Electronic Supplementary Information for

Visible Light-Driven Photogeneration of Hydrogen Sulfide

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<table>
<thead>
<tr>
<th>Experimental Details</th>
<th>S2</th>
</tr>
</thead>
<tbody>
<tr>
<td>References</td>
<td>S7</td>
</tr>
<tr>
<td>Scheme S1</td>
<td>Syntheses of DPBT S9</td>
</tr>
<tr>
<td>Table S1</td>
<td>Photophysical Data for DPBT S10</td>
</tr>
<tr>
<td>Figure S1</td>
<td>$^1$H NMR (300 MHz, CDCl$_3$) spectrum of DPBT S11</td>
</tr>
<tr>
<td>Figure S2</td>
<td>$^{13}$C($^1$H) NMR (126 MHz, CD$_2$Cl$_2$) spectrum of DPBT S12</td>
</tr>
<tr>
<td>Figure S3</td>
<td>Comparison of the $^1$O$_2$ reactivities of DPBF and DPBT S13</td>
</tr>
<tr>
<td>Figure S4</td>
<td>$^1$O$_2$-mediated fragmentation of DPBT into H$_2$S and 2-benzoylbenzophenone S14</td>
</tr>
<tr>
<td>Figure S5</td>
<td>Electrospray mass spectrum (positive mode) for an O$_2$-saturated acetonitrile solution containing 1.0 mM DPBT and 100 µM IrOMe after photoirradiation (300 W Xenon lamp) S15</td>
</tr>
<tr>
<td>Figure S6</td>
<td>Distributions of the diameter of polymersomes before and after photoirradiation S16</td>
</tr>
<tr>
<td>Figure S7</td>
<td>FE SEM images showing photostability of the H$_2$S-donating polymersomes S17</td>
</tr>
<tr>
<td>Figure S8</td>
<td>Comparison of the photochemical action spectrum for H$_2$S generation with the UV–vis absorption spectra of IrOMe and DPBT S18</td>
</tr>
<tr>
<td>Figure S9</td>
<td>Control experiments for the photogeneration of H$_2$S S19</td>
</tr>
<tr>
<td>Figure S10</td>
<td>Calibration curves for the estimation of the H$_2$S concentration ([H$_2$S]) from the integrated photoluminescence intensity of SF4 S20</td>
</tr>
<tr>
<td>Figure S11</td>
<td>Estimation of the concentration of total sulfides with employing the lead acetate assay S21</td>
</tr>
<tr>
<td>Figure S12</td>
<td>Estimation of the concentration of total sulfides with employing the methylene blue assay S22</td>
</tr>
<tr>
<td>Figure S13</td>
<td>MTT cell proliferation assays for HeLa cells treated with varying the concentration of polymersomes S23</td>
</tr>
<tr>
<td>Figure S14</td>
<td>Fluorescence visualization of intracellular H$_2$S photogenerated from the polymersomes S24</td>
</tr>
<tr>
<td>Figure S15</td>
<td>Inhibition of acute apoptosis of HeLa cells by photogenerating H$_2$S S25</td>
</tr>
<tr>
<td>Figure S16</td>
<td>Reduction in the H$_2$S concentration by the reaction with photosensitized $^1$O$_2$ S27</td>
</tr>
<tr>
<td>Figure S17</td>
<td>Photostability of SF4 S28</td>
</tr>
</tbody>
</table>
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. Na2S and 1,3-diphenylisobenzofuran were purchased from TCI. IrOMe1 and SF42 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.1H and 13C{1H} NMR spectra were collected with Ultrashield 500 and 300 plus NMR spectrometers (Bruker). Chemical shifts were referenced to (CH3)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 Irppy1 (11.3 mg, 13.7 μmol) were added into a 250 mL 1-neck round-bottom flask. After the addition of CH2Cl2 (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. Rf = 0.5 (hexane:EtOAc = 3:1, v/v). 1H NMR (300 MHz, CDCl3) 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). 13C{1H} NMR (126 MHz, CD2Cl2) 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 P2S5 (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 ºC 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. Rf = 0.6 (hexane:EtOAc = 4:1, v/v). 1H NMR (300 MHz, CDCl3) 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). 13C{1H} NMR (126 MHz,
CD$_2$Cl$_2$) $\delta$ (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 $\mu$L from a 10 mM DPBT solution in CH$_2$Cl$_2$, and 10 $\mu$L from a 10 mM $^1$O$_2$ PS solution in CH$_2$Cl$_2$ were added into a 20 mL glass vial, and fully dissolved in 10 mL CH$_2$Cl$_2$. The doping ratios of DPBT and the $^1$O$_2$ PS corresponded to 0.2 and 0.01 wt %, respectively. 20 $\mu$L from a 10 mM SF4 solution in DMSO (a 0.01 wt % doping ratio) was added for the fluorescent detection of H$_2$S. Slow removal of CH$_2$Cl$_2$ 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$_2$S photogeneration experiments.

**Steady-State UV–vis Absorption Measurements.** UV–vis absorption spectra were collected on a Cary 300 spectrophotometer (Agilent) at room temperature. 2 $\mu$M or 10 $\mu$M 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 $\mu$M IrOMe and 10 $\mu$M DPBF or 10 $\mu$M DPBT were photoirradiated at 350 nm (Xenon lamp equipped with a 350 nm bandpass filter). Decreases in the absorbance ($\Delta$ Abs) at 420 nm (DPBF and DPBT) were monitored during the continuous photoirradiation. Plots of $\Delta$ 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 ($9.6 \times 10^8$ M$^{-1}$ s$^{-1}$).5

**HPLC Experiments to Monitor the Reaction of DPBT.** An O$_2$-saturated CH$_3$CN solution (10 mL) containing 1.0 mM DPBT and 100 $\mu$M 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 $\times$ 100 mm, 2.7 $\mu$m); eluent condition: H$_2$O:CH$_3$CN = 50:50 to 100% CH$_3$CN). DPBT, IrOMe, and the $^1$O$_2$-oxidation product of DPBT were observed at elution times of 14.67, 1.67, and 4.00 min, respectively.
**H₂S 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. 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 Na₂S (0, 250, 500, and 1000 μM). Visual inspection of the treated papers indicated that a response was visible at [Na₂S]_{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 (Photanal 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 H₂S 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_{em} = 500–650 nm after the addition of increased concentrations of Na₂S. The H₂S concentration ([H₂S]) was calculated, considering the following protonation equilibria:

\[
\text{H}_2\text{S}(aq) \rightleftharpoons \text{HS}^-(aq) + \text{H}^+(aq) \quad pK_{a1} = 7.0
\]
HS\(^{-}(aq)\) ⇌ S\(^{2-}(aq)\) + H\(^{+}(aq)\) \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., \([\text{Na}_2\text{S}]_{\text{added}} = [\text{H}_2\text{S}] + [\text{HS}^{-}] + [\text{S}^{2-}]\)) yielded the following equation for [H\(_2\)S] at pH 7.4.

\[
[H_2S] = \frac{[\text{Na}_2\text{S}]_{\text{added}}}{1+10^{0.4}}
\]  

(eq S1)

**Determination of Photochemical Quantum Yields for H\(_2\)S Generation.** The quantum yields for photogeneration of H\(_2\)S were determined with employing the standard ferrioxalate actinometry. 500 \(\mu\)L 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 \(\mu\)L of a sodium acetate buffer and 500 \(\mu\)L 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 (\(\Delta A\)) into eq S2 returned photon flux(\(\lambda\)).

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

(eq S2)

In eq S2, \(\Phi(\lambda)\) was 1.1. Calculated photon flux values were 1.3 \(\times\) 10\(^{-8}\) einstein s\(^{-1}\) (350 nm), 7.4 \(\times\) 10\(^{-9}\) einstein s\(^{-1}\) (380 nm), 5.3 \(\times\) 10\(^{-9}\) einstein s\(^{-1}\) (410 nm), 3.7 \(\times\) 10\(^{-9}\) einstein s\(^{-1}\) (440 nm), 2.8 \(\times\) 10\(^{-9}\) einstein s\(^{-1}\) (470 nm), 2.6 \(\times\) 10\(^{-9}\) einstein s\(^{-1}\) (500 nm), 3.2 \(\times\) 10\(^{-9}\) einstein s\(^{-1}\) (550 nm), and 3.2 \(\times\) 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 (\(\lambda_{\text{em}} = 500–650\) nm). The integrated fluorescence intensity was converted into [H\(_2\)S], employing the calibration curve shown in Figure S10. Substituting the [H\(_2\)S] value into eq S3 yielded the quantum yields.

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

(eq S3)
**Amperometric Detection of Photogenerated H₂S.** Amperometric measurements were performed with employing a World Precision Instruments, ISO-H₂S-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 Na₂S. An O₂-saturated polymosome 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 polymosome suspension was photoirradiated, during which amperometric responses to H₂S were recorded through a World Precision Instruments, Lab-Trax-4/16.

**Preparation of Reactive Oxygen Species.**

1) H₂O₂: An aqueous solution of 30 wt % H₂O₂ (Sigma–Aldrich) was diluted in milli-Q water to a concentration of 500 μM. The molar absorbance of H₂O₂, ε = 44 M⁻¹ cm⁻¹, was employed to determine the concentration.

2) •OH: The 500 μM H₂O₂ solution was mixed with 2.5 mM FeSO₄ (aq), and the mixture was incubated for 10 min. The incubated solution was used without additional treatments.

3) O₂⁻: KO₂ 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, ε = 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 FeSO₄ (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 NaNO₂ (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 MnO₂, the solution was employed for H₂S 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% CO₂.

**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 °C. 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 Photogenerated Intracellular H₂S.** 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 (λirr ~ 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 H₂S.** 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 H₂S 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 λirr ~ 500 nm using green LEDs (1 W)
for 5 min. A 5 \( \mu L \) (7 nM) Annexin V-FITC and a 10 \( \mu L \) (2 \( \mu M \)) PI were added into the culture dish, and the cells were incubated for additional 10 min under dark. 0.2 wt % \( \text{H}_2\text{O}_2 \) (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 \( \lambda_{\text{ex}} = 485 \text{ nm} \) and \( \lambda_{\text{obs}} = 538–546 \text{ nm} \) for Annexin V-FITC and at \( \lambda_{\text{ex}} = 493 \text{ nm} \) and \( \lambda_{\text{obs}} = 625–634 \text{ nm} \) for PI. Fluorescence images were analyzed using the LSM 510 version 4.0 software.

References
Scheme S1. Synthesis of DPBT.
Table S1. Photophysical Data for DPBT$^a$

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<th>$\lambda_{\text{abs}}$ (nm; log$\varepsilon$)</th>
<th>$\lambda_{\text{ems}}$ (nm)$^b$</th>
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<td>DPBT</td>
<td>398 (4.11)</td>
<td>489</td>
<td>0.75</td>
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$^a$10 $\mu$M DMSO. $^b\lambda_{\text{ex}} = 398$ nm. $^c$9,10-Diphenylanthracene standard (PLQY = 1.0, toluene).
Figure S1. 1H NMR (300 MHz, CDC13) spectrum of DPBT.
Figure S2. $^{13}$C($^1$H) NMR (126 MHz, CD$_2$Cl$_2$) spectrum of DPBT.
Figure S3. Comparison of the $^1\text{O}_2$ reactivities of DPBF and DPBT. (a) Changes in the UV–vis absorption spectrum of an O$_2$-saturated DMSO solution containing 10 $\mu$M DPBF and 100 $\mu$M IrOMe upon continuous photoirradiation at 350 nm. (b) Changes in the UV–vis absorption spectrum of an O$_2$-saturated DMSO solution containing 10 $\mu$M DPBT and 100 $\mu$M 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 $^1\text{O}_2$ was known to be $9.6 \times 10^8$ M$^{-1}$ s$^{-1}$, the rate constant for a bimolecular reaction between DPBT and $^1\text{O}_2$ was estimated to be $7.4 \times 10^7$ M$^{-1}$ s$^{-1}$.
Figure S4. $^1$O$_2$-mediated fragmentation of DPBT into H$_2$S and 2-benzoylbenzophenone. Liquid chromatograms of an O$_2$-saturated CH$_3$CN 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 O₂-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 ($\lambda_{irr} = 350$ nm; 24 min).
Figure S7. FE SEM images showing photostability of the H₂S-donating polymersomes. FE SEM (100 k x; 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 ($\lambda_{irr} = 350$ nm; 24 min). Scale bar = 200 nm.
Figure S8. Comparison of the photochemical action spectrum for H₂S 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 ($1.3 \times 10^{-8}$ einstein s⁻¹), 380 nm ($7.4 \times 10^{-9}$ einstein s⁻¹), 410 nm ($5.3 \times 10^{-9}$ einstein s⁻¹), 440 nm ($3.7 \times 10^{-9}$ einstein s⁻¹), 470 nm ($2.8 \times 10^{-9}$ einstein s⁻¹), 500 nm ($2.6 \times 10^{-9}$ einstein s⁻¹), 550 nm ($3.2 \times 10^{-9}$ einstein s⁻¹), and 580 nm ($3.2 \times 10^{-9}$ einstein s⁻¹). 3.0 mL of O₂-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 H₂S. 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 ($\lambda_{\text{irr}} = 380 \text{ nm}$, 60 min), or after deaeration. In all cases, the polymersomes were additionally doped with a fluorescence H₂S probe, SF4 (0.01 wt %).
Figure S10. Calibration curves for the estimation of the H$_2$S concentration ([H$_2$S]) from the integrated photoluminescence intensity of SF4. (a) Integrated photoluminescence intensities ($\lambda_{\text{ex}} = 496$ nm; $\lambda_{\text{em}} = 500$–650 nm) of aqueous buffered solutions (pH 7.4; 10 mM PBS + 1.0 mM CTAB) containing 20 $\mu$M SF4 after the addition of increasing concentrations of Na$_2$S ([Na$_2$S]$_{\text{added}}$; 0–40 $\mu$M). (b) Integrated photoluminescence intensity as a function of the calculated concentration of [H$_2$S]. [H$_2$S] 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 Na₂S. (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 O₂-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 FeCl₃ (1.2 M HCl) for 20 min. Absorption changes at 670 nm were recorded, and the values were converted into total H₂S 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$_2$S 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 ($\lambda_{\text{irr}} \sim 500$ nm, 1 W) for 20 min. Conditions: $\lambda_{\text{ex}} = 488$ nm; $\lambda_{\text{obs}} = 503$–546 nm. Scale bar = 50 μm.
Figure S15. Inhibition of acute apoptosis of HeLa cells by photogenerating H$_2$S. (a & c) Live HeLa cells photoirradiated at 500 nm (1 W, 5 min) were subsequently treated with 0.2 wt % H$_2$O$_2$ 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 % H₂O₂. Green and red channels correspond to the fluorescence emission of an Annexin V-FITC apoptosis marker (\(\lambda_{\text{ex}} = 485 \text{ nm}, \lambda_{\text{obs}} = 535 \text{ nm}\)) and a propidium iodide (PI) necrosis marker (\(\lambda_{\text{ex}} = 493 \text{ nm}, \lambda_{\text{obs}} = 636 \text{ nm}\)), respectively. The cells shown in (a & b) were stained with 7 nM Annexin V-FITC and 2 \(\mu\text{M}\) PI. The cells shown in (c & d) were stained with 14 nM Annexin V-FITC and 4 \(\mu\text{M}\) PI. Scale bar = 100 \(\mu\text{m}\).
Figure S16. Reduction in the H₂S concentration by the reaction with photosensitized ¹O₂. O₂-saturated buffered aqueous solutions (pH 7.4, 10 mM PBS + 1.0 mM CTAB) containing 100 μM Na₂S were photoirradiated for 0, 10, 20, 30, and 40 min. 20 μM SF4 was subsequently delivered into the photoirradiation solutions to quantify residual H₂S. (a) Fluorescence spectral changes and (b) quantitated concentrations of residual H₂S after the photosensitization of O₂ 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 Na₂S was added into the solution to confirm the fluorescent H₂S response of SF4.