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Tuning H₂S Release by Controlling Mobility in a Micelle Core

Jeffrey C. Foster[‡], Ryan J. Carrazzone[‡], Nathan J. Spear, Scott C. Radzinski, Kyle J. Arrington, John B. Matson^{*}

Department of Chemistry, Center for Drug Discovery, and Macromolecules Innovation Institute, Virginia Tech, Blacksburg, VA, 24061, United States

Abstract

Drug delivery from polymer micelles has been widely studied, but methods to precisely tune rates of drug release from micelles are limited. Here, the mobility of hydrophobic micelle cores was varied to tune the rate at which a covalently bound drug was released. This concept was applied to cysteine-triggered release of hydrogen sulfide (H₂S), a signaling gas with therapeutic potential. In this system, thiol-triggered H₂S donor molecules were covalently linked to the hydrophobic blocks of self-assembled polymer amphiphiles. Because release of H₂S is triggered by cysteine, diffusion of cysteine into the hydrophobic micelle core was hypothesized to control the rate of release. We confirmed this hypothesis by carrying out release experiments from H₂S-releasing micelles in varying compositions of EtOH/H₂O. Higher EtOH concentrations caused the micelles to swell, facilitating diffusion in and out of their hydrophobic cores and leading to faster H₂S release from the micelles. To achieve a similar effect without addition of organic solvent, we prepared micelles with varying core mobility via incorporation of a plasticizing co-monomer in the core-forming block. The glass transition temperature (T_g) of the core block could therefore be precisely varied by changing the amount of the plasticizing co-monomer in the polymer. In aqueous solution under identical conditions, the release rate of H₂S varied over 20-fold ($t_{1/2} = 0.18 - 4.2$ h), with the lowest T_g hydrophobic block resulting in the fastest H₂S release. This method of modulating release kinetics from polymer micelles by tuning core mobility may be applicable to many types of physically encapsulated and covalently linked small molecules in a variety of drug delivery systems.

Graphical Abstract

^{*}Corresponding Author jbmatson@vt.edu.

[‡]These authors contributed equally

Supplementary Information. Electronic supplementary information (ESI) available. Synthetic and experimental details, characterization data and supplementary DLS, CMC, and H₂S release data.

Conflicts of Interest

There are no conflicts of interest to declare

Introduction

Polymeric micelles enhance the activity of conventional therapeutics by improving drug solubility, minimizing off-target effects, providing protection against rapid breakdown, enhancing circulation time (pharmacokinetics), reducing the volume of distribution, and imparting targeting capability such that the therapeutic agent accumulates in the desired tissue(s).^{1–3} Most of these enhancements derive from the difference in size of polymer micelles relative to their small molecule encapsulates and from the biocompatibility imparted by their hydrophilic polymer shells.⁴ Since the 1990s, numerous polymer assemblies have been developed to deliver assorted payloads, including small molecule anti-cancer drugs,^{5–6} contrast/imaging agents,^{7–8} proteins,⁹ RNA,¹⁰ and DNA,¹¹ among others.

Despite their numerous advantages, the delivery of drugs by polymeric micelles can be complicated by slow diffusion of triggering agents and/or encapsulated drugs both into and out of the micelle core. For example, Eisenberg and coworkers showed that hydrophobic model drug release from poly(caprolactone)-*b*-poly(ethylene oxide) micelles occurred under diffusion control, with release rates dependent on the structure and concentration of the encapsulated agent.¹² In a similar example, O'Reilly and coworkers demonstrated the inability of a hydrophilic probe to diffuse into the core of poly(triethyleneglycol acrylate)-*b*-poly(*tert*-butyl acrylate) micelles using a FRET experiment.¹³ Additionally, Appel and coworkers reported the dependence of the release rate of small molecule dyes from single-chain nanoparticles on core morphology, with low core glass transition temperature (T_g) leading to fast release and higher core T_g exhibiting sustained release.¹⁴ Most recently, Thayumanavan and coworkers established a system in which chain mobility facilitated the release of small molecule encapsulates from the glassy domain of supramolecular assemblies.¹⁵ Diffusion of molecules into micelles is particularly relevant for delivery systems in which the therapeutic is covalently attached to the polymer amphiphile and requires a chemical reaction to elicit drug release. In such systems, the rates of diffusion of small molecules into and out of the micelle core can compete with those of the drug-releasing reaction (i.e., hydrolysis). For example, Cui and coworkers recently demonstrated that supramolecular structure controlled the release of hydrophobic drugs covalently bound to short, self-assembling peptides by restricting access of the triggering molecule to the degradable group linking the drug to the peptide.¹⁶ Similarly, van Esch and coworkers showed that release of a model drug covalently linked to a gelling scaffold via an enzyme-labile linker was limited by the supramolecular structure of the hydrogel.¹⁷ These examples

highlight complexities that are often not easily addressed when formulating polymeric micelles and related supramolecular materials for drug delivery.

In our lab, we are interested in materials capable of delivering hydrogen sulfide (H_2S), a field of research garnering much attention recently.¹⁸ An endogenously produced gas that is tightly regulated in vivo, H_2S is extensively involved in cellular signaling and homeostasis.¹⁹ Moreover, the physiological activity of H_2S depends largely on its local concentration; therefore, to effect the desired biological response, H_2S must be delivered selectively (i.e., to the correct tissue), precisely, and on the appropriate timescale.^{20–21} H_2S -releasing small molecules and materials have been developed by our lab and others to deliver the gas both in vitro and in vivo,^{22–25} but better control over the timing, location, rate, and duration of release are still needed. Using polymer amphiphiles with reaction-triggered H_2S donors covalently bound to the hydrophobic block, we considered here whether the H_2S release rate could be controlled by modulating diffusion of the trigger into the micelles.

In recently published work, we prepared H_2S -releasing polymeric micelles based on block copolymers of poly(ethylene glycol) (PEG) and polymethacrylate.²⁴ To each constitutional unit of the polymethacrylate hydrophobic block was attached an *S*-aroylthiooxime (SATO) group, which releases H_2S in response to thiols such as cysteine (Scheme S1). We observed that H_2S release from SATO-functionalized micelles occurred over an order of magnitude more slowly than from analogous small molecule SATOs under identical experimental conditions (Table S1). Given that the SATO-functionalized hydrophobic blocks are sequestered within the hydrophobic core of the micelles, the triggering thiol must diffuse into the micelle to induce H_2S release. Therefore, the rate of diffusion of triggering molecules into micelle cores may greatly impact the H_2S release rate from these assemblies. In this contribution, we evaluate the effect of the properties of the polymer amphiphile (i.e., hydrophobic block glass transition temperature) on H_2S release from SATO-functionalized micelles. In addition, we consider how properties of the polymer amphiphile can be manipulated to tune characteristics of the micelle assemblies including their size and the kinetics of small molecule release. It should be noted that although we focus on triggered release of H_2S , the results discussed here may be applicable to a broad variety of drug-loaded polymer micelles.

Results and Discussion

Influence of Electronics on H_2S Release from Statistical and Block Copolymers

We showed in our initial publication on SATOs that the electronics of the SATO structure control the kinetics of thiol-triggered H_2S release (Scheme 1).²⁶ By changing substituents on the aryl thioester unit, the rate of release can be tuned over an order of magnitude for small molecule SATOs, with electron withdrawing substituents accelerating release and electron donating substituents retarding it. In fact, a linear free energy relationship was observed between the rate of the H_2S -releasing reaction and the *para* substituent Hammett constant (σ), allowing for precise chemical control over the release profile.

In further studies, we explored the rate of H_2S release from H_2O -soluble SATO-functionalized copolymers, which was also sensitive to electronic control and followed a

similar trend based on substituent electronics.²³ In the present work we initially asked if the rate of H₂S release could be manipulated in the same way for polymer assemblies prepared from SATO-functionalized block copolymer amphiphiles. Therefore, we prepared a series of three polymer amphiphiles with pendant SATOs that differed in their *para* substituents.

SATO-functionalized polymer amphiphiles were prepared in three steps (Scheme 2). First, 2-(4-formylbenzoyloxy)ethyl methacrylate (**FBEMA**) was polymerized via reversible addition–fragmentation chain transfer (RAFT) polymerization using a PEGylated chain transfer agent (macro-CTA; PEG = M_n 5,000 g/mol), yielding block copolymer **1** with a hydrophilic PEG block and a hydrophobic poly(methacrylate) segment bearing pendant aldehyde functionality on each constitutional unit. In the second step, the trithiocarbonate end group was displaced using azobisisobutyronitrile (AIBN) following a reported method,²⁷ making polymer **2**. Finally, SATO formation was conducted using three different *S*-aroylthiohydroxylamines (SATHAs), which differed in the *para* substituent of the aromatic ring, to prepare SATO-functionalized polymers **3a-c**.

With polymers **3a-c** in hand (molar mass and dispersity($\bar{D}$) values are provided in Table S2), polymer assemblies were prepared by the solvent switch method. Briefly, each polymer was first dissolved in THF; polymer solutions were then rapidly diluted with an equal volume of DI H₂O. The polymer solutions were then transferred to dialysis tubing and dialyzed against DI H₂O for 48 h. Analysis of the three samples by dynamic light scattering (DLS) revealed monomodal size distributions with intensity average diameters of 37 ± 4 nm, 43 ± 2 nm, and 40 ± 3 nm for polymers **3a**, **3b**, and **3c**, respectively (Figure S27). Based on these data, the size of the SATO polymer assemblies did not appear to depend on the *para*-substituent.

H₂S release from SATO-containing micelles was measured using the methylene blue assay, a common method for determining H₂S concentrations.²⁸ For each experiment, a solution of micelles with 250 μ M SATO groups was allowed to react with 1 mM Cys in the presence of Zn(OAc)₂ in phosphate buffer (pH = 7). Aliquots were withdrawn periodically and treated with FeCl₃ and *N,N*-dimethyl-*p*-phenylenediamine, leading to methylene blue formation. Kinetics plots were obtained by measuring the absorbance of methylene blue at 750 nm for each time point until a plateau was reached. In contrast to small molecule SATOs and SATO-functionalized linear polymers, half-lives measured by the methylene blue method for H₂S release from micelles prepared from polymers **3a-c** did not trend with R group electronics (Table S3). These results contradict our previous findings about the effect of substituent electronics on H₂S release rates, warranting further investigation into the origin of this apparent loss of electronic control (Figure 1).

Based on these data, we concluded that the reaction of Cys with the pendant SATOs of polymers **3a-c** was no longer the rate determining step of H₂S release. In our previous reports, H₂S release studies from small molecule SATOs and SATO statistical copolymers were conducted on single molecules dissolved in aqueous solution. Here, we speculated that the reaction likely occurred inside of the polymer micelles due to the covalent attachment of the SATO groups to the hydrophobic blocks of the polymer amphiphiles. Therefore, we hypothesized that the rate of diffusion of the triggering Cys molecules into the hydrophobic

micelle cores might determine the rate of H₂S release from the SATO-functionalized micelles.

Micelle Swelling using EtOH

To evaluate this hypothesis, we conducted H₂S release experiments from micelles made from polymer **3b** with varying amounts of organic solvent (EtOH). We reasoned that EtOH would swell the micelle cores, facilitating Cys diffusion into the micelles. We also realized that addition of EtOH could influence the rate of the H₂S-releasing reaction in addition to the rate of Cys diffusion. To determine whether EtOH altered the kinetics of H₂S release from SATOs, methylene blue assays were carried out on a small molecule SATO (C₆H₅COSN=CHC₆H₅) at 0, 10, 20, and 30 v/v% EtOH in phosphate buffer (pH = 7). Half-lives of H₂S release did not vary by more than 8% between the different experimental conditions, and there was no trend between EtOH concentration and half-life (Figure S29 and Table S4); therefore, we concluded that EtOH had no effect on the rate of the H₂S-releasing reaction.

After establishing that EtOH did not affect the rate of H₂S release from small molecule SATOs, we next investigated if EtOH would swell the polymer micelles and accelerate H₂S release kinetics due to increased diffusion of the Cys trigger (Figure 2A). Toward this end, DLS of micelles prepared from polymer **3b** was conducted with different amounts of EtOH, showing an increase in the diameter of the micelles from 39 ± 1 nm in DI H₂O to 114 ± 3 nm in 30 v/v% EtOH solution (Figure 2B; complete data set in Figure S32). As shown in Figure 2C, methylene blue experiments using the same series of EtOH concentrations as the small molecule study revealed a decrease in H₂S release half-lives from 4.2 ± 0.3 h (0 v/v% EtOH) to 1.8 ± 0.5 h (30 v/v% EtOH) (Figure 2C; complete data set in Figure S33 and Table S5). Taken together, these data are consistent with our assumption that EtOH could swell the polymer micelles, and that H₂S release would occur more rapidly in solvent-swollen micelles than in micelles that were not swollen. These conclusions support the hypothesis that Cys diffusion into micelles prepared from polymer **3b** affects the rate of H₂S release.

Tuning the Glass Transition Temperature of the H₂S-Releasing Block

Based on the DLS data on the polymer micelles, it is clear that EtOH causes swelling of the micelles, which may play a role in accelerating H₂S release. However, EtOH may also plasticize the micelle core, lowering the glass transition temperature (*T_g*) of the core block. Solvent swelling of micelle cores has been previously reported²⁹ and is associated with plasticization of the hydrophobic polymer block.³⁰ We hypothesized that a reduced *T_g* might increase the mobility of the micelle core, also contributing to enhanced diffusion of Cys and accelerated H₂S release kinetics. Therefore, to vary the rate of H₂S release without introducing organic solvents, we envisioned a copolymerization strategy to lower the *T_g* of the core block.

Copolymerization is an effective method to tune the *T_g* of a polymer because many copolymers obey statistical estimations of *T_g* developed by Fox. Using differential scanning calorimetry (DSC), we found that SATO-functionalized poly(FBEMA) has a *T_g* of 96 °C, while a dodecyl-oxime derivative of poly(FBEMA) has a much lower *T_g* of 18 °C. Statistical

copolymers of the two should therefore have T_g 's that are a function of the composition of the polymer in accordance with the Fox equation (Figure 3).

To prepare statistical copolymers functionalized with both SATO and alkyl oxime groups, it was necessary to understand the relative stability of these functional groups. Ideally, copolymers of the desired composition could be obtained simply by altering the ratio of SATHA and hydroxylamine added in a single post-polymerization reaction. Several model reactions were carried out to assess the relative stability of the SATO and oxime groups. Based on ^1H NMR spectral evidence, oxime formation dominated the equilibrium between these two functional groups (see the SI for a more detailed discussion). Therefore, to achieve the desired copolymer composition, poly(FBEMA) was treated with a mixture containing a sub-stoichiometric amount of hydroxylamine and an excess (3 equiv to aldehyde) of SATHA (e.g., to achieve 30 mol% oxime, a polymer with DP 100 would be treated with 30 equiv of hydroxylamine and 300 equiv of SATHA per polymer chain). This strategy, shown in Scheme 3, was employed to prepare a series of copolymers where the degree of oxime functionalization was varied from 0–100 mol% (Table 1).

DSC was employed to measure the T_g 's of the SATO/oxime statistical copolymers (**4a-e**). As shown in Figure 4, the T_g 's of the statistical copolymers fit well with predicted values, starting at 18 °C for a homopolymer functionalized with alkyl oximes and increasing to 96 °C for a homopolymer completely functionalized with SATOs.

Next, a PEG-*b*-poly(FBEMA) block copolymer (polymer **2**) was functionalized with different ratios of SATHA and hydroxylamine, using the strategy described above, to prepare polymers **5a-e** (Scheme 4). Based on the relationship we observed between the weight fraction of the SATO component and T_g for polymers **4a-e** (Figure 4B), we supposed that the trend of T_g in weight fraction of the SATO component would likely hold for the block copolymers **5a-e**. However, we were unable to measure T_g values for the block copolymers and we acknowledge that actual T_g values are likely lower than those obtained for statistical copolymers **4a-e** due to the relatively lower molar masses. Our characterization of this series of polymers is provided in Table 2.

As before, polymer assemblies of polymer amphiphiles **5a-e** were prepared using the solvent switch method. The sizes of the resulting polymer assemblies were then characterized by DLS and their solution stability measured using the Nile Red assay. Polymer assembly size did not appear to vary initially as a function of polymer composition, with assemblies prepared from polymers **5a-c** near 36 nm; however, as the alkyl oxime content was raised above 90 wt%, the size of the polymer assemblies increased significantly for polymers **5d** and **5e** (Table 2; see Figure S41 for DLS traces). Polymers **5d** and **5e** possess T_g values near rt. It is likely that the hydrophobic cores of the polymer assemblies prepared from these polymers have increased mobility relative to polymers **5a-c**, which are kinetically “frozen” in a glassy state. In contrast, the critical micelle concentrations (CMCs) for assemblies prepared from polymers **5a-e** measured using the Nile Red assay did not vary dramatically as a function of core composition (Table S7).

Effect of Core Mobility on Diffusion

Introduction of the alkyl oxime functionality into the polymer amphiphiles provided an effective strategy to modify the T_g of their hydrophobic blocks. In addition, this change in chemical structure led to differences in the size of polymer assemblies prepared from the alkyl-modified copolymers. Because the presence of EtOH enhanced diffusion of Cys into the micelles, we hypothesized that the alkyl-modification strategy would have a similar effect on the rate of diffusion of small molecules into and out of polymer assemblies. To quantify diffusion rates, a model study was conducted in which fluorescein was encapsulated in polymer assemblies of polymers **5a**, **5c**, and **5e**. The dye-loaded micelles were placed in dialysis tubes (6–8 kDa MWCO), and the fluorescence intensity of the incubation media was measured over time using fluorescence spectroscopy. The amount of fluorescein released was quantified (Figure 5), using a method similar to a previous report.¹⁴ Briefly, the fluorescence intensity at each time point was compared to the equilibrium intensity measured after 48 h. Release rates were extracted from these data using the Ritger-Peppas equation.³¹

Micelles prepared from polymer **5a** (highest core T_g) exhibited the slowest release kinetics, with a release rate of 3.1% / h. An intermediate release rate was obtained for polymer assemblies prepared from polymer **5c** (10% / h), while the fastest release rate of 17% / h was measured for assemblies prepared from polymer **5e**. This observed trend in fluorescein release rates is related to the T_g of the block copolymers utilized in the study, with the fastest release rates occurring from the polymer assemblies with the lowest T_g cores. Since release rate in this case only depends on diffusion of fluorescein molecules out of the micelle cores, it is reasonable to assume that the enhanced release kinetics observed for polymer **5e** originate from faster fluorescein diffusion facilitated by increased core mobility and void volume within the polymer assemblies.

Effect of Core Mobility on H₂S Release

Ultimately, we endeavored to modulate the rate of H₂S release from SATO-functionalized micelles. We showed in our earlier study that SATO moieties covalently bound inside the cores of polymer assemblies released H₂S considerably slower than SATO moieties bound to molecularly dissolved (unassembled) polymers.²⁴ We showed here that this decreased release rate is likely due to slow diffusion of Cys into the glassy core of the polymer micelles. Based on these results, we expected that it would be possible to achieve fast H₂S release by lowering the T_g of the micelle cores by introducing plasticizing alkyl chains to the copolymer structures. To evaluate if this strategy would be useful for modulating H₂S release rates, we conducted methylene blue assays on polymer assemblies prepared from polymers **5a-e**. We prepared micelle samples with identical SATO concentrations (250 μ M) and triggered release in all cases with 1 mM Cys to maintain consistent concentrations of the reacting species across all experiments. As shown in Figure 6, H₂S release occurred rapidly from assemblies prepared from polymer **5d** (most alkyl content, lowest T_g) with a half-life of release of 0.18 h. The rate of H₂S release then decreased with increasing T_g , similar to the trend observed for fluorescein release rates measured above. For polymer **5a** (no alkyl content, highest T_g), the release half-life was 4.2 h.

We attribute the >20-fold difference in rate between the fastest and slowest releasing micelles to increasingly faster diffusion of the triggering Cys molecules into the cores of assemblies with increasingly lower T_g . We also considered that a high rate of unimer exchange for the micelles with plasticized cores could be responsible for the observed rate differences, but previous reports on micelle exchange kinetics indicate that this process is generally much slower than the timescale of H_2S release observed here.^{32–33} Our conclusion that core mobility affects Cys diffusion (and therefore H_2S release kinetics) is supported by the solvent swelling experiments, in which H_2S release occurred more rapidly from swollen, plasticized micelles and was attributed to faster Cys diffusion. Further support comes from the fluorescein release experiments that showed more rapid release of fluorescein for micelles functionalized with the highest content of alkyl oxime (lowest T_g , most mobile). Indeed, at the highest alkyl content (91 wt. %), the rate of H_2S release from the micelles approached the rate of H_2S release from small molecules dissolved in solution, implying that the reaction kinetics have been decoupled from Cys diffusion and again depend on the rate of reaction of Cys with the SATO moieties.

Conclusions

The origin of slow release of H_2S from SATO-functionalized micelles was investigated and attributed to limitations in the rate of diffusion of triggering Cys molecules into the micelle cores. Swelling studies with EtOH showed that greater swelling in micelle cores led to faster H_2S release, likely due to faster Cys diffusion. To increase Cys diffusion without adding organic solvent, SATO-containing amphiphilic block copolymers were functionalized with plasticizing alkyl chains to increase micelle core mobility. Incorporation of an alkyl comonomer was shown to decrease T_g of the hydrophobic block in a systematic fashion. Evidence of enhanced diffusion in lower T_g micelles was provided by release of sequestered fluorescein from micelles prepared from these alkyl-modified block copolymers, with faster release observed from micelles with increased weight fractions of the alkyl comonomer. When triggered with Cys, the half-life of H_2S release could be tuned over more than an order of magnitude, from 0.18 – 4.2 h, by increasing the weight fraction of alkyl comonomer. We attribute this dramatic difference in release rate for block copolymer micelles with high alkyl content to faster diffusion of the triggering Cys molecules into the micelle cores. Furthermore, we anticipate that this strategy could be broadly applied in other drug delivery systems to precisely modulate drug release kinetics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Kataoka K; Harada A; Nagasaki Y, Block copolymer micelles for drug delivery: Design, characterization and biological significance. *Adv. Drug Deliv. Rev* 2012, 64, 37–48.
2. Rösler A; Vandermeulen GWM; Klok H-A, Advanced drug delivery devices via self-assembly of amphiphilic block copolymers. *Adv. Drug Deliv. Rev* 2001, 53 (1), 95–108. [PubMed: 11733119]
3. Duncan R, The dawning era of polymer therapeutics. *Nat. Rev. Drug. Discov* 2001, 53 (5), 347–360.
4. Allen TM; Cullis PR, Drug Delivery Systems: Entering the Mainstream. *Science* 2001, 53 (5665), 1818–1822.
5. Nishiyama N; Okazaki S; Cabral H; Miyamoto M; Kato Y; Sugiyama Y; Nishio K; Matsumura Y; Kataoka K, Novel Cisplatin-Incorporated Polymeric Micelles Can Eradicate Solid Tumors in Mice. *Cancer Res* 2001, 53 (24), 8977–8983.
6. Peer D; Karp JM; Hong S; Farokhzad OC; Margalit R; Langer R, Nanocarriers as an emerging platform for cancer therapy. *Nat. Nanotechnol* 2001, 53 (12), 751–760.
7. Matson JB; Grubbs RH, Synthesis of Fluorine-18 Functionalized Nanoparticles for use as in vivo Molecular Imaging Agents. *J. Am. Chem. Soc* 2001, 53 (21), 6731–6733.
8. Robin MP; Mabire AB; Damborsky JC; Thom ES; Winzer-Serhan UH; Raymond JE; O'Reilly RK, New Functional Handle for Use as a Self-Reporting Contrast and Delivery Agent in Nanomedicine. *J. Am. Chem. Soc* 2001, 53 (25), 9518–9524.
9. Lee Y; Ishii T; Cabral H; Kim HJ; Seo J-H; Nishiyama N; Oshima H; Osada K; Kataoka K, Charge-Conversional Polyionic Complex Micelles—Efficient Nanocarriers for Protein Delivery into Cytoplasm. *Angew. Chemie. Int. Ed* 2001, 53 (29), 5413–5416.
10. Smith AE; Sizovs A; Grandinetti G; Xue L; Reineke TM, Diblock Glycopolymers Promote Colloidal Stability of Polyplexes and Effective pDNA and siRNA Delivery under Physiological Salt and Serum Conditions. *Biomacromolecules* 2001, 53 (8), 3015–3022.
11. Kakizawa Y; Kataoka K, Block copolymer micelles for delivery of gene and related compounds. *Adv. Drug Deliv. Rev* 2001, 53 (2), 203–222.
12. Lim Soo P; Luo L; Maysinger D; Eisenberg A, Incorporation and Release of Hydrophobic Probes in Biocompatible Polycaprolactone-block-poly(ethylene oxide) Micelles: Implications for Drug Delivery. *Langmuir* 2001, 53 (25), 9996–10004.
13. Robin MP; Osborne SAM; Pikramenou Z; Raymond JE; O'Reilly RK, Fluorescent Block Copolymer Micelles That Can Self-Report on Their Assembly and Small Molecule Encapsulation. *Macromolecules* 2001, 53 (2), 653–662.
14. Chan D; Yu AC; Appel EA, Single-Chain Polymeric Nanocarriers: A Platform for Determining Structure–Function Correlations in the Delivery of Molecular Cargo. *Biomacromolecules* 2001, 53 (4), 1434–1439.
15. Molla MR; Rangadurai P; Antony L; Swaminathan S; de Pablo JJ; Thayumanavan S, Dynamic actuation of glassy polymersomes through isomerization of a single azobenzene unit at the block copolymer interface. *Nature Chemistry* 2001, 53 (6), 659–666.
16. Cheetham AG; Zhang P; Lin Y-a.; Lock, L. L.; Cui, H., Supramolecular Nanostructures Formed by Anticancer Drug Assembly. *J. Am. Chem. Soc* 2001, 53 (8), 2907–2910.
17. van Bommel KJC; Stuart MCA; Feringa BL; van Esch J, Two-stage enzyme mediated drug release from LMWG hydrogels. *Org. Biomol. Chem* 2001, 53 (16), 2917–2920.
18. Urquhart MC; Ercole F; Whittaker MR; Boyd BJ; Davis TP; Quinn JF, Recent advances in the delivery of hydrogen sulfide via a macromolecular approach. *Polym. Chem* 2001, 53 (35), 4431–4439.
19. Wang R, Physiological Implications of Hydrogen Sulfide: A Whiff Exploration That Blossomed. *Physiol. Rev* 2001, 53 (2), 791–896.
20. Zheng Y; Yu B; De La Cruz LK; Roy Choudhury M; Anifowose A; Wang B, Toward Hydrogen Sulfide Based Therapeutics: Critical Drug Delivery and Developability Issues. *Med. Res. Rev* 2001, 53 (1), 57–100.
21. Powell CR; Dillon KM; Matson JB, A review of hydrogen sulfide (H₂S) donors: Chemistry and potential therapeutic applications. *Biochem. Pharm* 2001, 53, 110–123.

22. Carter JM; Qian Y; Foster JC; Matson JB, Peptide-based hydrogen sulphide-releasing gels. *Chem. Commun* 2001, 53 (66), 13131–13134.
23. Foster JC; Matson JB, Functionalization of Methacrylate Polymers with Thiooximes: A Robust Postpolymerization Modification Reaction and a Method for the Preparation of H₂S-Releasing Polymers. *Macromolecules* 2001, 53 (15), 5089–5095.
24. Foster JC; Radzinski SC; Zou X; Finkielstein CV; Matson JB, H₂S-Releasing Polymer Micelles for Studying Selective Cell Toxicity. *Mol. Pharm* 2001, 53 (4), 1300–1306.
25. Connal LA, The benefits of macromolecular hydrogen sulfide prodrugs. *Journal of Materials Chemistry B* 2001, 53 (44), 7122–7128.
26. Foster JC; Powell CR; Radzinski SC; Matson JB, S-Aroylthiooximes: A Facile Route to Hydrogen Sulfide Releasing Compounds with Structure-Dependent Release Kinetics. *Org. Lett* 2001, 53 (6), 1558–1561.
27. Chen M; Moad G; Rizzardo E, Thiocarbonylthio end group removal from RAFT-synthesized polymers by a radical-induced process. *J. Poly. Sci. Part A: Polym. Chem* 2001, 53 (23), 6704–6714.
28. Zhao Y; Wang H; Xian M, Cysteine-activated hydrogen sulfide (H₂S) donors. *J. Am. Chem. Soc* 2001, 53, 15–17.
29. Löbbling TI; Ikkala O; Gröschel AH; Müller AHE, Controlling Multicompartment Morphologies Using Solvent Conditions and Chemical Modification. *ACS Macro Lett* 2001, 53 (9), 1044–1048.
30. Evans CM; Henderson KJ; Saathoff JD; Shull KR; Torkelson JM, Simultaneous Determination of Critical Micelle Temperature and Micelle Core Glass Transition Temperature of Block Copolymer–Solvent Systems via Pyrene-Label Fluorescence. *Macromolecules* 2001, 53 (10), 4131–4140.
31. Ritger PL; Peppas NA, A simple equation for description of solute release II. Fickian and anomalous release from swellable devices. *J. Control. Release* 2001, 53 (1), 37–42.
32. Won Y-Y; Davis HT; Bates FS, Molecular Exchange in PEO–PB Micelles in Water. *Macromolecules* 2001, 53 (3), 953–955.
33. Lund R; Willner L; Richter D; Dormidontova EE, Equilibrium Chain Exchange Kinetics of Diblock Copolymer Micelles: Tuning and Logarithmic Relaxation. *Macromolecules* 2001, 53 (13), 4566–4575.

$\text{R} = \text{CN}, \text{CF}_3, \text{Cl}, \text{F}, \text{H}, \text{CH}_3, \text{OCH}_3$

$t_{1/2} = 8 \text{ min} \longrightarrow 82 \text{ min}$

Scheme 1.

H_2S release from small molecule SATOs.

Scheme 2.

Preparation of SATO-functionalized polymer amphiphiles.

Scheme 3.
Preparation of alkyl-modified homopolymers.

Scheme 4.
Preparation of PEG-*b*-(SATO-*co*-alkyl) polymer amphiphiles.

Figure 1.
Ranges of H₂S release rates from previously studied small molecule SATO and SATO-functionalized statistical copolymers, compared with SATO containing block copolymer micelles.

Figure 2.

A) Schematic illustration of the effect of added EtOH on micelle size and diffusion of Cys into the micelle cores. B) DLS intensity average size distributions of micelles prepared from polymer **3b** in H₂O (red trace) and 30 v/v% EtOH in H₂O (green trace). C) H₂S release kinetics from micelles prepared from polymer **3b** in H₂O (red filled circles and line) and 30 v/v% EtOH in H₂O (green open circles and line). The lines correspond to pseudo-first-order kinetics fits. A SATO concentration of 250 μM was utilized for each experiment, and 1 mM Cys was applied to trigger H₂S release. Release reactions were conducted in EtOH/phosphate buffer (pH = 7.0) mixtures at rt. The error bars represent the standard deviation in conversion of n=3 replicates.

Figure 3.
Tuning the T_g of the hydrophobic block by introducing a plasticizing co-monomer.

Figure 4.

DSC analysis of poly(FBEMA) functionalized with different ratios of SATO and oxime. A) DSC of **polymer 4a** (blue trace), **4b** (purple trace), **4c** (red trace), **4d** (orange trace), and **4e** (green trace). B) Plot of $1/T_g$ as a function of weight fraction of SATO-containing repeat unit. The solid black line represents a theoretical fit using the Fox equation and the T_g 's of the homopolymers.

Figure 5. Fluorescein release from polymer assemblies **5a** (blue triangles), **5c** (red squares), and **5e** (green circles) at various time points. Release experiments were conducted in phosphate buffer (pH = 7.0) at 25 °C. The colored lines represent fits of the data using the Ritger-Peppas equation. Error bars represent the standard deviation for n=3 replicates.

Figure 6.

Effect of polymer composition and T_g on kinetics of H_2S release from micelles of polymers **5a** (blue stars), **5b** (purple triangles), **5c** (red squares), and **5d** (orange circles). Solid lines represent pseudo-first-order kinetics fits to each data set. A SATO concentration of $250 \mu M$ was utilized for each experiment, and 1 mM Cys was applied to trigger H_2S release. Release reactions were conducted in phosphate buffer ($pH = 7.0$) at rt. The error bars represent the standard deviation in conversion of $n=3$ replicates.

Table 1.Characterization of SATO-*co*-alkyl statistical copolymers.

Polymer	$M_{n, SEC}$ (kg mol ⁻¹) ^a	$M_{n, theo}$ (kg mol ⁻¹) ^b	$\bar{D}^a$	Wt. Fraction SATO ^c	T _g (°C) ^d
4a	38	39.7	1.29	1.00	96
4b	39	40.9	1.16	0.72	66
4c	43	42.3	1.21	0.43	49
4d	45	43.6	1.18	0.18	32
4e	44	44.6	1.14	0.00	18

^a Absolute molar mass measured by SEC with light scattering.^b Calculated using the molar masses of the two repeating units (SATO and oxime) in the statistical copolymer derived from the poly(FBEMA) block and mol fractions of each unit obtained via ¹H NMR spectroscopy.^c Weight fraction of SATO comonomer in the statistical copolymer determined from theoretical molar mass values and mol fractions of SATO and oxime units, demonstrated in Equation S1.^d Measured by DSC with a 20 °C/min heating and cooling ramp; data reported from second heat cycle.

Table 2.

Characterization of PEG-*b*-(SATO-*co*-alkyl) polymer amphiphiles.

Polymer	$M_{n, SEC} \text{ (kg mol}^{-1}\text{)}^a$	$M_{n, theo} \text{ (kg mol}^{-1}\text{)}^b$	$\bar{D}^a$	Wt. Fraction SATO ^c	$D_h \text{ (nm)}^d$
5a	12.4	12.9	1.19	1.00	36 ± 5
5b	13.3	13.2	1.13	0.66	36 ± 1
5c	13.6	13.5	1.14	0.40	36 ± 1
5d	13.8	13.8	1.17	0.09	65 ± 2
5e	14.0	13.9	1.28	0.00	68 ± 5

^a Absolute molar mass measured by light scattering.

^b Calculated using the molar masses of the two repeating units (SATO and oxime) in the statistical copolymer derived from the poly(FBEMA) block and mol fractions of each unit obtained via ¹H NMR spectroscopy.

^c Weight fraction of SATO comonomer in the statistical copolymer block determined from theoretical molar mass values and mol fractions, demonstrated in equation S1.

^d Intensity average hydrodynamic diameter measured using DLS in DI H₂O at 25 °C with block copolymer concentrations of 1 mg/mL.