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

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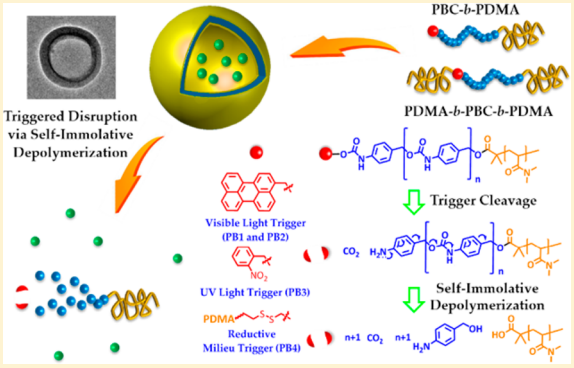
Supporting Information

ABSTRACT: Stimuli-triggered disassembly of block copolymer vesicles or polymersomes has been conventionally achieved via solubility switching of the bilayer-forming block, requiring cooperative changes of most of the repeating units. Herein we report an alternative approach by incorporating hydrophobic blocks exhibiting stimuli-triggered head-to-tail cascade depolymerization features. Amphiphilic block copolymers bearing this motif self-assemble into self-immolative polymersomes (SIPsomes). By modular design of terminal capping moieties, visible light, UV light, and reductive milieu can be utilized to actuate SIPsomes disintegration into water-soluble small molecules and hydrophilic blocks. The design of SIPsomes allows for triggered drug co-release and controllable access toward protons, oxygen, and enzymatic substrates. We also demonstrate programmed (OR-, AND-, and XOR-type logic) enzymatic reactions by integrating SIPsome encapsulation and trigger/capping moiety-selective cascade depolymerization events.



INTRODUCTION

Lipid vesicles (liposomes) and polymeric vesicles (polymersomes) are artificial self-assemblies inspired by complex biological systems such as cells and viral capsids.[1] Both of them consist of an aqueous interior entrapped by a hydrophobic bilayer membrane. Since discovery, they have been frequently utilized to construct drug delivery nanocarriers,[2] nanoreactors,[3] and artificial organelles.[4] Note that all of these functions are related to the exchange of substances between the outside and inside milieu. Although polymersomes are more robust and stable than liposomes, they suffer from severe membrane permeability concerns and are almost nonpermeable to small substances, ions, and even water molecules.[5]

To address this issue, block copolymers with the core-forming block being responsive to external milieu (e.g., pH,[6] temperature,[7] light,[8] redox,[9] sugar,[10] and CO_2[11]) have been employed to construct stimuli-responsive polymersomes. However, solubility switching of the responsive block typically relies on physicochemical changes of most repeating units and requires considerable input of external stimuli (energy and concentration),[12] just as those occurred in pH titration experiments.[12b] Although polymersomes containing biodegradable polyester block[13] or labile linkages[14] can be disrupted via spontaneous or random hydrolysis, multiple cleavage events are needed, and the process is less controlled; in addition, irregular aggregates might form due to the insolubility of degradation intermediates.[14b]

Since 2003, self-immolative dendrimers[15] and polymers[16] have emerged as a new design paradigm, featuring cascade chain depolymerization and release of small molecule building blocks upon removal of a single capping moiety at the focal point or chain terminal. Shabat et al.[16a] originally reported the synthesis of poly(benzyl carbamate) (PBC) self-immolative polymers terminally caged with cleavable moieties (e.g., 4-hydroxy-2-butanone); bovine serum albumin (BSA) can actuate trigger cleavage and release either fluorescent or colorimetric reporters. The nonlinear amplification nature renders them ideal candidates for drug delivery[17] and diagnostic[18] functions. For instance, microcapsules with triggered release characteristics have been fabricated from self-immolative homopolymers via either emulsification or microfluidic techniques.[17c,d] However, the controlled modular synthesis and hierarchical self-assembly of block copolymers bearing self-immolative motifs have been far less explored.[16c] We envisage that supramolecular aggregates of self-immolative block copolymers can impart advanced properties such as minimum cleavage events required for disintegration and robust aggregate morphology with rich responsive modes.

Herein we report on the fabrication of self-immolative polymersomes (SIPsomes) via self-assembly from amphiphilic block copolymers consisting of a triggered degradable PBC block and a hydrophilic poly(*N*,*N*-dimethylacrylamide) (PDMA) block (Scheme 1). The self-immolative block was caged with perylen-3-yl, 2-nitrobenzyl, or disulfide moieties, which are responsive to visible light (420 nm),[19] UV light (365

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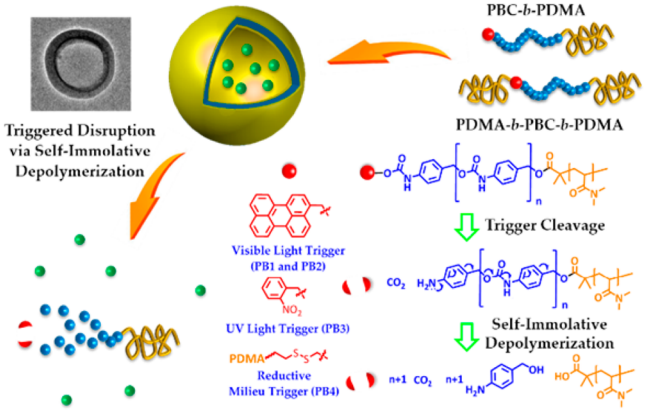
Scheme 1. Schematic Illustration of SIPsomes Self-Assembled from Poly(benzyl carbamate)-*b*-poly(*N*,*N*-dimethylacrylamide), PBC-*b*-PDMA, Amphiphilic Block Copolymers Containing Hydrophobic PBC Blocks, Which Are Subjected to Self-Immolative Depolymerization into Small Molecules upon Cleavage of "Capping" Moieties (i.e., Perylen-3-yl, 2-nitrobenzyl or Disulfide for Stimuli of Visible Light, UV Light, And Reductive Milieu, Respectively)[a]



[a]Various encapsulated bioactive agents are released from polymersomes upon stimuli-triggered disintegration.

nm)[20] or reductive milieu,[21] respectively. Upon removal of caging moiety triggered by appropriate stimulus, the block copolymer depolymerized into water-soluble 4-aminobenzyl alcohol (ABA), carbon dioxide, and PDMA. Triggered drug co-release and controllable access toward protons, oxygen, and enzymatic substrates can be achieved for guest-loaded SIPsomes, and further applications in programmed (OR, AND and XOR-type logic) enzymatic catalysis are also demonstrated.

RESULTS AND DISCUSSION

Amphiphilic block copolymers containing self-immolative hydrophobic blocks were synthesized by combining condensation polymerization and reversible addition−fragmentation chain transfer (RAFT) polymerization (Figure 1a and Scheme S1). First, the self-immolative PBC block (**P1**−**P5**) was synthesized through the polymerization of caged isocyanate **1** at 110 °C,[16a] followed by chain terminal functionalization with a variety of capping moieties, e.g., perylen-3-yl methanol, 2-nitrobenzyl alcohol, diethanol disulfide, or noncleavable benzyl alcohol. Next, PBC was transformed into a macroRAFT agent via hydroxyl esterification reaction with RAFT agent **2** (Figure S1). Finally, AB-type diblock (**PB1**−**PB3** and **PB5**) and BAB-type triblock (**PB4**) copolymers were prepared by RAFT polymerization. The molecular parameters of all polymer precursors and self-immolative block copolymers are summarized in Table 1.

The self-assembly of self-immolative diblock copolymer **PB1** in aqueous media was then investigated. TEM and SEM observations revealed the formation of robust polymersomes or SIPsomes (Figure 1b,c); dynamic light scattering (DLS) measurement revealed an intensity-average hydrodynamic diameter, $\langle D_h \rangle$, of ∼250 nm and a polydispersity of 0.13 (Figure S2). The vesicular nanostructure of SIPsomes was also verified using confocal laser scanning microscope (CLSM) by encapsulating green-emitting calcein in the aqueous interior (Figure 1d).

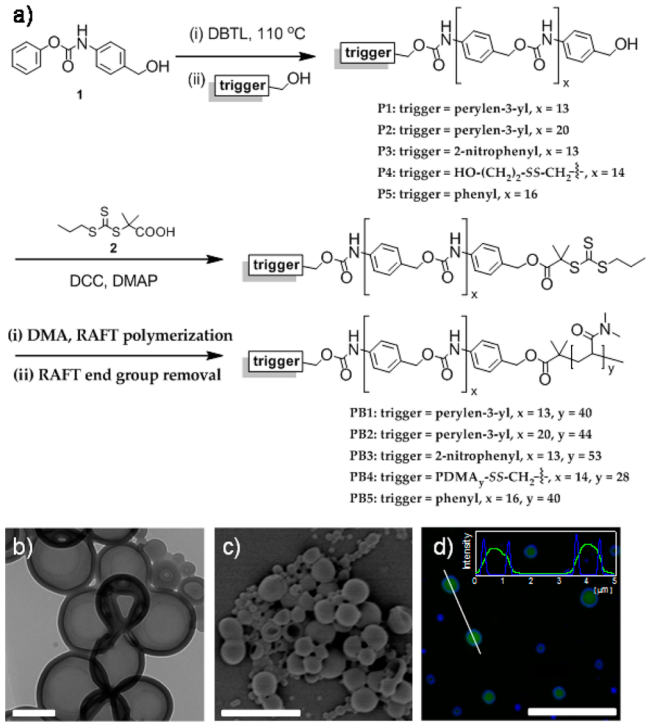


Figure 1. (a) Schematics employed for the synthesis of PBC−PDMA amphiphilic block copolymers containing hydrophobic self-immolative blocks. Abbreviations: DBTL, di-*n*-butyltin dilaurate; DCC, dicyclohexyl carbodiimide; DMAP, 4-dimethylaminopyridine. (b) Typical TEM (scale bar: 200 nm), (c) SEM image (scale bar: 2 μm), and CLSM images (scale bar: 5 μm; blue channel: perylene emission, 405 nm excitation; green channel: calcein, 488 nm excitation) recorded for **PB1** SIPsomes. Polymersomes encapsulating calcein were used for CLSM imaging, and inset in (d) shows blue and green channel emission intensity profiles.

Table 1. Molecular Parameters of Polymer Precursors and Self-Immolative Diblock Copolymers

entry	sample	$M_{n,\,NMR}$ (kDa)[a]	$M_{n,\,GPC}$ (kDa)[b]	M_w/M_n[b]
P1	*per*-PBC$_{13}$	2.1	4.8	1.45
P2	*per*-PBC$_{20}$	3.2	6.2	1.48
P3	*NB*-PBC$_{13}$	2.0	4.5	1.38
P4	HO-*SS*-PBC$_{14}$	2.2	4.8	1.39
P5	*Ph*-PBC$_{16}$	2.5	5.4	1.44
PB1	*per*-PBC$_{13}$-*b*-PDMA$_{40}$	6.3	10.9	1.22
PB2	*per*-PBC$_{20}$-*b*-PDMA$_{44}$	7.6	12.9	1.24
PB3	*NB*-PBC$_{13}$-*b*-PDMA$_{53}$	7.3	12.1	1.23
PB4	PDMA$_{28}$-*SS*-PBC$_{14}$-*b*-PDMA$_{28}$	7.8	12.7	1.21
PB5	*Ph*-PBC$_{16}$-*b*-PDMA$_{40}$	6.8	11.5	1.25

[a]Calculated from ^{1}H NMR results. [b]Determined by GPC using DMF as the eluent (1.0 mL/min).

The driving force for SIPsome formation from **PB1** with a high hydrophilic fraction f (67 wt %), which is much higher than the typical value (<35 wt % ± 10 wt %)[5b] required for generating block copolymer vesicles, should be ascribed to strong hydrogen-bonding interactions between carbamate moieties.[8] Possessing similar hydrophilic fractions, **PB3** and **PB4** also self-assemble into polymersomes, as confirmed by TEM (Figure 2g,i) and CLSM images (Figure S3). The average diameter of **PB4** SIPsomes (∼580 nm) are much larger than

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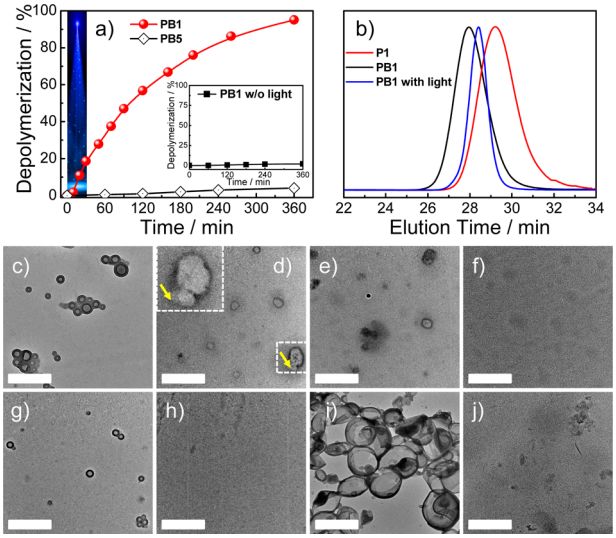

Figure 2. (a) Time-dependent depolymerization profiles recorded for **PB1** and **PB5** SIPsomes without or with 420 nm irradiation (25 °C); following 30 min blue light irradiation (blue region), the extent of spontaneous release of ABA was monitored by HPLC. (b) DMF GPC traces recorded for **P1** (red line), **PB1** (black line), and **PB1** subjected to complete self-immolative depolymerization for the hydrophobic block (blue line). (c–f) Typical TEM images recorded for **PB1** SIPsomes without irradiation (c) and upon incubating for 0 h (d), 1 h (e), and 12 h (f) after being irradiated for 30 min (420 nm light); yellow arrows indicate pore formation. (g,h) TEM images recorded for **PB3** SIPsomes without UV irradiation (g) and upon incubating for 12 h (h) after 30 min UV 365 nm irradiation. (i,j) TEM images recorded for **PB4** SIPsomes without DTT coincubation (i) and after being treated with 10 mM DTT for 24 h (j). Scale bars: 1 μm.

SIPsomes of **PB1** (~250 nm) and **PB3** (~205 nm), which should be ascribed to BAB triblock-type topology (Figure S2). Possessing longer hydrophobic PBC block compared to **PB1**, **PB2** self-assembles into large compound vesicles (LCVs) (Figure S4).

Next, we examined visible light responsiveness of **PB1** SIPsomes. After 30 min of light irradiation at 420 nm to fully decage the perylen-3-yl capping moiety (Figures S5 and S6), the self-immolative depolymerization process was traced by GPC and HPLC via monitoring the concentration of released ABA (Figure S7). Without irradiation, **PB1** SIPsomes are highly stable (inset in Figure 2a). For the irradiated dispersion, ~6 h is required for the PBC block of SIPsomes to almost completely depolymerize (>95% ABA release, Figure 2a). In addition, for the control sample (**PB5** SIPsomes), negligible ABA release can be discerned over the same time period due to that 420 nm irradiation cannot cleave the terminal benzyl moiety of **PB5**. The molecular weight of resultant residual polymer fragments was consistent with that of PDMA block in the original **PB1** block copolymer (M_n ~ 6500 Da) (Figure 2b), confirming the full spontaneous decomposition of PBC block. In addition, the self-immolative degradation of PBC block was also verified by ^{1}H NMR studies (Figure S8).

After 30 min visible light irradiation, the spontaneous SIPsome disintegration process was then monitored by TEM and CLSM experiments. The shells of **PB1** SIPsomes changed from thick continuous bilayer membranes (Figures 2c and S9a) to pore-embedded thinner ones (Figure 2d). Upon further extending incubation duration, vesicular nanostructures were disrupted and rearranged into irregular aggregates (Figure 2e), which finally disappeared after 12 h incubation (Figures 2f and S9b). The above process was also confirmed by time-dependent DLS measurements (Figure S10). The UV irradiation or reductive milieu triggered disintegration of **PB3** and **PB4** SIPsomes was also explored. Quite comparable spontaneous self-immolative depolymerization processes for the hydrophobic PBC block occurred after being subjected to 30 min UV irradiation (Figures 2g,h and S11) or by treating with 10 mM DTT (Figures 2i,j, S3, and S12), respectively.

Polymersomes are excellent drug nanocarriers that can encapsulate both hydrophobic and hydrophilic drugs for combinational therapy.[1,22] **PB4** SIPsomes respond to intracellular reductive milieu, which are ideally biological relevant,[21] and thus can serve as combinational therapeutic delivery nanovehicles upon co-encapsulating hydrophobic camptothecin (CPT) and hydrophilic doxorubicin (DOX·HCl). In the absence of glutathione (GSH), only ~4% DOX and ~19% CPT were released in 20 h; whereas upon addition of 10 mM GSH, the release rates considerably increased, and ~86% DOX and ~82% CPT were released in the same time scale (Figure 3). This was obviously caused by GSH-triggered SIPsome

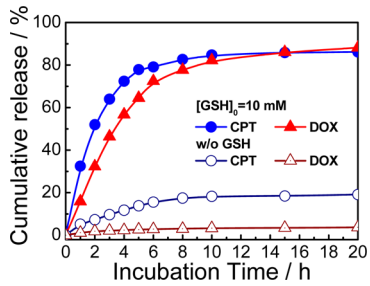

Figure 3. In vitro co-release profiles of hydrophobic CPT and hydrophilic Dox·HCl at 37 °C from CPT/DOX co-loaded **PB4** SIPsomes (0.1 g/L; 0.1 M PBS buffer, pH 7.4) in the absence or presence of GSH (10 mM).

disruption (Scheme 1). Considering the potential applications of SIPsomes in the field of drug delivery, we further conducted cell viability assays to check the cytotoxicity of SIPsomes. It was found that both irradiated and nonirradiated (420 nm, 30 min) **PB1** SIPsome dispersions exhibited negligible cytotoxicity up to a concentration of 1.0 g/L (Figure S13).

PB1 SIPsomes can also serve as drug delivery vehicle for photodynamic therapy agents (eosin Y), and the $^1O_2$ generation rate is directly linked to SIPsome integrity (Figure 4). For intact eosin-loaded **PB1** SIPsomes before triggered disassembly, the release of photosensitizer (eosin) is slow (Figure 4a), and diffusion rate of oxygen and 1,9-di(aminoethyloxyl)anthracene dihydrochloride (*An*-2NH$_2$) across hydrophobic SIPsome bilayers is also restricted, thus, $^1O_2$ generation rate is retarded (Figure 4b). However, upon SIPsome disintegration, the $^1O_2$ generation rate is considerably enhanced, as evidenced by the fast decay of *An*-2NH$_2$ emission. Besides, the catalytic activities of alkaline phosphatase (ALP)-loaded **PB1** and **PB4** SIPsomes can be modulated via visible light irradiation or GSH addition, respectively, due to altered rates of substrate access (Figures S14 and S15).

Can protons diffuse freely across the SIPsome bilayers? To answer this question, we measured the change of pH discrepancy, ΔpH, across the bilayer membrane of **PB1** SIPsomes using a ratiometric fluorescent internal pH probe (NTEA, see Figure S16 for chemical structure). By comparison,

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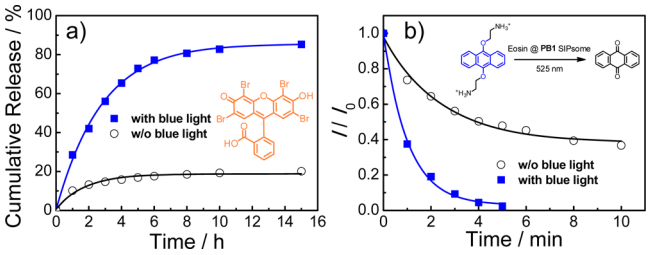

Figure 4. (a) In vitro release profiles of encapsulated eosin from **PB1** SIPsomes (0.1 g/L; phosphate buffer, pH 7.4) without and after being subjected to 420 nm blue light irradiation for 30 min. (b) Time-dependent decomposition of *An*-$2NH_2$ (i.e., $^1O2$ generation rate) upon 525 nm excitation recorded for eosin@**PB1** SIPsomes (0.1 g/L, phosphate buffer 0.1 M, pH 7.4) before and after blue light (420 nm)-triggered complete SIPsome disruption.

ΔpH of NTEA-loaded PEO_{15}-*b*-PS_{60} vesicles was also examined, where PS is polystyrene. Due to the existence of polar carbamate moieties in the bilayer membrane, protons can diffuse across **PB1** SIPsome membranes at a faster rate than PEO_{15}-*b*-PS_{60} polymersomes,[5a] therefore the ΔpH of **PB1** SIPsome was always smaller than that the latter (Figure 5a–c).

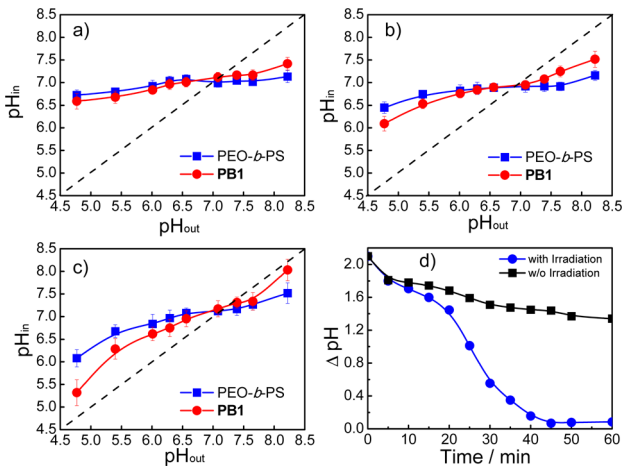

Figure 5. (a–c) The measured local pH within the interior of NTEA-loaded PEO_{15}-*b*-PS_{60} polymersomes and **PB1** SIPsomes upon adjusting the external buffer pH in the range of pH 4.8–8.2 and incubating for 5 min (a), 1 h (b), and 10 h (c) (25 °C; the original inside and outside pH is 7.0). The error bars indicate standard deviations from three parallel experiments. (d) Time-dependent variation of pH discrepancy, ΔpH, between the inside and outside of NTEA-loaded **PB1** SIPsomes in aqueous media without or upon blue light irradiation (420 nm); the original pH inside SIPsomes was 7.0, whereas the outside pH was adjusted to pH 5.0 using 0.1 M buffer just prior to light irradiation.

When subjected to blue light irradiation, the ΔpH of **PB1** SIPsomes decreased from ~2 to ~0 (Figure 5d) due to SIPsome disintegration, whereas the ΔpH of PEO_{16}-*b*-PS_{60} polymersomes was almost insensitive to light irradiation (Figure S17).

The modular design of block copolymers containing self-immolative blocks has allowed for the construction of three types of SIPsomes responsive to different triggering stimuli (visible light, UV light, and reductive milieu). Based on a mixture of different types of SIPsomes selectively encapsulating enzymes/substrates/inhibitors, we further designed OR, AND, and XOR logic gate-type enzymatic reactions (Figure 6). The

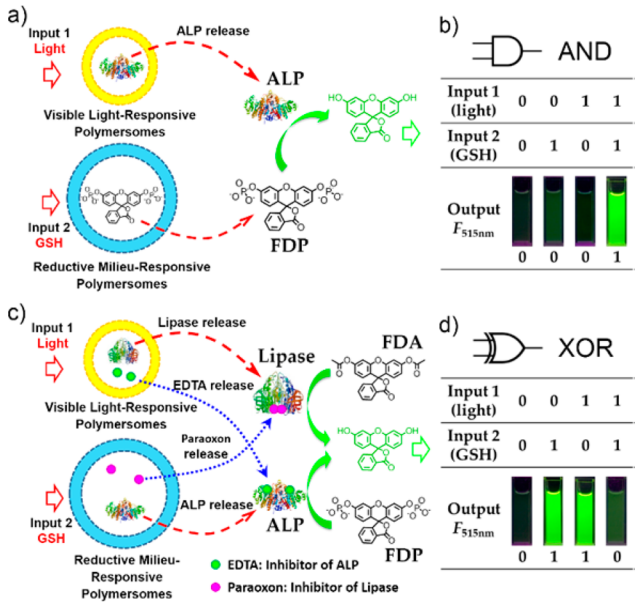

Figure 6. Construction of AND- (a,b) and XOR-type (c,d) logic gates based on a mixture of SIPsomes responsive to visible light irradiation (30 min) and reductive milieu, respectively. (b) Images taken under UV lamp for the aqueous dispersion containing 0.2 g/L ALP@**PB1** SIPsomes and 0.2 g/L FDP@**PB4** SIPsomes (0.04 M Tris-HCl buffer, pH 8.0) exhibiting AND logic gate output with dual inputs of visible light irradiation and GSH addition. (d) Images taken under UV lamp for the aqueous dispersion containing 0.2 g/L (Lipase&EDTA)@**PB1** SIPsomes and 0.2 g/L (ALP&Paraoxon)@PB4 SIPsomes exhibiting XOR-type logic gate output (0.04 M Tris-HCl buffer, pH 8.0). (a) and (c) show design principles. In all cases, [FDP] = [FDA] = 30 μM.

AND logic gate is composed of ALP-loaded **PB1** (ALP@**PB1**) SIPsomes and fluorescein diphosphate (FDP)-loaded **PB4** (FDP@ **PB4**) SIPsomes. After being subjected to both visible light irradiation and GSH treatment, ALP catalyzes the hydrolysis of FDP substrate to produce intense fluorescein emission (i.e., "1" output signal) (Figure 6a,b).

As for the XOR logic gate, the two events triggered by two inputs respectively must be mutually exclusive, thus, we loaded two enzymes and their corresponding inhibitors into different types of responsive SIPsomes: lipase/EDTA (ethylene diamine tetraacetic acid, inhibitor of ALP)@**PB1** SIPsome and ALP/paraoxon (inhibitor of lipase)@**PB4** SIPsome. FDP and fluorescein diacetate (FDA) were chosen as externally added substrates of ALP and lipase, respectively (Figure 6c). When subjected to (1, 0) or (0, 1) input, only one kind of enzyme can be released from SIPsomes, while its inhibitor was still trapped within another type of SIPsomes, so the released enzyme can act on its corresponding substrate to generate strong emission ("1" output). However, with (1, 1) input, both enzymes as well as their inhibitors were released, thus generating much weaker fluorescein emission ("0" output) (Figures 6d and S18). OR logic gate was also fabricated by encapsulating ALP within two types of SIPsomes responsive to two different stimuli, all exhibiting "1" output with (1, 0), (0, 1), or (1, 1) input (Figure S19).

CONCLUSIONS

In summary, we have developed the concept of SIPsomes based on amphiphilic block copolymers containing hydrophobic self-immolative blocks, which were terminally modified with three

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types of capping moieties. Upon triggered deprotection by the corresponding stimuli (visible and UV light, reductive milieu), SIPsomes disintegrate due to cascade depolymerization of the hydrophobic block. The modular responsive modes of SIPsomes allow for triggered drug co-release and switchable access toward protons, oxygen, and enzymatic substrates. Moreover, we also constructed OR, AND and XOR logic gate-type programmed enzymatic reactions on the basis of polymersomes with different types of stimuli responsiveness. The modular design and responsive selectivity/specificity of SIPsomes open a new platform for the fabrication of next generation drug delivery nanovehicles and smart devices, when further integrated with more biorelevant triggering motifs, targeting ligands, and therapeutic/imaging agents.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Detailed experimental procedures, NMR, UV−vis, and FL spectra, HPLC traces, TEM and CLSM images, and DLS. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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