Supporting Information:

Light-Responsive AIE Nanoparticles with Cytosolic Drug Release to Overcome Drug Resistance in Cancer Cells

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Supplementary Methods

Materials and chemicals. Methoxypolyethylene glycol amine (PEG-NH_2, average molecule weight of 2,000) was purchased from Nanocs Inc. (United States). Doxorubicin hydrochloride (DOX•HCl) was purchased from Alfa Aesar. Nile Red, Triton X-100, Tris buffer, 9,10-anthracenediyl-bis(methylene)dimalonic acid (ABDA), ascorbic acid (Asc), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 2′,7′-dichlorofluorescin diacetate (DCF-DA), acridine orange (AO) and other chemicals were all purchased from Sigma-Aldrich and used as received without further purification.

Dulbecco's Modified Essential Medium (DMEM) was purchased from Invitrogen. Milli-Q water was supplied by Milli-Q Plus System (Millipore Corporation, Breford, United States). 10× phosphate-buffer saline (PBS) buffer with pH = 7.4 (ultrapure grade) is a commercial product of 1st BASE (Singapore). Milli-Q water was used to prepare the 1× PBS buffer solutions from the 10× PBS stock buffer. 1× PBS contains NaCl (137 mM), KCl (2.7 mM), Na_2HPO_4 (10 mM), and KH_2PO_4 (1.8 mM). Fetal bovine serum (FBS), Hoechst 33342, LysoTracker® Green, MitoTracker® Green and trypsin-EDTA solution were purchased from Life Technologies.

Characterization. NMR spectra were measured on a Bruker ARX 400 NMR spectrometer. Mass spectra were recorded on Agilent 5975 DIP-MS for electron impact (EI) and the AmaZon X LC-MS for electrospray ionization (ESI). Gel permeation chromatography (GPC) analysis was conducted with

a Waters 2690 liquid chromatography system equipped with Waters 996 photodiode detector and Phenogel GPC columns, using polystyrenes as the standard and DMF as the eluent at a flow rate of 1.0 mL min^{-1} at 35 °C. Particle size and size distribution were determined by laser light scattering (LLS) with a particle size analyzer (90 Plus, Brookhaven Instruments Co., USA) at a fixed angle of 90° and room temperature. TEM images were obtained from a JEOL JEM-2010 transmission electron microscope with an accelerating voltage of 200 kV. Visible light (400-700 nm) generated from Cold Light L-150A at a power density of 0.10 W cm^{-2} was used as the irradiation source. The intensities of incident beams were checked by a power and energy meter. 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 (developed by NIH, http://rsbweb.nih.gov/ij/). UV-vis absorption spectra were taken on a Shimadzu Model UV-1700 spectrometer. Photoluminescence (PL) spectra were measured on a Perkin-Elmer LS 55 spectrofluorometer. All UV and PL spectra were collected at 24 ± 1 °C.

Synthesis of compound 1. To the solution of 4-bromobenzophenone (5.30 g, 20.3 mmol) and 4,4'-dimethoxybenzophenone (3.80 g, 15.7 mmol) in dry THF (80 mL) was added zinc powder (5.90 g, 90.8 mmol). Then the suspension was cooled down to −78 ℃. Titanium tetrachloride (5.0 mL) was added to the above mixture dropwise. After addition, the mixture was slowly warmed up to room temperature, which was followed by refluxing for 8 hours. The mixture was subsequently cooled down in an ice-water bath and saturated sodium bicarbonate aqueous solution (50 ml) was added slowly. The mixture was extracted with ethyl acetate (100 mL × 3). The combined organic phase was washed with brine (100 mL × 2), and dried over $MgSO_4$. Then the mixture was filtered and the filtrate was concentrated under reduced pressure. The desired residue was purified with chromatography (hexane/ethyl acetate = 100/1-30/1) to give the desired product as white solid (3.10 g, 41.8%).

Synthesis of TPETP: 5-Acetyl-2-thienylboronic acid (204 mg, 1.20 mmol) and compound **1** (500 mg, 0.96 mmol) were dissolved in THF (8.0 mL), and 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 °C 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 with a solution of malononitrile (165 mg, 2.50 mmol) and ammonium acetate (192 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 hours. The mixture was cooled down and subsequently separated with chromatography (hexane/ethyl acetate = 20/1) to give the desired product as red solid (390 mg, 72.3%). ^{1}H 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. EI-MS, m/z: $[M-1]^+$ calcd 564.1, found 565.2.

Synthesis of TPETP-NH_2(Boc): TPETP (200 mg, 0.360 mmol), 3-(boc-amino)propylbenzaldehyde (131 mg, 0.468 mmol, 1.2 eq.), piperidine (3.4 mg, 0.04 mmol, 0.1 eq.) 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 TPETP-NH_2(Boc) as a red solid (67.9 mg, 26.2%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (s, 1H), 8.35 (d, *J* = 8.4 Hz, 1H), 8.24 (d, *J* = 8.4 Hz, 1H), 7.54 (d, *J* = 4.0 Hz, 1H), 7.43 – 7.24 (m, 6H), 7.07 – 6.85 (m, 9H), 6.77 (d, *J* = 8.9 Hz, 2H), 6.59 (dd, *J* = 14.5, 8.9 Hz, 3H), 5.61 (d, *J* = 7.3 Hz, 1H), 3.97 (t, *J* = 6.0 Hz, 2H), 3.68 (d, *J* = 6.4 Hz, 6H), 3.43 – 3.36 (m, 2H), 2.05 – 1.99 (m, 2H), 1.19 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.150, 161.883, 158.355, 151.913, 147.919, 145.711, 143.856, 141.199, 138.206, 136.042, 134.327, 132.621, 132.245, 131.413, 130.719, 130.212, 127.857, 127.449, 126.362, 125.433, 124.016, 122.330, 115.533, 115.223, 114.646, 144.062, 113.909, 113.199, 64.841, 60.393, 55.124 53.411, 50.886, 48.087, 33.821, 31.645, 30.206, 29.546, 28.960, 28.661, 22.690, 21.040, 14.109. $[M-Boc]^+$ calcd 725.5, found 725.5.

Synthesis of TPETP-TK: To the TPETP-NH_2(Boc) (29 mg, 0.040 mmol) in dichloromethane (3 ml) was added TFA (20%). The reaction was stirred at room temperature for 4 hours. Then the solvent was removed under reduced pressure. The resulting solid was washed by ethyl acetate and recrystallization from hot ethanol to afford TPETP-NH_2 as a red powder. Subsequently, the red powder, HBTU (1.2 eq), DIPEA (2.0 eq) and dialkyl bis-(carboxyethylmercapto)-methanes[1] (50 mg, 0.20 mmol) were stirred in dry DCM for 8 h. The crude product was separated with chromatography to give the desired product as red solid (12 mg, 31.5%) ESI-MS, m/z: $[M-1]^+$ calcd 958.3, found 958.1.

Synthesis of TPETP-TK-PEG. TPETP-TK (5 mg, 0.005 mmol) and PEG-NH_2 (20.8 mg, 0.014 mmol) were dissolved in DMF (1 mL). Then HBTU (1.2 eq) and DIPEA (2.0 eq) were added and stirred at room temperature for 8 h. The mixture was then concentrated and precipitated into a mixture of methanol and diethyl ether (v/v = 1/10) three times to give red powders. The crude product was re-dissolved in DMF and further purified by dialysis against distilled water using a Spectra/Por dialysis tubing (molecular weight cutoff of 12,000 Da, Spectrum Laboratories, Rancho Dominguez, CA, United States) for 48 h with changes of water. After freeze-drying, TPETP-TK-PEG (5.2 mg, 35%) was obtained as red powders.

ROS detection in solution. The ROS generation was studied using ABDA as an indicator as the absorbance of ABDA decreases upon reaction with ROS.[2] For ROS detection, the ABDA (200 μM) was mixed with the AIE-NPs (0.1 mg mL^{-1}) in PBS and exposed to white light (λ = 400–700 nm) irradiation. The decomposition of ABDA was monitored by the absorbance decrease at 400 nm.

DOX loading in AIE-NPs (AIE-NPs/DOX). The NPs self-assembled from TPETP-TK-PEG loaded with DOX were prepared by a dialysis method. In a typical process, 10 mg of TPETP-TK-PEG and 1.0 mg of DOX were first dissolved in 1 mL of DMSO. Then 5 mL of Milli-Q water was added slowly under moderate stirring. The AIE-NPs/DOX were obtained after organic solvent removal through dialysis (MWCO 3,500 Da) against water. Drug loading capacity of DOX (%) was calculated as

follows: loading capacity = [amount of DOX in the TPETP-TK-PEG]/[amount of TPETP-TK-PEG] × 100.

In vitro drug release. The drug release from AIE-NPs/DOX upon white light irradiation was studied by using a dialysis membrane tubing (MWCO 12,000 Da) in a release medium. The AIE-NPs/DOX in the dialysis membrane tubing was first exposed to white light irradiation. At predetermined intervals, the release medium was collected and equal volume of fresh medium was replaced. The concentration of DOX in the release medium was measured by HPLC using an Agilent HPLC system with acetonitrile-water (50:50, v/v) as the mobile phase. Linear calibration curve was constructed using the peak areas for concentrations in the range of 0.5 - 500 μg mL^{-1}.

Cell culture. Human breast cancer cell line MDA-MB-231 cells was obtained from the American Type Culture Collection (ATCC, MD, USA) and from which doxorubicin resistant MDA-MB-231/DOX cells were developed in our laboratory using a stepwise increase in drug concentration protocol according to the previous methods.[3] The cells were cultured in DMEM (Invitrogen, Carlsbad, CA) containing 10% heat-inactivated FBS (Invitrogen), 100 U mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin (Thermo Scientific) and maintained in a humidified incubator at 37 °C with 5% CO_2. MDA-MB-231/DOX cells were maintained with free doxorubicin at 1 μg mL^{-1}. Before experiments, the cells were precultured until confluence was reached.

Confocal imaging. MDA-MB-231/DOX cells were cultured in the chambers at 37 °C. After 80% confluence, the culture medium was removed and washed twice with PBS buffer. Following incubation with AIE-NPs/DOX for 2 h, cells were washed twice with PBS and then exposed to white light irradiation for 2 min at the power density of 0.1 W cm^{-2}. After the irradiation, the medium was replaced with fresh medium and the cells were incubated for another 2 h to ensure the released DOX to migrate into the cell nuclei. After that, the cell nucleus was living stained with Hoechst 33342 (Life Technologies). The cells were then imaged immediately by confocal laser scanning microscope (CLSM, Zeiss LSM 410, Jena, Germany). For Hoechst 33342 detection, the excitation wavelength was 405 nm, and the emission filter was 430−470 nm; for DOX detection, the excitation wavelength was

488 nm, and the emission filter was 610−640 nm; for AIE-NPs detection, the excitation was 488 nm, and the emission was collected above 650 nm.

Intracellular ROS detection. The intracellular ROS generation was detected by using 2′,7′-dichlorofluorescein diacetate (DCF-DA) as an indicator and studied by confocal images. MDA-MB-231/DOX cells in 8-well chambers (Thermo Scientific) were firstly incubated with AIE-NPs (10 μg mL^{-1}) for 2 h in the dark, then the cells were rinsed with PBS for 3 times and stained with 2 μM of DCF-DA. After 10 min incubation, the cells were washed with 1× PBS and exposed to visible light (λ = 400–700 nm) irradiation (30 s, 0.10 W cm^{-2}). After irradiation, the cells were washed with 1× PBS and studied by confocal microscope (CLSM, Zeiss LSM 410, Jena, Germany). For DCF detection, the excitation wavelength was 488 nm, and the emission filter was 505−525 nm.

Flow cytometry quantification of DOX in MDA-MB-231/DOX cell nuclei. The cells in 24-well plate (Costar, IL, USA) were incubated with AIE-NPs/DOX for 2 h in dark conditions. After the white light irradiation, the cells were trypsinized and suspended at a concentration of 5 $\times 10^6$ cells mL^{-1} for 10 min at 4 °C in 100 mM NaCl solution with 1 mM EDTA, 1% Triton X-100 and 10 mM Tris buffer (pH 7.4). After the incubation, the suspension was centrifuged (15 min, 800 g) and the precipitate of cell nuclei were separated from the supernatant cell cytosol. The precipitate was resuspended in 1 mL of 1× PBS and subjected to flow cytometry analysis (Cyan-LX, DakoCytomation) with an excitation wavelength of 488 nm. The cells without any treatment were used as control. The mean fluorescence was determined by counting 10,000 events.

Cytotoxicity studies. MTT assays were used to assess the metabolic activity of the MDA-MB-231/DOX cells. The cells were seeded in 96-well plates. After overnight incubation, the cells were incubated with AIE-NPs/DOX in fresh medium for 2 h in dark conditions. Then the cells were washed with PBS and exposed to white light irradiation for 2 min at a power density of 0.1 W cm^{-2}. With further incubation in fresh medium for designated time, the cells were washed with PBS and incubated with 100 μL of MTT solution (0.5 mg mL^{-1}) in culture medium for 3 h. Then DMSO was added to

dissolve the formed precipitates and the absorbance at 570 nm was monitored by a microplate reader. Cells without any treatment were used as controls.

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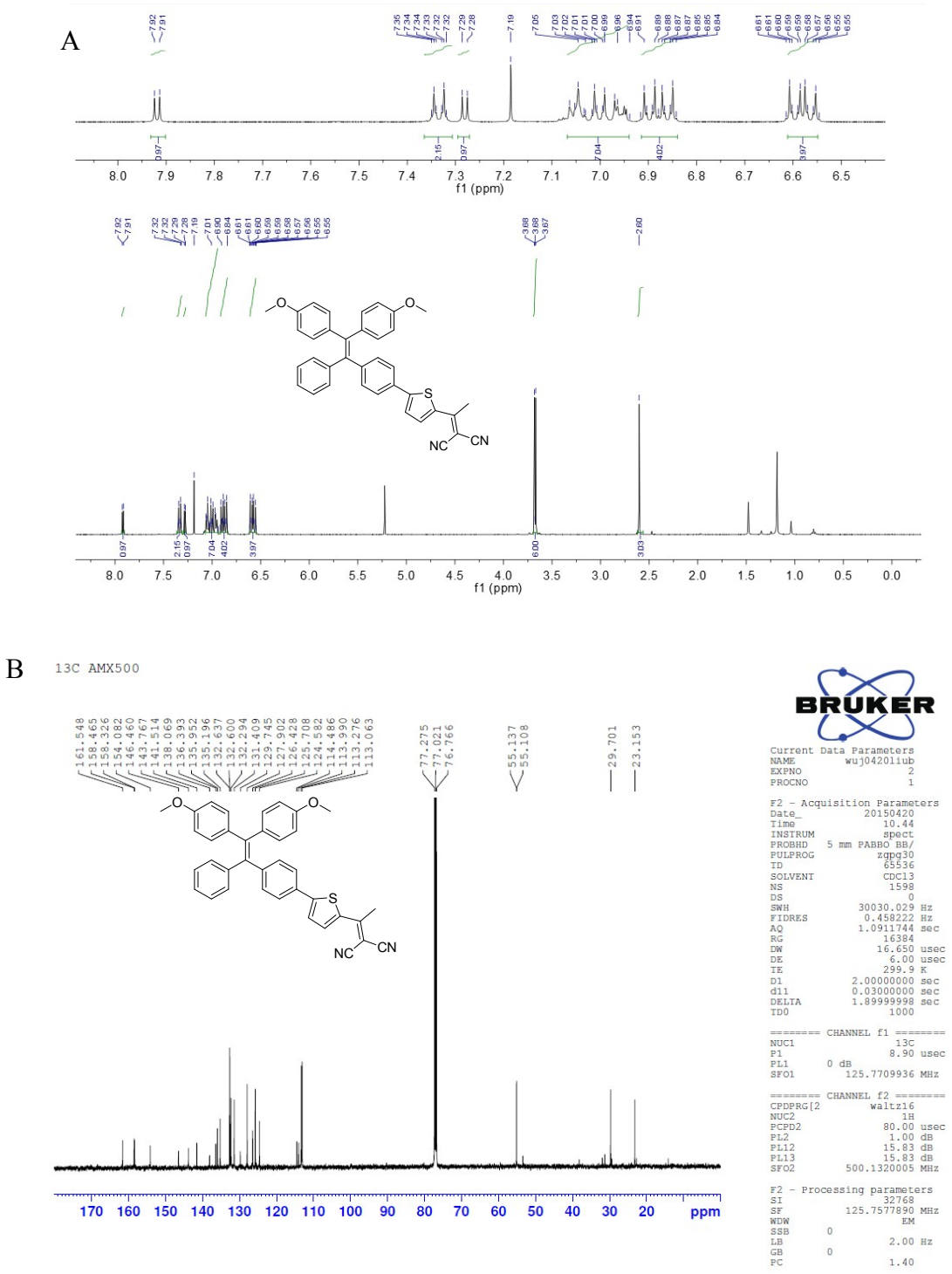

Figure S1. Characterization of TPETP. (A) ^{1}H NMR spectrum of TPETP; (B) ^{13}C NMR spectrum of TPETP; (C) mass spectrum of TPETP.

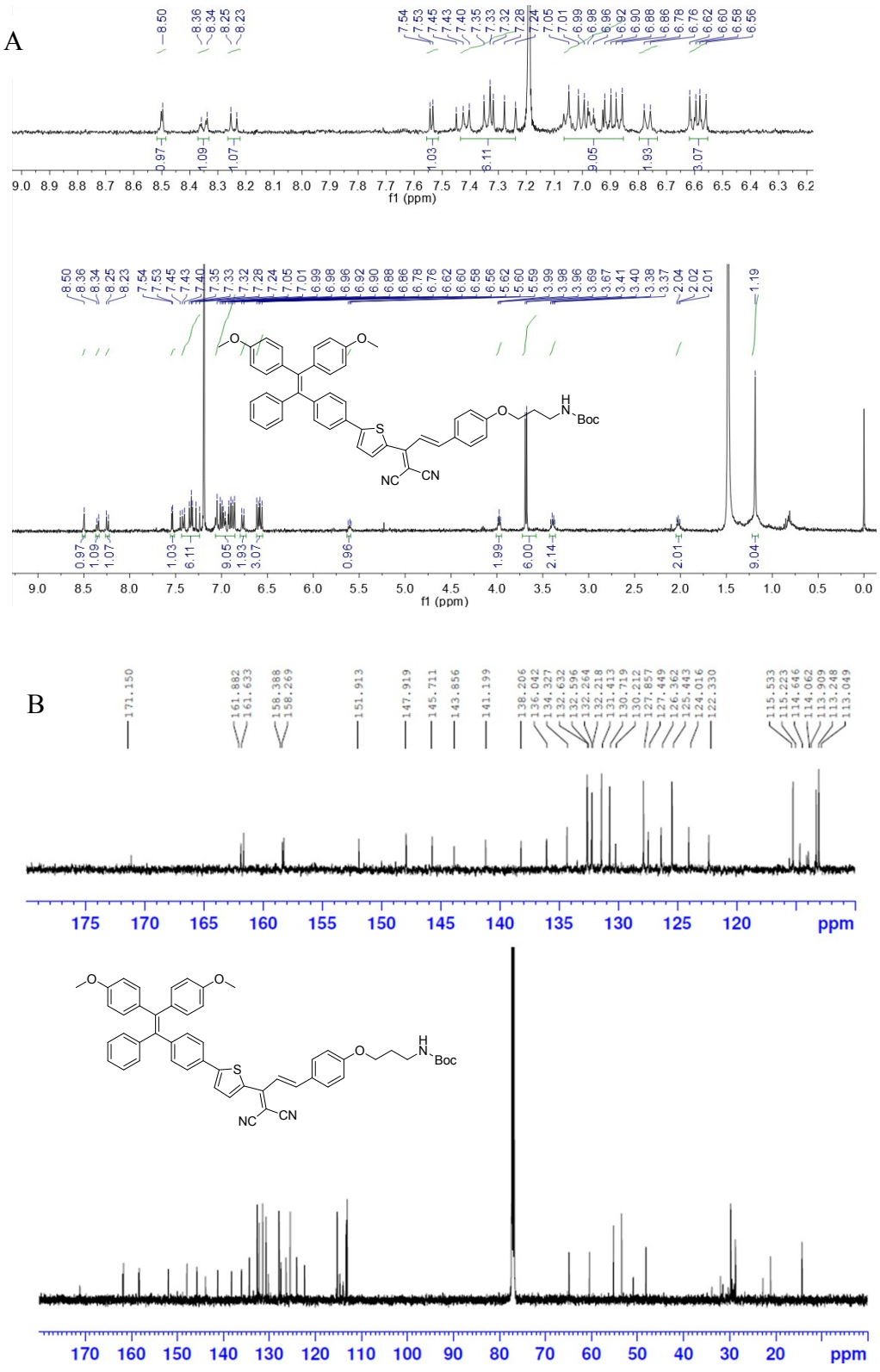

Figure S2. Characterization of TPETP-NH_2(Boc). (A) ^{1}H NMR spectrum of TPETP-NH_2(Boc); (B) ^{13}C NMR spectrum of TPETP-NH_2(Boc); (C) mass spectrum of TPETP-NH_2(Boc).

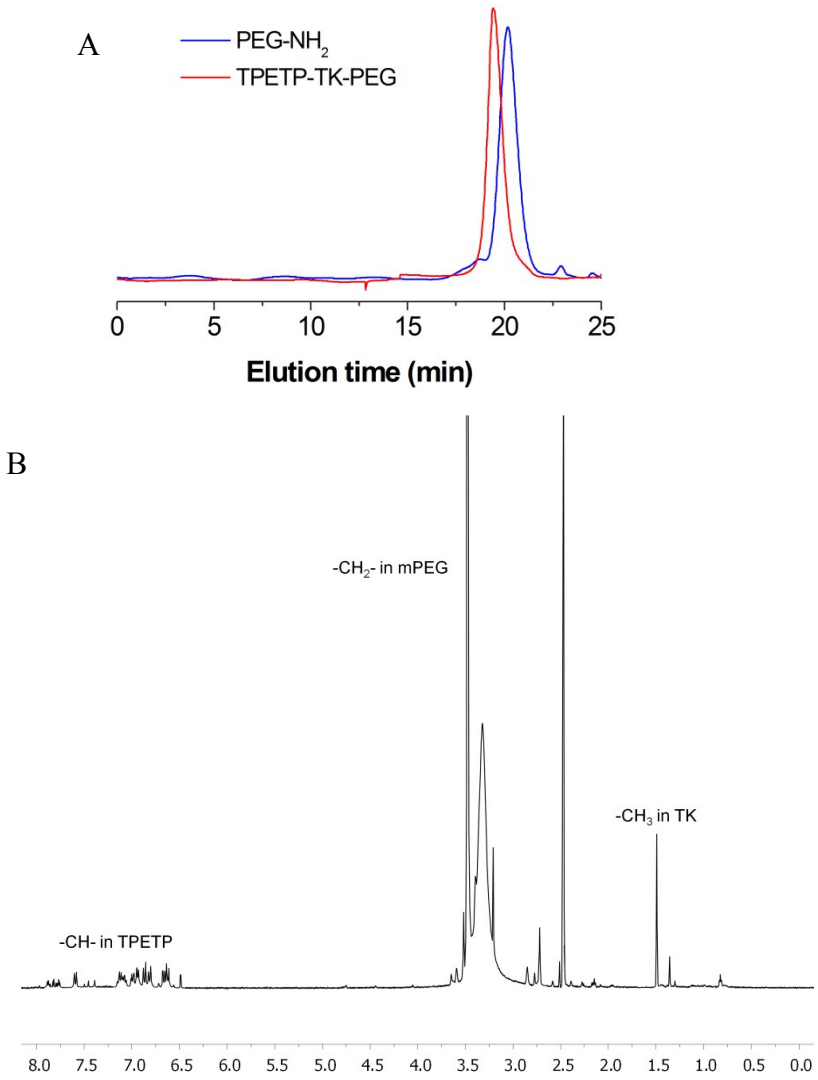

Figure S3. (A) GPC spectrum of PEG-NH_2 and TPETP-TK-PEG. (B) ^{1}H NMR spectrum of TPETP-TK-PEG.

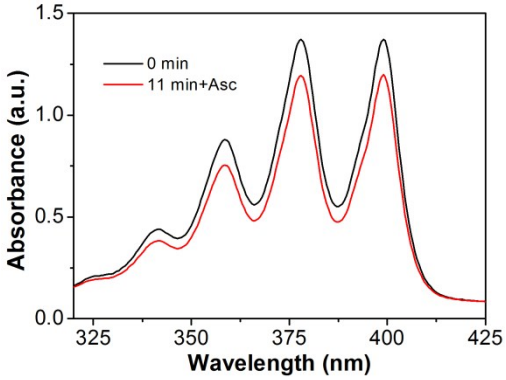

Figure S4. The absorption changes of ABDA when mixed with AIE-NPs (0.1 mg mL^{-1}) upon white light (λ = 400–700 nm, 0.10 W cm^{-2}) irradiation for 11 min with or without ROS scavenger ascorbic acid (Asc).

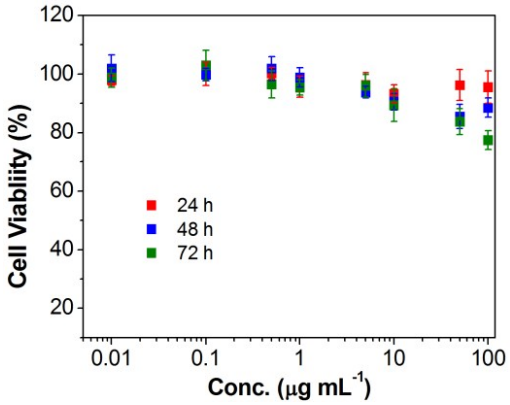

Figure S5. Vability of MDA-MB-231/DOX cells after treatment with AIE-NPs at different concentrations with light irradiation (2 min, 0.1 W cm^{-2}) and further incubation in fresh medium for different time duration.