Electronic Supplementary Information for

Visible Light-Driven Photogeneration of Hydrogen Sulfide

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Experimental Details

Materials and General Methods

Commercially available chemicals were used as received unless otherwise stated. Pluronic F-127, phosphate buffered saline (PBS), cetrimonium bromide (CTAB), lead(II) acetate, and platinum(II) octaethylporphyrin (PtOEP) were purchased from Sigma-Aldrich. Na_2S and 1,3-diphenylisobenzofuran were purchased from TCI. IrOMe[1] and SF4[2] were prepared following the literature procedures. PBS buffered solution (10 mM) was prepared in milli-Q grade water from a Milli-Q Direct 16 system (18.2 MΩ·cm; Merck KGaA, Darmstadt, Germany), and by adjusting the pH to 7.4 with standard KOH (45 wt %, Sigma-Aldrich) and HCl (1 N, Fluka) solutions. 1.0 mM CTAB was included in the PBS solution.[3] Trace metal contamination was removed from the buffered solution by treating with Chelex resin (Bio-Rad), prior to the addition of CTAB. Stock solutions of DPBT were dissolved in DMSO (Sigma-Aldrich, biotech grade) to 10 mM. ^{1}H and ^{13}C{^{1}H} NMR spectra were collected with Ultrashield 500 and 300 plus NMR spectrometers (Bruker). Chemical shifts were referenced to $(CH_3)_4Si$. High resolution mass spectra (positive mode, FAB, *m*-NBA) were obtained by employing a JMS-600W mass spectrometer (JEOL).

Synthesis of DPBT. DPBT was synthesized through the modified procedure of the two-step synthesis reported by Lin and a co-worker.[4] 1,3-Diphenylisobenzofuran (3.70 g, 13.7 mmol) and Irppy[1] (11.3 mg, 13.7 μmol) were added into a 250 mL 1-neck round-bottom flask. After the addition of CH_2Cl_2 (150 mL) into the flask, the solution was stirred under continuous photoirradiation at 365 nm under an aerobic condition for 2 d. The reaction mixture was concentrated *in vacuo*. Column purification was performed on silica gel using hexane:EtOAc = 3:1 (v/v) as an eluent to afford 2-benzoylbenzophenone as a white powder in a 64% yield. R_f = 0.5 (hexane:EtOAc = 3:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.38 (t, J = 7.8 Hz, 4H), 7.52 (t, J = 7.5 Hz, 2H), 7.62 (s, 4H), 7.70 (m, 4H). ^{13}C{^{1}H} NMR (126 MHz, CD_2Cl_2) δ (ppm): 128.47, 129.81, 129.97, 130.50, 133.14, 137.32, 140.16, 196.72. 2-Benzoylbenzophenone (0.500 g, 1.75 mmol) and P_2S_5 (0.389 g, 1.75 mmol) were added into a 50 mL 2-neck round-bottom flask. Anhydrous pyridine (20 mL) was delivered into the flask under an Ar atmosphere, and then, the reaction mixture was heated at 115 °C for 24 h. After cooling to room temperature, the solution was concentrated under a reduced pressure. Column purification was performed on silica gel using hexane:EtOAc = 19:1 (v/v) as an eluent to give DPBT as a yellowish white powder in a 76% yield. R_f = 0.6 (hexane:EtOAc = 4:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.10 (m, 2H), 7.39 (t, J = 3.9 Hz, 2H), 7.48 (t, J = 8.1 Hz, 4H), 7.70 (m, 4H), 7.84 (m, 2H). ^{13}C{^{1}H} NMR (126 MHz,

CD_2Cl_2) δ (ppm): 121.61, 124.83, 128.06, 129.61, 129.70, 134.66, 134.78, 135.75. HR MS (FAB, positive, *m*-NBA): Calcd for $C_{20}H_{14}S$ ($[M+H]^+$), 287.0894; found: 287.0848.

Preparation of Polymersomes. 736 mg Pluronic F-127, 500 μL from a 10 mM DPBT solution in CH_2Cl_2, and 10 μL from a 10 mM $^1O_2$ PS solution in CH_2Cl_2 were added into a 20 mL glass vial, and fully dissolved in 10 mL CH_2Cl_2. The doping ratios of DPBT and the $^1O_2$ PS corresponded to 0.2 and 0.01 wt %, respectively. 20 μL from a 10 mM SF4 solution in DMSO (a 0.01 wt % doping ratio) was added for the fluorescent detection of H_2S. Slow removal of CH_2Cl_2 with employing a rotavap yielded a thin film of a polymer solid solution. Then, 10 mL of a buffered aqueous solution (pH 7.4; 10 mM PBS + 1.0 mM CTAB) was delivered into the vial. The solution was vortexed vigorously for 10 min. The hazy suspension was used for the H_2S photogeneration experiments.

Steady-State UV–vis Absorption Measurements. UV−vis absorption spectra were collected on a Cary 300 spectrophotometer (Agilent) at room temperature. 2 μM or 10 μM solutions were used for the measurements unless otherwise stated.

Comparison of the Reactivity of DPBF and DPBT with Singlet Oxygen. O_2-saturated DMSO solutions containing 100 μM IrOMe and 10 μM DPBF or 10 μM DPBT were photoirradiated at 350 nm (Xenon lamp equipped with a 350 nm bandpass filter). Decreases in the absorbance (Δ *Abs*) at 420 nm (DPBF and DPBT) were monitored during the continuous photoirradiation. Plots of Δ *Abs* as a function of photoirradiation time were constructed, and the initial four data points were fit to a linear line. A ratio of slope values of the linear fits for DPBF over DPBT was 13. The rate constant for the reaction of DPBT with singlet oxygen was calculated from the ratio and the literature value of the rate constant for the reaction of DPBF with singlet oxygen (9.6×10^8 M^{-1} s^{-1}).[5]

HPLC Experiments to Monitor the Reaction of DPBT. An O_2-saturated CH_3CN solution (10 mL) containing 1.0 mM DPBT and 100 μM IrOMe was photoirradiated at 380 nm with a Xenon lamp. An aliquot of 1.0 mL was taken from the solution at five-minute intervals, and was subjected to HPLC-ESI-MS analysis (Agilent, 6120DW LC/MSD equipped with a Poroshell, EC-C18 (2.1 × 100 mm, 2.7 μm); eluent condition: H_2O:CH_3CN = 50:50 to 100% CH_3CN). DPBT, IrOMe, and the $^1O_2$-oxidation product of DPBT were observed at elution times of 14.67, 1.67, and 4.00 min, respectively.

H_2S Assay with Employing Filter Papers Adsorbed with $Pb(OAc)_2$. Whatman filter papers were soaked in 3.0 mL milli-Q grade water saturated with $Pb(OAc)_2$.[6] The papers were dried in a convection oven. Colorimetric responses to sulfides were visualized by dipping the paper into milli-Q grade water having different concentrations of Na_2S (0, 250, 500, and 1000 μM). Visual inspection of the treated papers indicated that a response was visible at $[Na_2S]_{added} > 250$ μM. The $Pb(OAc)_2$-treated filter paper was employed to estimate the concentration of sulfides in a photoirradiated (λ_{irr} = 380 nm; 30 min) aqueous solution containing polymersomes doped with 0.2 wt % DPBT and 0.01 wt % IrOMe.

Steady-State Photoluminescence Measurements. Photoluminescence spectra were obtained using a Quanta Master 400 scanning spectrofluorometer (Photon Technology International, Edison, NJ, USA) at room temperature (organic solutions) or at 37 °C (buffered aqueous solutions). The temperature was maintained by employing a water circulator. The excitation wavelength for recording the SF4 fluorescence was 496 nm.

Dynamic Light Scattering Experiments. Distributions of the diameter of 10 μM polymersome suspensions were determined through dynamic light scattering (DLS) experiments by using an ELS-Z1000 instrument (Photal Otsuka Electronics, Japan) at room temperature. Data analyses were performed with employing the software provided by the manufacturer.

Field-Emission Scanning Electron Microscopy Experiments. An aliquot of the polymersome suspension was placed on a 1 cm × 1 cm glass substrate, and dried under dark. The field-emission scanning electron micrographs of the polymersomes were obtained using a SUPRA 55VP equipment (Carl Zeiss, Germany), after platinum coating (120 s) using an EM ACE200 (Leica, Austria).

Construction of the Calibration Curve for Converting the Integrated Photoluminescence Intensity Curve into the H_2S Concentration. Photoluminescence spectra of 20 μM SF4 (buffered solution containing 10 mM PBS and 1.0 mM CTAB; pH 7.4) were integrated over λ_{ems} = 500–650 nm after the addition of increased concentrations of Na_2S. The H_2S concentration ($[H_2S]$) was calculated, considering the following protonation equilibria:

$$H_2S(aq) \rightleftharpoons HS^-(aq) + H^+(aq) \qquad pK_{a1} = 7.0$$

$HS^-(aq) \rightleftharpoons S^{2-}(aq) + H^+(aq)$ $\quad\quad\quad\quad$ $pK_{a2} > 14.0$

Generation of S^{2-} is neglected, because its pK_{a2} is significantly larger than pK_{a1}. Combining the equilibrium equation for the first protonation and a mass balance equation for the total sulfide (i.e., $[Na_2S]_{added} = [H_2S] + [HS^-] + [S^{2-}]$) yielded the following equation for $[H_2S]$ at pH 7.4.

$$[H_2S] = [Na_2S]_{added} / (1+10^{0.4}) \quad \text{(eq S1)}$$

Determination of Photochemical Quantum Yields for H_2S Generation. The quantum yields for photogeneration of H_2S were determined with employing the standard ferrioxalate actinometry. 500 μL of 6.0 mM ferrioxalate solutions were photoirradiated at 350, 380, 410, 440, 470, 500, 550, or 580 nm for 30 s. These excitation wavelengths were selected from Xenon lamp after passing motorized bandpass filters. The photoirradiated solutions were mixed with 500 μL of a sodium acetate buffer and 500 μL of a phenanthroline solution (1.0 wt % in milli-Q grade water), and then, incubated for 1 h under dark. Absorbance at 510 nm was recorded, and inserting the value (ΔA) into eq S2 returned photon flux(λ).

$$\text{Photon flux}(\lambda)\ (\text{einstein s}^{-1}) = (\Delta A \times 1.5 \times 10^{-3}) / (\Phi(\lambda) \times 11100\ \text{M}^{-1}\ \text{cm}^{-1} \times 30\ \text{s}) \quad \text{(eq S2)}$$

In eq S2, $\Phi(\lambda)$ was 1.1. Calculated photon flux values were 1.3×10^{-8} einstein s^{-1} (350 nm), 7.4×10^{-9} einstein s^{-1} (380 nm), 5.3×10^{-9} einstein s^{-1} (410 nm), 3.7×10^{-9} einstein s^{-1} (440 nm), 2.8×10^{-9} einstein s^{-1} (470 nm), 2.6×10^{-9} einstein s^{-1} (500 nm), 3.2×10^{-9} einstein s^{-1} (550 nm), and 3.2×10^{-9} einstein s^{-1} (580 nm).

A 3.0 mL of an O_2-equilibrated, buffered aqueous solution (pH 7.4; 10 mM PBS + 1.0 mM CTAB) of Pluronic F-127 polymersomes containing 0.2 wt % DPBT, 0.01 wt % IrOMe, and 0.01 wt % SF4 was photoirradiated for 20 min at each excitation wavelength. Photoluminescence spectrum of the photoirradiated solution was measured and integrated over the SF4 emission range (λ_{ems} = 500–650 nm). The integrated fluorescence intensity was converted into $[H_2S]$, employing the calibration curve shown in Figure S10. Substituting the $[H_2S]$ value into eq S3 yielded the quantum yields.

$$\text{Quantum yield}(\lambda) = ([H_2S] \times 3 \times 10^{-3}) / (\text{photon flux}(\lambda) \times 1200\ \text{s}) \quad \text{(eq S3)}$$

Amperometric Detection of Photogenerated H_2S. Amperometric measurements were performed with employing a World Precision Instruments, ISO-H_2S-100 microsensor. Prior to measurements, the amperometer was calibrated using an aqueous solution buffered with PBS (pH 7.2; 10 mL) with increasing the concentration of Na_2S. An O_2-saturated polymersome suspension (5 mL) containing 0.2 wt % DPBT and 0.01 wt % IrOMe was delivered into a 20 mL vial which was sealed with a rubber septum. The microsensor was inserted into the solution through a hole of the septum to prevent gas exchange. The polymersome suspension was photoirradiated, during which amperometric responses to H_2S were recorded through a World Precision Instruments, Lab-Trax-4/16.

Preparation of Reactive Oxygen Species.

1) H_2O_2: An aqueous solution of 30 wt % H_2O_2 (Sigma–Aldrich) was diluted in milli-Q water to a concentration of 500 μM. The molar absorbance of H_2O_2, $\varepsilon = 44\ M^{-1}\ cm^{-1}$, was employed to determine the concentration.
2) •OH: The 500 μM H_2O_2 solution was mixed with 2.5 mM $FeSO_4$ (*aq*), and the mixture was incubated for 10 min. The incubated solution was used without additional treatments.
3) $O_2^{\bullet-}$: KO_2 was dissolved in milli-Q water to a concentration of 500 μM.
4) OCl^-: A NaOCl solution was purchased from Sigma–Aldrich, and diluted to a 500 μM concentration in milli-Q water. The concentration was determined, using the molar absorbance, $\varepsilon = 360\ M^{-1}\ cm^{-1}$.
5) *t*-BuOOH: A commercially available Luperox® TBH70X, *t*-BuOOH solution (Sigma–Aldrich) was diluted in milli-Q water to a concentration of 500 μM.
6) *t*-BuO•: The 500 μM *t*-BuOOH solution was mixed with 2.5 mM $FeSO_4$ (*aq*), and the mixture was incubated for 10 min. The incubated solution was used without additional treatments.
7) GSNO: GSNO (Sigma–Aldrich) was dissolved in milli-Q water to a concentration of 500 μM.
8) NO: Diethylamine NONOate sodium salt hydrate was purchased from Sigma–Aldrich, and dissolved in milli-Q water to a concentration of 500 μM.
9) HNO: Angeli's salt was purchased from Calbiochem, and dissolved in milli-Q water to a concentration of 500 μM.
10) $ONOO^-$: 0.6 M $NaNO_2$ (*aq*) was acidified with 0.6 M HCl, and 1.5 M KOH (*aq*) was added subsequently. After the addition of an excess amount of MnO_2, the solution was employed for H_2S generation without additional treatments

Cell Culture. HeLa cells purchased from the Korean Cell Line Bank were cultured in RPMI (Gibco) supplemented with 10% fetal bovine serum and penicillin (100 units/mL) at 37 °C in a humidified incubator under 5% CO_2.

MTT Cell Proliferation Assay. Cell proliferation assays were performed, following the protocol provided by the manufacturer (Promega, CellTiter 96® Non-Radioactive Cell Proliferation Assay). HeLa cells were seeded into 96-well plates two days prior to the experiment. The cells were incubated with polymersome (0–61 mg mL^{-1}, 100 μL per well, 15 min incubation). 15 μL of the MTT reagent was added to each well, and the cells were further incubated for 3 h at 37 ºC. Finally, a 100 μL stop solution was delivered to the wells. After additional 1 h incubation, absorbance at 570 nm was recorded using a MiniMax 300, microplate reader.

Fluorescence Microscopic Visualization of Photoproduced Intracellular H_2S. Polymersome suspensions (containing 0.2 wt % DPBT and 0.01 wt % PtOEP) were diluted in serum-free RPMI (Gibco) to a 1:10 ratio (v/v). A stock solution of 10 mM SF4 was prepared in DMSO (Sigma-Aldrich, biotech grade). One day prior to imaging, HeLa cells were plated onto glass-bottom culture dishes (SPL Life Sciences, Korea). The cells were washed and supplemented with fresh PBS (Welgene, Korea). The polymersomes diluted in serum-free RPMI were added into the culture dish, and the cells were incubated for 15 min at 37 °C. The cells were washed twice with fresh PBS to remove remaining polymersomes. The cells were further treated with 10 μM SF4 for 15 min at 37 °C, and washed with PBS. Confocal laser-scanning microscopy experiments were performed before and after the green-LEDs photoirradiation (λ_{irr} ~ 500 nm, 1 W). A Carl Zeiss LSM 510 META confocal laser scanning microscope was used to obtain fluorescence images. An excitation beam (488 nm) was focused onto the dish, and the signals were acquired through 30 emission channels covering the range 410–691 nm. Fluorescence images and mean intensities were analyzed using the LSM 510 version 4.0 software.

Inhibition of Acute Apoptosis by Intracellular Photogeneration of H_2S. A fluorescent apoptosis marker, Annexin V-FITC, and a fluorescent necrosis marker, propidium iodide (PI), were purchased from Sigma-Aldrich. HeLa cells were treated with polymersomes, following the methods identical to those for the fluorescence H_2S visualization experiments described above. After washing with fresh PBS (twice), 1.0 mL serum-free RPMI and 102 μL binding buffer (Sigma-Aldrich) were added. Then, the cells were photoirradiated at λ_{irr} ~ 500 nm using green LEDs (1 W)

for 5 min. A 5 μL (7 nM) Annexin V-FITC and a 10 μL (2 μM) PI were added into the culture dish, and the cells were incubated for additional 10 min under dark. 0.2 wt % H_2O_2 (Sigma-Aldrich, 30 wt %) was delivered into the dish to induce acute apoptosis. Control cells were prepared identically, except the treatment with the polymersomes. Fluorescence micrographs were obtained using a Carl Zeiss LSM 510 META confocal laser scanning microscope. Fluorescence emissions were acquired at λ_{ex} = 485 nm and λ_{obs} = 538–546 nm for Annexin V-FITC and at λ_{ex} = 493 nm and λ_{obs} = 625–634 nm for PI. Fluorescence images were analyzed using the LSM 510 version 4.0 software.

References

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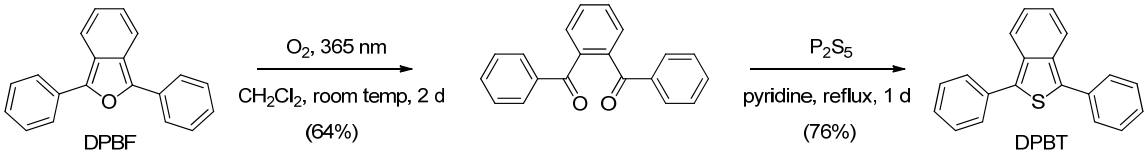

Scheme S1. Synthesis of DPBT.

Table S1. Photophysical Data for DPBT[a]

	λ_{abs} (nm; logε)	λ_{ems} (nm)[b]	PLQY[c]
DPBT	398 (4.11)	489	0.75

[a]10 μM DMSO. [b]λ_{ex} = 398 nm. [c]9,10-Diphenylanthracene standard (PLQY = 1.0, toluene).

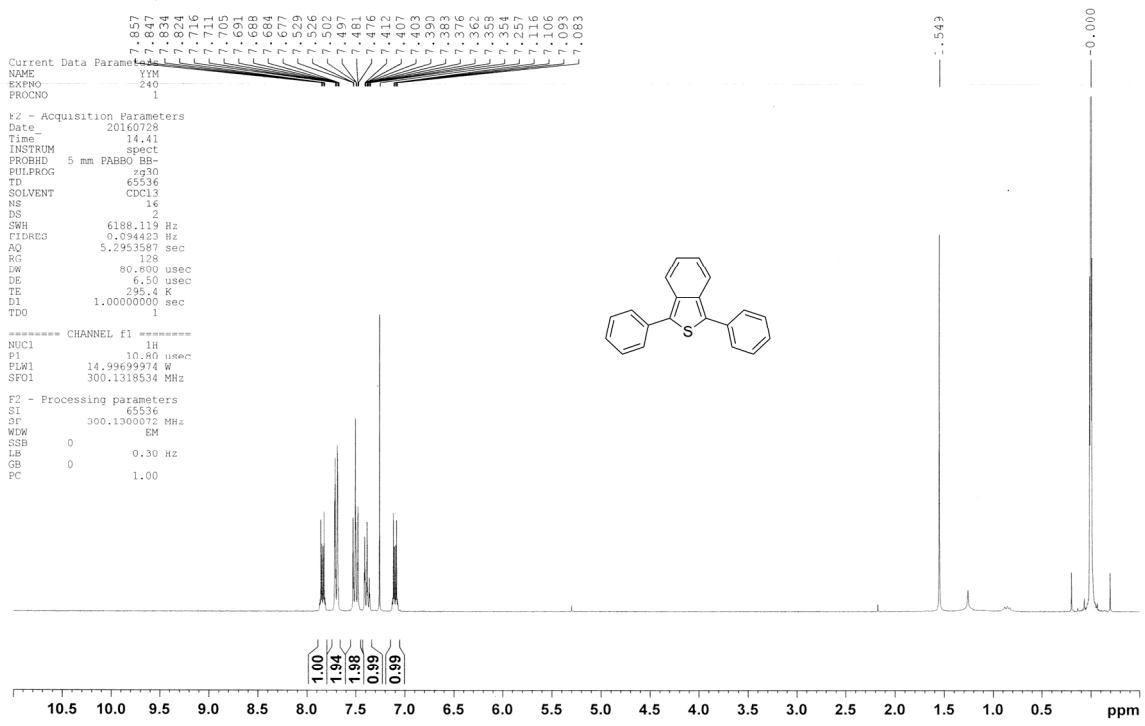

Figure S1. ^{1}H NMR (300 MHz, $CDCl_3$) spectrum of DPBT.

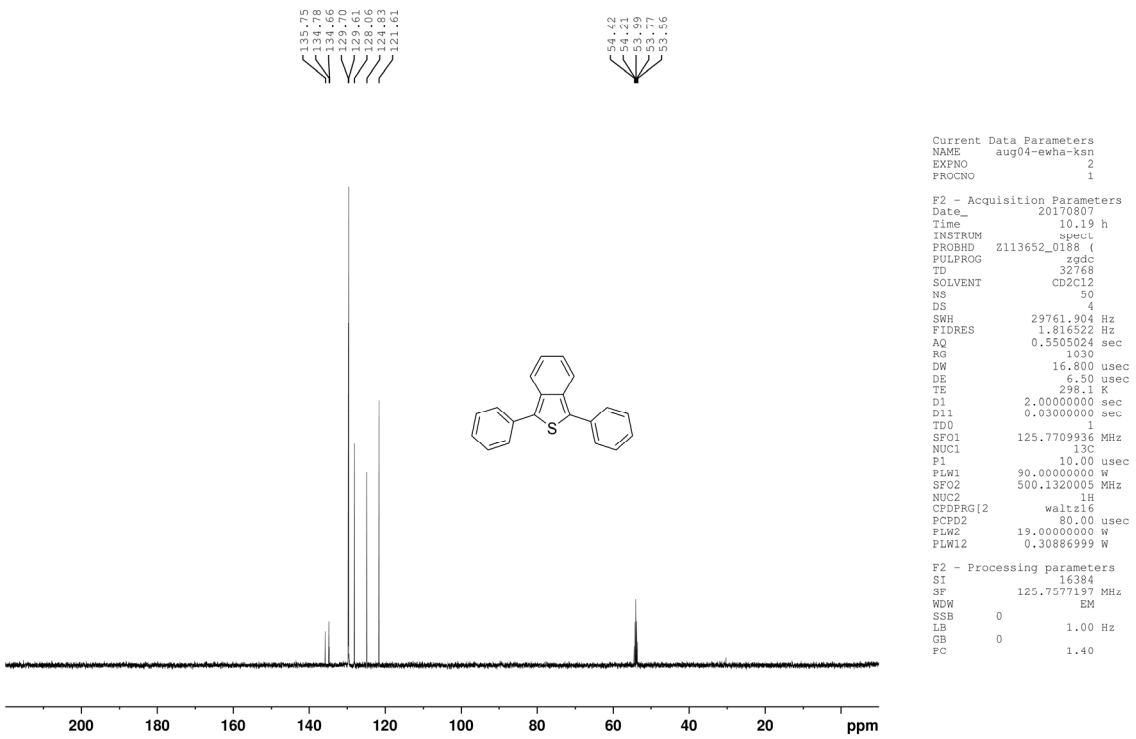

Figure S2. $^{13}C\{^{1}H\}$ NMR (126 MHz, CD_2Cl_2) spectrum of DPBT.

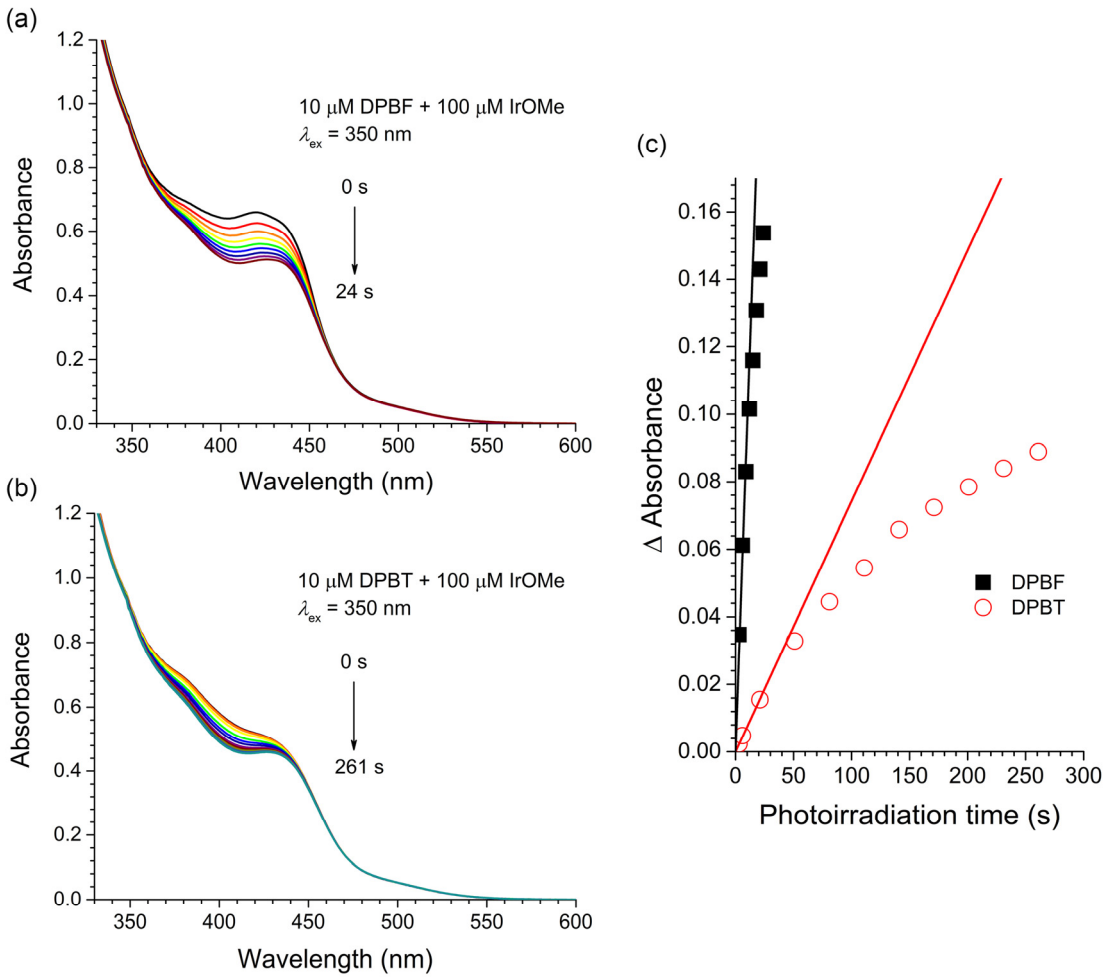

Figure S3. Comparison of the $^1O_2$ reactivities of DPBF and DPBT. (a) Changes in the UV–vis absorption spectrum of an O_2-saturated DMSO solution containing 10 μM DPBF and 100 μM IrOMe upon continuous photoirradiation at 350 nm. (b) Changes in the UV–vis absorption spectrum of an O_2-saturated DMSO solution containing 10 μM DPBT and 100 μM IrOMe upon continuous photoirradiation at 350 nm. (c) Plots of the absorbance changes at 420 nm of DPBF (black squares) and DPBT (red circles) as a function of photoirradiation time. The back and red lines are linear fits of the initial four data points. A ratio of the slopes of two linear fits is 13 (DPBF/DPBT). Since the rate constant for a bimolecular reaction between DPBF and $^1O_2$ was known to be 9.6×10^8 M^{-1} s^{-1},[4] the rate constant for a bimolecular reaction between DPBT and $^1O_2$ was estimated to be 7.4×10^7 M^{-1} s^{-1}.

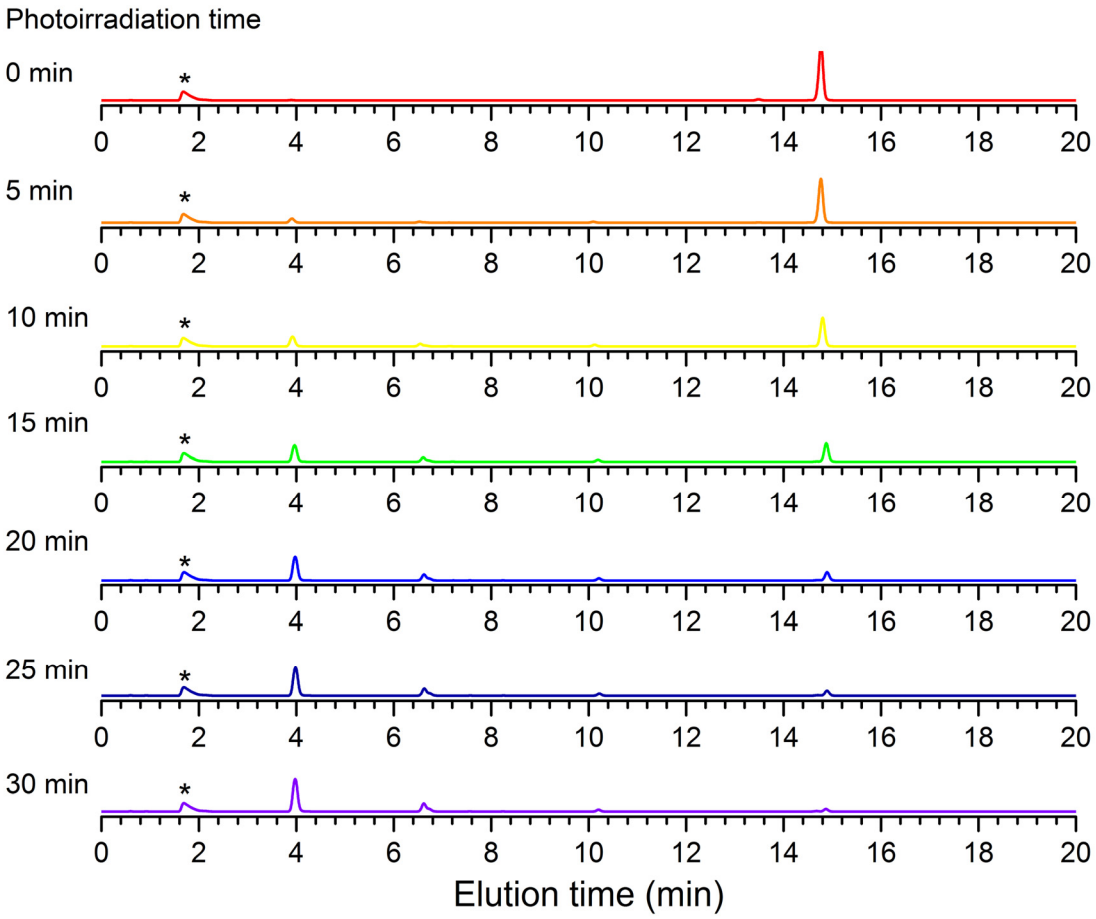

Figure S4. $^1O_2$-mediated fragmentation of DPBT into H_2S and 2-benzoylbenzophenone. Liquid chromatograms of an O_2-saturated CH_3CN solution containing 1.0 mM DPBT and 100 μM IrOMe upon continuous photoirradiation at 380 nm (0–30 min). The peaks marked with asterisks (*) are IrOMe.

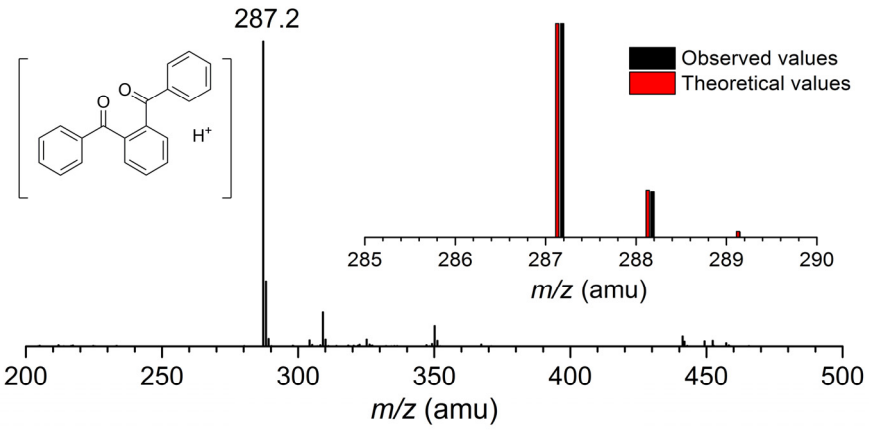

Figure S5. Electrospray mass spectrum (positive mode) for an O_2-saturated acetonitrile solution containing 1 mM DPBT and 100 μM IrOMe after photoirradiation (300 W Xenon lamp) for 30 min. The inset graph is a comparison of the base peak and the theoretical values of the protonated form of 2-benzoylbenzophenone (the structure shown on the left).

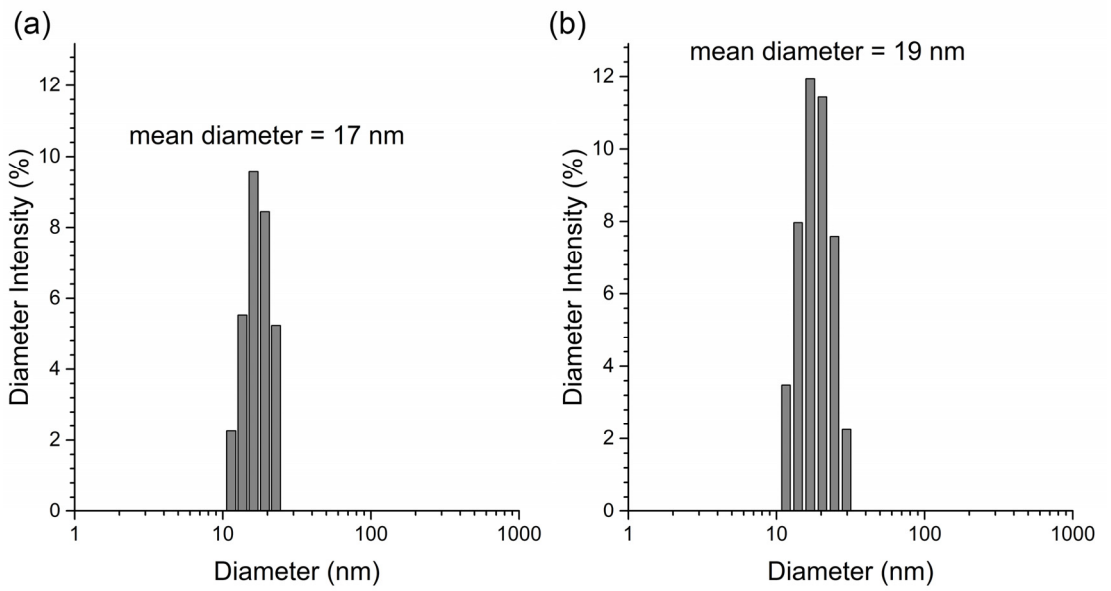

Figure S6. Distributions of the diameter of polymersomes before (a) and after (b) photoirradiation. The diameters were determined for milli-Q grade water containing Pluronic F-127 polymersomes doped with 0.2 wt % DPBT and 0.01 wt % IrOMe before (a) and after (b) photoirradiation (λ_{irr} = 350 nm; 24 min).

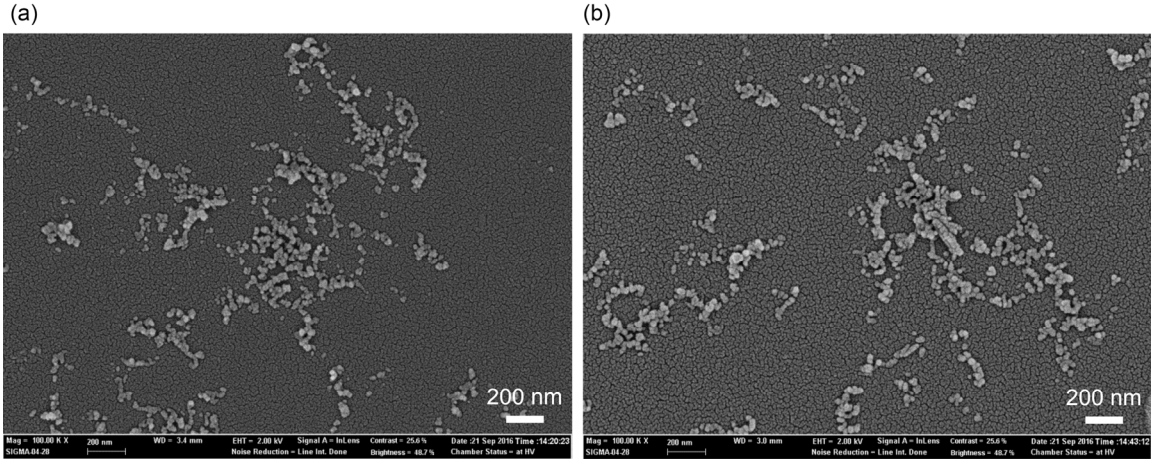

Figure S7. FE SEM images showing photostability of the H_2S-donating polymersomes. FE SEM (100 k ×; 2.00 kV) images of Pluronic F-127 polymersomes doped with 0.2 wt % DPBT and 0.01 wt % IrOMe before (a) and after (b) photoirradiation (λ_{irr} = 350 nm; 24 min). Scale bar = 200 nm.

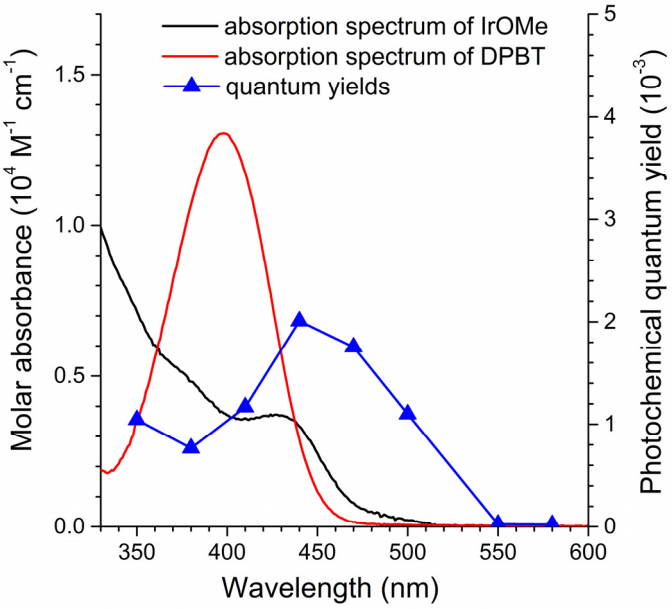

Figure S8. Comparison of the photochemical action spectrum for H_2S generation (blue triangles) with the UV–vis absorption spectra of IrOMe (black) and DPBT (red). Standard ferrioxalate actinometry was employed to determine photon flux at 350 nm (1.3×10^{-8} einstein s^{-1}), 380 nm (7.4×10^{-9} einstein s^{-1}), 410 nm (5.3×10^{-9} einstein s^{-1}), 440 nm (3.7×10^{-9} einstein s^{-1}), 470 nm (2.8×10^{-9} einstein s^{-1}), 500 nm (2.6×10^{-9} einstein s^{-1}), 550 nm (3.2×10^{-9} einstein s^{-1}), and 580 nm (3.2×10^{-9} einstein s^{-1}). 3.0 mL of O_2-equilibrated, buffered aqueous solutions (pH 7.4; 10 mM PBS + 1.0 mM CTAB) containing Pluronic F-127 polymersomes doped with 0.2 wt % DPBT and 0.01 wt % IrOMe, and 0.01 wt % SF4 were photoirradiated at monochromated excitation wavelengths, 350, 380, 410, 440, 470, 500, 550, and 580 nm for 20 min. The photochemical quantum yields were calculated employing eq S3.

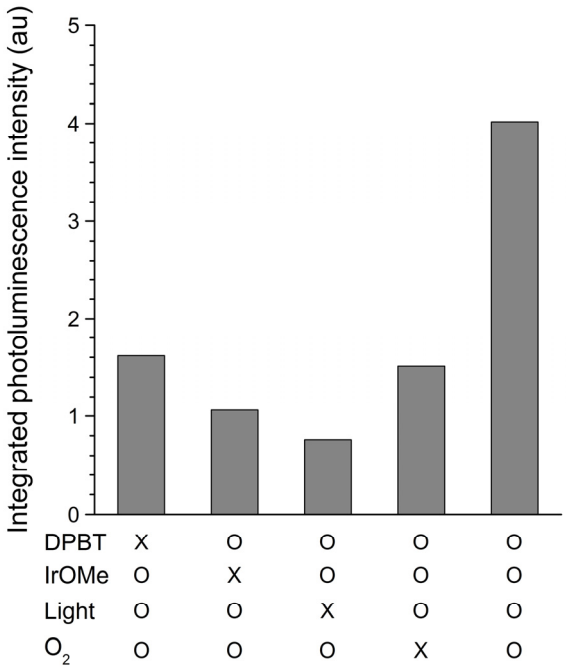

Figure S9. Control experiments for the photogeneration of H_2S. Integrated photoluminescence intensities of buffered solutions (pH 7.4; 10 mM PBS + 1.0 mM CTAB) of Pluronic F-127 polymersomes were measured in the absence of 0.2 wt % DPBT, 0.01 wt % IrOMe, or photoirradiation (λ_{irr} = 380 nm, 60 min), or after deaeration. In all cases, the polyersomes were additionally doped with a fluorescence H_2S probe, SF4 (0.01 wt %).

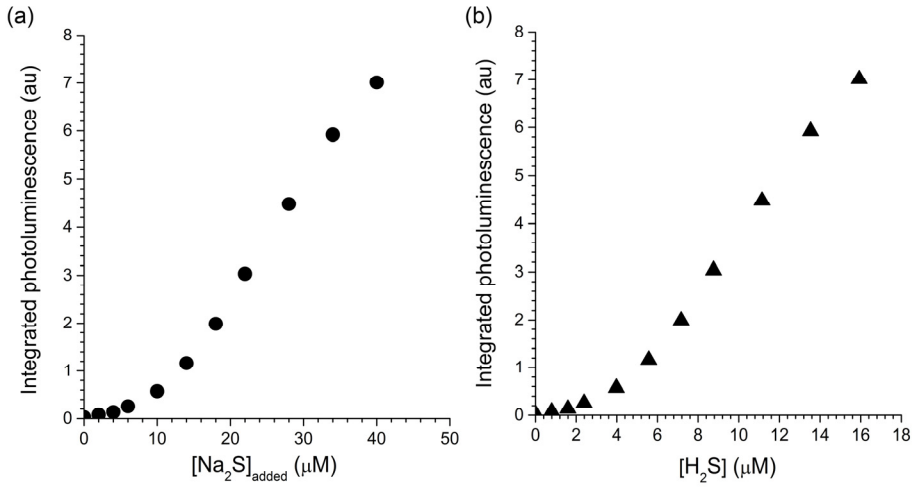

Figure S10. Calibration curves for the estimation of the H_2S concentration ([H_2S]) from the integrated photoluminescence intensity of SF4. (a) Integrated photoluminescence intensities (λ_{ex} = 496 nm; λ_{em} = 500–650 nm) of aqueous buffered solutions (pH 7.4; 10 mM PBS + 1.0 mM CTAB) containing 20 μM SF4 after the addition of increasing concentrations of Na_2S ([Na_2S]$_{added}$; 0–40 μM). (b) Integrated photoluminescence intensity as a function of the calculated concentration of [H_2S]. [H_2S] was calculated employing eq S1.

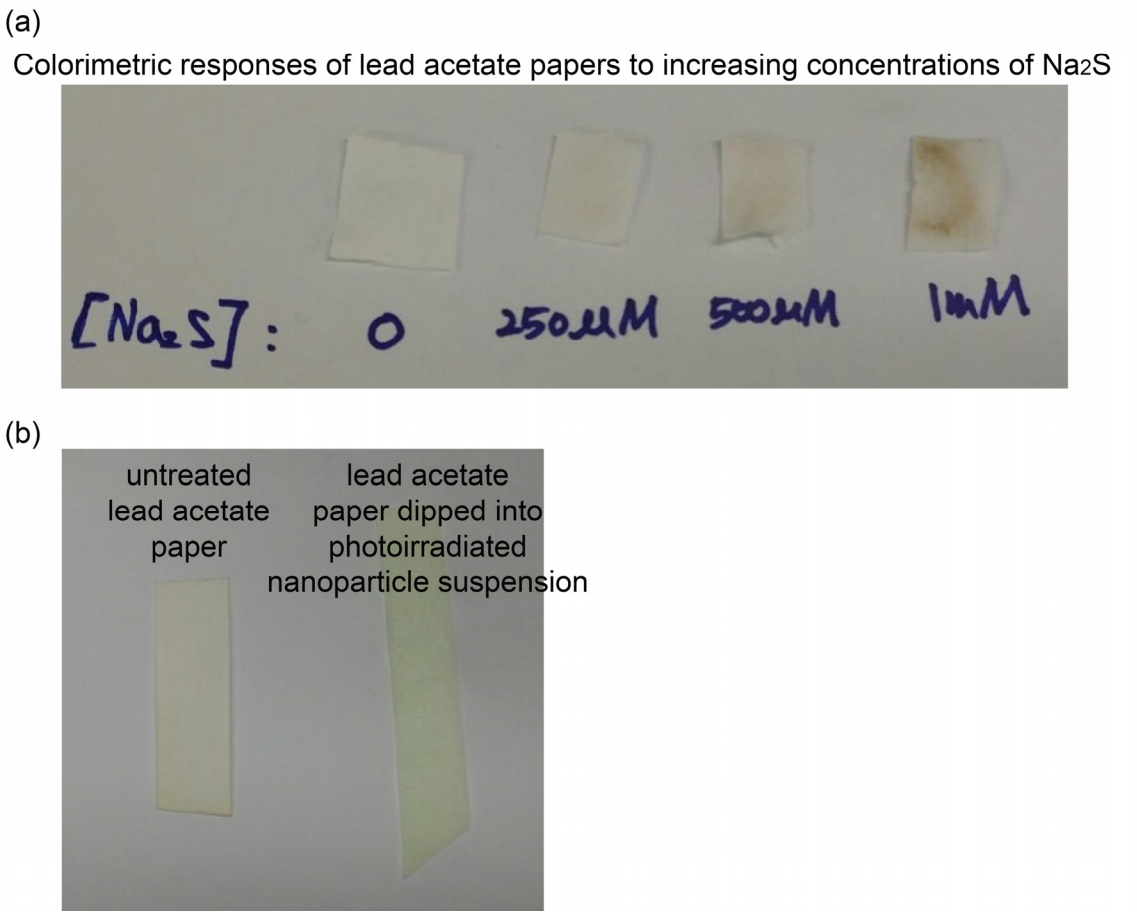

Figure S11. Estimation of the concentration of total sulfides through the lead acetate assay. (a) Photos showing color changes of Whatman filter papers adsorbed with $Pb(OAc)_2$ to different concentrations of Na_2S. (b) Photos showing $Pb(OAc)_2$-treated filter papers before (left) and after (right) exposure to a photoirradiated aqueous solution containing Pluronic F-127 polymersomes doped with 0.2 wt % DPBT and 0.01 wt % IrOMe. The pale green color of the right paper was due to the absorption color of DPBT.

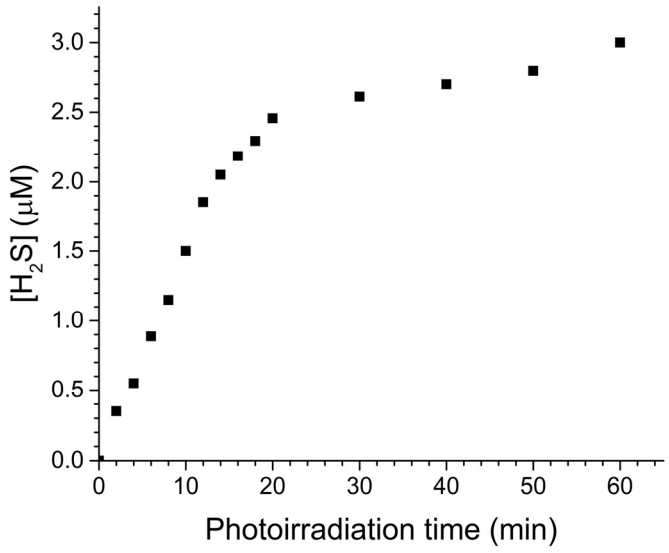

Figure S12. Estimation of the concentration of total sulfides through the methylene blue assay. An O_2-saturated aqueous buffered solution (pH 7.4, 10 mM PBS + 1.0 mM CTAB) containing Pluronic F-127 polymer vesicles doped with 0.2 wt % DPBT and 0.01 wt % IrOMe were photoirradiated at 380 nm for 60 min. A 500 μL of the solution was taken during the photoirradiation, and incubated with a 150 μL of 20 mM *N*,*N*-dimethylaniline (7.2 M HCl) and a 150 μL of 30 mM $FeCl_3$ (1.2 M HCl) for 20 min. Absorption changes at 670 nm were recorded, and the values were converted into total H_2S concentrations.

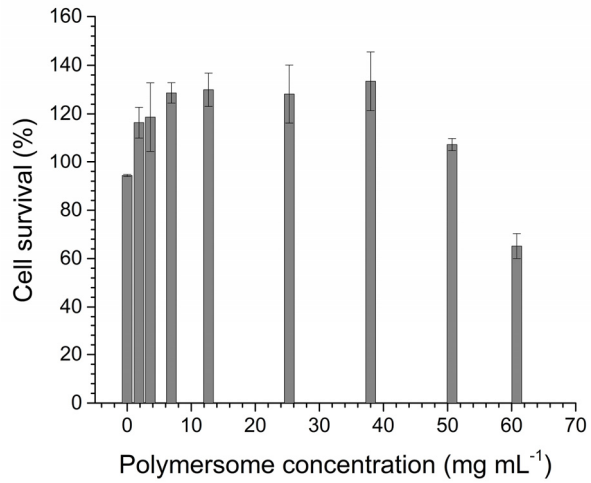

Figure S13. MTT cell proliferation assays for HeLa cells treated with varying the concentration of polymersomes. Incubation time = 15 min.

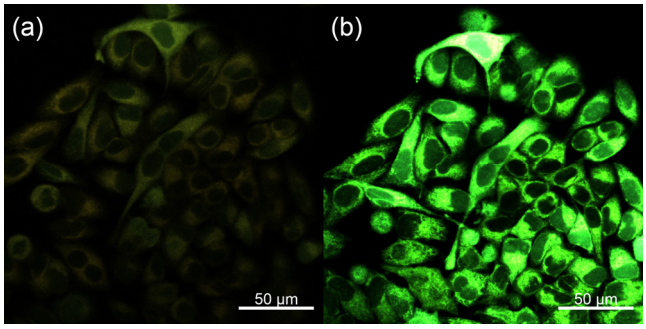

Figure S14. Fluorescence visualization of intracellular H_2S photogenerated from the polymersomes. HeLa cells were treated with the Pluronic F-127 polymersomes doped with 0.2 wt % DPBT and 0.01 wt % PtOEP (15 min incubation), followed by subsequent treatment of 10 μM SF4 (10 min incubation). Fluorescence micrographs were taken before (a) and after (b) green-LEDs photoirradiation (λ_{irr} ~ 500 nm, 1 W) for 20 min. Conditions: λ_{ex} = 488 nm; λ_{obs} = 503–546 nm. Scale bar = 50 μm.

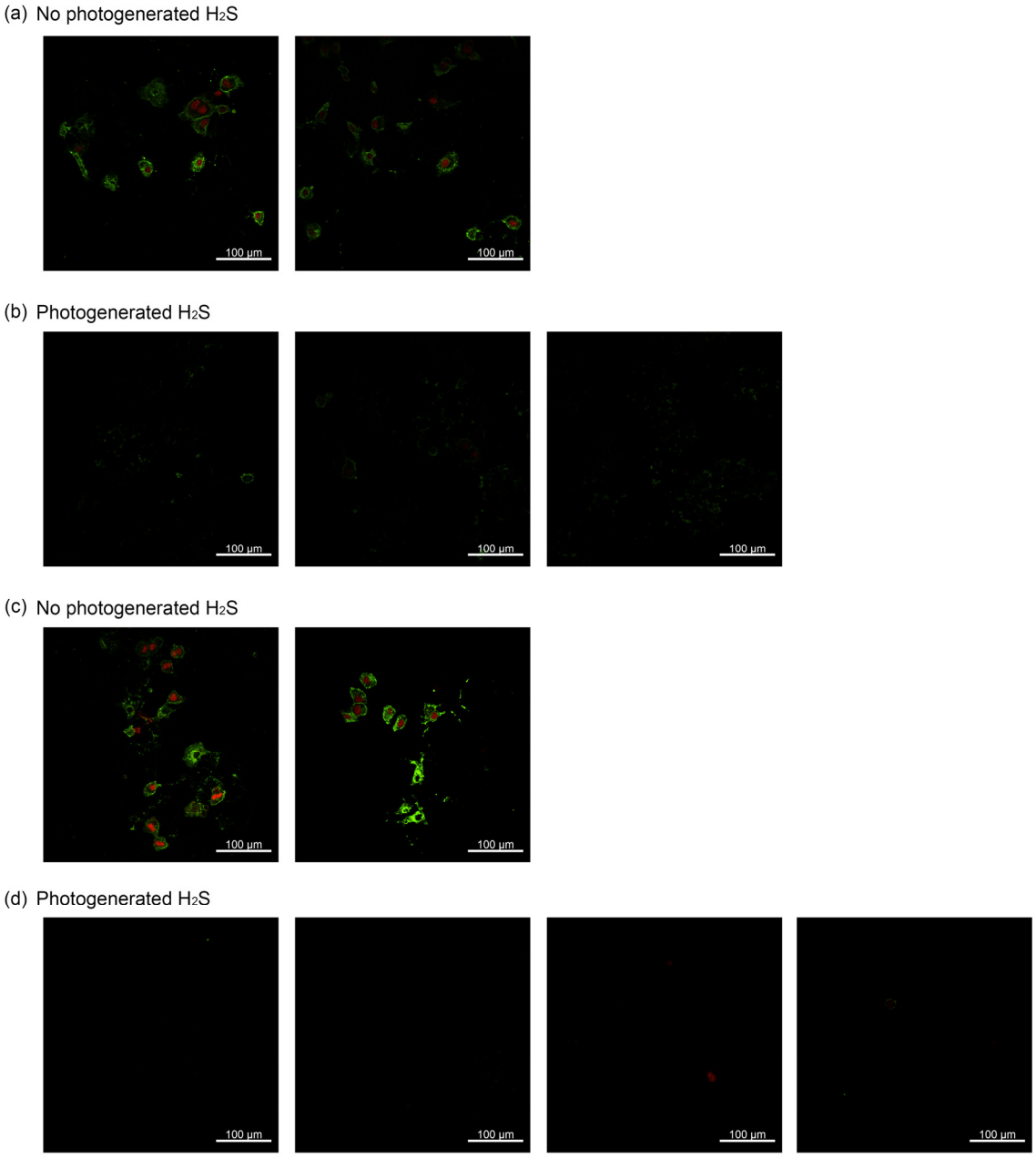

Figure S15. Inhibition of acute apoptosis of HeLa cells by photogenerating H_2S. (a & c) Live HeLa cells photoirradiated at 500 nm (1 W, 5 min) were subsequently treated with 0.2 wt % H_2O_2 to induce acute apoptosis. (b & d) Live HeLa cells photoirradiated at 500 nm (1 W, 5 min) after incubation with the polymersomes containing 0.2 wt % DPBT and 0.01 wt % PtOEP were

subsequently treated with 0.2 wt % H_2O_2. Green and red channels correspond to the fluorescence emission of an Annexin V-FITC apoptosis marker (λ_{ex} = 485 nm, λ_{obs} = 535 nm) and a propidium iodide (PI) necrosis marker (λ_{ex} = 493 nm, λ_{obs} = 636 nm), respectively. The cells shown in (a & b) were stained with 7 nM Annexin V-FITC and 2 μM PI. The cells shown in (c & d) were stained with 14 nM Annexin V-FITC and 4 μM PI. Scale bar = 100 μm.

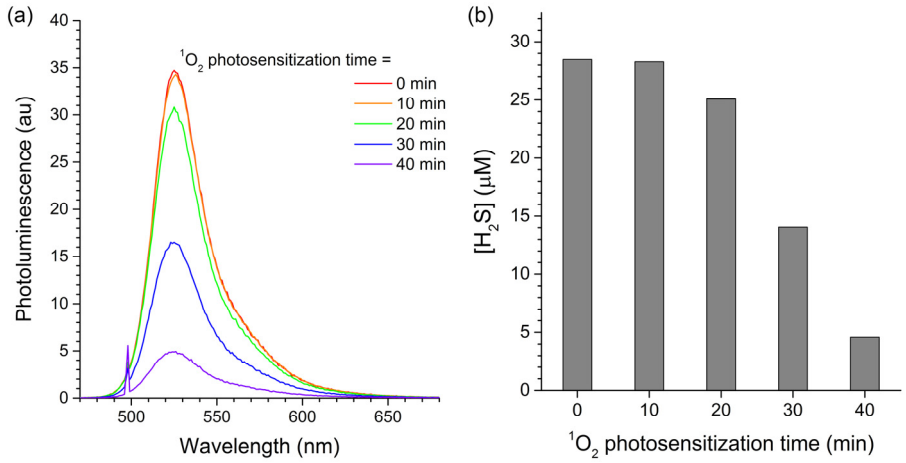

Figure S16. Reduction in the H_2S concentration by the reaction with photosensitized $^1O_2$. O_2-saturated buffered aqueous solutions (pH 7.4, 10 mM PBS + 1.0 mM CTAB) containing 100 μM Na_2S were photoirradiated for 0, 10, 20, 30, and 40 min. 20 μM SF4 was subsequently delivered into the photoirradiation solutions to quantify residual H_2S. (a) Fluorescence spectral changes and (b) quantitated concentrations of residual H_2S after the photosensitization of O_2 for the indicated times.

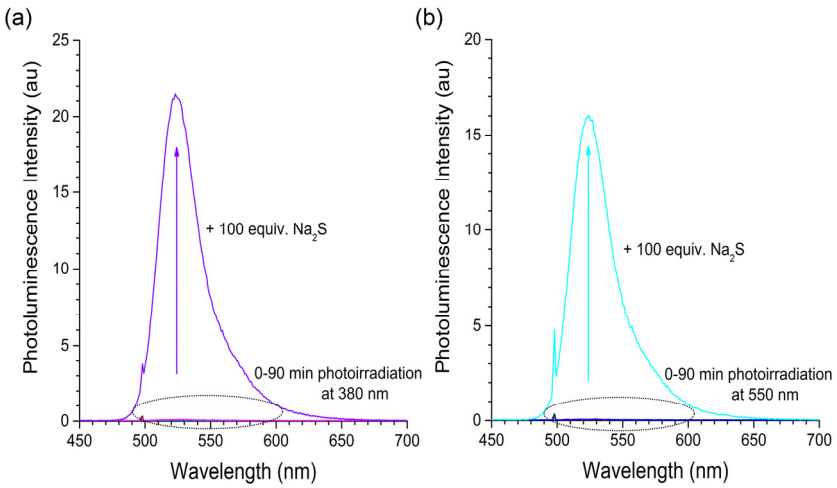

Figure S17. Photostability of SF4. Fluorescence spectra of 10 μM SF4 in buffered aqueous solutions (pH 7.4; 10 mM PBS + 1.0 mM CTAB) were recorded during continuous photoirradiation at 380 nm (a) and 550 nm (b). After photoirradiation for 90 min, 1.0 mM Na_2S was added into the solution to confirm the fluorescent H_2S response of SF4.