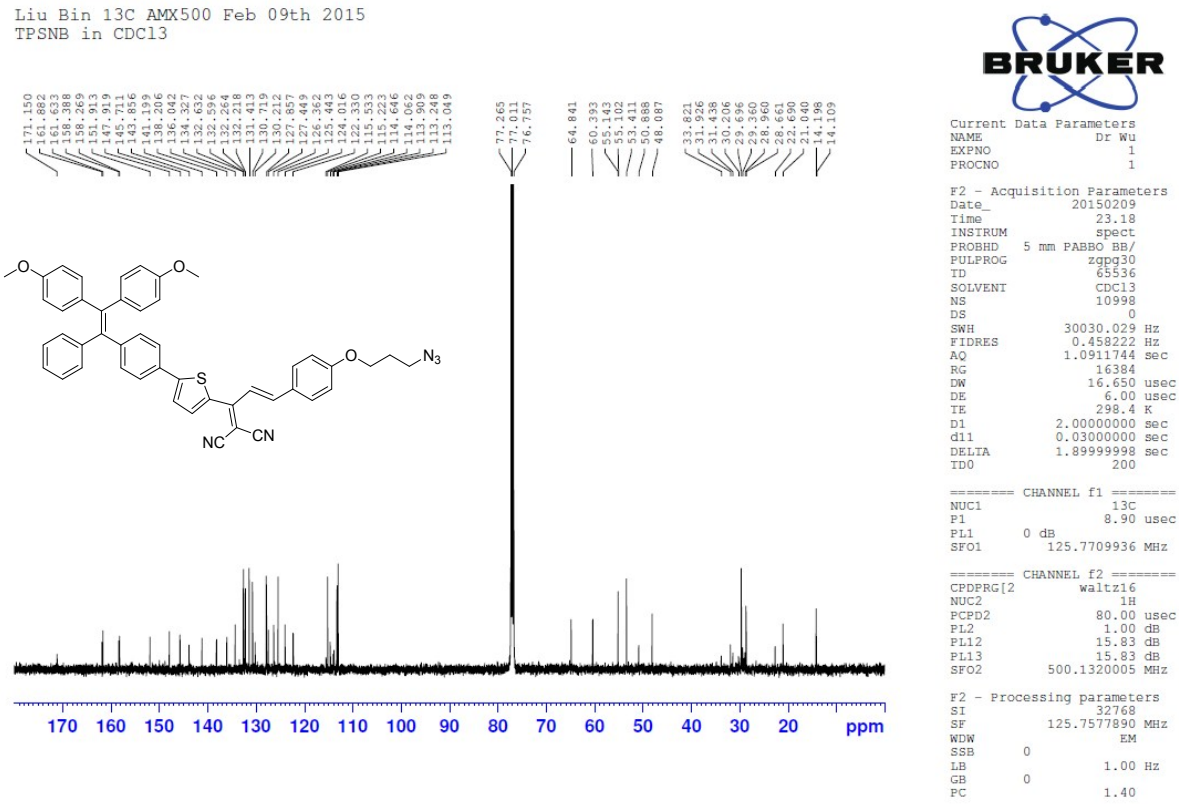

Figure S5. ^{13}C NMR spectrum of **TPETF-N_3** in $CDCl_3$.

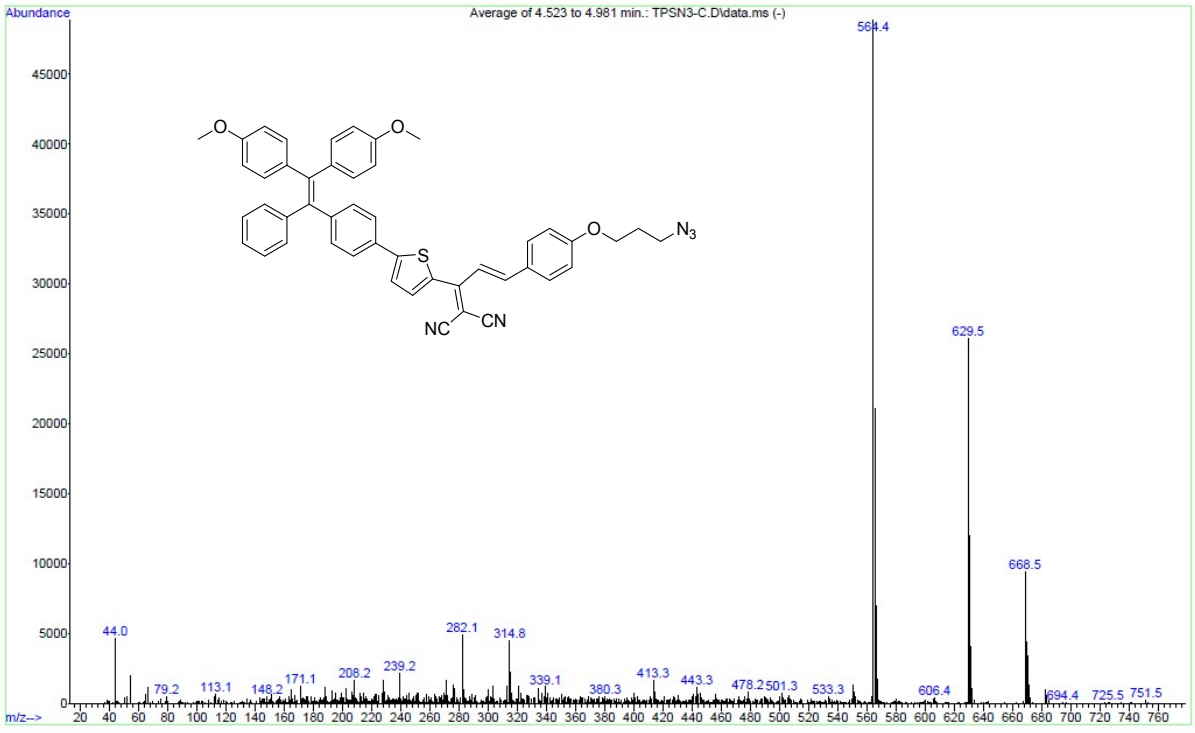

Figure S6. Mass spectrum of **TPETF-N_3**.

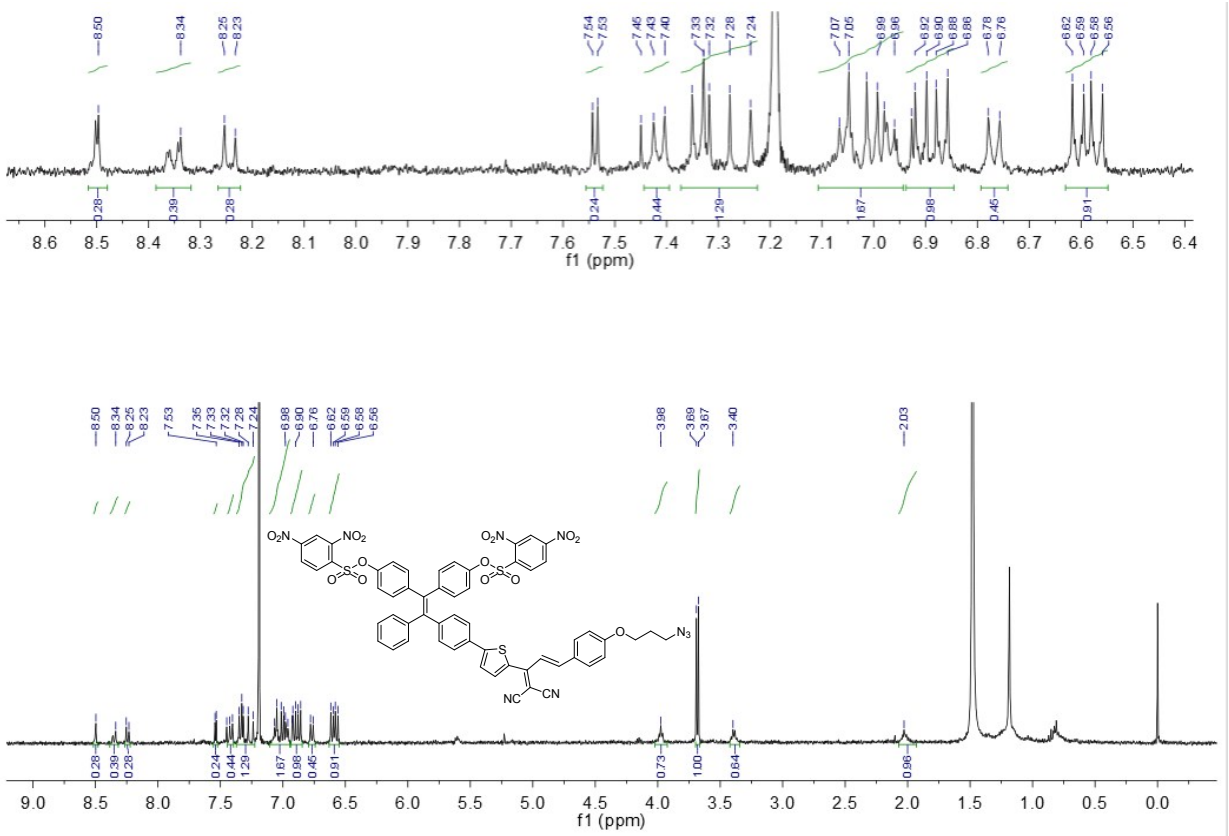

Figure S7. ^{1}H NMR spectrum of **TPETF-NQ** in $CDCl_3$.

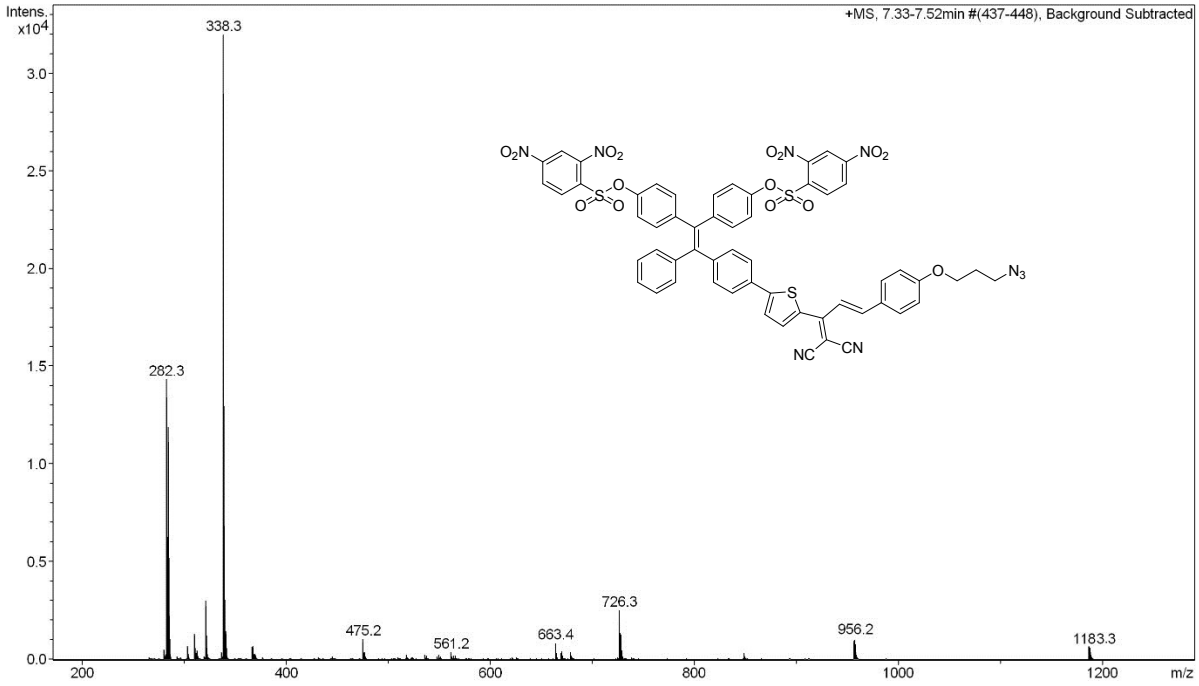

Figure S8. Mass spectrum of **TPETF-NQ**.

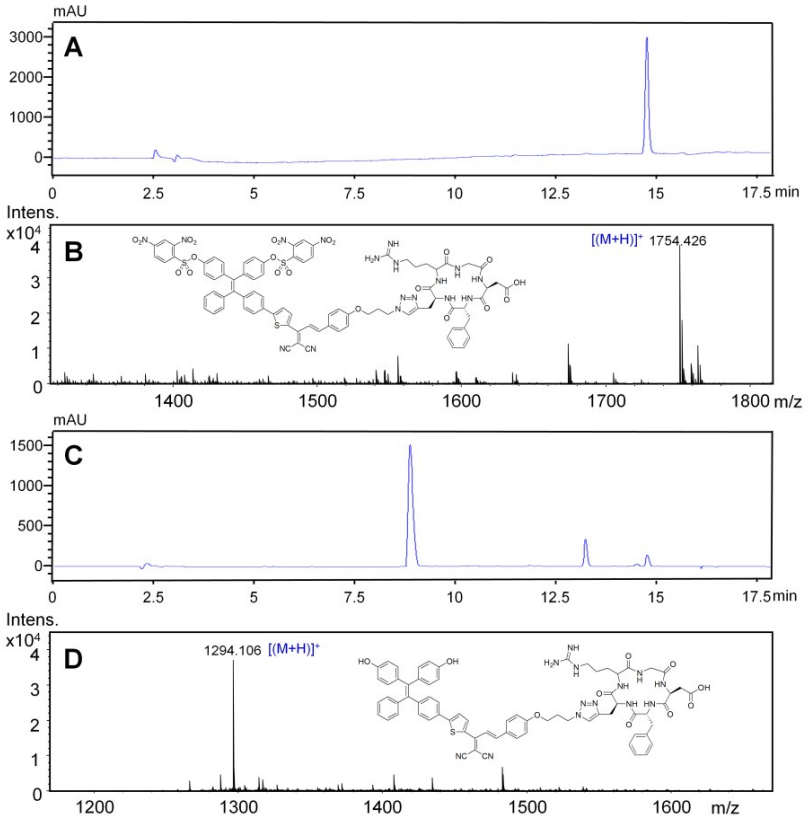

Figure S9. HPLC and mass spectra of the probe before (A, B) and after (C, D) treatment with GSH (0.1 mM) for 24 h.