Supporting Information

Self-Immolative Polymersomes for High-Efficiency Triggered Release and Programmed Enzymatic Reactions

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Experimental Section

Materials. *N*,*N*-Dimethylacrylamide (DMA) purchased from Aldrich was distilled under vacuum and then stored at -20 °C prior to use. Dimethyl sulfoxide (DMSO) purchased from Sinopharm Chemical Reagent Co. Ltd. was dried with CaH_2 and distilled under vacuum. Di-*n*-butyltin dilaurate (DBTL, Aldrich), Nile Red (NR, Aldrich), eosin (TCI), 2-nitrobenzyl alcohol (TCI), paraoxon (Aldrich), 2,2'-dithiodiethanol (Aldrich), camptothecin (CPT, Aldrich), and doxorubicin hydrochloride (Dox-HCl, Iffect Chemphar) were used as received. Dicyclohexyl carbodiimide (DCC), 4-dimethylaminopyridine (DMAP), ethylenediamine-tetraacetic acid (EDTA), 1,4-dioxane, dimethylfomamide (DMF), and all other reagents were purchased from Sinopharm Chemical Reagent Co. Ltd. and used as received. Fluorescein diphosphate, tetraammonium salt (FDP) and fluorescein diacetate (FDA) purchased from Invitrogen and used as received. PEO_{16}-*b*-PS_{60} diblock copolymer (Polymer Source, Inc.) was used as received. Water was deionized with a Milli-Q SP reagent water system (Millipore) to a specific resistivity of 18.4 MΩ cm. Phenyl (4-(hydroxymethyl)phenyl) carbamate,[1] perylen-3-yl methanol,[2] RAFT chain transfer agent, *S*-1-propyl-*S'*-(α,α'-dimethyl-α"-acetic acid) trithiocarbonate (PDMAT),[3] 1,9-di(aminoethyloxyl)anthracene dihydrochloride (*An*-$2NH_2$),[4] and the fluorescent ratiometric pH probe, 2-(2-aminoethyl)pyrido[3,4,5-gh][1,2,4]triazolo[1,5-a]perimidine-1,3(2*H*,6*H*)-dione hydrochloride (NTEA)[5] were synthesized according to literature procedures.

Sample Preparation. Synthetic routes employed for the preparation of amphiphilic block copolymers, **PB1-PB5**, are shown in Figure 1a and Scheme S1. As a typical example, the detailed procedures for **PB1** synthesis are described below.

*Synthesis of Perylen-3-yl Caped Poly(Benzyl Carbamate) (per-PBC, **P1**).* Phenyl-(4-(hydroxymethyl)phenyl) carbamate (0.486 g, 2 mmo1), DBTL (63.1 mg, 0.1 mmol), and dry DMSO (1 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 °C, perylen-3-yl methanol (0.572 g, 2 mmol) was added, and the solution was further stirred for 1 h at 110 °C. After that, the reaction tube was quenched into liquid N_2, and the reaction solution was precipitated into an excess of methanol. The above dissolution-precipitation cycle was repeated for three times. The

final product was obtained as a yellow solid (0.23 g, yield: 49.1%) after being dried in a vacuum oven overnight at room temperature. The molecular weight and molecular weight distribution of the product were determined by GPC using DMF as the eluent, revealing an M_n of 4,800 and M_w/M_n of 1.45. The DP was determined to be ~13 by ^{1}H NMR analysis in DMSO-d_6 (Figure S1).

*Synthesis of macroRAFT Agent, **P1**-CTA.* **P1** (0.2 g, 87 μmol) was dissolved in dry DMF, then RAFT agent PDMAT (41 mg, 174 μmol), DCC (41 mg 200 μmol) and DMAP (1 mg) were added. After being stirred for 48 h under N_2 at room temperature, the mixture was precipitated into an excess of methanol. The above dissolution-precipitation cycle was repeated for three times. The final product was dried in a vacuum oven overnight at room temperature, yielding a yellow solid (0.18 g, yield: 90 %). The extent of end group functionalization was determined to be ~96% by ^{1}H NMR analysis in DMSO-d_6 (Figure S1).

*Synthesis of **PB1** Diblock Copolymer. **P1**-*CTA macro-RAFT agent (0.1 g, 40 μmol), DMA (0.2 g, 2 mmol), AIBN (6 mg, 4 μmol) and 1 mL 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 70 ^{o}C, 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 (0.21 g, yield: 70 %). The molecular weight and molecular weight distribution of the product were determined by GPC using DMF as the eluent, revealing an M_n of 10,900 and M_w/M_n of 1.22. The DP of PDMA block was determined to be 40 by ^{1}H NMR analysis in DMSO-d_6 (Figure S1).

*Removal of RAFT End Group in **PB1** Block Copolymer. **PB1**-CTA* (0.1 g 15 μmol), excess AIBN (55 mg, 0.3 mmol), and 1 ml dry DMSO were charged into a glass ampoule equipped with a magnetic stirring bar. The ampoule was degassed by three freeze-pump-thaw cycles and backfilled with nitrogen. After being stirred for 2 h at 70 ^{o}C, 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 final product was obtained as a yellow solid (89 mg, yield: 89 %)

after being dried in a vacuum oven overnight at room temperature.

Preparation of SIPsomes. A typical procedure is described as follows: **PB1** (2 mg) was dissolved in 1 mL solvent mixture of DMSO and dioxane (1/9 v/v) 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 ^{o}C under stirring (500 rpm). The dispersion was stirred for 5 h followed by purification by dialysis for 24 h.

Preparation of Drug-Loaded SIPsomes. Typical procedures employed for the co-encapsulation of chemotherapeutic drugs, camptothecin (CPT, within the bilayer membrane) and doxorubicin (DOX, within the internal aqueous compartment) are as follows. **PB4** (10 mg) and CPT (2 mg) was dissolved in a mixture of DMSO and dioxane (2 mL, 1/9 v/v). Then the solution of DOX in DI water (1 g/L, 2 mL) was added into the organic solution by a syringe pump at a speed of 1 mL/h at 25 ^{o}C under stirring (500 rpm). Then 16 mL DI water was added. The dispersion was stirred for 5 h, and then purified by dialysis for 24 h. To determine the drug loading efficiency, an aliquot of polymersome solution was lyophilized and the powder was dissolved in DMSO to be subjected to UV spectrophotometry. Based on the standard calibration curves, the loading contents of CPT and DOX were calculated to be ~6.0 and ~4.2 wt% respectively.

Enzyme Encapsulation in SIPsomes. A typical procedure for the encapsulation of enzymes is described below. **PB1** (2 mg) was dissolved in a solvent mixture of DMSO and dioxane (1 mL, 1/9 v/v). After the solution was stirred for 3 h at room temperature, solution of alkaline phosphatase (ALK) in DI water (1 g/L, 1 mL) was added into the organic solution by a syringe pump at a speed of 1 mL/h at 25 ^{o}C under stirring (500 rpm). Then, 8 mL DI water was added, the dispersion was stirred for 5 h and then purified by repeated ultracentrifugation using a centrifugal filter (MWCO is 100 KDa).

Monitoring Triggered Disintegration of SIPsomes. PB1 SIPsome dispersion (0.2 g/L, 0.5 mL) was placed in a dialysis tube (cellulose membrane; MWCO is 3,500 Da) and then immersed into 5 mL of PBS medium (0.02 M, pH 7.4) with added external standard (10 μM benzyl alcohol) for HPLC. Next, the dialysis tube was irradiated with 420 nm blue light for 30 min. The concentration of released small molecule 4-aminobenzyl alcohol (ABA) was then

quantified by HPLC (260 nm) against a standard curve (In Figure 2a, "100% fragmentation" was defined as the calculated amount of 4-aminobenzyl alcohol (ABA) release assuming complete depolymerization). After 12 h, sediment in the dialysis tube was collected by centrifugation and analyzed by HPLC. The supernatant solution collected from centrifugation was lyophilized and analyzed by GPC. For TEM observation, 1 mL of **PB1** SIPsome dispersion was charged into a cuvette, and then irradiated with 420 nm light for 30 min. The samples for TEM observations were prepared upon further incubating for different time periods.

In Vitro Drug Release Measurements. Typically, 150 μL CPT and DOX co-loaded **PB4** SIPsome dispersion (0.2 g/L) was placed in a dialysis tube (cellulose membrane; MWCO is 3,500 Da) and then immersed into 5 mL of PBS medium (0.02 M, pH 7.4) with GSH (10 mM) at 37 °C. Periodically, the external buffer solution was removed and replaced with fresh medium. Upon each sampling, the 1 mL buffer solution was dissolved in 9 ml DMSO. The drug concentration was then quantified by measuring the UV-vis absorbance against a standard curve.

Measurement of Transmembrane pH Discrepancy of Polymersomes. PB1 SIPsome and PEO_{15}-*b*-PS_{60} polymersomes loaded with NTEA as the pH probe were prepared according to following procedures. Briefly, **PB1** (2 mg) was dissolved in a mixture of DMSO and dioxane (1 mL, 1/9 v/v), then NTEA (1 mg) in water (1 mL, pH 7.0) was added by a syringe pump at a speed of 1 mL/h at 25 °C under stirring (500 rpm). At last, after addition of 8 mL water (pH 7.0), the dispersion was stirred for 5 h and then purified by dialysis with water (pH 7.0) for 72 h.

To measure the transmembrane pH difference, 900 μL of the polymersome solution was charged into a cuvette, then 100 μL of concentrated buffer solution (1 mM) with target pH was added and vortex mixed. Transmembrane ΔpH was monitored by changes in fluorescence intensity ratios ($I_{480\ nm}/I_{510\ nm}$).

Activity Assay for Enzymes Loaded within Polymersomes. The activity of enzyme encapsulated in the polymersomes can be regained by disassembly of the polymersomes via appropriate trigger stimuli. For the light responsive **PB1** SIPsomes, the polymersome dispersion (2 mL, 0.2 g/L) was irradiated with blue light (420 ± 5 nm) for 30 min, and then stirred for another 12 h at room temperature. For the reductive responsive **PB4** SIPsome, a concentrated

solution of GSH in the buffer (0.1 mL, 0.1 M) was added into the SIPsome dispersion (2 mL, 0.2 g/L), so the final GSH concentration in the solution was 1 mM. This solution was allowed to stand for 12 h at room temperature.

1 mL of SIPsome dispersion and 20 μL Tris-HCl buffer (1 M, pH 8.0) was charged into a cuvette, and then 10 μL of FDP (3 mM in buffer solution) or FDA (3 mM in DMSO) was added and vortex mixed. The reaction was monitored by following the fluorescence emission intensity at 515 nm.

Logic Gate Construction. AND, OR and XOR-type logic gates were constructed. Input 1: addition of 10 μL GSH buffer solution (0.1 M); Input 2: irradiation with blue light (420 nm) for 30 min. Output: fluorescence emission at 515 nm.

AND Logic Gate. The mixture of ALP loaded light-responsive **PB1** SIPsome solution (0.2 g/L, 500 μL) and FDP loaded reductive responsive **PB4** SIPsome solution (0.2 g/L, 500 μL) was used for the preparation of AND logic gate.

OR Logic Gate. The mixture of ALP loaded light responsive **PB1** SIPsome solution (0.2 g/L, 500 μL) and the reductive responsive **PB4** SIPsome solution (0.2 g/L, 500 μL) was used to construct OR logic gate.

XOR Logic Gate. The mixture of Lipase and EDTA (ALP inhibitor) co-loaded light responsive **PB1** SIPsome dispersion (0.2 g/L, 500 μL), as well as the ALP and paraoxon (Lipase inhibitor) co-loaded reductive responsive **PB4** SIPsome dispersion (0.2 g/L, 500 μL) were used to perform OR logic gate.

In Vitro Cytotoxicity Assay. HepG2 cells were seeded in a 96-well plate at an initial density of ca. 5000 cells/well in 100 μL of complete DMEM medium. After incubating for 24 h, DMEM was replaced with fresh medium, and the cells were treated with PB1 SIPsome dispersion at varying concentrations. The treated cells were incubated in a humidified environment with 5% CO_2 at 37 ^oC for 12 h. Next, the 96-well plate was either irradiated with green light (420 nm) or kept in dark for 30 min, and then incubated for another 24 h. MTT reagent (in 20 μL PBS, 5 mg/mL) was added to each well. The cells were further incubated for 4 h at 37 ^oC. The medium in each well was then removed and replaced by 150 μL DMSO. The plate was gently agitated for 15 min before the absorbance at 570 nm was recorded by a

microplate reader (Thermo Fisher). Each experiment condition was done in quadruple and the data are shown as the mean value plus a standard deviation (± SD).

Characterization. All nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AV300 NMR 300 MHz spectrometer operated in the Fourier transform mode. DMSO-d_6 and D_2O were used as the solvents. Molecular weights and molecular weight distributions were determined by gel permeation chromatography (GPC) equipped with Waters 1515 pump and Waters 2414 differential refractive index detector (set at 30 ^oC). It used a series of two linear Styragel columns (HR2 and HR4) at an oven temperature of 45 ^oC. The eluent was DMF at a flow rate of 1.0 mL/min. A series of low polydispersity polystyrene standards were employed for calibration. Dynamic laser light scattering (LLS) measurements were conducted on a commercial spectrometer (ALV/DLS/SLS-5022F) equipped with a multi-tau digital time correlator (ALV5000) and a cylindrical 22 mW UNIPHASE He-Ne laser (λ_0= 632 nm) as the light source. Scattered light was collected at a fixed angle of 90^o for duration of ~5 min. Distribution averages and particle size distributions were computed using cumulants analysis and CONTIN routines. All data were averaged over three measurements. Transmission electron microscopy (TEM) observations were conducted on a Hitachi H-800 electron microscope at an acceleration voltage of 200 kV. The sample for TEM observations was prepared by placing 10 μL of mixed micellar solution (0.5 g/L) on copper grids coated with thin films of Formvar and carbon successively. Confocal laser scanning microscopy (CLSM) images were acquired using a Leica TCS SP5 microscope. HPLC analysis was performed with a Shimadzu HPLC system, equipped with a LC-20AP binary pump, a SPD-20A UV−vis detector, and a Symmetry C18 column. The UV-vis detector was set at 260 nm linked to the software for data analysis. Fluorescence spectra were recorded on a F-4600 (Hitachi) spectrofluorometer. The slit widths were both set at 5 nm for excitation and emission.

References

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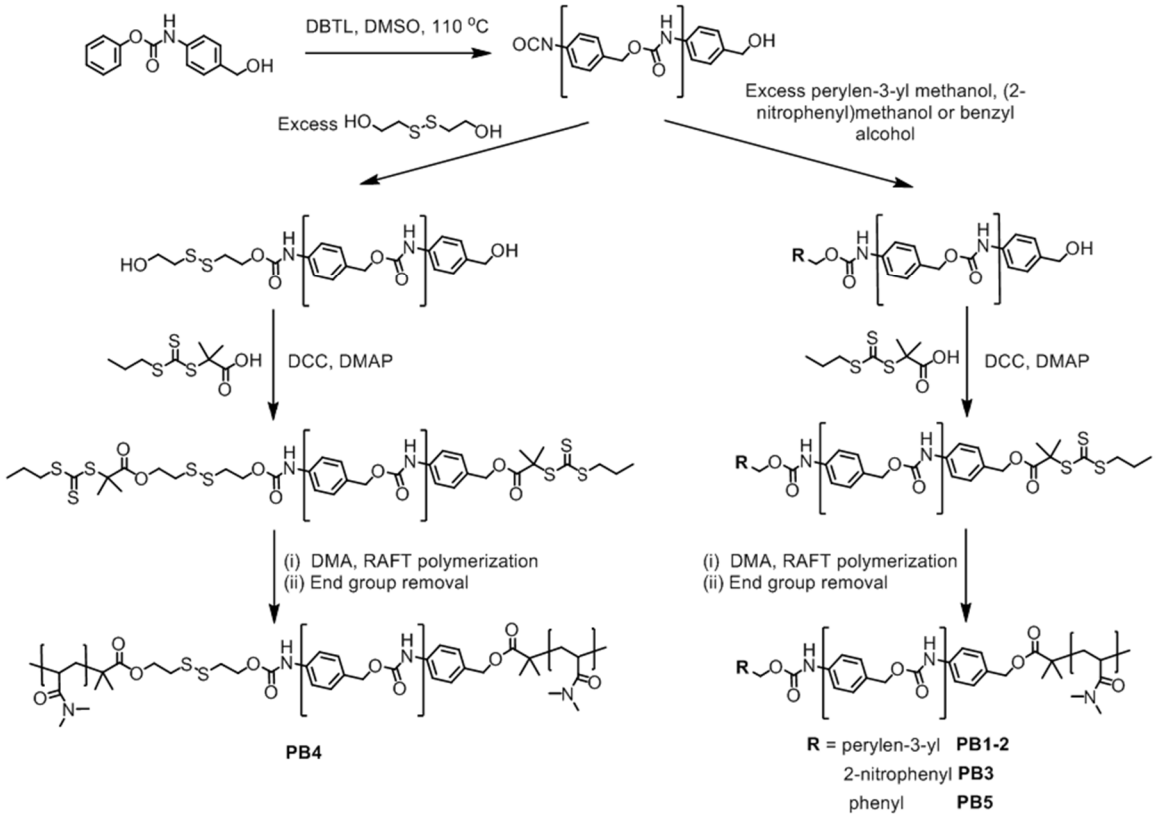
(2) Jana, A.; Devi, K. S.; Maiti, T. K.; Singh, N. D. *J. Am. Chem. Soc.* **2012**, *134*, 7656-9.
(3) Qiu, X.-P.; Tanaka, F.; Winnik, F. M. *Macromolecules* **2007**, *40*, 7069-7071.
(4) Arian, D.; Kovbasyuk, L.; Mokhir, A. *J. Am. Chem. Soc.* **2011**, *133*, 3972-80.
(5) Zhou, J.; Fang, C.; Chang, T.; Liu, X.; Shangguan, D. *J. Mater. Chem. B* **2013**, *1*, 661.



Scheme S1. Synthetic schemes employed for the preparation of amphiphilic block copolymers, PBC-*b*-PDMA (**PB1**-**PB3** and **PB5**) and PDMA-*b*-PBC-*b*-PDMA (**PB4**), containing hydrophobic self-immolative PBC blocks via the combination of condensation polymerization and RAFT polymerization.

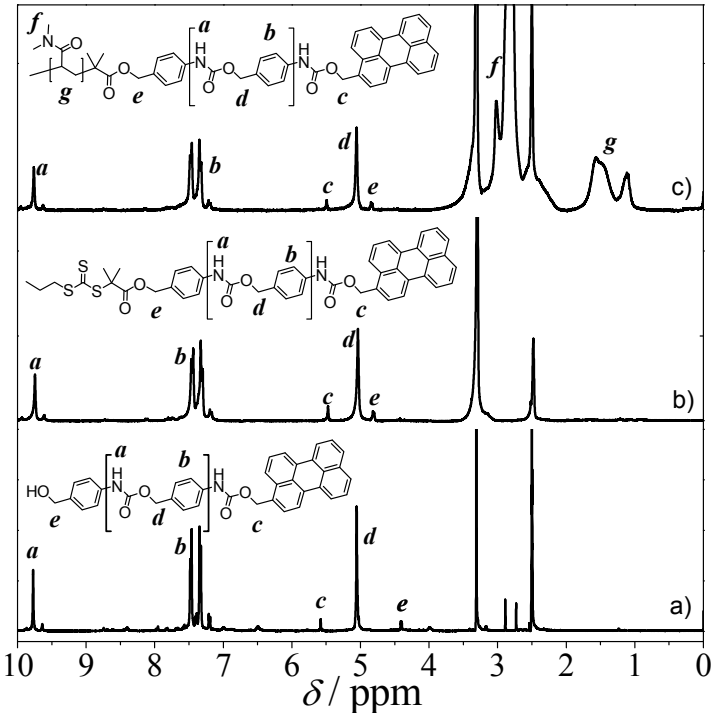


Figure S1. ^{1}H NMR spectra recorded in DMSO-d_6 for **P1** (a), **P1**-RAFT agent (b), and **PB1** block copolymer (c).

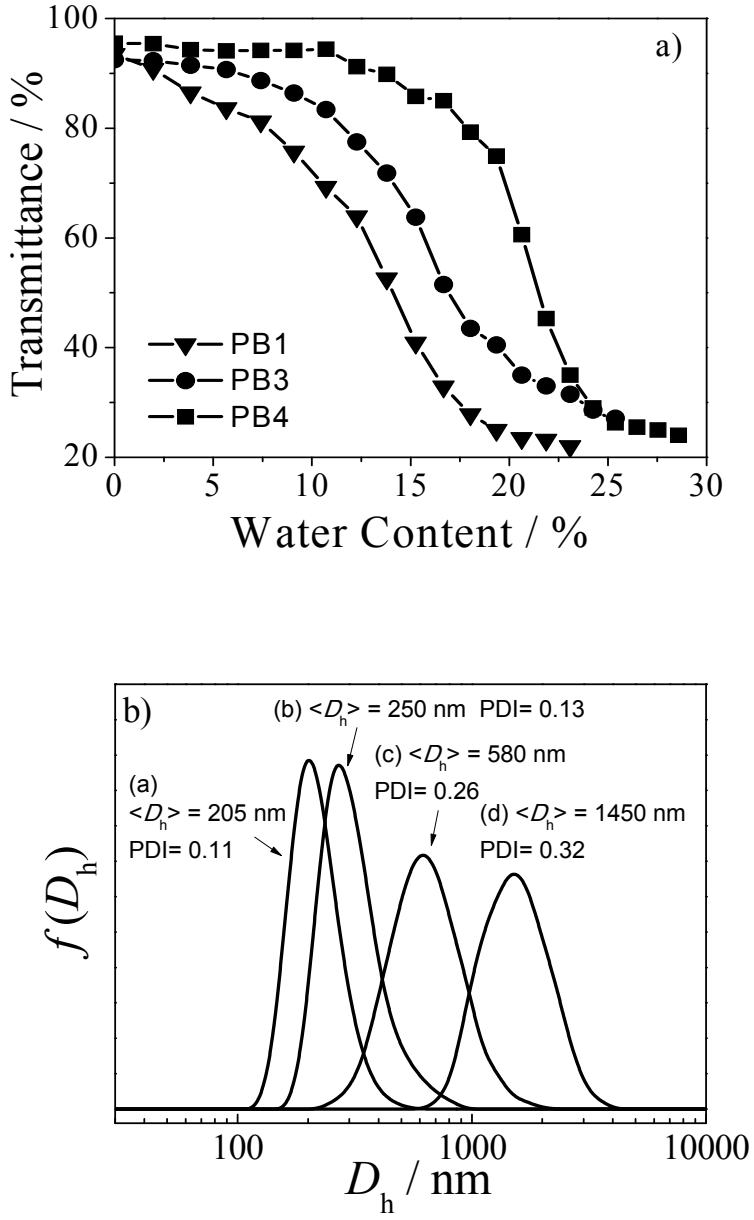


Figure S2. (a) The variation of optical transmittance at 800 nm as a function of water contents recorded upon water addition into the dioxane/DMSO solution (9/1 v/v, 2.0 g/L) of three types of amphiphilic block copolymers (**PB1**, **PB3**, and **PB4**). (b) Intensity-average hydrodynamic diameter distributions, $f(D_h)$, recorded for aqueous dispersions of (a) **PB3**, (b) **PB1**, (c) **PB4,** and (d) **PB2** SIPsomes.

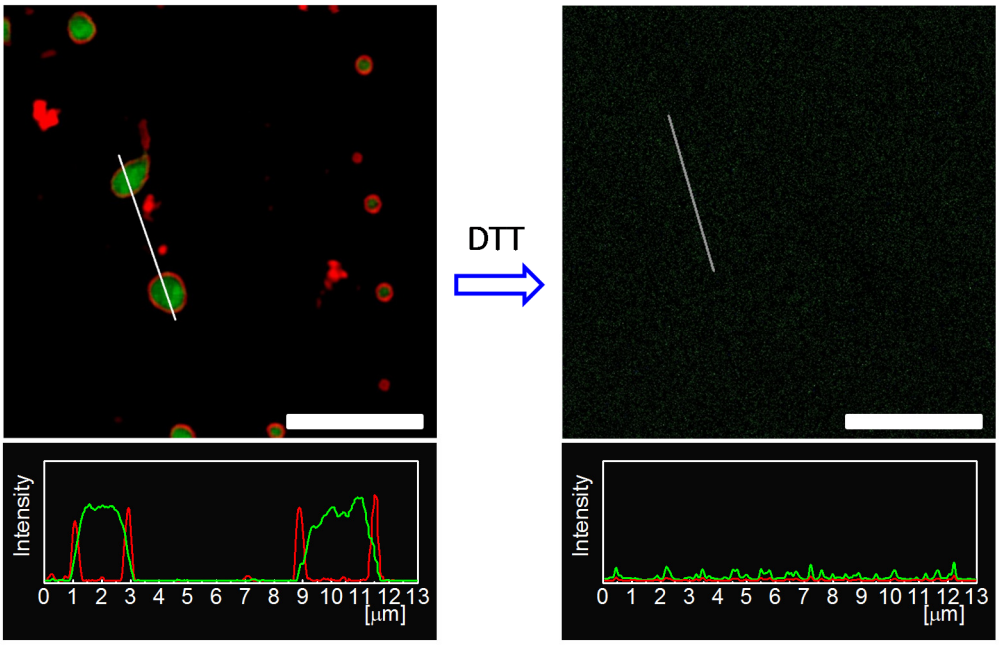


Figure S3. CLSM images (scale bar: 10 µm; red channel: Nile Red emission; green channel: calcein emission) and blue and green channel emission intensity profiles recorded for 0.1 g/L aqueous dispersion of **PB4** SIPsomes (left) before and (right) after co-incubation with 10 mM DTT for 10 h.

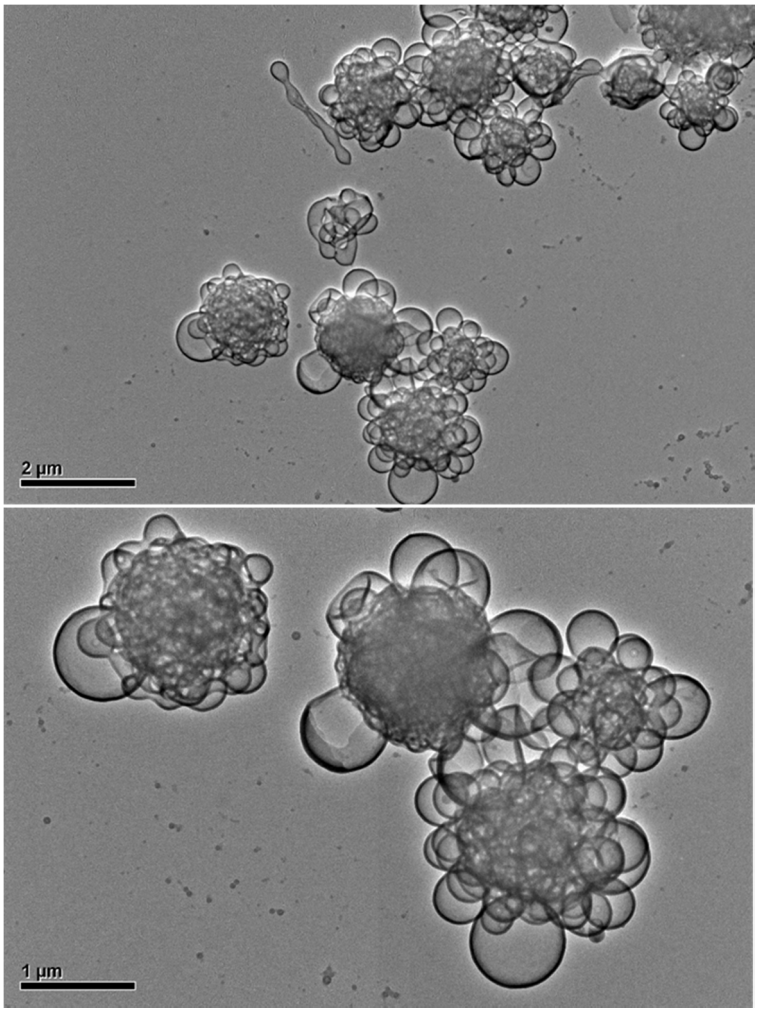


Figure S4. Typical TEM image recorded for **PB2** SIPsomes by drying the aqueous dispersion on copper grids.

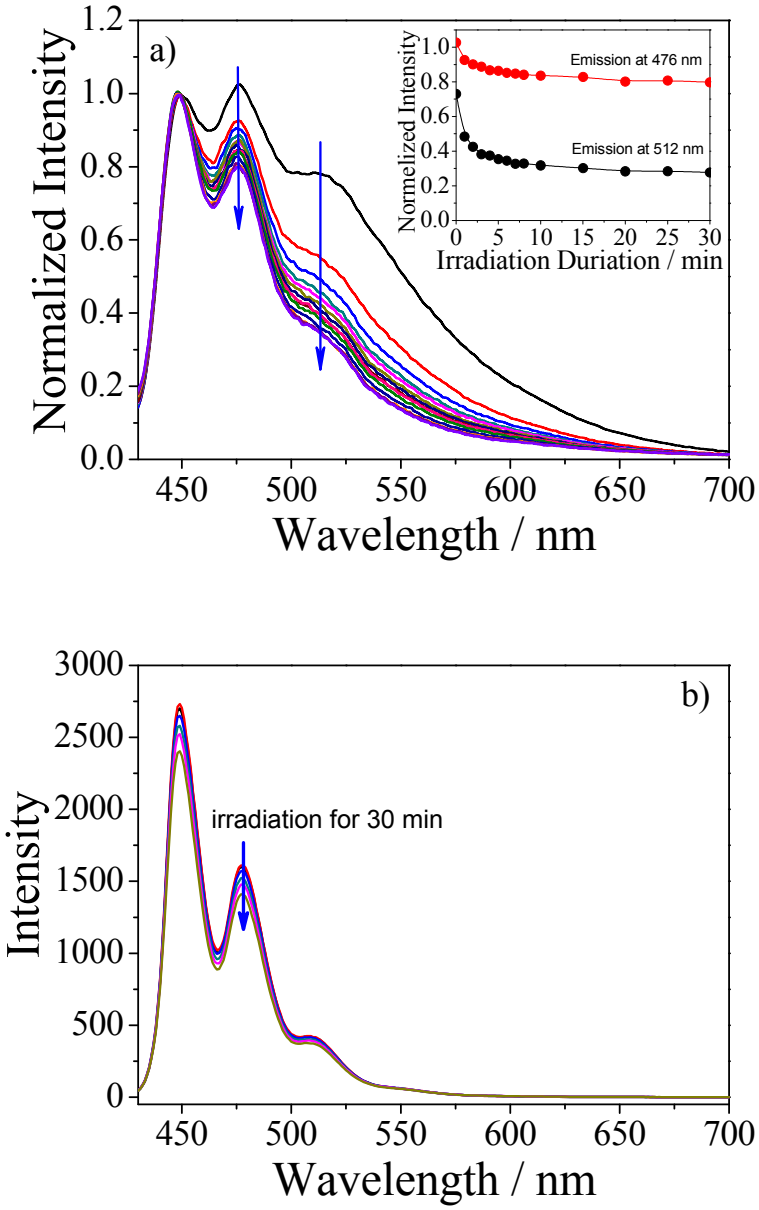


Figure S5. Evolution of fluorescence emission spectra (λ_{ex} = 400 nm, 25 °C) recorded for the (a) aqueous and (b) DMSO solution of **PB1** block copolymer (0.2 g/L) upon blue light irradiation (420 nm) for varying durations (0-30 min). The inset in (a) shows the variation of normalized emission intensity with irradiation duration.

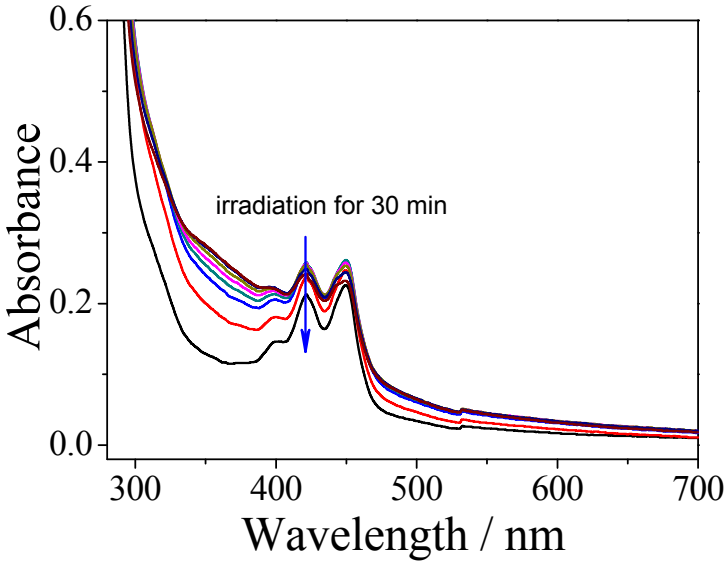


Figure S6. Evolution of absorbance spectra recorded at 25 °C for the aqueous dispersion of **PB1** SIPsomes (0.2 g/L) upon blue light irradiation (420 nm) for varying time periods (0-30 min).

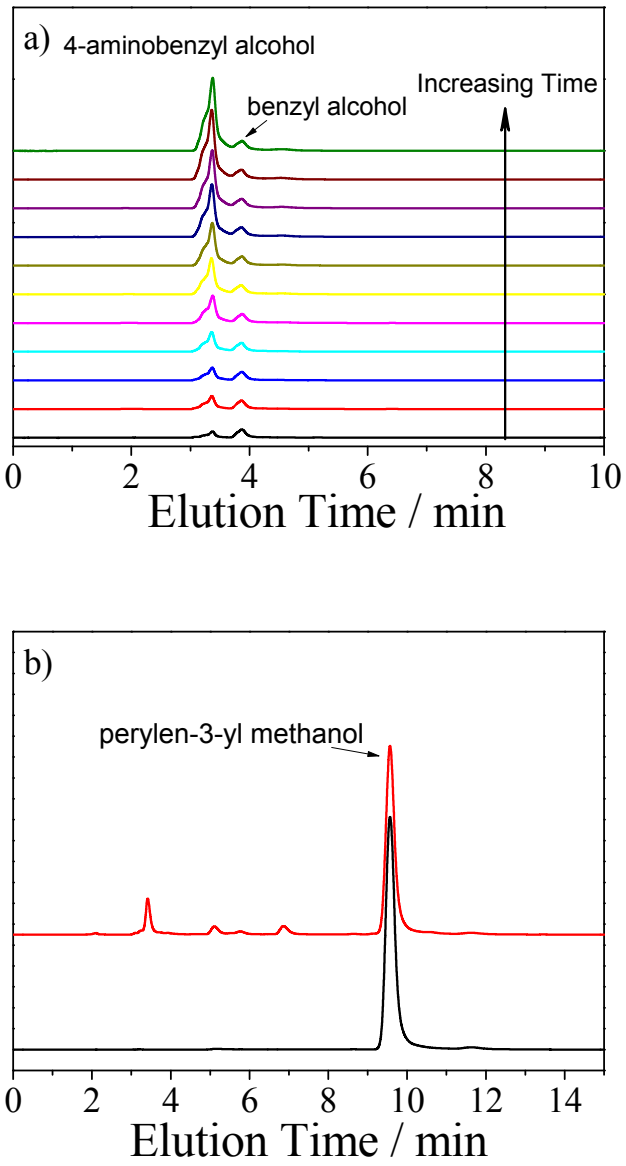


Figure S7. (a) HPLC traces (MeOH/H_2O 8/2 (v/v); 260 nm absorbance) recorded at different storage time intervals for the aqueous dispersion of **PB1** SIPsomes (0.2 g/L) after being subjected to blue light irradiation for 30 min. Benzyl alcohol was used as the external standard. (b) HPLC analysis (MeOH/H_2O 8/2 (v/v); 260 nm absorbance) of the sediment obtained after complete PBC block depolymerization for **PB1** SIPsomes (black line, HPLC trace of perylen-3-yl methanol standard).

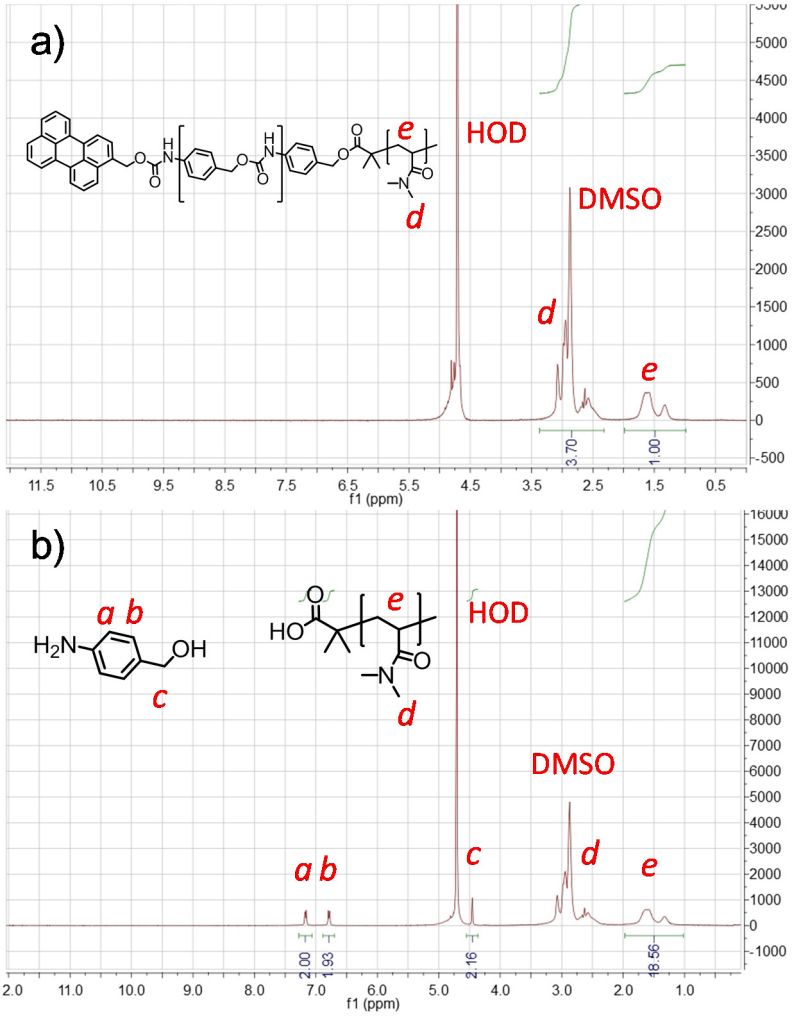


Figure S8. ^{1}H NMR spectra recorded in D_2O/DMSO-d_6 (9/1 v/v) dispersion of **PB1** SIPsomes (a) before and (b) after blue light-triggered complete self-immolative depolymerization.

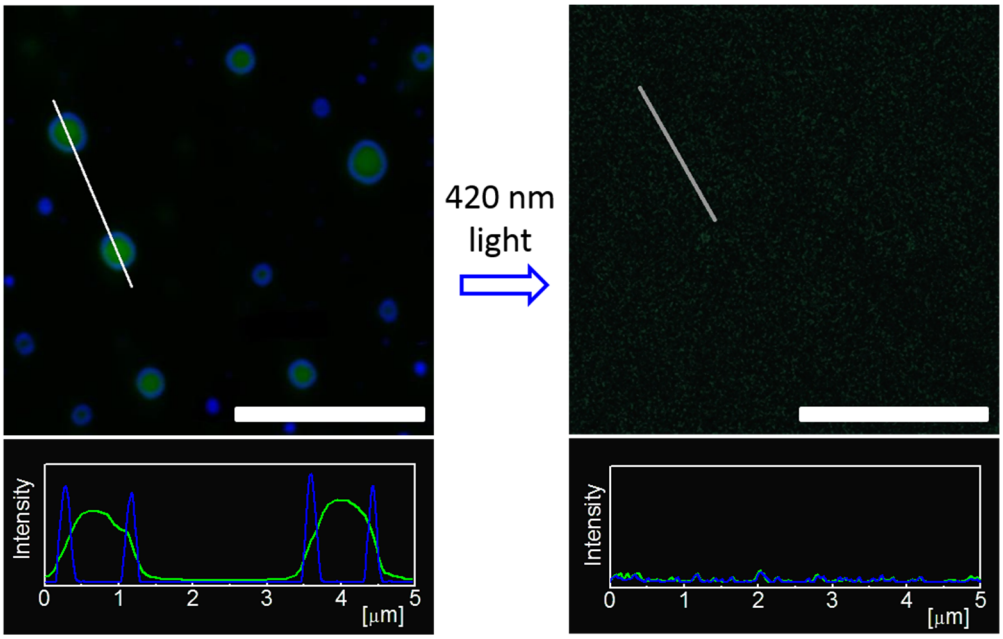


Figure S9. CLSM images (scale bars: 5 µm; blue channel: perylene emission, green channel: calcein emission) and blue and green channel emission intensity profiles recorded for 0.1 g/L aqueous dispersion of **PB1** SIPsomes before irradiation (left) and upon 12 h storage after being subjected to 420 nm irradiation for 30 min.

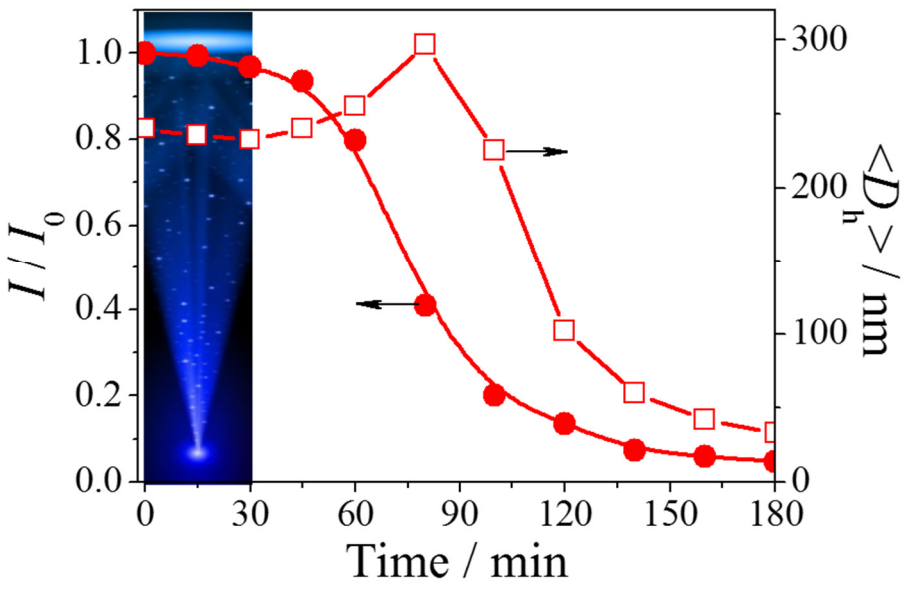


Figure S10. Evolution of normalized scattered light intensities and intensity-average hydrodynamic diameter, $<D_h>$, recorded at different time intervals for the aqueous dispersion of **PB1** SIPsomes (0.2 g/L, 25 °C) after being subjected to 30 min blue light irradiation (420 nm).

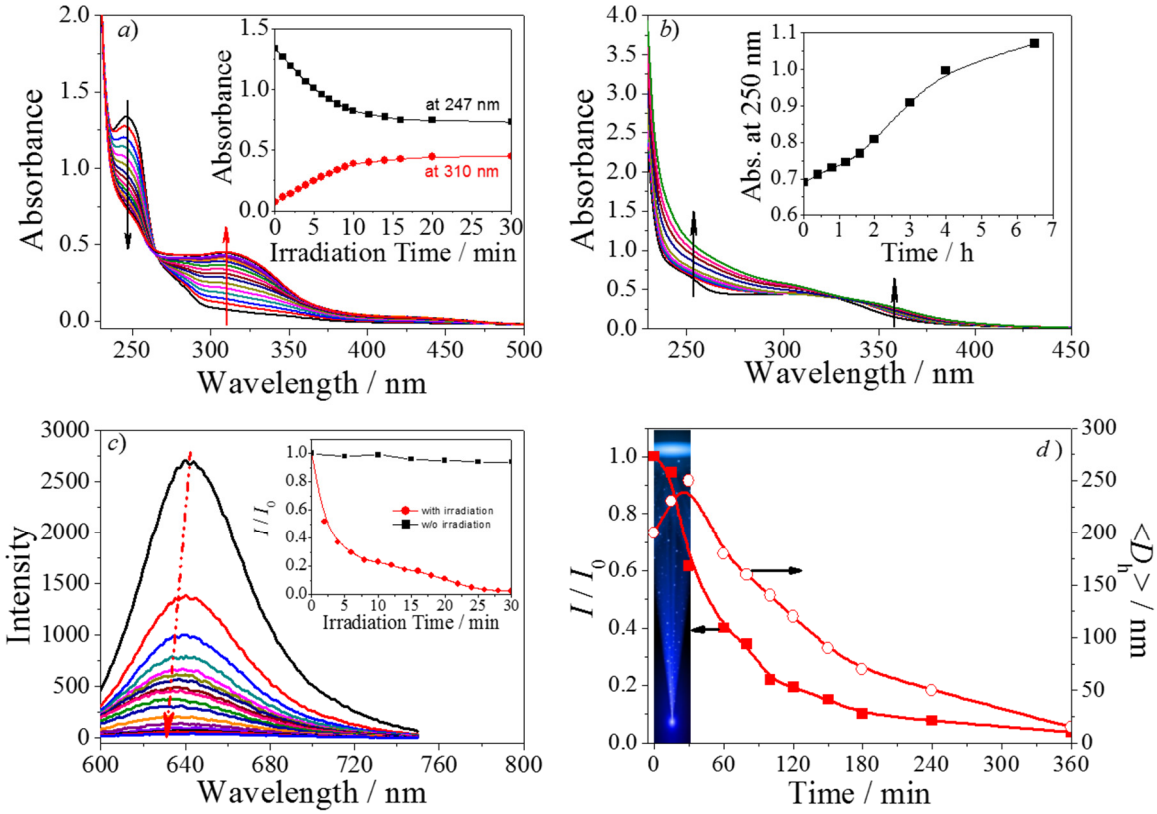


Figure S11. UV-vis absorbance spectra recorded for **PB3** SIPsome dispersion (a) during UV irradiation (0-30 min) and (b) upon storage for varying time intervals (0-20 h) after 30 min UV irradiation. (c) Fluorescence emission spectra (λ_{ex} = 550 nm) recorded for the aqueous dispersion of **PB3** SIPsomes encapsulating Nile red (NR) during UV irradiation (0-30 min); the inset shows NR release profiles from SIPsome bilayers. (d) Normalized scattered light intensity and intensity-average hydrodynamic diameters, $\langle D_h \rangle$, recorded at 25 °C for the aqueous dispersion of **PB3** SIPsomes upon 30 min UV irradiation followed by extended incubation.

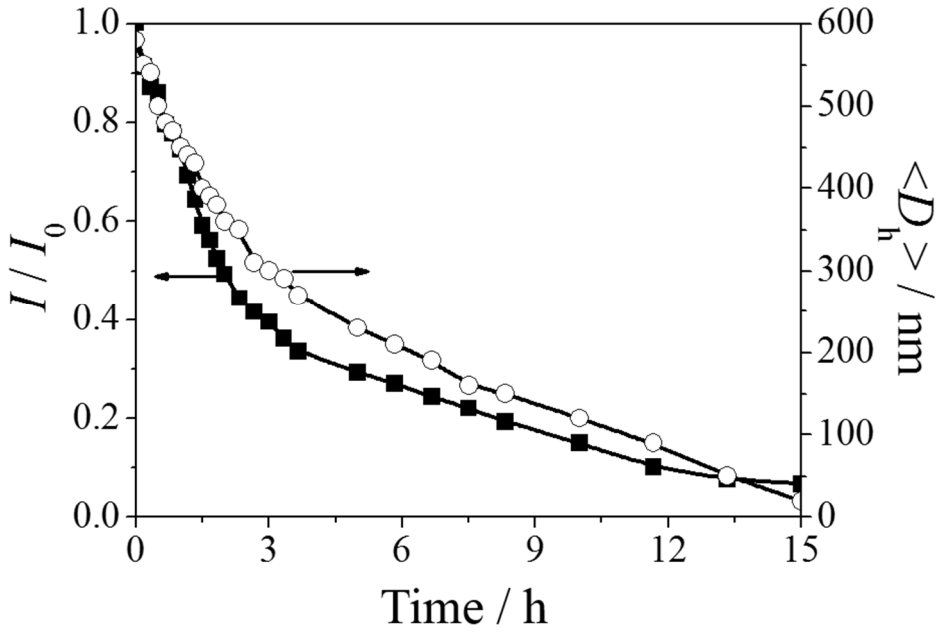


Figure S12. Evolution of normalized scattered light intensity and intensity-average hydrodynamic diameters, $<D_h>$, with incubation duration recorded at 25 °C for the aqueous dispersion of **PB4** SIPsomes (0.2 g/L) upon addition of DTT (10 mM).

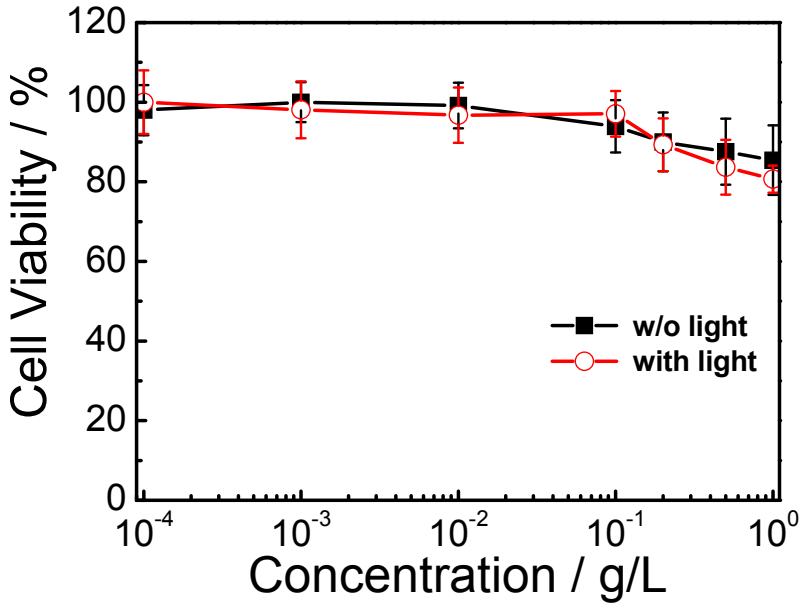


Figure S13. In vitro cytotoxicity determined by MTT assay against HepG2 cells for **PB1** SIPsome dispersions with or without light irradiation (420 nm, 30 min). The error bars indicate standard deviations from four parallel experiments.

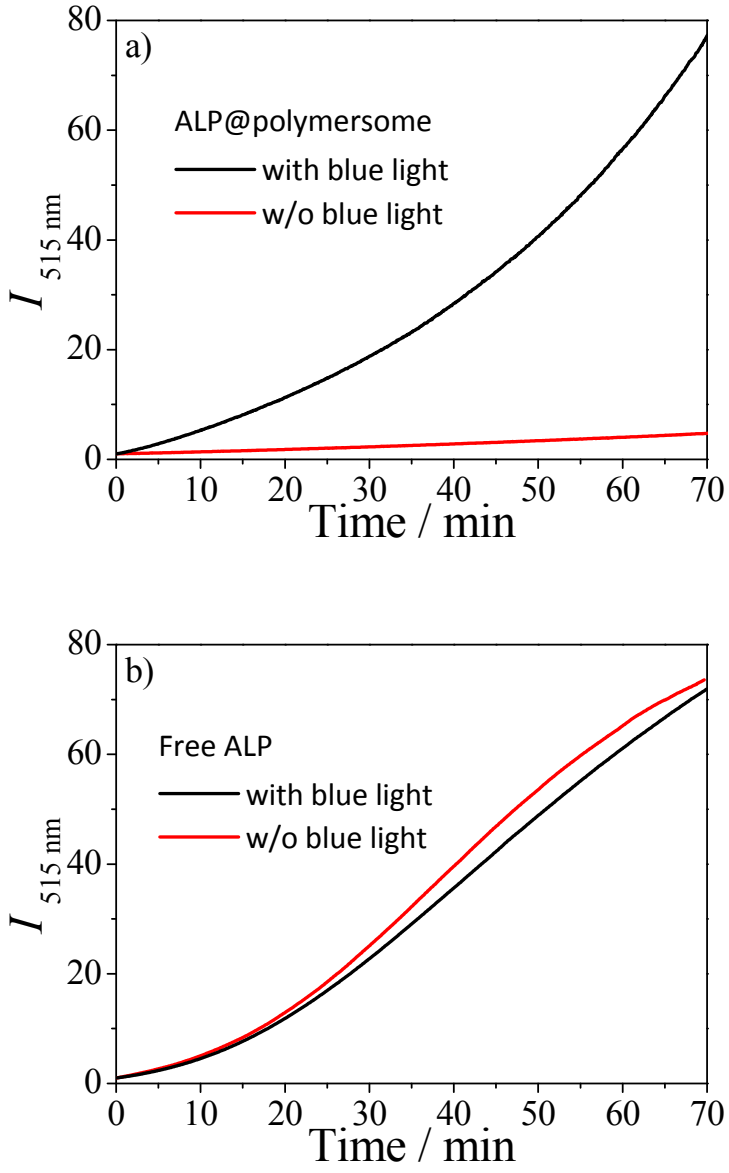


Figure S14. (a) Time-dependent evolution of fluorescence emission intensity (λ_{ex} = 480 nm, λ_{em} = 515 nm) of FDP (30 μM) in the presence of alkaline phosphatase (ALP) loaded **PB1** SIPsomes (0.2 g/L, 37 °C; 0.02 M Tris-HCl buffer, pH 8) before and after complete blue light triggered SIPsome disintegration. (b) Time-dependent emission intensity recorded for the aqueous solution of free ALP (5 mg/L, 37 °C; 0.02 M Tris-HCl buffer, pH 8) upon addition of FDP (30 μM); before FDP addition, the enzyme solution was either subjected to 30 min blue light irradiation (420 nm) or kept in dark.

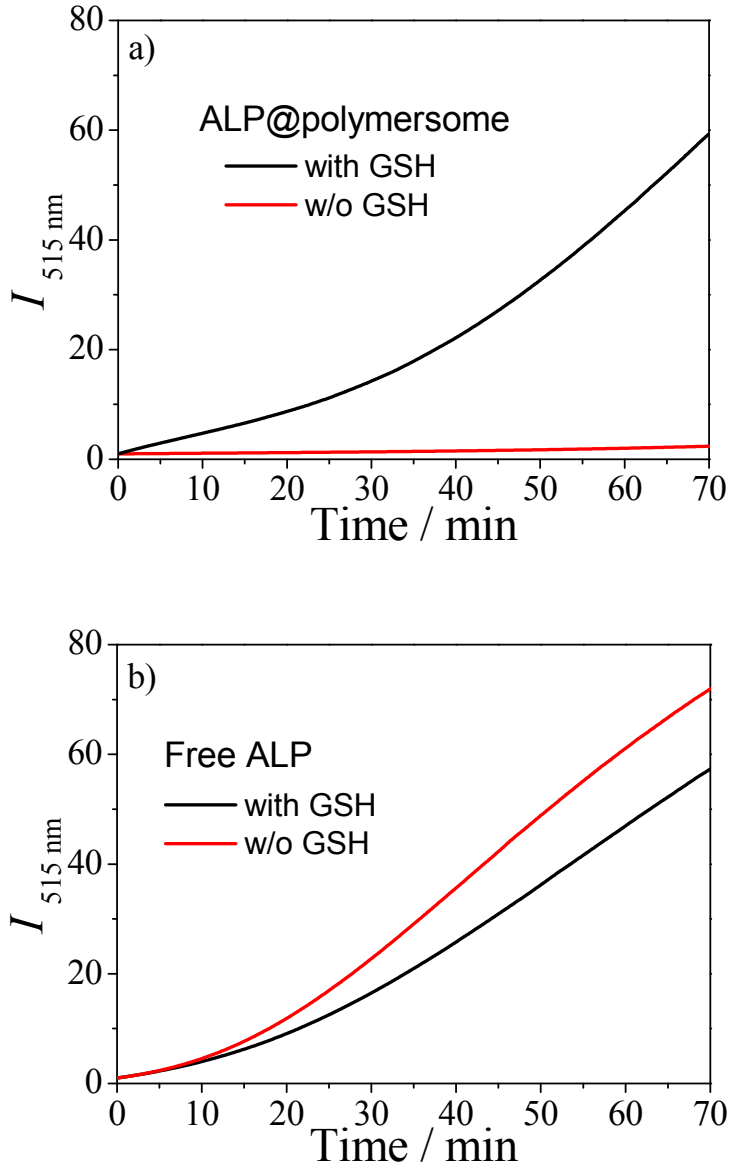


Figure S15. (a) Time-dependent evolution of fluorescence emission intensity (λ_{ex} = 480 nm, λ_{em} = 515 nm) of FDP (30 μM) in the presence of alkaline phosphatase (ALP) loaded **PB4** SIPsomes (ALP@**PB4** SIPsomes; 0.2 g/L, 37 °C; 0.02 M Tris-HCl buffer, pH 8) before and after complete GSH-triggered SIPsome disintegration. (b) Time-dependent emission intensity recorded for the aqueous solution of free ALP (5 mg/L, 37 °C; 0.02 M Tris-HCl buffer, pH 8) upon addition of FDP (30 μM); before FDP addition, the enzyme solution was co-incubated without or with GSH for 10 h.

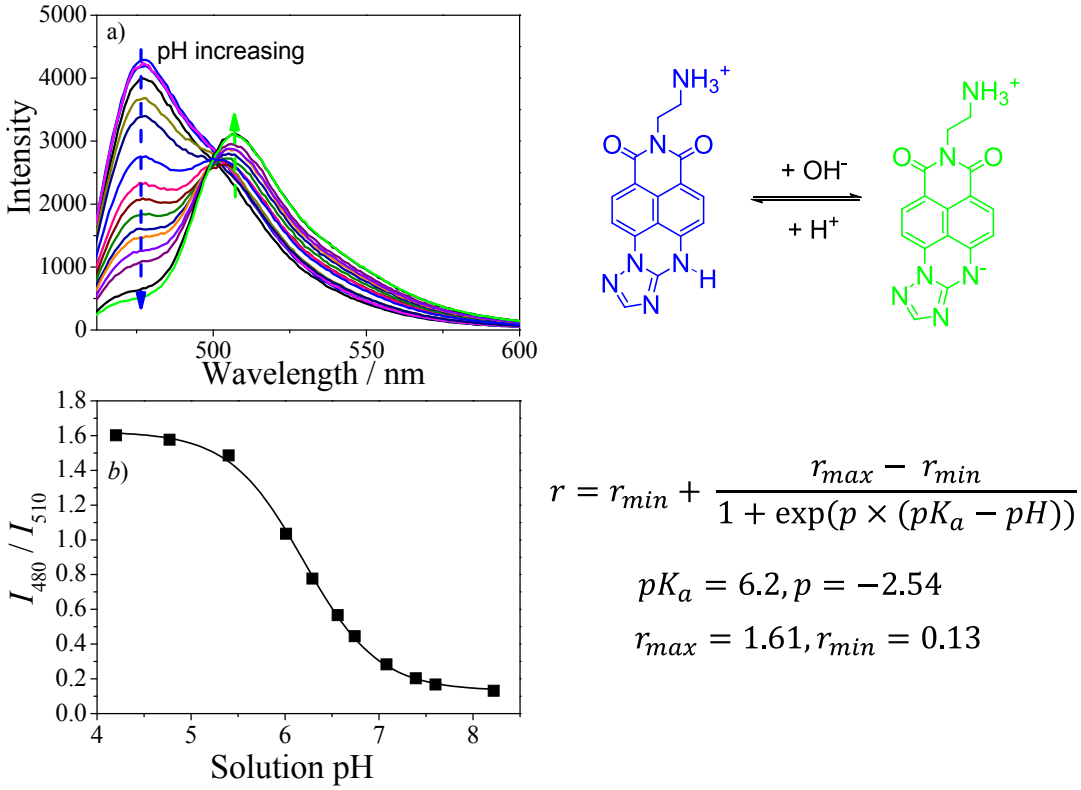


Figure S16. pH-dependent (a) fluorescence emission spectra (λ_{ex} = 455 nm) and (b) emission intensity ratios, I_{480}/I_{510}, recorded for the aqueous solution of NTEA (10 μM).

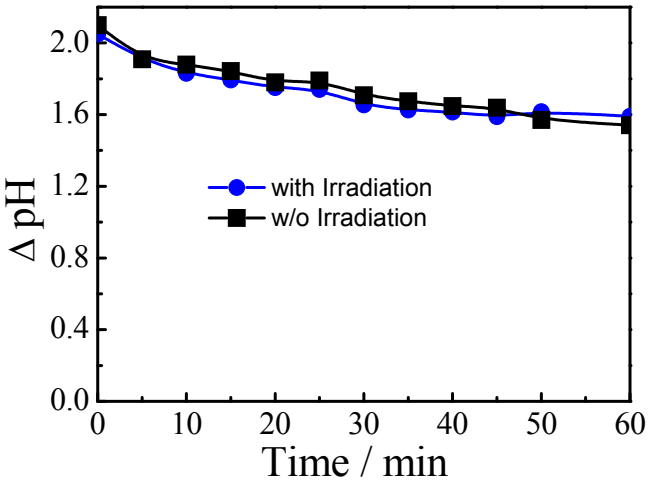


Figure S17. Time-dependent variation of pH discrepancy, ΔpH, between the inside and outside of NTEA-loaded PEO_{15}-*b*-PS_{60} polymersomes in aqueous media without or upon blue light irradiation (420 nm); the original pH inside polymersomes was 7.0, whereas the outside pH was adjusted to pH 5.0 using 0.1 M buffer just prior to light irradiation.

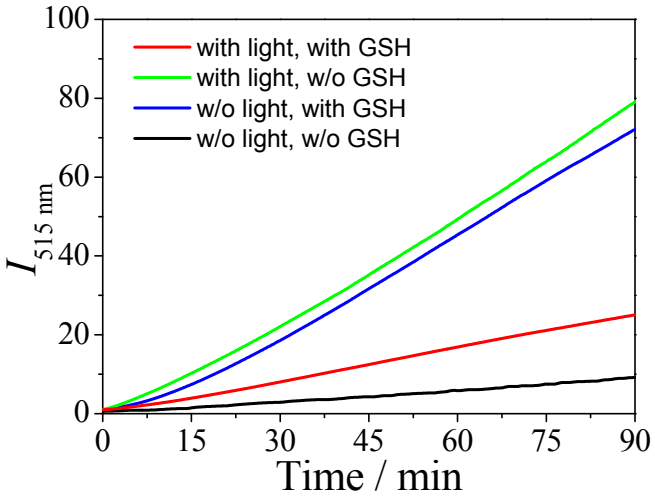


Figure S18. Time-dependent fluorescence emission intensities (λ_{ex} = 480 nm, λ_{em} = 515 nm) recoded for the aqueous mixture of 0.2 g/L (Lipase&EDTA)@PB1 SIPsomes and 0.2 g/L (ALP&Paraoxon)@PB4 SIPsomes (37 ºC, 0.04 M Tris-HCl buffer, pH 8) upon addition of 30 μM FDP and incubating for 90 min; before FDP addition, the SIPsome mixture was subjected to blue light irradiation (30 min) and/or GSH actuation.

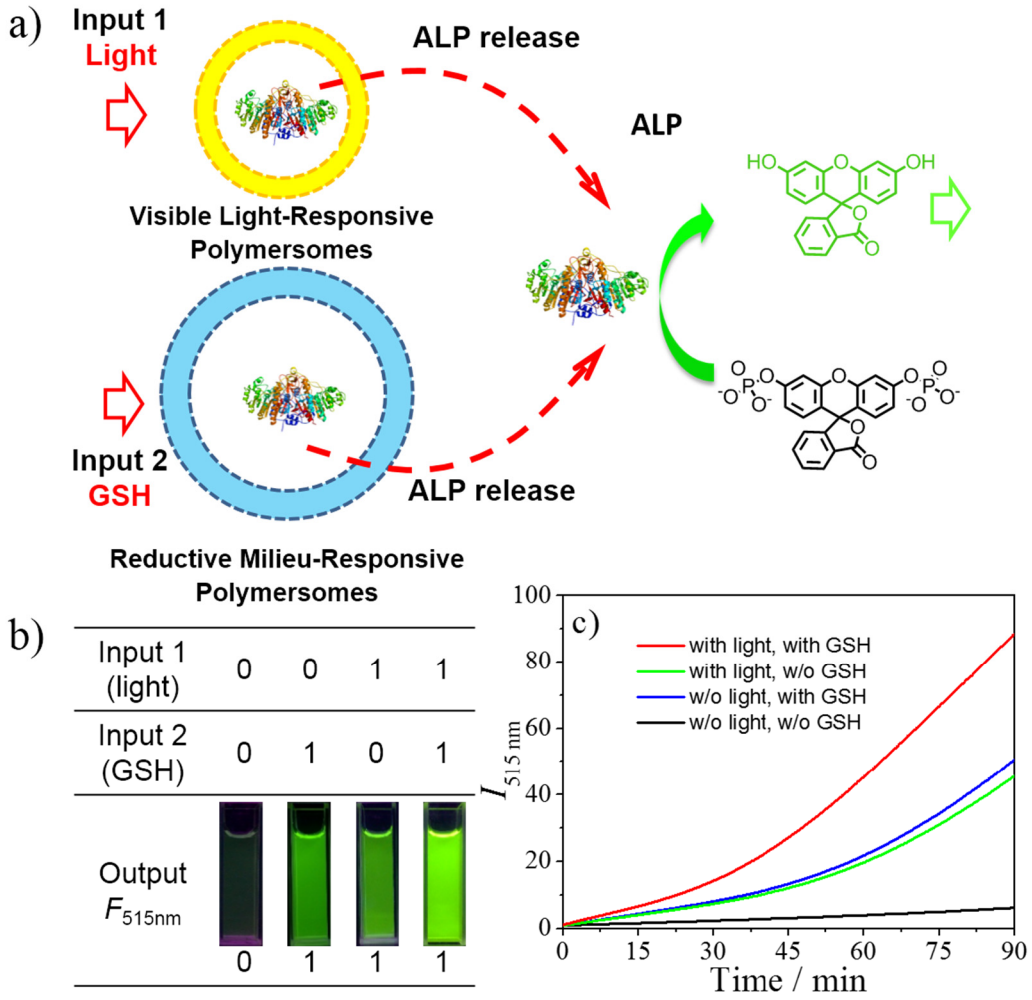


Figure S19. (a) Design principle of systems exhibiting OR-type logic gate output features on the basis of dual inputs of light and GSH addition. (b) Fluorescence photographs and (c) corresponding time-dependent fluorescence emission intensities (λ_{ex} = 480 nm, λ_{em} = 515 nm) recorded for the aqueous mixture of 0.2 g/L ALP@**PB4** SIPsomes and 0.2 g/L ALP@**PB1** SIPsomes (37 ºC, 0.04 M Tris-HCl buffer, pH 8) upon addition of 30 μM FDP and incubating for 90 min; before FDP addition, the SIPsome mixture was subjected to blue light or/and GSH actuation.