Supporting Information

Type I Photosensitizers Responsive Self-Immolative Polymers: Combining Drug Release with Photodynamic Therapy

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Experimental Section

Materials.

4-aminobenzyl alcohol (Innochem), phenyl chloroformate (Innochem), di-nbutyltin dilaurate (DBTL, Macklin), 4-(hydroxymethyl)phenylboronic acid pinacol ester (Innochem), 1-propanethiol (Aladdin), carbon disulfide (Innochem), 2-bromoisobutyric acid (Innochem), oxalyl chloride (TCI) were used as received. N,N-dimethylacrylamide (DMA, Alfa Aesar) was purified through a column of neutral alumina to remove polymerization inhibitors. 2,2'-Azobis(2-methylpropionitrile (AIBN, Innochem), was purified by recrystallization prior to use. Doxorubicin (DOX, Innochem), Evodiamine (Evo, Macklin), thiazolyl blue tetrazolium bromide (MTT, Innochem), 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA, solid, Macklin), Hoechst 33342 (Beyotime) were used as received. A549 cells were purchased from Shanghai An Wei Technology Co., Ltd. (Shanghai, China).

Ultrapure water (18.2 M Ω cm) was obtained from a Milli-Q reference system. Ultra-dry solvents were purchased from Innochem. Other solvents were purchased from Tong Guang Chemical Reagent Beijing Co., Ltd.

White light (PLS-SXE300 Xenon lamp, 75 or 40 $mW \cdot cm^{-2}$) and red light (20 W, 10-18 V, 1200 mA, λ = 660-665 nm, 45.6 or 31.2 $mW \cdot cm^{-2}$) were used as the irradiation light source for photocatalytic experiment.

Synthesis.

Full synthetic details are provided in Figures S1. The obtained polymers and intermediates were confirmed via ^1H NMR spectrum (Fig. S2−S7).

Synthesis of phenyl (4-(hydroxymethyl)phenyl)carbamate (Compound 1)[1]. 4-aminobenzyl alcohol (1.8 g, 15 mmol) was dissolved in a 28 mL mixture of THF: sat. $NaHCO_3$: water (ratio 2:2:1), and phenylchloroformate (2 mL, 15.3 mmol) was added dropwise over 10 minutes. The reaction was monitored by TLC (EtOAc: Hex 1:1) until it went to completion. EtOAc was then added and the organic phase was washed twice with saturated NH_4Cl solution. The solvents were removed under reduced pressure and recrystallized to give compound 1 (3.2 g, 88%) as a white solid.

Synthesis of Compound 2[1]. Compound 1 (0.973 g, 4 mmol), DBTL (126.2 mg, 0.2 mmol), and dry DMSO (2 mL) were charged into a reaction flask. The solution was deoxygenated by bubbling with dry N_2 for 60 min. After being stirred for 15 min at 110 ℃, 4-(Hydroxymethyl)phenylboronic acid pinacol ester (0.936 g, 4 mmol) was added, and the solution was further stirred for 3 h at 110 ℃. The reaction mixture was rapidly quenched by immersion in liquid nitrogen, followed by precipitation into an excess of methanol. The above dissolution-precipitation cycle was repeated for three times. Compound 2 was obtained as a yellow solid (0.5 g, yield: 75%) after being dried in a vacuum oven overnight at room temperature. The DP was determined to be ~10 and the extent of end group functionalization was determined to be ~100% by ^1H NMR analysis in DMSO-d_6 (Figure S3).

Synthesis of Carboxylic Trithiocarbonate RAFT Agent (CTA)[2]. 1-Propanethiol (2.74 g, 35.94 mmol) was added into a stirred suspension of K_3PO_4 (7.63 g, 35.94 mmol) in acetone (50 mL). 10 min later, CS_2 (8.21 g, 107.78 mmol) was added to the reaction falsk. After another 10 min, 2-bromo-2-methylpropionic acid (5 g, 29.94 mmol) was added. The reaction mixture was kept to stir for overnight. After that, the solvent was removed and the residue was extracted into CH_2Cl_2 (2×100 mL) from 1M HCl aqueous solution (100 mL). After the organic phase was washed with water (2 × 100 mL) and dried by anhydrous Na_2SO_4, the DCM was removed by rotary evaporation. The pure product was obtained by recrystallization from ethyl acetate and hexane (4.7 g, yield: 66%).

Synthesis of Compound 3. Under a nitrogen atmosphere at 0 °C, oxalyl chloride (120 μL, 1.4 mmol) was added dropwise to a solution of chain transfer agent (CTA, 56.4 mg, 0.24 mmol) in dry dichloromethane (500 μL). The reaction mixture was then heated to 45 °C and refluxed for 2 hours. The excess oxalyl chloride and DCM were evaporated under vacuum to obtain CTA-Cl[3]. Under a N_2 atmosphere, dry triethylamine (20 μL, 0.144 mmol) was added to a solution of compound 2 (50 mg, 0.029 mmol) in dry DMF (300 μL). The mixture was cooled to below 10°C, followed by dropwise addition of the CTA-Cl solution (synthesized in the previous step) in dry DMF (200 μL) under maintained N_2 protection. The resulting mixture was then stirred at room temperature overnight. The reaction solution was precipitated into an excess of methanol. The above dissolution-precipitation cycle was repeated for three times. Compound 3 was obtained as a yellow solid (45 mg, yield: 80%) after being dried in a vacuum oven overnight at room temperature.

Synthesis of Self Immolative Polymer (SIP)[4]. Compound 3 (25 mg, 0.013 mmol), N,N-dimethylacrylamide (DMA, 74.35 mg, 0.75 mmol), 2,2'-Azobis(2-methylpropionitrile (AIBN, 0.49 mg, 0.003 mmol) and 400 μL dry DMSO were charged into a glass ampoule equipped with a magnetic stirring bar. The mixture was degassed by three freeze-pump-thaw cycles and backfilled with nitrogen. After being stirred for 2 h at 75 ℃, the reaction tube was quenched into liquid N_2, opened and exposed to air, and the reaction mixture was precipitated into an excess of diethyl ether. The above dissolution-precipitation cycle was repeated for three times. The final precipitate was dried in a vacuum oven overnight at room temperature to afford a yellow solid (55 mg, yield: 75 %). The DP of PDMA block was determined to be 40 by ^{1}H NMR analysis in DMSO-d_6 (Figure S7).

Preparation of SIPMs.

A typical procedure is described as follows: SIP (2 mg) was dissolved in 1 mL DMSO in a 15 mL capped vial with a magnetic stirrer. The solution was stirred for 3 h at room temperature. DI water (9 mL) was added into the organic solution by a syringe pump at a speed of 1 mL/h at 25 ℃ under stirring (500 rpm). The dispersion was stirred for 5 h followed by dialysis for 24 h.

Preparation of Drug-Loaded SIPMs.

Typical procedures are described as follows: SIP (4 mg) and DOX (0.1 mg) or Evo (0.01 mg) was dissolved in DMSO (0.2 mL). Then DI water (1.8 mL) was added into the organic solution by a syringe pump at a speed of 1 mL/h at 25 ℃ under stirring (500 rpm). The dispersion was stirred for 5 h, and then purified by dialysis for 24 h. The drug loading efficiency was determined by comparing the UV absorption (for DOX) or fluorescence intensity (for Evo) of the dialyzed system

against their respective standard calibration curves (Figure S21, S22). Based on the standard calibration curves, the loading contents of DOX and Evo were determined to be approximately 1.4 wt% and 0.15 wt%, with corresponding encapsulation efficiencies of 58% and 60.7%, respectively.

Monitoring Triggered Disintegration of SIPMs.

For ^{1}H NMR and GPC analysis, SIPMs dispersion (0.4 mg/mL) was treated under two distinct conditions: (a) incubation with 10 equiv. H_2O_2, or (b) exposure to 0.5 equiv. PS1 under xenon lamp irradiation (75 mW·cm^{-2}, 3 h). After stirring for 24 h, all samples were lyophilized and subsequently analyzed. For TEM observation, SIPMs dispersion (0.2 mg/mL) was mixed with either (a) 0.5 equiv. PS1 followed by xenon lamp irradiation (75 mW·cm^{-2}, 1 h or 3 h), or (b) 0.5 equiv. PS2 with subsequent red light irradiation (45.6 mW·cm^{-2}, 1 h or 3 h). All samples were then stirred for 24 h, after which 10 μL aliquots were deposited onto copper grids and stained with phosphotungstic acid for 30 seconds.

Cell Culture.

A549 cells were seeded at an initial density of approximately 5,000 cells per well in 96-well plates (100 μL per well) or at 50,000 cells per dish in confocal dishes (1 mL per dish), using DMEM: F12 medium supplemented with 10% FBS and 1% penicillin/streptomycin. All cells were incubated in a humidified incubator at 37 °C with 5% CO_2.

In Vitro Cytotoxicity Assay.

After incubating for 24 h, DMEM: F12 was replaced with fresh medium. For PS1-treated cells: the cells were treated with SIPMs dispersions (SIPMs alone, SIPMs+PS1, or DOX@SIPMs+PS1) at varying concentrations. The treated cells were incubated in a humidified atmosphere with 5% CO_2 at 37 °C for 12 h. The 96-well plate was then either irradiated with a xenon lamp (40 mW·cm^{-2}, 12 min) or kept in darkness, followed by an additional 12 h incubation; For PS2-treated cells: the cells were treated with SIPMs dispersions (SIPMs alone, SIPMs+PS2, or Evo@SIPMs+PS2) at specified concentrations. After 12 h of incubation under standard conditions (37 °C, 5% CO_2), the treated cells were either exposed to red light irradiation (31.2 mW·cm^{-2}, 7 min) or maintained in darkness, and subsequently incubated for another 12 h. Thiazolyl blue tetrazolium bromide (MTT, 5 mg/mL PBS solution) was added to each well. The cells were further incubated for 4 h at 37 °C. The medium in each well was then removed and replaced by 100 μL DMSO. The plate was gently agitated for 10 min before the absorbance at 570 nm was recorded by a microplate reader (BioTek Epoch, USA). Data are presented individually and as the mean ± SD (column with error bar) of n = 3 independent measurements.

Confocal laser scanning microscopy (CLSM) measurements.

After incubating for 24 h, DMEM: F12 was replaced with fresh medium, and the cells were treated with PS1 or PS2 system with 5% CO_2 at 37 °C for 12 h (SIPMs: 0.2 mg/mL; PS1: 5 μM,

Doxorubicin: 5 μM; PS2: 62.5 nM, Evodiamine: 1 μM). Next, the confocal dishes were either irradiated with light (for PS1: xenon lamp, 40 $mW \cdot cm^{-2}$ for 12 min; for PS2: red light, 31.2 $mW \cdot cm^{-2}$ for 7 min) or kept in dark, and then incubated for another 24 h. The DMEM: F12 medium was then replaced with fresh serum-free medium containing ROS probe DCFH-DA, followed by incubation at 37 °C for 30 min. After removal of the medium, the cells were washed with PBS and incubated with fresh medium containing Hoechst 33342 dye. Cell imaging was performed using confocal laser scanning microscopy (CLSM). ROS probe scan excitation wavelength: 488 nm. Detection wavelength: 510−550 nm. Hoechst 33342 scan excitation wavelength: 405 nm. Detection wavelength: 420−480 nm.

Characterization.

All nuclear magnetic resonance (NMR) spectra were recorded on JEOL JNM-ECX600P and JNM-ECS600 (400 MHz or 600 MHz) spectrometers at room temperature with DMSO-d_6 (δ = 2.50 ppm) or $CDCl_3$ (δ = 7.26 ppm) or D_2O (δ = 4.79 ppm) or Methanol-d_4 (δ = 3.31 ppm) as solvent. Gel Permeation Chromatography (GPC) was conducted in an instrument of Waters 1515 Isocratic HPLC Pump with a Waters 2414 refractive index detector at 25 °C. The solvent was DMF and the calibration was proceeded with narrow dispersity polystyrenes. Transmission electron microscopic (TEM) images were recorded using a FEI Tecnai Spirit 120 kV TEM at Tsinghua University. The zeta potential and dynamic light scattering (DLS) size of the nanoparticles in water was obtained on a Zetasizer Lab (Malvern Panalytical) with all data averaged over three measurements. Confocal laser scanning microscopy (CLSM) images were acquired using a Nikon A1R microscopy system at Beijing Normal University. ESR analysis was performed on a Bruker E 500 spectrometer. UV-Vis absorbance measurement was carried out on a Cary 60 UV-Vis Spectrophotometer (Agilent Technologies). Fluorescence spectra were recorded in quartz cuvettes (light path 1 mm) on fluorescence spectrophotometer (FS5, UK, and Agilent eclipse, Malaysia). For all the samples, the EX slit and EM slit were set as 5 nm, and the PMT voltage was 600 V.

Supplementary Figures

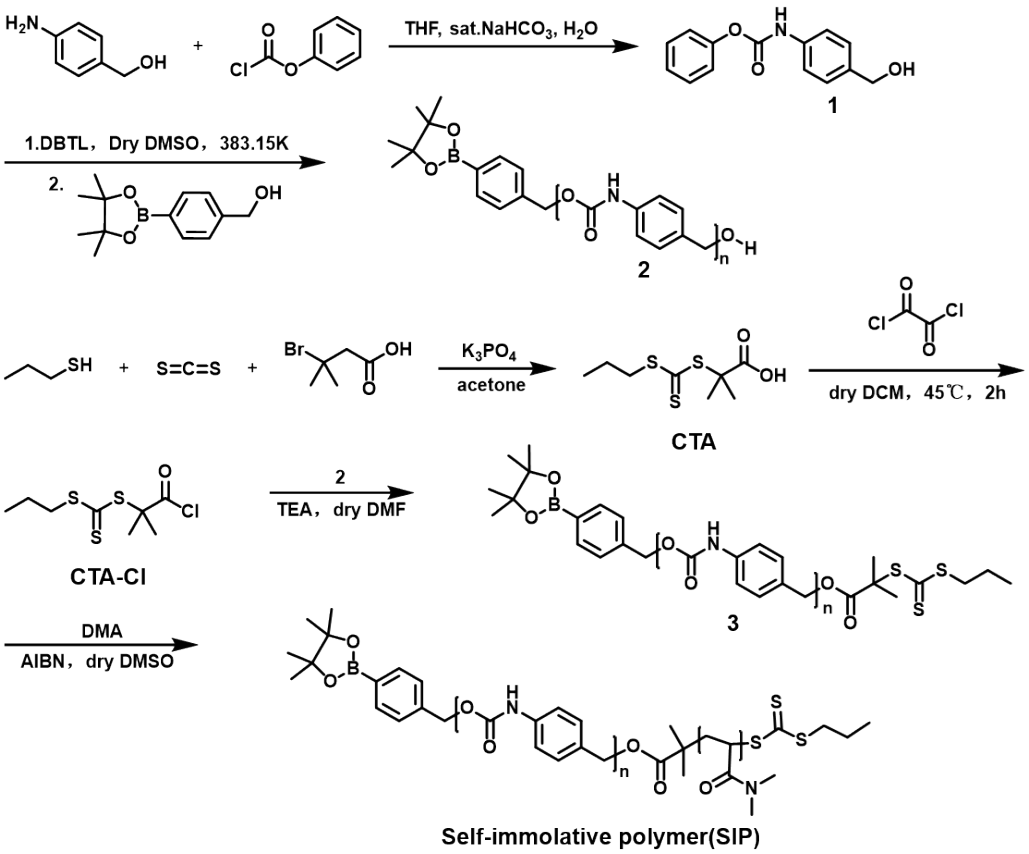

Figure S1. Synthetic route of Self-immolative polymer (SIP).

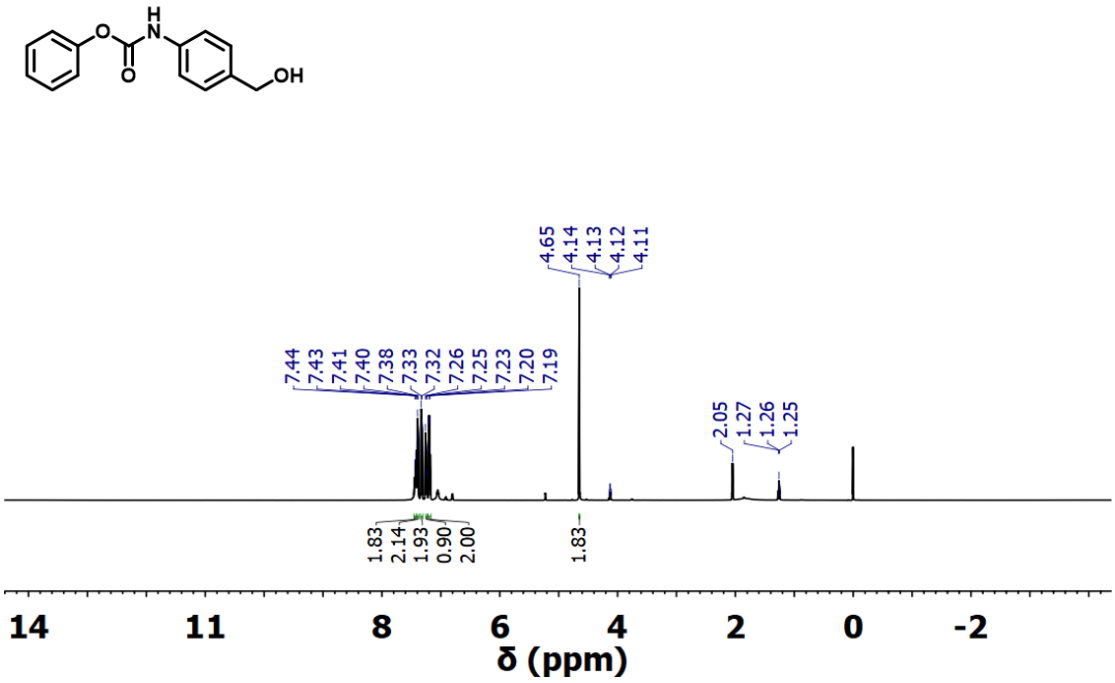

Figure S2. ^{1}H NMR spectrum recorded in $CDCl_3$ of Compound 1. ^{1}H NMR (400 MHz, $CDCl_3$): δ =7.44 (d, J = 5.3 Hz, 2H), 7.40 (t, J = 4.1 Hz, 2H), 7.33 (d, J = 5.6 Hz, 2H), 7.24 (d, J = 5.0 Hz, 1H), 7.20 (d, J = 5.7 Hz, 2H), 4.65 (s, 2H). The signals at δ= 7.26 ppm are attributed to the solvent residual peak. The signals at δ = 4.13 (q), 2.05 (s), 1.26 (t) are assigned to ethyl acetate.

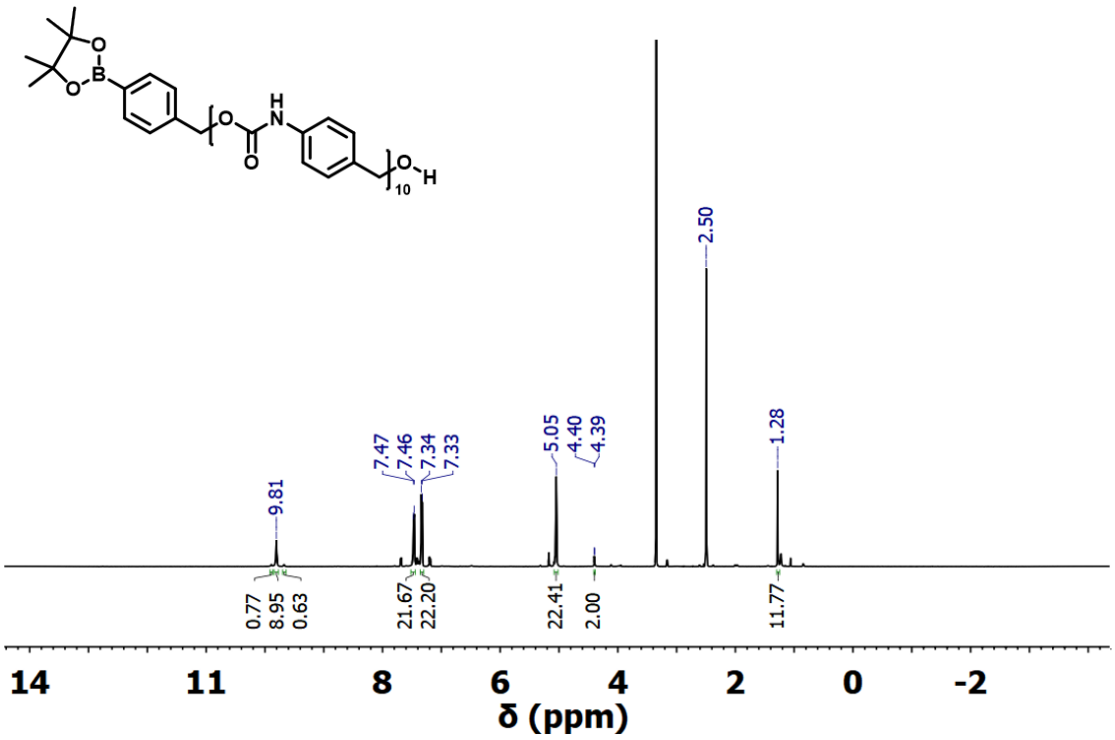

Figure S3. ^{1}H NMR spectrum recorded in DMSO-d_6 of Compound 2. ^{1}H NMR (600 MHz, DMSO-d_6): δ = 9.81 (s, 10H), 7.47 (d, *J* = 8.0 Hz, 22H), 7.34 (d, *J* = 8.3 Hz, 22H), 5.05 (s, 22H), 4.40 (d, *J* = 5.6 Hz, 2H), 1.28 (s, 12H). The signals at δ = 3.33 and 2.50 ppm are attributed to the H_2O and solvent residual peak.

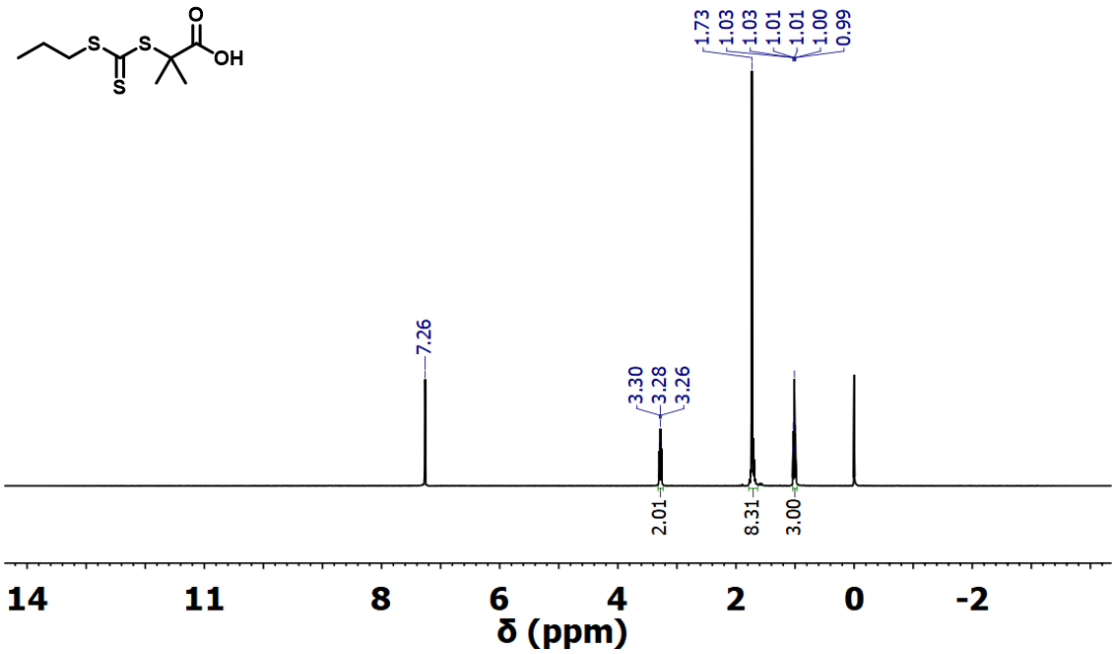

Figure S4. ^{1}H NMR spectrum recorded in $CDCl_3$ of CTA. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.28 (t, *J* = 7.3 Hz, 2H), 1.73 (m, 8H), 1.00 (t, *J* = 7.3 Hz, 3H). The signal at δ = 7.26 ppm is correspond to solvent residual peak.

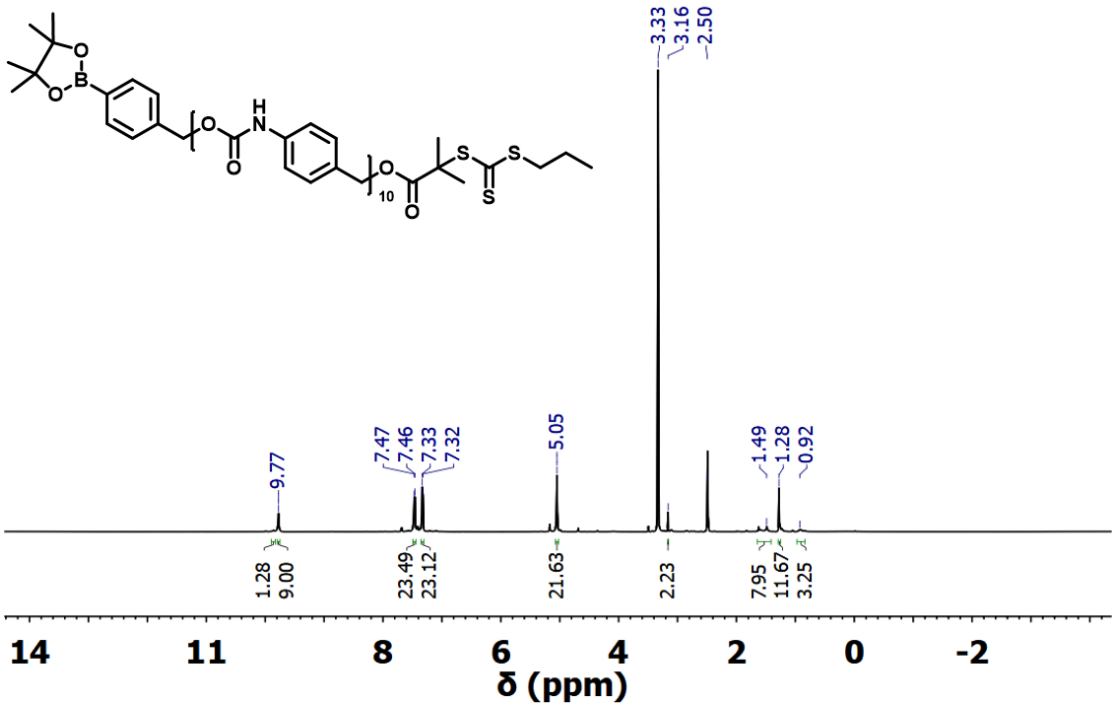

Figure S5. ^{1}H NMR spectrum recorded in DMSO-d_6 of Compound 3. ^{1}H NMR (600 MHz, DMSO-d_6): δ = 9.77 (s, 10H), 7.47 (d, *J* = 8.0 Hz, 23H), 7.33 (d, *J* = 8.3 Hz, 23H), 5.05 (s, 22H), 3.16 (m, *J* = 5.2 Hz, 2H), 1.49 (m, 8H), 1.28 (s, 12H), 0.92 (m, 3H). The signals at δ = 3.33 and 2.50 ppm are attributed to the H_2O and solvent residual peak.

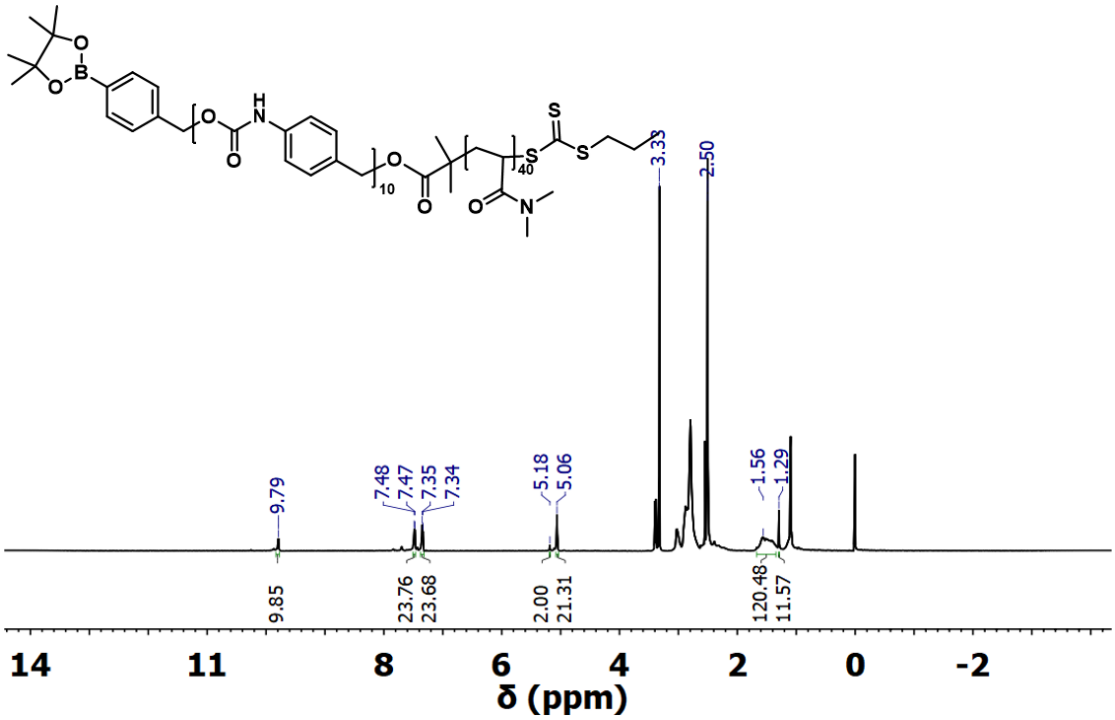

Figure S6. ^{1}H NMR spectrum recorded in DMSO-d$_6$ of SIP. ^{1}H NMR (600 MHz, DMSO-d$_6$): δ = 9.79 (s, 10H), 7.48 (d, *J* = 6.9 Hz, 24H), 7.35 (d, *J* = 6.9 Hz, 24H), 5.18 (s, 2H), 5.06 (s, 21H) 1.56 (m, 120H), 1.29 (s, 12H). The signals at δ = 3.33 and 2.50 ppm are attributed to the H_2O and solvent residual peak.

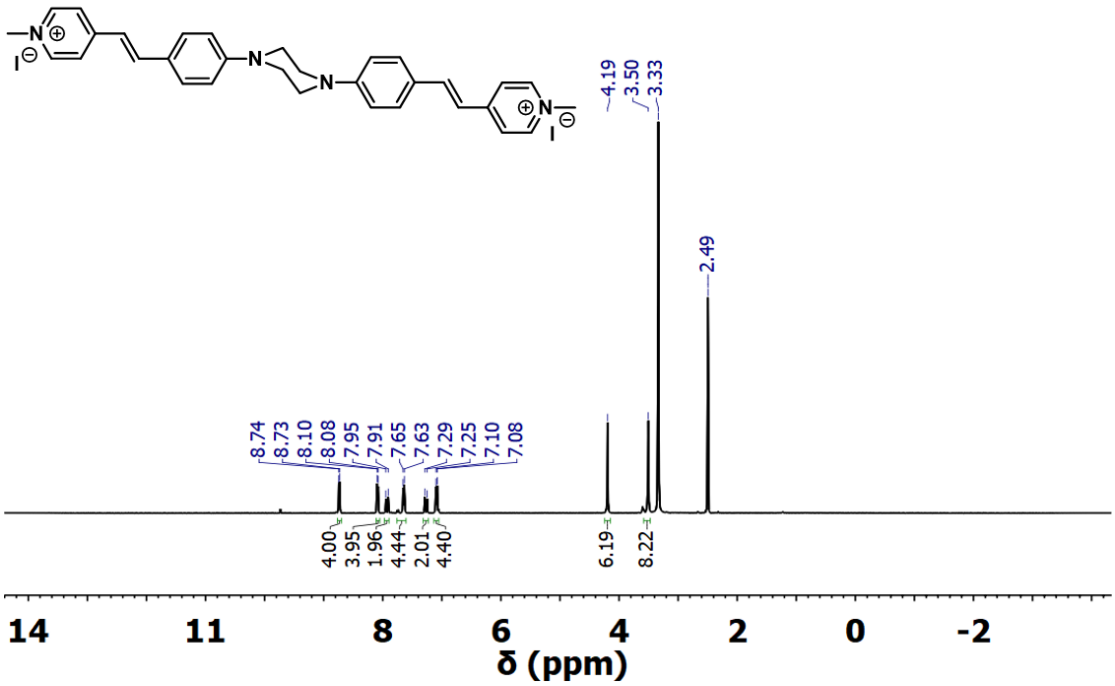

Figure S7. ^{1}H NMR spectrum of PS1 in DMSO-d$_6$. ^{1}H NMR (400 MHz, DMSO-d$_6$): δ = 8.74 (d, *J* = 6.6 Hz, 4H), 8.09 (d, *J* = 6.6 Hz, 4H), 7.93 (d, *J* = 16.2 Hz, 2H), 7.64 (d, *J* = 8.5 Hz, 4H), 7.27 (d, *J* = 16.1 Hz, 2H), 7.09 (d, *J* = 8.7 Hz, 4H), 4.19 (s, 6H), 3.50 (s, 8H). The signals at δ = 3.33 and 2.49 ppm are attributed to the H_2O and solvent residual peak.

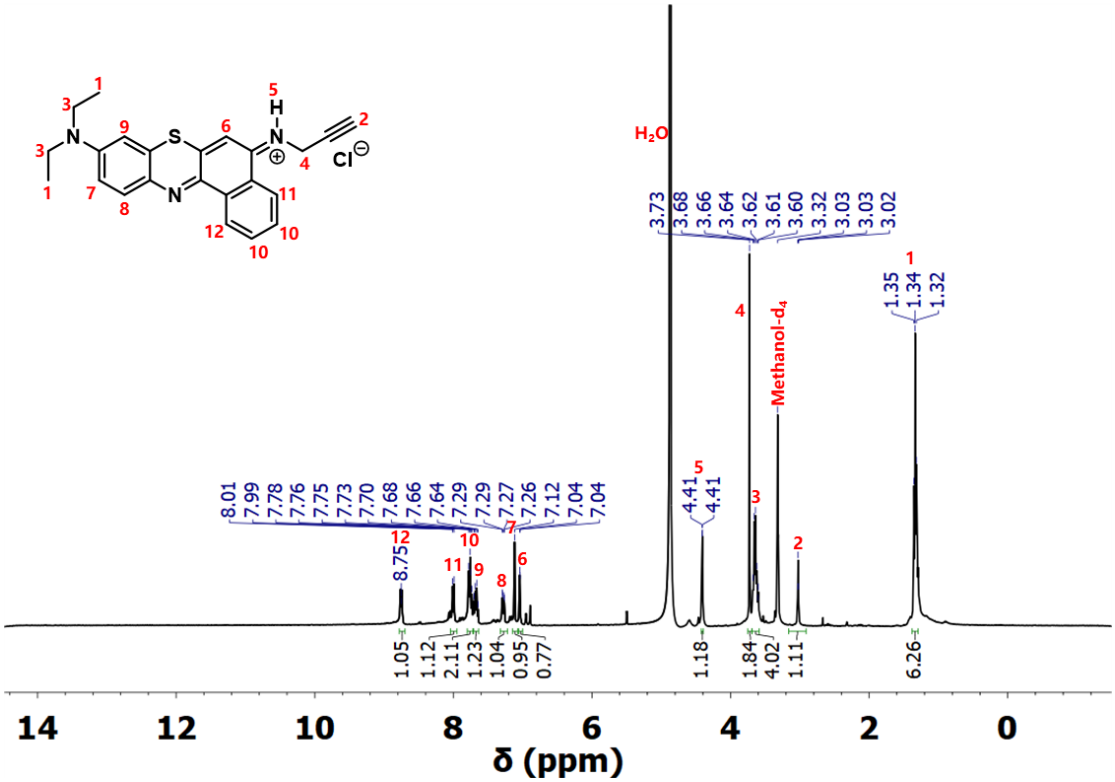

Figure S8. ^{1}H NMR spectrum of PS2 in Methanol-d$_4$. ^{1}H NMR (400 MHz, Methanol-d4): δ = 8.75 (s, 1H), 8.00 (d, *J* = 8.2 Hz, 1H), 7.76 (dd, *J* = 10.4, 8.4 Hz, 2H), 7.67 (dd, *J* = 15.9, 7.5 Hz, 1H), 7.28 (dd, *J* = 9.6, 2.7 Hz, 1H), 7.12 (s, 1H), 7.04 (d, *J* = 2.4 Hz, 1H), 4.41 (d, *J* = 2.4 Hz, 1H), 3.73 (s, 2H), 3.63 (dt, *J* = 9.4, 5.1 Hz, 4H), 3.03 (t, *J* = 2.3 Hz, 1H), 1.34 (t, *J* = 7.2 Hz, 6H). The signals at δ = 4.87 and 3.32 ppm are attributed to the H_2O and solvent residual peak.

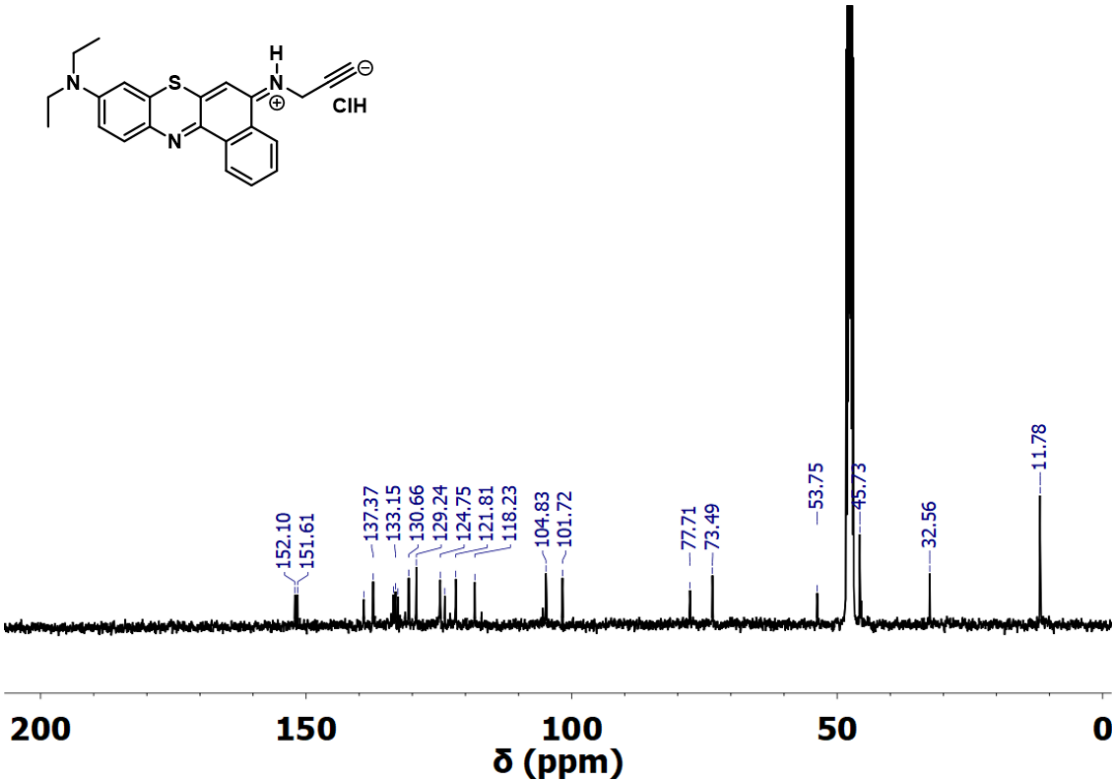

Figure S9. ^{13}C NMR spectrum of PS2 in Methanol-d_4. ^{13}C NMR (101 MHz, Methanol-d_4): δ = 152.10, 151.61, 139.13, 137.37, 133.55, 133.15, 132.71, 130.66, 129.24, 124.75, 123.89, 121.81, 118.23, 104.83, 101.72, 77.71, 73.49, 53.75, 45.73, 32.56, 11.78. The signals at δ = 49.0 ppm are attributed to the solvent residual peak.

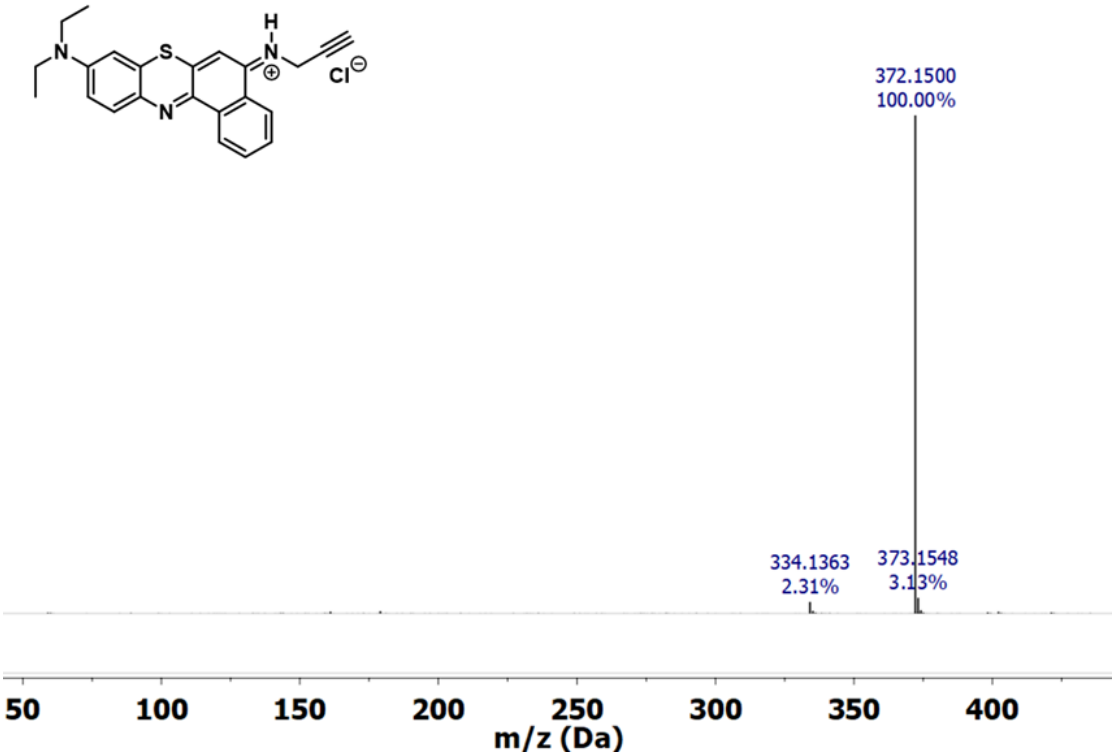

Figure S10. HRMS spectrum of PS2. m/z ($[C_{23}H_{22}N_3S]^+$) = 372.1500; calcd =372.1529.

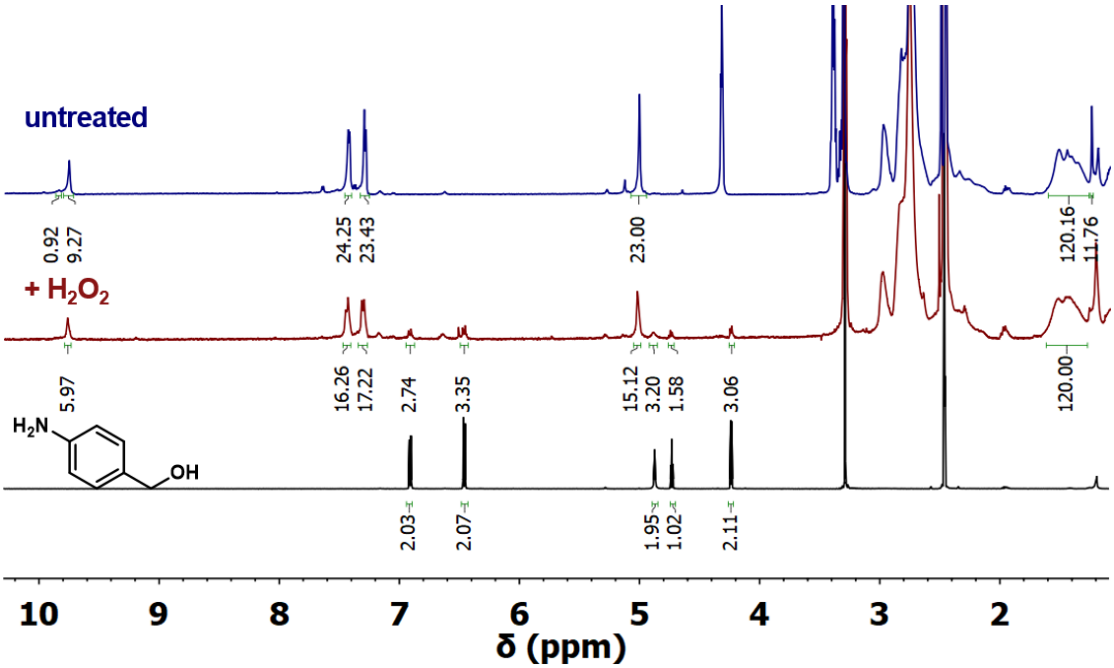

Figure S11. ^{1}H NMR spectra recorded in DMSO-d_6 of 4-aminobenzyl alcohol and PB-PBC$_{10}$-PDMA$_{40}$ before and after H_2O_2 treatment. SIPMs dispersion (0.4 mg/mL) was incubated with or without 10 equiv. H_2O_2 for 24 h under stirring.

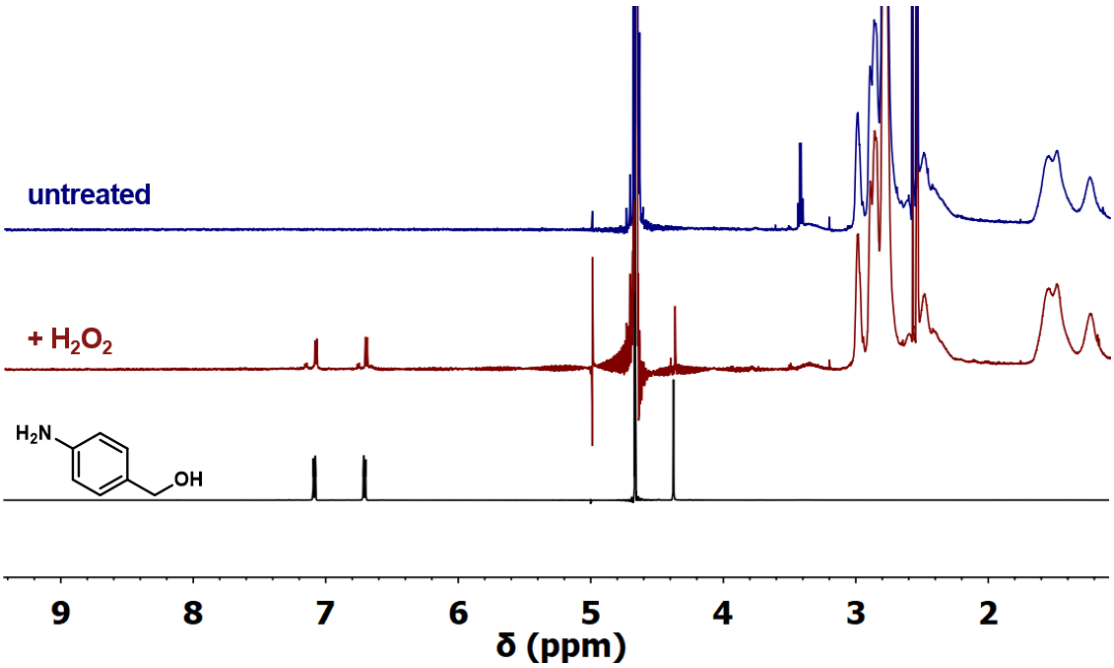

Figure S12. ^{1}H NMR spectra recorded in D_2O/DMSO-d_6 (9/1 v/v) dispersion of 4-aminobenzyl alcohol and PB-PBC$_{10}$-PDMA$_{40}$ before and after H_2O_2 treatment. SIPMs dispersion (0.4 mg/mL) was incubated with or without 10 equiv. H_2O_2 for 24 h under stirring.

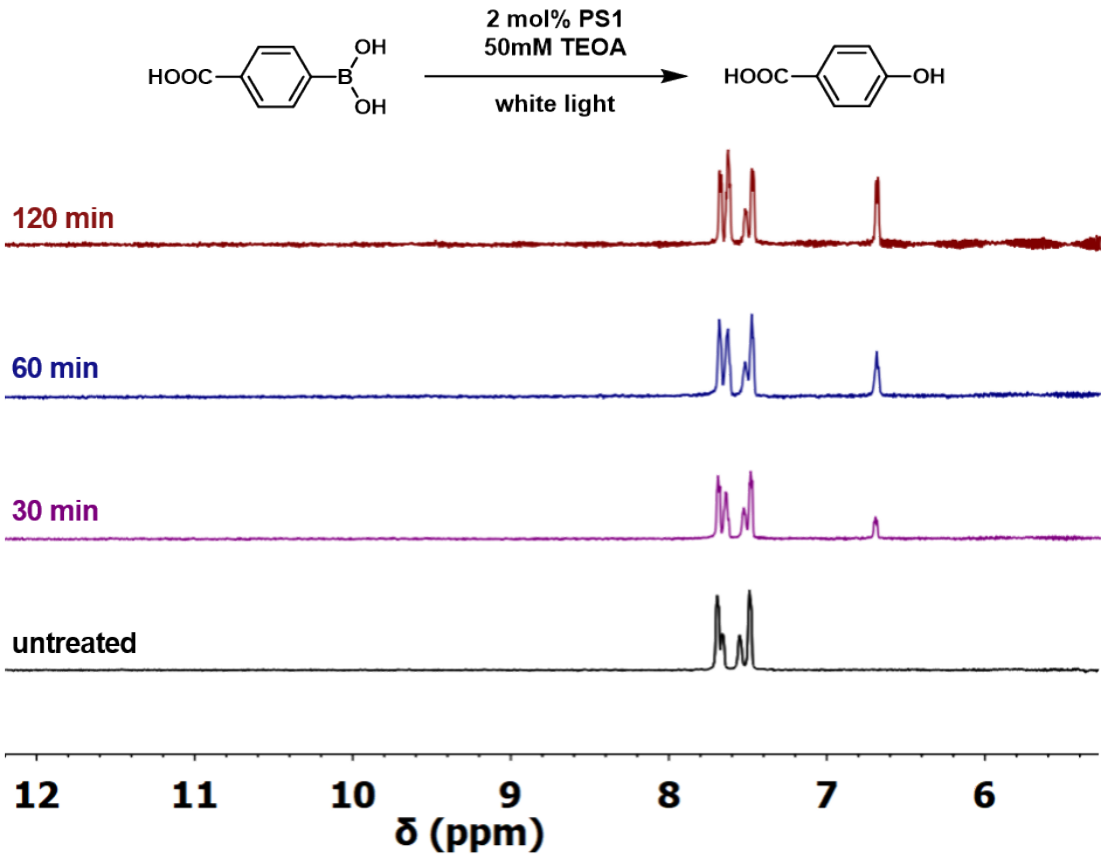

Figure S13. Time-dependent profile of PS1-catalyzed oxidative hydroxylation of 4-carboxyphenylboronic acid. Substrate conversions reached 25%, 41%, and 48% at 30, 60, and 120 min, respectively.

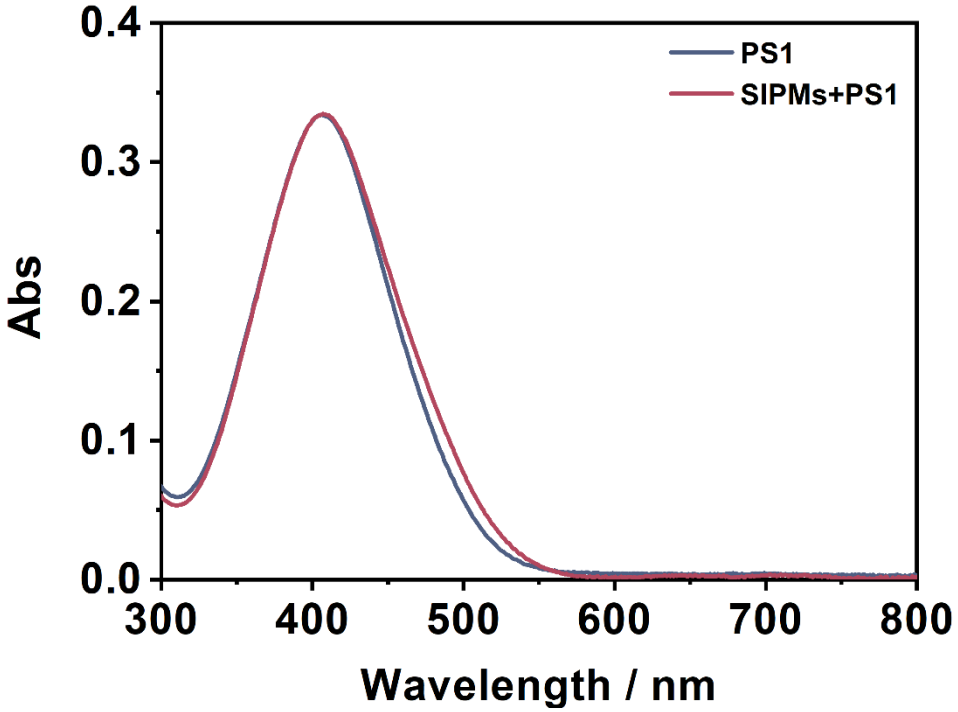

Figure S14. UV-Vis absorption spectra of PS1 (25 μM in DI water) in the absence and presence of SIPMs (0.5 mg/mL).

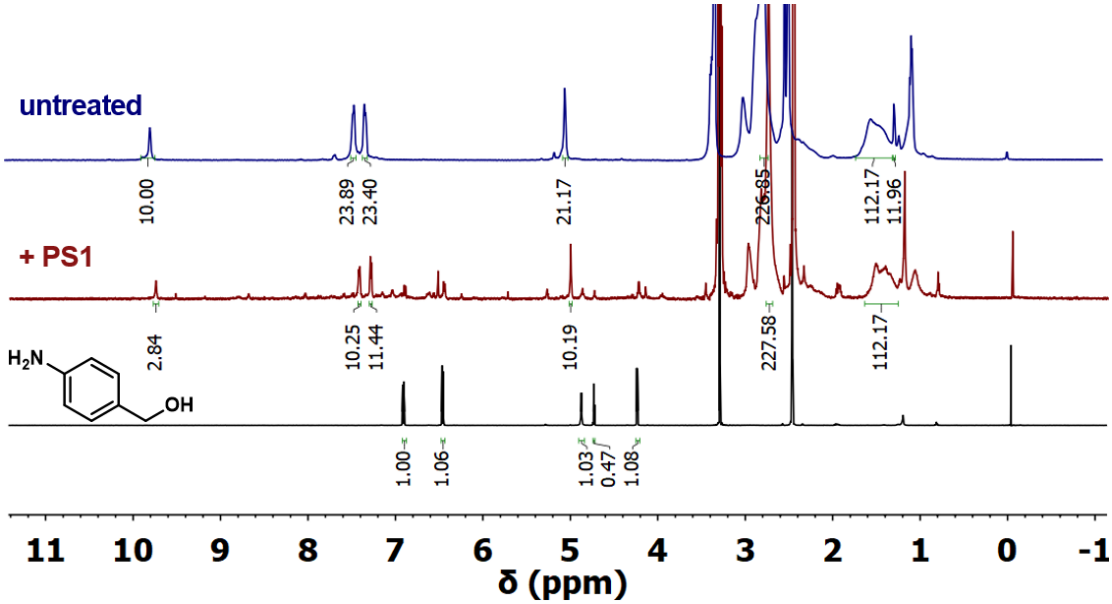

Figure S15. ^{1}H NMR spectra recorded in DMSO-d_6 of 4-Aminobenzyl alcohol and SIPMs under different treatment conditions. SIPMs dispersion (0.4 mg/mL) with or without 0.5 equiv. PS1 was subjected to xenon lamp irradiation (75 mW·cm^{-2}, 3 h) followed by 24 h stirring.

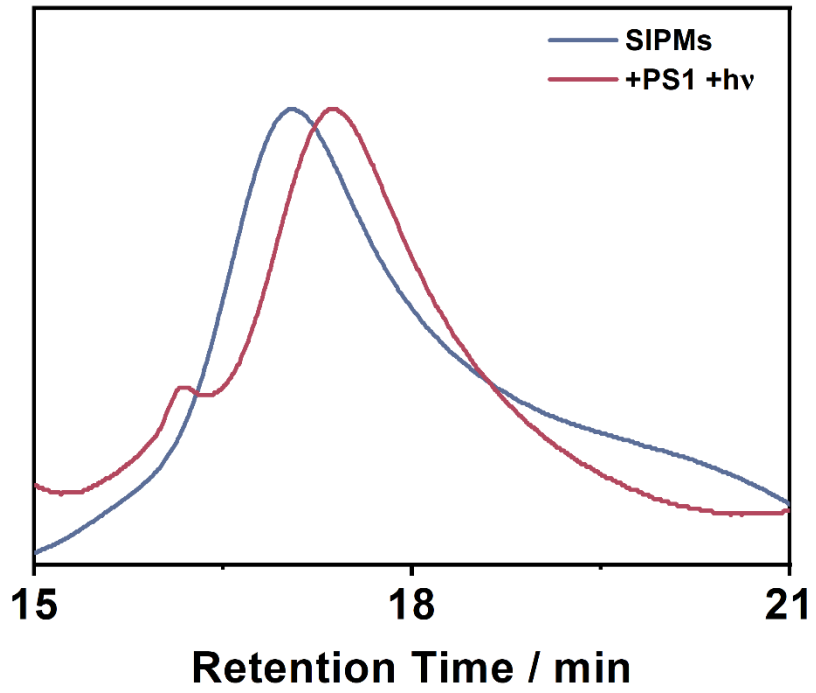

Figure S16. GPC trace of SIPMs under different treatment conditions. SIPMs dispersion (0.4 mg/mL) with or without 0.5 equiv. PS1 was subjected to white light irradiation (75 mW·cm^{-2}, 3 h) followed by 24 h stirring.

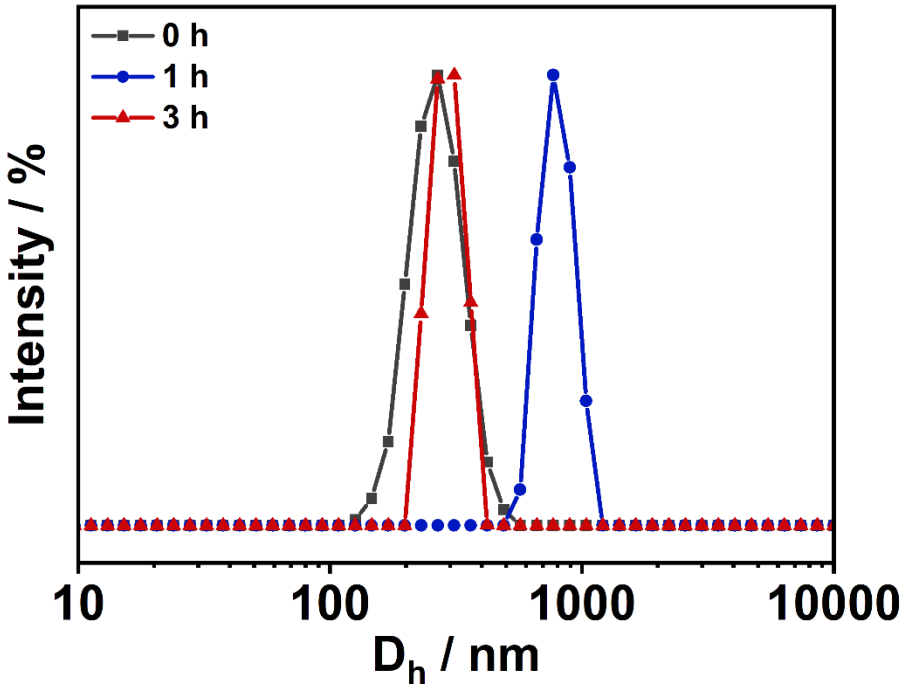

Figure S17. DLS plots of SIPMs mixed with PS1 upon light irradiation (75 mW·cm^{-2}) at different time.

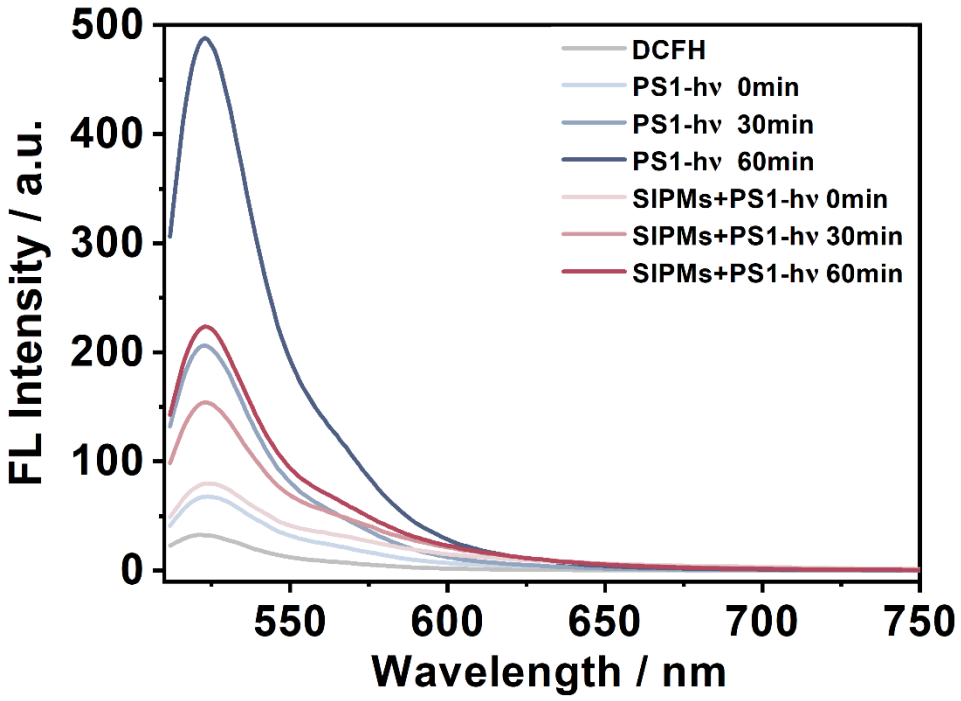

Figure S18. ROS generation from PS1 with or without SIPMs upon white light irradiation (75 mW·cm^{-2}), using DCFH (40 μM) as an indicator.

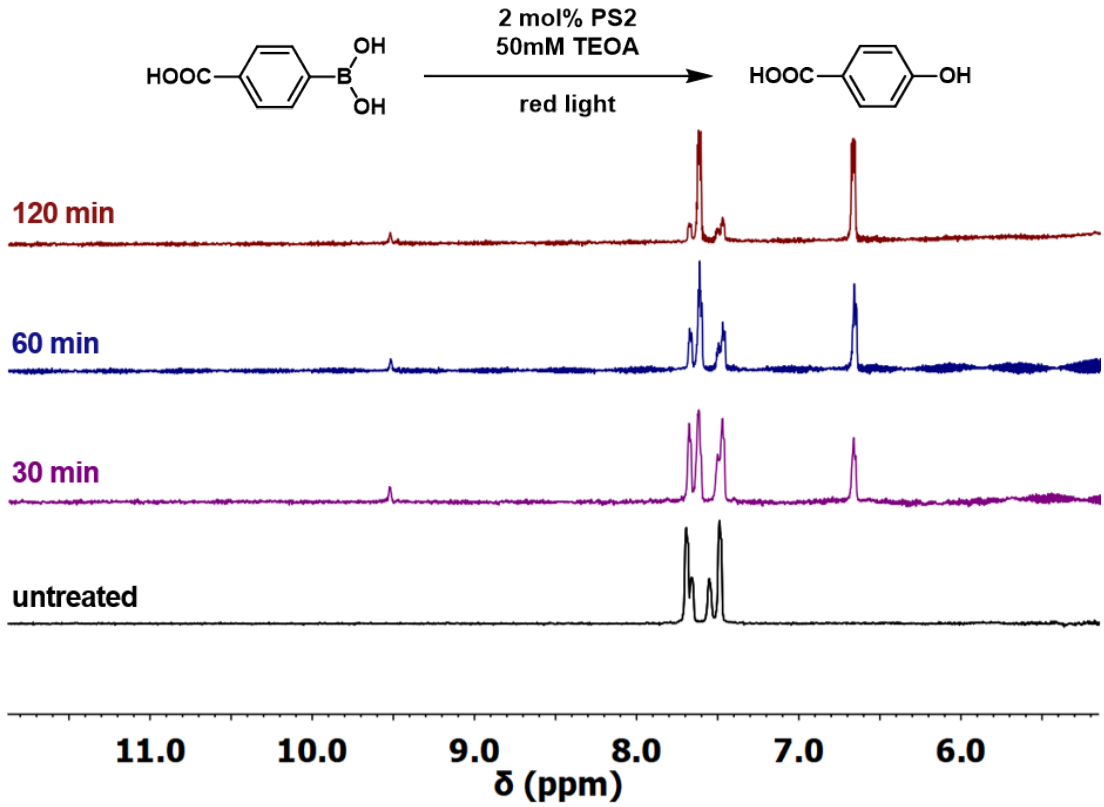

Figure S19. Time-dependent profile of PS2-catalyzed oxidative hydroxylation of 4-carboxyphenylboronic acid. Substrate conversions reached 46%, 68%, and 88% at 30, 60, and 120 min, respectively.

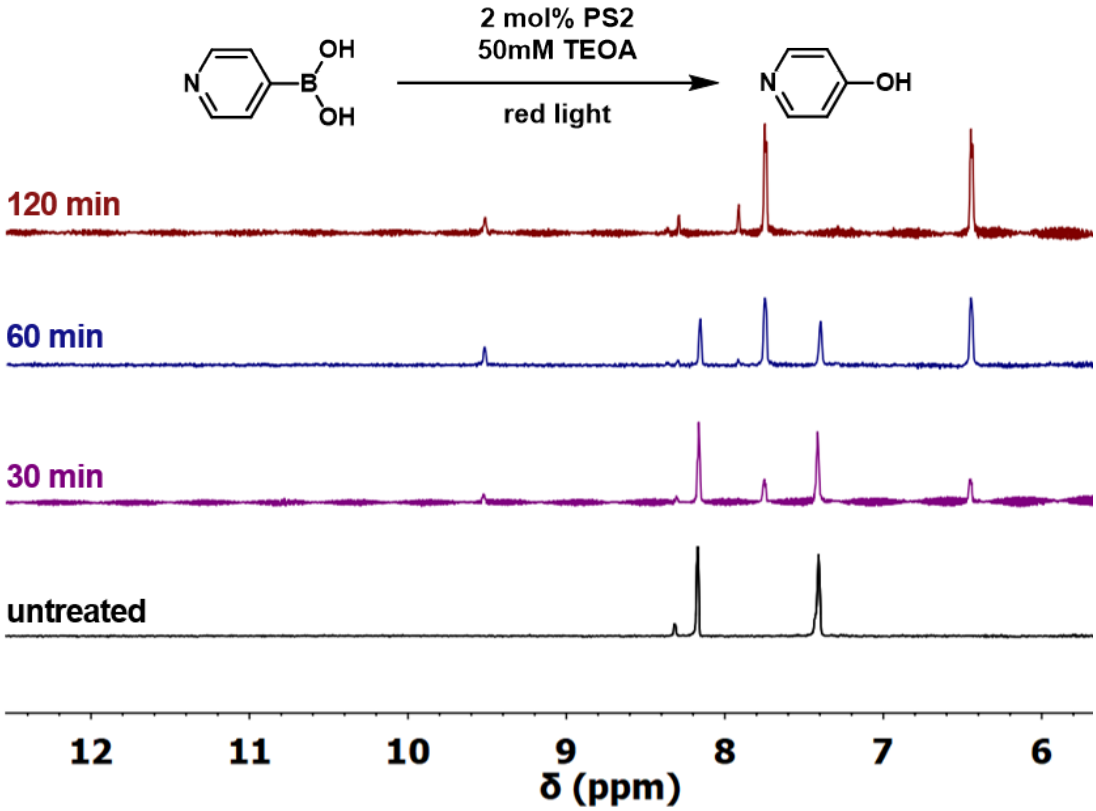

Figure S20. Time-dependent profile of PS2-catalyzed oxidative hydroxylation of 4-Pyridineboronic acid. Substrate conversions reached 27%, 65%, and 99% at 30, 60, and 120 min, respectively.

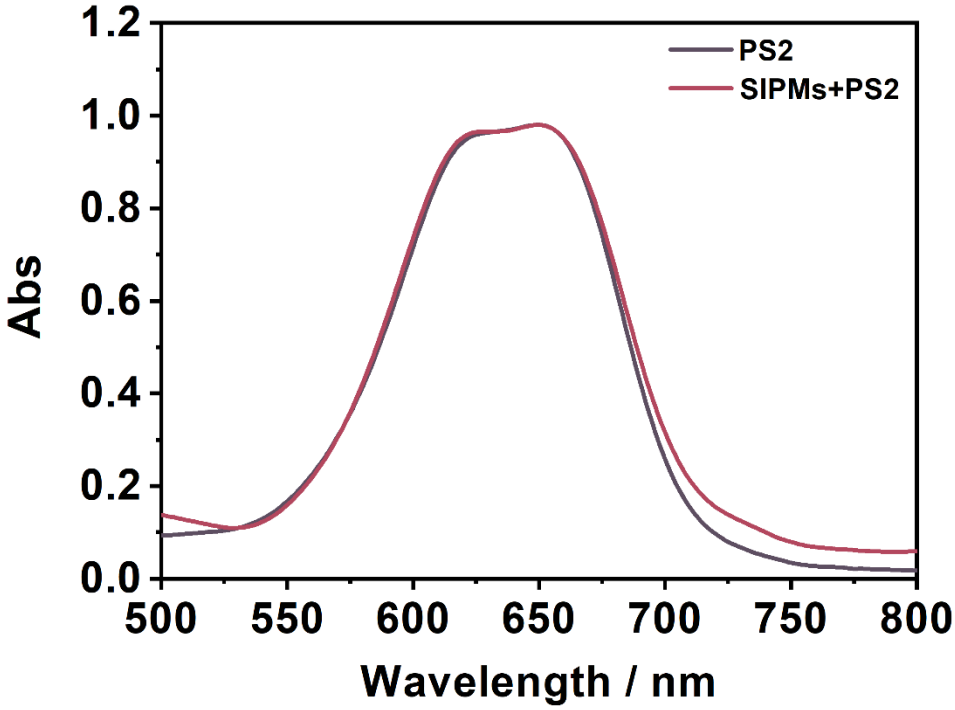

Figure S21. UV-Vis absorption spectra of PS2 (25 μM in DI water) in the absence and presence of SIPMs (0.5 mg/mL).

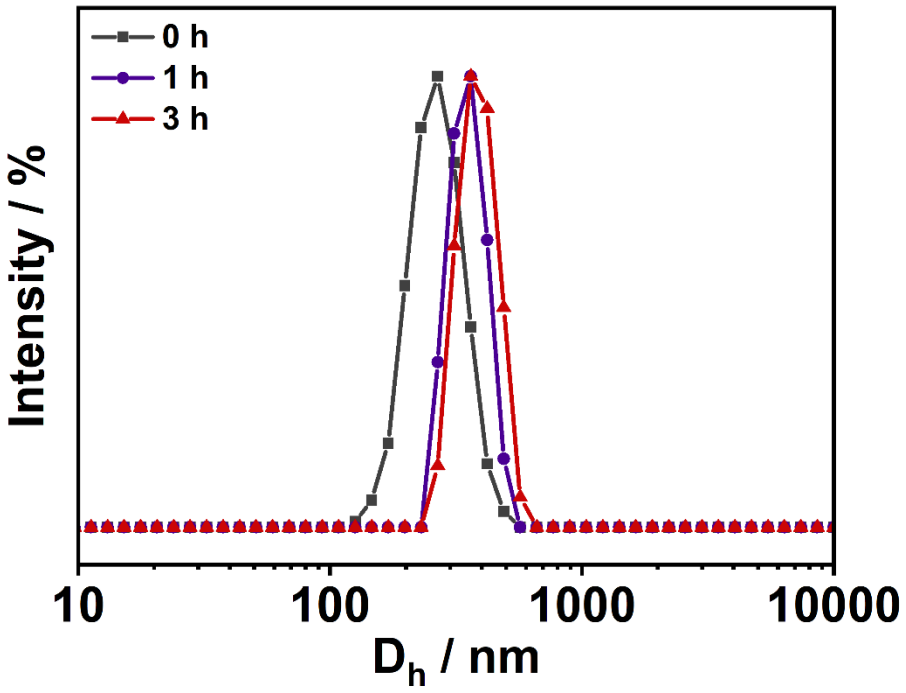

Figure S22. DLS image of SIPMs mixed with PS2 upon red light irradiation (45.6 mW·cm^{-2}) at different time.

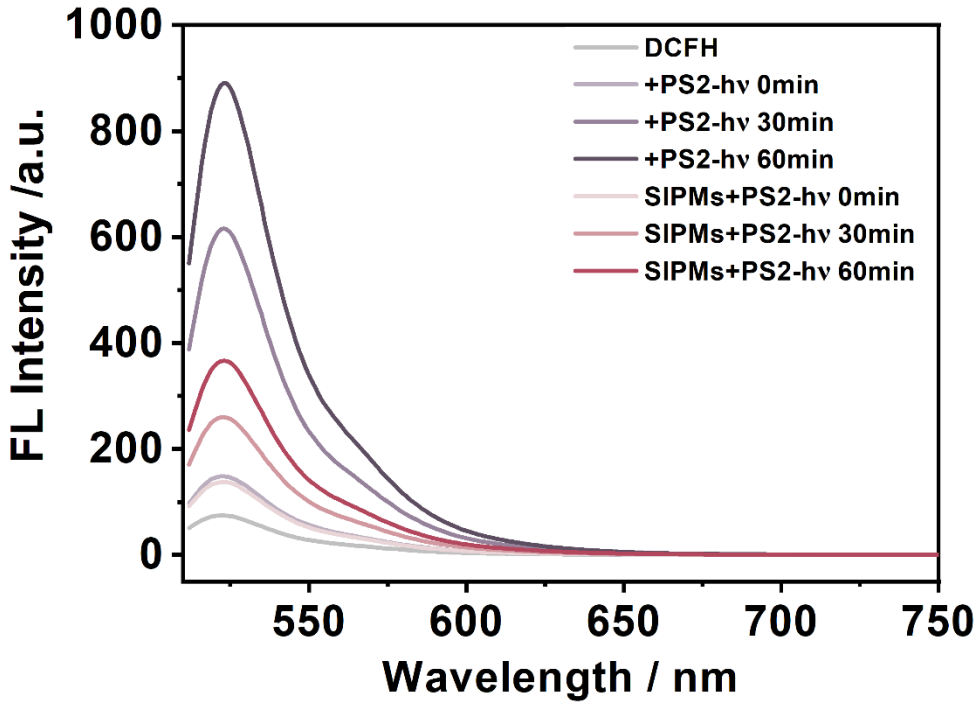

Figure S23. ROS generation from PS2 with or without SIPMs upon red light irradiation, using DCFH (40 μM) as an indicator.

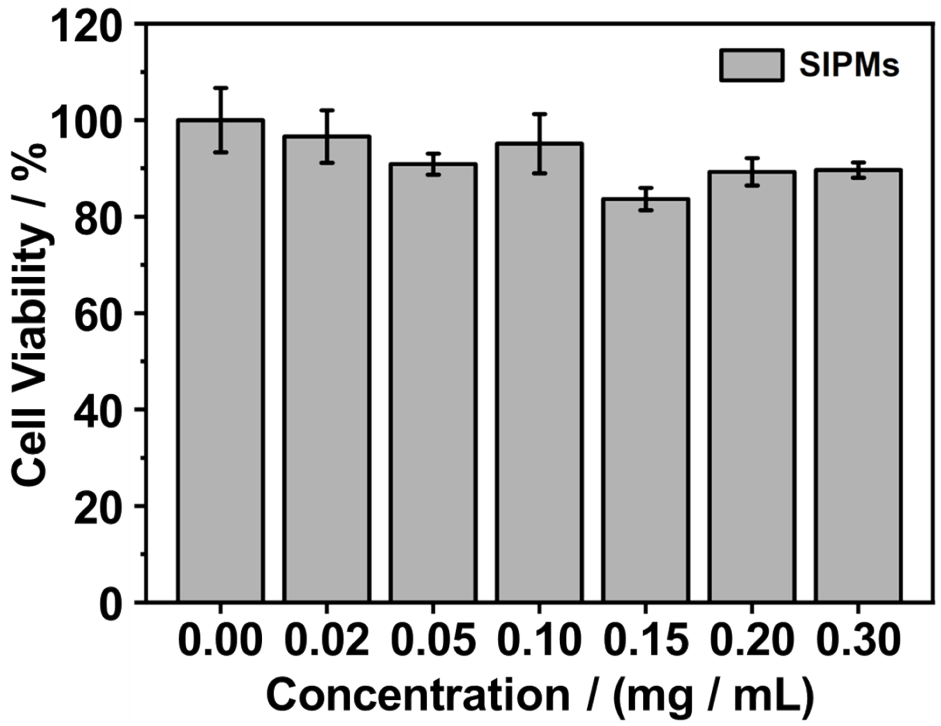

Figure S24. Cytotoxicity assay of SIPMs at different concentrations in A549 cells.

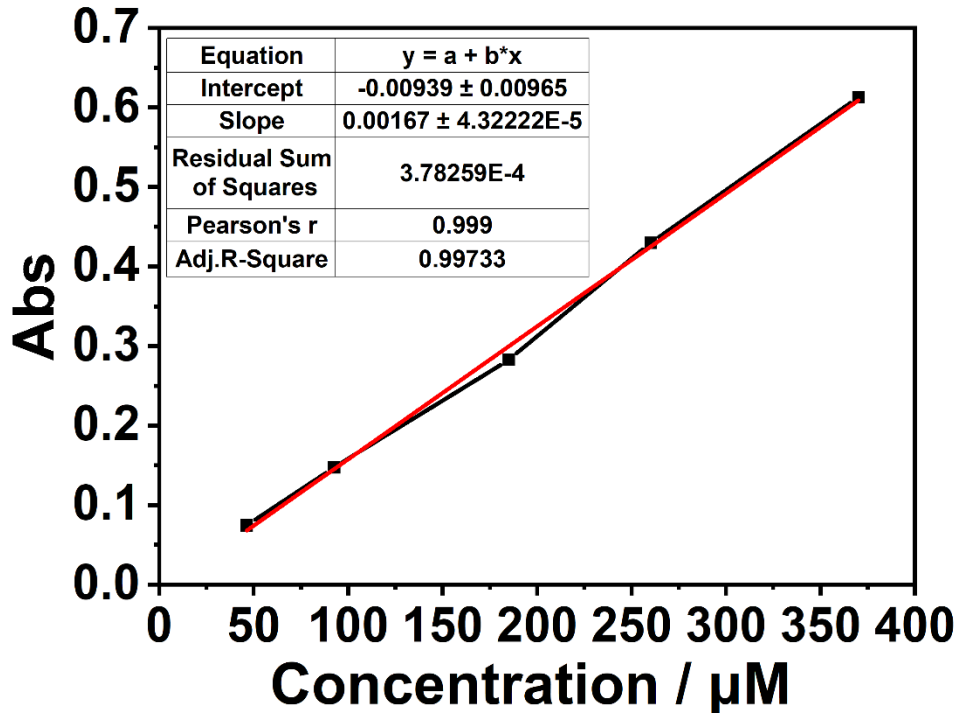

Figure S25. UV–Vis absorption of doxorubicin in DMSO/water solution (prepared by dispersing a DMSO stock solution into deionized water).

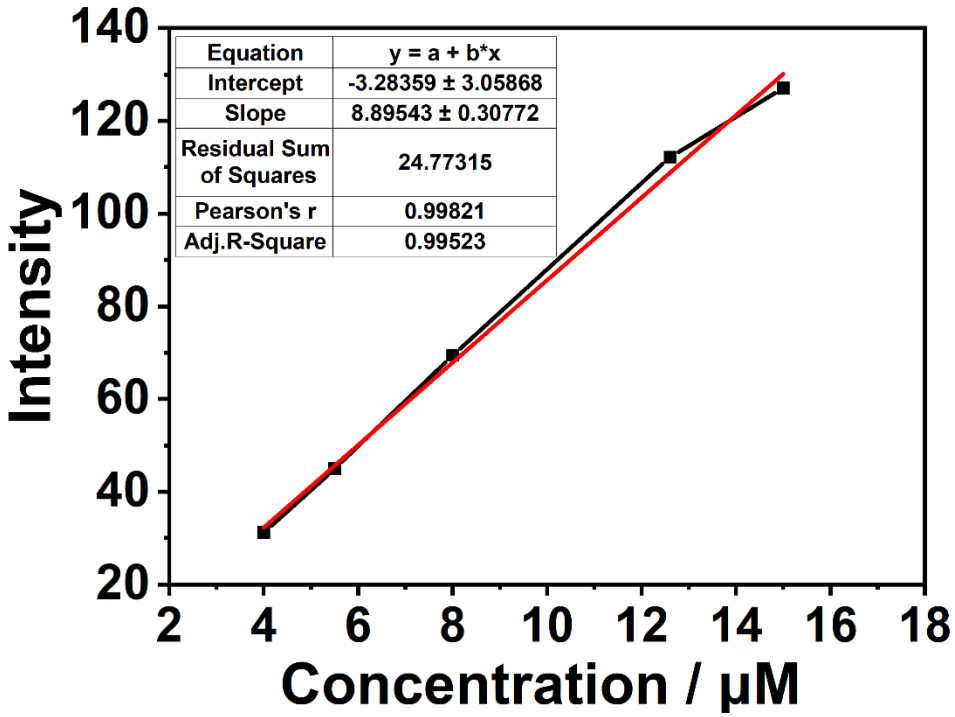

Figure S26. Fluorescence standard curve of Evodiamine in DMSO.

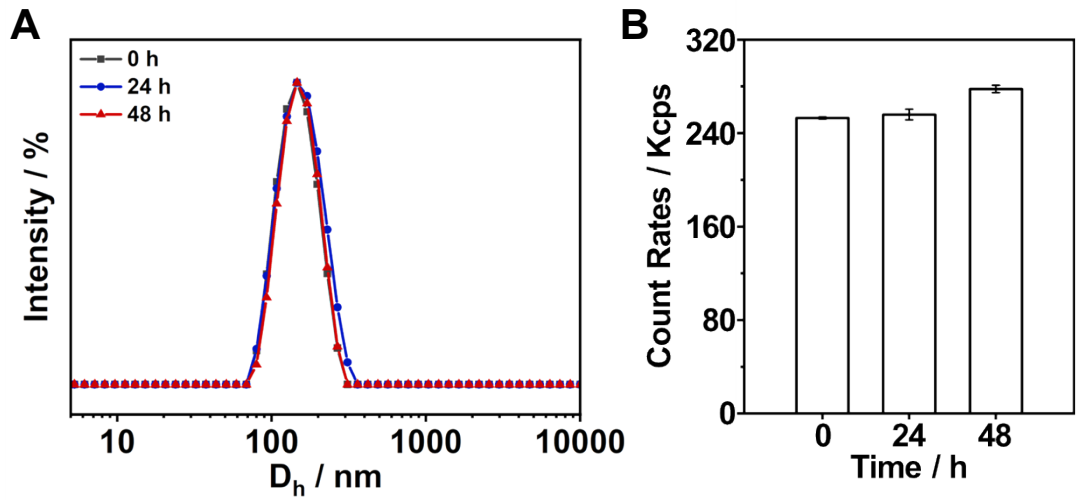

Figure S27. Stability of DOX-loaded and PS1-incorporated SIPMs in 10% FBS-containing medium. (A) Hydrodynamic diameter distribution; (B) Count rates image of the micelles measured by DLS at 0 h, 24 h, and 48 h of incubation. SIPMs: 0.2mg/mL, PS1: 5 μM.

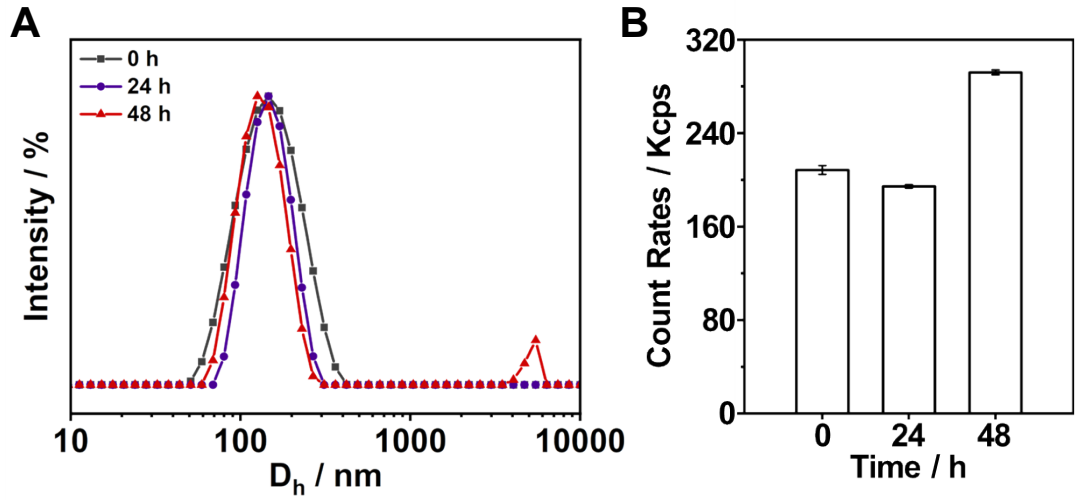

Figure S28. Stability of Evo-loaded and PS2-incorporated SIPMs in 10% FBS-containing medium. (A) Hydrodynamic diameter distribution; (B) Count rates image of the micelles measured by DLS at 0 h, 24 h, and 48 h of incubation. SIPMs: 0.2mg/mL, PS2: 62.5 nM.

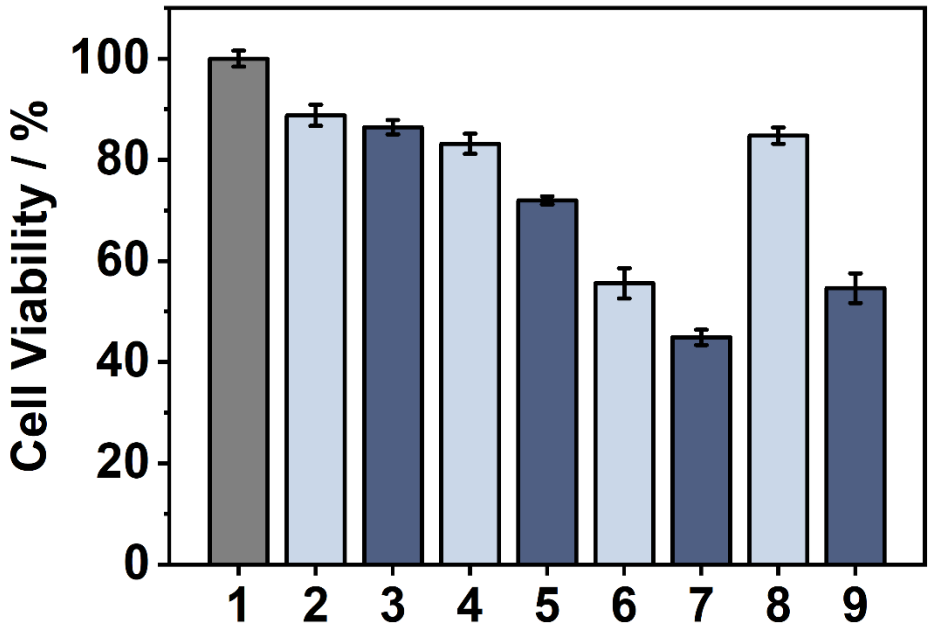

Figure S29. Cytotoxicity assay of various PS1-based formulations. 1: untreated; 2: DOX@SIPMs; 3: DOX@SIPMs+hv; 4: SIPMs+PS1; 5: SIPMs+PS1+hv; 6: DOX+PS1; 7: DOX+PS1+hv; 8: DOX@SIPMs+PS1; 9: DOX@SIPMs+PS1+hv. White light: 40 $mW \cdot cm^{-2}$, 12 min. Concentrations: DOX: 5 μM; PS1: 5 μM.

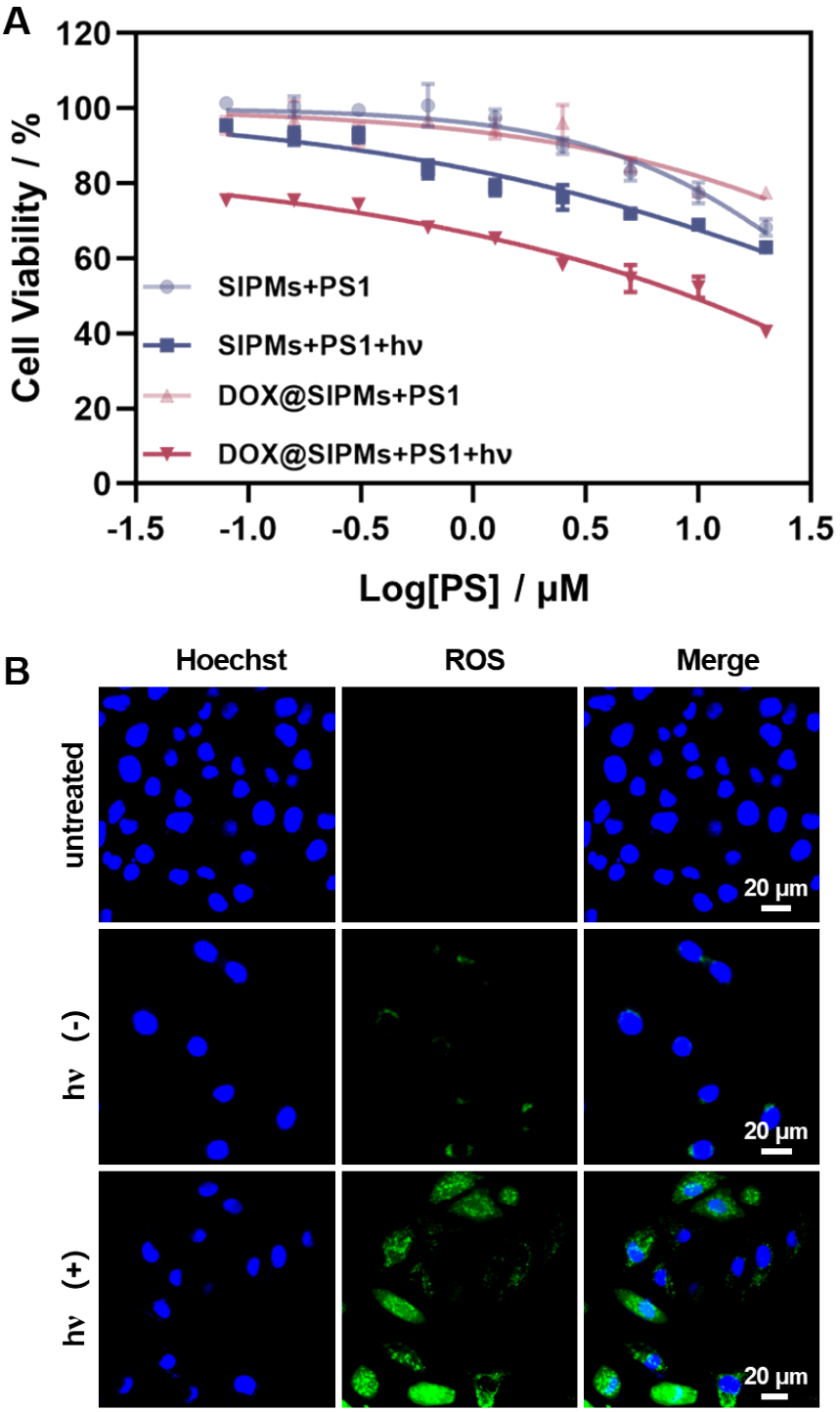

Figure S30. Cytotoxicity and therapeutic efficacy evaluation of drug-loaded SIPMs with Type I PS1 in A549 cells. (A) Cytotoxicity assay of SIPMs+PS1, DOX@SIPMs+PS1 with and without white light irradiation (40 mW·cm^{-2}, 12 min). (B) CLSM images of intracellular reactive oxygen species (ROS) of DOX@SIPMs+PS1 with and without white light irradiation in cells, PS1: 5 μM, DOX: 5 μM. Green: oxidized DCF (ROS); Blue: Hoechst 33342 (cell nuclei). Scale bar, 20 μm.

Time / h	PDI (DOX@SIPMs+PS1)	PDI (Evo@SIPMs+PS2)
0	0.19±0.01	0.22±0.01
24	0.23±0.01	0.28±0.03
48	0.32±0.04	0.40±0.01

Table S1. Polydispersity Index (PDI) of DOX@SIPMs+PS1 and Evo@SIPMs+PS2 after incubation in 10% FBS-containing medium for 0, 24, and 48 h. SIPMs: 0.2mg/mL, PS1: 5 μM, PS2: 62.5 nM.

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