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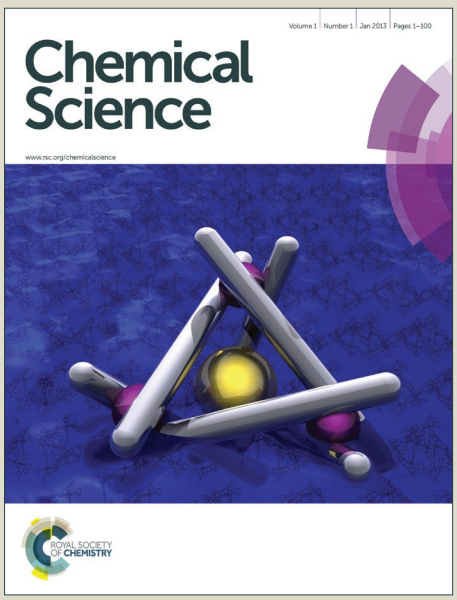





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# $H_2S$ gasotransmitter-responsive polymer vesicles

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Building biomimetic polymer vesicles that can sense a biological signaling molecule is a tremendous challenge in the cross-frontier of chemistry and biology. We develop a new class of *o*-azidomethylbenzoate (AzMB)-containing block copolymer that can respond to an endogenous signaling molecule, hydrogen sulfide ($H_2S$). Such a gasotransmitter can trigger cascade chemical reactions to sever the AzMB side functionalities, which alters the polymer amphiphilicity and further leads to a controllable disassembly of their self-assembly vesicular nanostructure. Moreover, if we introduce cystathionine γ-lyase (CSE), a specific enzyme converting cysteine into $H_2S$, onto the vesicle membrane, the polymersomes can extend their responsive scope from $H_2S$ to specific amino acid bioactivator. We anticipated that this polymer model would open up a new avenue to constructing biosignal-triggered nanocapsules for intracellular applications.

## Introduction

Stimuli-responsive polymer vesicles, regarded as a kind of smart nanovehicles, have sparked great attention in recent years since they hold promising prospective in drug delivery systems and nanomedicine.[1] In general, these nanovehicles can respond to external stimuli and then undergo drastic chemical structural changes,[2] thus inducing controlled dissociation so as to release cargos for target therapy. To date, a variety of external signals including temperature,[3] light,[4] and electric field,[5] as well as physiological factors, such as redox,[6] pH,[7] and enzyme[8] have been exploited for building responsive polymersomes. Recently, more and more intracellular applications require us to directly utilize bioactive molecules as triggers to manipulate payload release. This new strategy has two particular advantages: endogenous stimuli are conducive to enhancing specific drug biodistribution in diseased cells[1a] and prevent cell damage from chemical agent accumulation. In this respect, despite an imperative to simulate bioresponsive modes, building macromolecular assemblies that can sense a particular bioactivator remains elusive.[9]

Hydrogen sulfide ($H_2S$), known as a toxic gas in atmosphere, is also an important neuromodulator and cell signaling molecule. In cell, $H_2S$ can be generated from L-cysteine via cystathionine γ-lyase (CSE) mediated decomposition.[10] Latest reports have demonstrated that it plays a pivotal role in tuning blood vessel dilation, resisting inflammation and inducing cell apoptosis.[11] Metabolic disturbance of $H_2S$ is directly related to numerous diseases including angiocardiopathy and neurodegeneration. Taking into account the importance of $H_2S$ on cell, exploring how to use this bioactivator as a stimulation mode to activate the polymer self-assembly behaviors for targeted drug delivery is quite challenging.

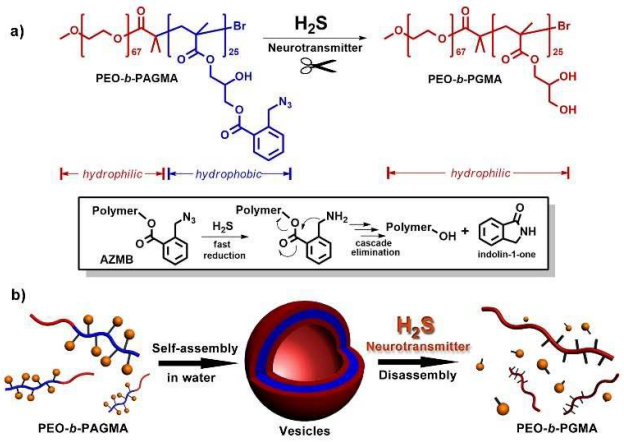


Fig. 1 (a) $H_2S$-responsive cleavage of *o*-azidomethylbenzoate (AzMB)-containing diblock copolymer (PEO-*b*-PAGMA) and $H_2S$-induced cascade reaction mechanism. (b) Schematic illustration of their polymer self-assembly into vesicles and $H_2S$-responsive controlled disassembly process.

At present, some nascent studies have devoted to embedding biosignals into polymer systems. A successful example is Yuan and Zhao et al. proposed $CO_2$-sensitive polymer assemblies.[12] Davis group designed nitric oxide (NO)-tuning copolymer.[13] In spite of the progress on this frontier, the polymers that can feedback $H_2S$ gasotransmitter have not been synthesized so far. Here we developed the new idea of using a class of peculiar *o*-azidomethylbenzoate-containing block copolymers to fabricate $H_2S$-responsive polymer vesicles. Their disassembly process is dependent on the stimulus concentration. Actually, *o*-Azido-methylbenzoate (AzMB) is a

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sensitive self-cleavable precursor, which can convert to benzylamine by $H_2S$, and the latter is able to cause a series of intramolecular cascade reactions, finally to sever the benzoyl bond (Fig. 1a). Based on this, we speculated that the AzMB pendants covalently linked onto the copolymer could be cleaved by low concentration of $H_2S$. Thereby, this will drive an amphiphilicity change of this block copolymer, further triggering a desirable disassembly of the polymer aggregates, as shown in Fig. 1b.

## Results and discussion

### Designing $H_2S$-responsive block copolymer

To fulfil the goal, we designed and synthesized a kind of diblock copolymer, consisting of a biocompatible poly(ethylene oxide) (PEO) chain as the hydrophilic block and a poly(*o*-azidomethyl benzoyl glycerol methacrylate) chain (termed as PAGMA) as the $H_2S$-sensitive hydrophobic segment. This target copolymer, PEO-*b*-PAGMA, was prepared by atom transfer radical polymerization with well-defined molecular weight ($M_w$ = 11.2 kDa) and near-monodispersion ($M_w/M_n$ = 1.09, the details of the synthesis and characterization in the ESI†).

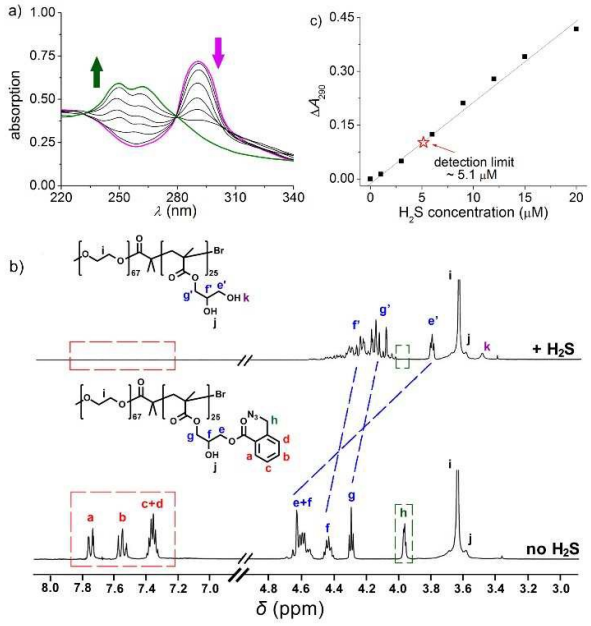


Fig. 2 (a) UV-Vis absorption changes of PEO-*b*-PAGMA under different $H_2S$ concentrations (from pink to green curve): 0, 2, 4, 6, 10, 12, 16, 20 $\mu$M. (b) $^1$H NMR spectra comparison of PEO-*b*-PAGMA before and after $H_2S$ treatment ($d_6$-DMSO). (c) The UV-Vis absorbed intensity changes of PEO-*b*-PAGMA upon different $H_2S$ levels ($\lambda_{max}$ = 290 nm). All the experiment were carried out at the polymer concentration of $9\times10^{-3}$ g $L^{-1}$, containing 20 $\mu$M AzMB groups.

We investigated $H_2S$-responsiveness of this copolymer by use of UV-Vis spectroscopy. With increasing the $H_2S$ concentration, the absorption changes of PEO-*b*-PAGMA can be monitored. We found that in the absence of external stimulus, the polymer solution ($9\times10^{-3}$ g $L^{-1}$, containing 20 $\mu$M concentration of AzMB groups) exhibited a characteristic absorption at 290 nm ascribed to the AzMB species (Fig. 2a, pink curve). Upon gradual addition of $H_2S$ from 0 to 20 $\mu$M, interestingly, the intensity of AzMB absorption band declined by 94%, whereas a group of new double peaks at 248 nm and 259 nm appeared and was slowly strengthened (Fig. 2a, green curve), suggesting that $H_2S$ can react with the AzMB functionality. To further elucidate this chemical process, before and after gas treatment, all the reactive components in solution were separated by liquid chromatography and analysed by $^1$H NMR spectroscopy (Fig. 2b). Before stimulus, it is clear that the $^1$H NMR spectrum of PEO-*b*-PAGMA showed a group of typical aromatic region proton shifts ($H_a$—$H_d$, $\delta$ = 7.30—7.76 ppm) and a benzyl peak ($H_h$, $\delta$ = 3.95 ppm) ascribed to AzMB species. After the copolymer solution was exposure upon $H_2S$, however, all the above peaks in the final products totally vanished, indicating the cleavage of the benzoyl bond. In addition, the three groups of methylene peaks ($H_e$, $H_f$ and $H_g$) belonged to the glycerol motif relatively shifted to upfield ($H_e\rightarrow H_{e'}$: $\delta$ = 4.63→3.81, $H_f\rightarrow H_{f'}$: $\delta$ = 4.43→4.36, $H_g\rightarrow H_{g'}$: $\delta$ = 4.31→4.19), which is in line with the spectrum of poly(glycerol methacrylate) (PGMA, Fig. S3 in ESI†). These results provided key evidence of the detachment of AzMB pendants from the copolymer main chain. Furthermore, we attempted to reveal the reaction mechanism of $H_2S$ cutting off the AzMB group. To this end, we separated and purified other byproduct from the reactive solution. A cyclic benzolactam compound was detected in the filtrate residue. Its $^1$H NMR spectrum perfectly matched with commercial indolin-1-one (Fig. S4 in ESI†) and its UV-Vis absorbance is in accord with the double-peak curve (Fig. S5 in ESI†), indicating that $H_2S$ can facilitate an intramolecular cyclization of benzoyl bond to yield indolin-1-one byproduct.

Infrared spectra (IR, Fig. S6 in ESI†) corroborated this reactive mechanism: the initial PEO-*b*-PAGMA solution displayed strong azido group stretching vibration (2102 $cm^{-1}$); upon 6 min of $H_2S$ gas stimulus, the benzylazide peak was greatly depressed but a shoulder peak at 3465 $cm^{-1}$ ascribed to benzylamine reinforced. However, this new vibration band vanished after 30 min of $H_2S$ treatment and the final PEO-*b*-PGMA generated, as indicated by the disappearance of phenyl group (1614 $cm^{-1}$) and the appearance of broad hydroxyl band (3240–3640 $cm^{-1}$). All these findings demonstrates that $H_2S$ fast transforms benzylazide into a high-reactive nucleophilic benzylamine intermediate, and the latter is capable of attacking intramolecularly on the adjacent benzoyl to induce cascade self-elimination reaction, leading to a site-specific chemical scission (Fig. 1a).

Since the designed PEO-*b*-PAGMA can respond to $H_2S$, we next aimed to quantitatively test its sensitivity. If the detection limit of $H_2S$ is defined as a 10% change in UV-Vis absorbance,[13] it is worth noting that PEO-*b*-PAGMA is determined to be extremely high sensitive to $H_2S$ stimulus (detection limit is 5.1 $\mu$M, Fig. 2c). Recent studies indicated that $H_2S$ concentration in the brain is likely less than 9.2 $\mu$M,[14] but which is capable of activating the cleavage reaction. Additionally, we discovered

that this polymer system showed linearly $H_2S$ concentration-dependent responsiveness, which means that the cleaved ratio of the polymer is adjustable.

**$H_2S$-triggered controlled disassembly of the polymersomes**

To further explore whether this polymer system is adapt to use as drug nanovehicles, we studied their self-assembly behaviors. Owing to the amphiphilicity, PEO-*b*-PAGMA can spontaneously form aggregates in aqueous solution. The critical aggregate concentration (CAC) is determined to be 0.02 g $L^{-1}$, as measured by fluorescent probe method (Fig. S7 in ESI†).[15] Transmission electron microscope (TEM) was employed to visualize the size and morphology of these polymeric aggregates. It is clear that PEO-*b*-PAGMA can self-assemble into sphere-like nanostructure (Fig. 3a). The legible contrast between the dark periphery and hollow center indicates that these globular aggregates are typical vesicular architecture and their membrane thickness is evaluated to be 8 nm by TEM statistics (Fig. 3a, inset). Normally, in the vesicles, PEO block chains serve as the hydrophilic outer and inner layers while PAGMA portion as the hydrophobic core layer. TEM images displayed that the average diameter of these aggregates is 62 nm, corresponding to the hydrodynamic radius ($R_h$) of 34.6 nm determined by dynamic light scattering (DLS, Fig. S8 in ESI†). The small deviation (7.2 nm) between TEM and DLS results contributes to the fact that DLS measures the hydrated state while TEM, using dried samples, does not. In the absence of any stimuli, these aggregates are stable in water, and either their size or shape has no change over two months.

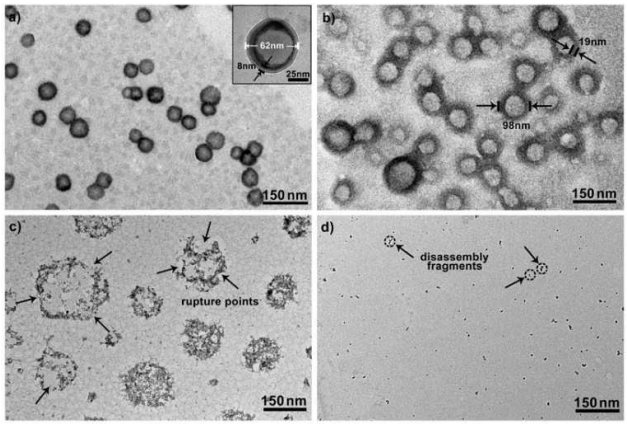


Fig. 3 TEM images of PEO-*b*-PAGMA aggregates in various levels of $H_2S$ stimulus: (a) 0 $\mu$M, (b) 15 $\mu$M, (c) 30 $\mu$M and (d) 45 $\mu$M. (The polymer concentration is 0.02 g $L^{-1}$, which contains AzMB group concentration of 45 $\mu$M).

Since the PAGMA block chain can react with $H_2S$ and further remove the AzMB moieties to yield PGMA block, we speculated that their polymer assemblies could disassemble in $H_2S$ atmosphere. As expected, when $H_2S$ was passed through the polymer solution (0.02 g $L^{-1}$, containing 45 $\mu$M of AzMB groups), their assembling structures began to change. In the presence of a small amount of $H_2S$ gas (15 $\mu$M), these vesicles expanded remarkably. As shown in Fig. 3b, much larger vesicles were dominant in the solution. Their diameter rose averagely 160% from 62 nm to 98 nm and their volume increased 410%. The reason is that one-third hydrophobic PAGMA block chains convert to hydrophilic PGMA block, which results in a strong hydration effect within the core layer; as a consequence, these vesicles swell fast in order to lower the interaction free energy. Nearly a double thickening of these vesicle walls from 8 nm to 19 nm supported this hydration-driven expansion mechanism. Similar results have be found in other responsive vesicles.[12a,c] When $H_2S$ level was increased to 30 $\mu$M, a great amount of PAGMA block chains transformed. Because the sharp enhancement of water solubility of the polymer chains result in an insupportable interfacial tension, these swollen vesicles started to crack and their membrane presented many random fractured points (Fig. 3c). With injecting $H_2S$ to 45 $\mu$M, the majority of AzMB groups were disconnected and the amphiphilic PEO-*b*-PAGMA changed to water-soluble PEO-*b*-PGMA, finally leading to complete vesicle dissociation. Only a little of tiny nanofragments remained in the solution, whose size (<10 nm) is in line with that of dried PEO-*b*-PGMA unimers (Fig. 3d). DLS monitoring confirmed this disassembly process. By changing the $H_2S$ aeration time, we can tune the $H_2S$ stimulus level. Under a lower $H_2S$ level (15 $\mu$M), the $R_h$ of these aggregates climbed up to 54.3 nm, being consistent with the early vesicle expansion. Then their $R_h$ underwent an abrupt decrease from a maximum value to 4.3 nm with $H_2S$ increase to 45 $\mu$M, corresponding to the later vesicular burst process (Fig. 4a).

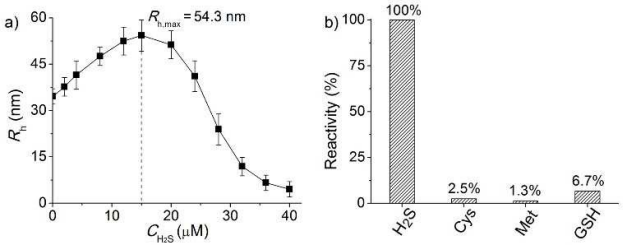


Fig. 4 (a) The polymersome radius changing as a function of $H_2S$ concentration monitored by DLS counting (after gas treatment for 30 min, the polymer solution was incubated in 30 min and then tested by DLS). (b) The responsive specificity comparison among other sulphur-containing bioactivators ($H_2S$: 20 $\mu$M; Cys, Met and GSH: 45 $\mu$M. $H_2S$ as a reference of 100% activity and the treatment time of all stimulants keep in 60 min).

On the other hand, one may doubt that other factors such as mechanical effects could bring about this vesicle disruption. In a control experiment, we prepared a copolymer counterpart, PEO-*b*-poly(benzoyl glycerol methacrylate) (PEO-*b*-PBGMA) lack of the azido group. It can form analogous nanoobject in aqueous solution as well but is unable to disaggregate even in higher $H_2S$ level (100 $\mu$M), thus eliminating this possibility and proving that the vesicular disassembly mechanism arises from $H_2S$-sensitive macromolecular structural alteration (Fig. S9 in ESI†).

### The specificity of $H_2S$-triggered polymersome disassembly

To better adapt to cell environment, it is desirable that this disassembly is of $H_2S$ specificity and selectivity. In biological cells, besides the $H_2S$ gasotransmitter, there are other sulphur-containing bioactivators to guide cell activities such as cysteine (Cys), methionine (Met), and glutathione (GSH).[16] According to the above experiments, we know that the cleavage of polymer leads to an absorption change. Based on this characteristic, we surveyed the effects of these sulphur-containing chemicals by UV-Vis spectroscopy. A higher level of bioactivators (45 $\mu$M) was injected into the polymer solution and the system was incubated for 60 min. Regarding $H_2S$-induced spectral change as a 100% active reference, other bioactivators (Cys, Met and GSH) showed negligible activities (<7%, Fig. 4b and Fig. S10 in ESI†). It points out that these polymersomes entering the cells can selectively respond to $H_2S$ gasotransmitter but prevent from the influence of other sulphur interferents.

### Extending the bioresponsive scope of the polymersomes

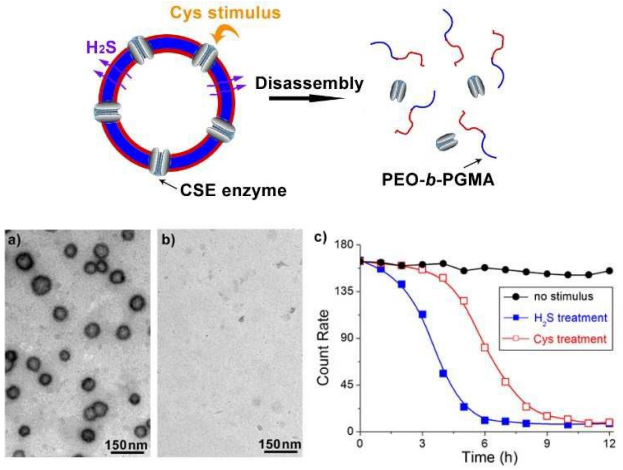


Fig. 5 Schematic illustration of Cys-responsive polymer vesicle disruption (top). (a) and (b) TEM images showing Cys-triggered disassembly of CSE/PEO-b-PAGMA hybrid polymersomes: (a) no stimulus, (b) injecting 45 $\mu$M Cys into the aggregate solution (The polymer concentration is 0.02 g $L^{-1}$, containing the AzMB group of 45 $\mu$M). (c) The disassembly rate of the polymersomes upon various conditions: no stimulus (●), $H_2S$ treatment (■) and Cys treatment (□). The test was conducted by DLS counting rate.

In view of the excellent $H_2S$-responsiveness of our vesicles, we expected that we could extend their responsive range to a variety of biomolecules or biological metabolites. In cell, $H_2S$ can be endogenously produced by many kinds of proteases. For example, CSE enzyme is capable of converting intracellular Cys into $H_2S$.[10] Therefore, introducing CSE into the vesicle system can broaden the responsiveness from $H_2S$ to a specific amino acid, Cys. Based on this principle, a trace amount of CSE protein (20 nM) was co-assembled with PEO-*b*-PAGMA. After the vesicle formed, there should be a part of CSE disperse in the solution. To remove these residues, the aggregate solution was centrifuged for 5 min at 11000 rpm at certain intervals (2 h), and 2 mL of the supernatant was withdrawn and replaced by fresh medium. Through measuring the UV-Vis spectra of the supernatant until no obvious CSE absorption, it indicated that the unassembled CSE were removed and only 35% of proteins were noncovalently anchored on the vesicular membrane (Fig. S11 in ESI†). Adding Cys (45 $\mu$M) in place of $H_2S$, this amino acid can penetrate across the membrane and further be decomposed into $H_2S$ by CSE. Afterwards the generated $H_2S$ can in-situ react with PAGMA block chains to provoke a similar vesicular disruption (Fig. 5a–b). In comparison to $H_2S$ stimulus, Cys-triggered disassembly rate is 45% slower than that of $H_2S$ treatment, as indicated by DLS counting experiments (Fig. 5c). It is understandable since the CSE-mediated enzymatic reaction needs an induction period for Cys transmembrane penetration and $H_2S$ production. In a similar way, if one can introduce other $H_2S$-producing proteins into the polymer vesicle, the responsive scope will be expanded to the corresponding biomolecules.

### $H_2S$-responsive controlled drug release

Finally, to assess the feasibility of these polymersomes as drug delivery vectors, we performed the payload release tests. It is known that the overproduction of $H_2S$ gasotransmitter can result in the blood vessel overexpansion and hypotension.[11a] Epinephrine (EP), as a water-soluble vasoconstrictor, is used for resisting vessel dilation. Encapsulated EP into our vesicles, it is anticipated when these drug-loaded nanocarriers enter the $H_2S$-overproducing cells, they can rapidly rupture to liberate internal cargos for in-situ inhibiting the vessel dilation.

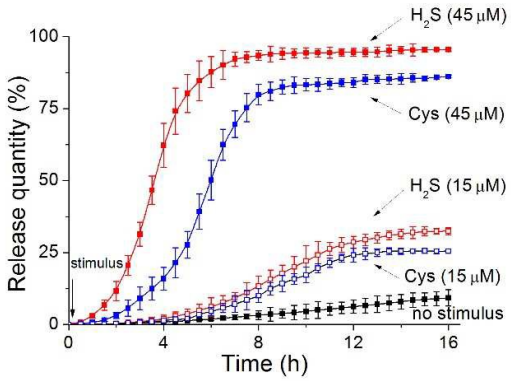


Fig. 6 Controlled drug release of EP from PEO-*b*-PAGMA vesicles or CSE/PEO-*b*-PAGMA hybrid vesicles in different conditions: no stimulus (■), non-hybrid vesicles upon 45 $\mu$M $H_2S$ stimulus (■), non-hybrid vesicles upon 15 $\mu$M $H_2S$ stimulus (□), hybrid vesicles upon 45 $\mu$M Cys stimulus (■) and hybrid vesicles upon 15 $\mu$M Cys stimulus (□). The polymer aggregate concentration is 0.02 g $L^{-1}$, containing AzMB group of 45 $\mu$M.

In our experiments, the EP-loaded polymersomes were packed in semipermeable bag (MWCO = 3.0 kDa), which was dialyzed against phosphate buffer (pH = 7.2). The quantity of

release was recorded through the intensity of EP diagnostic excitation[17] ($\lambda_{ex}$ = 317 nm) by fluorescent spectra. The release amount plotted against release time in various stimulation cases are depicted in Fig. 6. Without any trigger the nanocapsules showed a low-level free release process (less than 10% over 16 h). When applying a low concentration of $H_2S$ stimulus (15 $\mu$M), these vesicles only expand but not crack, thus leading to an increase of the number of membrane nanoporous defects in the vesicles. Such a result further enhances the leak of internal EP payloads, thereby the release rate had a modest ascent (nearly 32% within 16 h). In contrast, when adding a high concentration of $H_2S$ stimulus (45 $\mu$M), the complete disruption of these polymer vesicles caused a rapid cargo release. The maximum release amount reaches 94% in a shorter time (6 h).

In another case, we utilized Cys as an alternative stimulant to trigger the CSE-anchored hybrid polymersomes. In comparison to those corresponding entities without enzymes, their release speed were decelerated down (15 $\mu$M Cys caused a 25% release within 16 h and 45 $\mu$M Cys caused a 86% release within 10 h: blue dash line and blue solid line, respectively). The results can be drawn that our vesicles can serve as intelligent nanovehicles for realizing controllable drug delivery by modulating biosignal strength.

## Conclusions

In summary, we have designed and developed a new class of *o*-azidomethylbenzoate (AzMB)-containing block copolymers. They can spontaneously form vesicular architecture in aqueous media on the basis their amphiphilicity. Particular functionality in the polymer endow these vesicles with unique sensitivity to a gaseous signaling molecule, $H_2S$. $H_2S$ can motivate a controlled disassembly of the polymersomes by site-specific cleavage of the AzMB groups. Moreover, we propose a concept, by means of installing various $H_2S$-producing proteins onto the vesicle membrane, to reasonably extend the polymer responsive scope from simple $H_2S$ to complex biomolecules. In the respect of drug delivery, due to this endogenous stimulus mode, the nanovectors are promising for intracellular targeted release and therapy. We envisage that this kind of polymer model will open up a new avenue to construct bioresponsive nanocapsules for more biological applications.

## Acknowledgements

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## Notes and references

1 (a) S. Mura, J. Nicolas and P. Couvreur, *Nat. Mater.*, **2013**, 12, 991-1003. (b) C. J. F. Rijcken, O. Soga, W. E. Hennink and C. F. van Nostrum, *J. Controlled Release*, **2007**, 120, 131-148. (c) P. Tanner, P. Baumann, R. Enea, O. Onaca, C. Palivan and W. Meier, *Acc. Chem. Res.*, **2011**, 44, 1039-1049.

2 (a) F. H. Meng, Z. Y. Zhong and J. Feijen, *Biomacromolecules*, **2009**, 10, 197-209. (b) M.-H. Li and K. Patrick, *Soft Matter*, **2009**, 5, 927-937.

3 (a) Y. T. Li, B. S. Lokitz and C. L. McCormick, *Angew. Chem. Int. Ed.*, **2006**, 45, 5792-5795. (b) Y. Cai, K. B. Aubrecht and R. B. Grubbs, *J. Am. Chem. Soc.*, **2010**, 133, 1058-1056. (c) A. O. Mougton and R. K. O'Reilly, *Chem. Commun.*, **2010**, 46, 1091-1093.

4 (a) B. Yan, X. Tong, P. Ayotte and Y. Zhao, *Soft Matter*, **2011**, 7, 10001-10009. (b) Y. Liu, C. Y. Yu, H. B. Jin, B. B. Jiang, X. Y. Zhu, Y. F. Zhou, Z. Y. Lu and D. Y. Yan, *J. Am. Chem. Soc.*, **2013**, 135, 4765-4470. (c) Y. P. Wang, P. Han, H. P. Xu, Z. Q. Wang, X. Zhang and A. V. Kabanov, *Langmuir*, **2010**, 26, 709-715.

5 Q. Yan, J. Y. Yuan, Z. N. Cai, Y. Xin, Y. Kang and Y. W. Yin, *J. Am. Chem. Soc.*, **2010**, 132, 9268-9270.

6 (a) A. Napoli, M. Valentini, N. Tirelli, M. Müller, J. A. Hubbell, *Nat. Mater.*, **2004**, 3, 183-189. (b) N. Ma, Y. Li, H. Xu, Z. Wang and X. Zhang, *J. Am. Chem. Soc.*, **2010**, 132, 442-443. (c) W. Cao, Y. Gu, M. Meineck, T. Li and H. P. Xu, *J. Am. Chem. Soc.*, **2014**, 136, 5132-5137.

7 (a) J. Z. Du, Y. Q. Tang, A. L. Lewis and S. P. Armes, *J. Am. Chem. Soc.*, **2005**, 127, 17892-17893. (b) J. Rodríguez-Hernández and S. Lecommandoux, *J. Am. Chem. Soc.*, **2005**, 127, 2026-2027. (c) L. Shen, J. Z. Du, S. P. Armes and S. Y. Liu, *Langmuir*, **2008**, 24, 10019-10025. (d) J. Yin, D. Dupin, J. F. Li, S. P. Armes and S. Y. Liu, *Langmuir*, **2008**, 24, 9334-9340. (e) W. Q. Chen and J. Z. Du, *Sci. Rep.*, **2013**, 3, 2162. (f) K. E. B. Doncom, C. F. Hansell, P. Theato, R. K. O'Reilly, *Polym. Chem.*, **2012**, 3, 3007-3015.

8 (a) G. H. Liu, X. R. Wang, J. M. Hu, G. Y. Zhang and S. Y. Liu, *J. Am. Chem. Soc.*, **2014**, 136, 7492-7497. (b) A. R. Rodriguez, J. R. Kramer and T. J. Deming, *Biomacromolecules*, **2013**, 14, 3610-3614. (c) J. Y. Rao, C. Hottinger and A. Khan, *J. Am. Chem. Soc.*, **2014**, 136, 5872-5875. (d) M. R. Molla, P. Prasad and S. Thayumanavan, *J. Am. Chem. Soc.*, **2015**, 137, 7286-7289.

9 (a) Q. Yan and Y. Zhao, *Chem. Sci.*, **2015**, 6, 4343-4349. (b) S. Biswas, K. Kinbara, T. Niwa, H. Taguchi, N. Ishii, S. Watanabe, K. Miyata, K. Kataoka and T. Aida, *Nat. Chem.*, **2013**, 5, 613-620.

10 L. Li, P. Rose and P. K. Moore, *Annu. Rev. Pharmacol. Toxicol.*, **2011**, 51, 169-187.

11 (a) L. Li and P. K. Moore, *Trends Pharmacol. Sci.*, **2008**, 29, 84-90. (b) L. Li, M. Bhatia, Y. Z. Zhu, Y. C. Zhu, R. D. Ramnath, Z. J. Wang, F. B. Anuar, M. Whiteman, M. Salto-Tellez and P. K. Moore, *FASEB J.*, **2005**, 19, 1196-1198. (c) G. Yang, L. Wu and R. Wang, *FASEB J.*, **2006**, 20, 553-555.

12 (a) Q. Yan, R. Zhou, C. K. Fu, H. J. Zhang, Y. W. Yin and J. Y. Yuan, *Angew. Chem. Int. Ed.*, **2011**, 50, 4923-4927. (b) H. L. Che, M. Huo, L. Peng, T. Fang, N. Liu, L. Feng, Y. Wei and J. Y. Yuan, *Angew. Chem. Int. Ed.*, **2015**, 54, 8934-8938. (c) B. Yan, D. H. Han, O. Biossière, P. Ayotte and Y. Zhao, *Soft Matter*, **2013**, 9, 2011-2016. (d) S. J. Lin, P. Theato, *Macromol. Rapid Commun.*, **2013**, 34, 1118-1133.

13 J. M. Hu, M. R. Whittaker, H. Duong, Y. Li, C. Boyer and T. P. Davis, *Angew. Chem. Int. Ed.*, **2014**, 126, 7913-7918.

14 H. Kimura, *Antioxid. Redox Signal*, **2010**, 12, 1111-1123.

15 A. Mohr, P. Talbiersky, H.-G. Korth, R. Sustmann, R. Boese, D. Bläser and H. Rehage, *J. Phys. Chem. B.*, **2007**, 111, 12985-12992.

16 (a) T. Finkel, *J. Cell Biol.*, **2011**, 194, 7-15. (b) M. H. Stipanuk, *Annu. Rev. Nutr.*, **1986**, 6, 179-209.

17 S. Siva, G. Venkatesh, A. Antony Muthu Prabhu, R. K. Sankaranarayanan, N. Rajendiran, *Phys. Chem. Liq.*, **2012**, 50, 434-452.