Supporting Information

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, and John B. Matson

Department of Chemistry and Macromolecules Innovation Institute, Virginia Tech, Blacksburg, VA, 24061, United States
**Materials**

All reagents were obtained from commercial vendors and used as received unless otherwise stated. 2,2’-Azobis(2-methylpropionitrile) (AIBN) was recrystallized from methanol prior to use. S-Aroylthiohydroxylamine (SATHA) and SAT01 ((C₆H₅)C=OSN=CH(C₆H₄)COOH were prepared according to previously reported procedures.¹ The monomer 2-(4-formylbenzoyloxy)ethyl methacrylate (FBEMA) was also synthesized as previously reported.² Dry solvents were purified by passage through a solvent purification system (MBraun).

**Methods**

NMR spectra were measured on an Agilent 400 MHz spectrometer. ¹H and ¹³C NMR chemical shifts are reported in ppm relative to internal solvent resonances. Yields refer to chromatographically and spectroscopically pure compounds unless otherwise stated. Size exclusion chromatography (SEC) was carried out in THF at 1 mL/min at 30 °C on two Agilent PLgel 10 µm MIXED-B columns connected in series with a Wyatt Dawn Heleos 2 light scattering detector and a Wyatt Optilab Rex refractive index detector. No calibration standards were used, and dn/dc values were obtained by assuming 100% mass elution from the columns. UV-Vis absorbance spectra were recorded on a Spectramax M2 plate reader (Molecular Devices) from 800 to 550 nm. Dynamic light scattering (DLS) was conducted using a Malvern Zetasizer Nano operating at 25 °C. A solution of micelles was prepared at 1 mg/mL and filtered with a 0.2 µm filter prior to scanning. The calculations of the particle size distributions and distribution averages were conducted using CONTIN particle size distribution analysis routines with intensity, volume and number averages. Measurements were made in triplicate and errors reflect standard deviations. In cases where EtOH/H₂O mixtures were used the solvent viscosity and
refractive index were set according to literature values\textsuperscript{3-4}. Fluorescence spectroscopy was performed on a Varian Cary Eclipse Fluorescence Spectrophotometer. Fluorescence measurements were conducted with $\lambda_{\text{ex}} = 460$ nm and slit widths of 5 nm for both excitation and emission slits. The emission intensity was then monitored at 540 nm. Differential scanning calorimetry (DSC) studies were carried out on a Q-2000 DSC in aluminum pans operated with a dry nitrogen purge from -50 °C to 140 °C with a heating and cooling rate of 20 °C/min. Results are reported from the second heat cycle and figures are shown as exo down.

**Synthesis of Alkyl Hydroxylamine**

![Synthesis of Alkyl Hydroxylamine](image)

Alkyl hydroxylamine was prepared using a modified literature procedure.\textsuperscript{5} A round bottom flask was charged with 1-bromoundecane (10.40 g, 41.7 mmol), hydroxyphthalimide (10.21 g, 62.6 mmol), K$_2$CO$_3$ (8.65 g, 62.6 mmol), and DMF (60 mL). The reaction mixture was heated at 80 °C for 48 h. The reaction mixture was then cooled to rt. The reaction mixture was diluted with H$_2$O (100 mL), and the product was extracted with EtOAc (2 x 100 mL). The combined EtOAc layers were washed with brine (5x), dried over Na$_2$SO$_4$, and rotovapped to afford the crude dodecyl phthalimide product as a yellow oil. This was used in the next step without further purification.

Dodecyl phthalimide(14.9 g, 45.0 mmol) was dissolved in CH$_2$Cl$_2$ (200 mL) in a round bottom flask. The flask was placed in an ice bath. Hydrazine (40% in H$_2$O) (17.48 mL, 225 mmol) was added in one portion, and the reaction mixture was allowed to warm to rt and was stirred for 2 h. The reaction mixture was transferred to a separatory funnel and washed consecutively with H$_2$O, satd. NaHCO$_3$, and brine. The organic layer was dried over Na$_2$SO$_4$ and rotovapped to afford the
pure product as a colorless oil (5.88 g, 65% yield). $^1$H NMR (CDCl$_3$, 400 MHz) δ: 5.34 (s, 2H), 3.65 (t, $J = 8$ Hz, 2H), 1.56 (q, $J = 8$ Hz, 2H), 1.28 (m, 18H), 0.88 (t, $J = 7$ Hz, 3H). $^{13}$C NMR (CDCl$_3$, 100 MHz) δ: 76.24, 31.90, 29.64, 29.62, 29.58, 29.57, 29.50, 29.33, 28.39, 25.99, 22.67, 14.10.

**Synthesis of 4-cyano-4-(dodecylsulfanylthiocarbonyl)sulfanyl pentanoic acid (CTA)**

**CTA** was prepared according to a previously reported procedure: Dodecanethiol (10 mL, 41.7 mmol) was dissolved in hexanes (150 mL) in a round bottom flask. To the flask was added a solution of potassium tert-butoxide (4.68 g, 41.7 mmol) in THF (50 mL). The reaction mixture was stirred at rt for 20 min. CS$_2$ was added (2.7 mL, 44.7 mmol), and the reaction mixture was stirred for an additional 1 h. I$_2$ was then added in portions until the reaction mixture maintained a persistent dark brown color. The reaction mixture was stirred overnight at rt. The reaction mixture was transferred to a separatory funnel and washed consecutively with 10% Na$_2$S$_2$O$_3$, H$_2$O, and brine. The organic layer was then dried over Na$_2$SO$_4$ and rotovapped.

The resulting viscous yellow oil (22.8 g, 41.1 mmol) was redissolved in EtOAc (100 mL) in a round bottom flask. To the flask was added 4,4'-azobis(4-cyanovaleric acid) (11.6 g, 41.4 mmol). The reaction mixture was heated at reflux overnight. Silica gel was poured into the reaction flask, and the silica slurry was rotovapped to dryness. The silica was then dry-loaded onto a silica gel column, eluting with 9:1 hexanes/EtOAc. The product-containing fractions ($R_f \sim 0.3$ in the mobile phase) were combined and rotovapped to give the product as a yellow solid (9.50 g, 57% yield). $^1$H NMR (CDCl$_3$, 400 MHz): δ 3.33 (t, $J = 4$ Hz, 2H), 2.68 (t, $J = 4$ Hz, 2H), 2.54 (m, 1H), 2.39 (m, 1H), 1.88 (s, 3H), 1.69 (q, $J = 8$ Hz, 2H), 1.45-1.19 (m, 18H), 0.88 (t, $J = 4$ Hz, 3H). $^{13}$C NMR (CDCl$_3$, 100 MHz): δ 216.88, 177.63, 118.99, 46.30, 37.21, 33.58, 32.03, 29.74, 30.75.
29.67, 29.66, 29.54, 29.46, 29.19, 29.05, 27.78, 24.96, 22.81, 14.25. HR-MS: [M + H]$^+$ calculated 404.1746; found 404.1749.

**Synthesis of macroCTA**

**MacroCTA** was prepared according to a previously reported procedure:⁶ A round bottom flask was charged with **CTA** (0.81 g, 2.01 mmol), polyethylene glycol monomethyl ether ($M_n = 5,000$ g/mol, 2.0 g, 0.400 mmol), 4-dimethylamino pyridine (0.12 g, 0.982 mmol), and anhydrous CH$_2$Cl$_2$ (40 mL). N,N'-Dicyclohexylcarbodiimide (DCC) (0.41 g, 1.99 mmol) was dissolved in 5 mL of anhydrous CH$_2$Cl$_2$ in a vial. The DCC solution was added dropwise to the flask containing the other reagents, and the reaction mixture was stirred at rt overnight. The precipitated solids were removed by filtration. The desired product was isolated via precipitation from diethyl ether and was purified by repeated precipitations (2-4) from CH$_2$Cl$_2$ into diethyl ether to afford the product as a yellow solid (1.50 g, 69% yield). $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 4.24 (t, $J = 4$ Hz, 2H), 3.80 (t, $J = 4$ Hz, 2H), 3.71-3.51 (m, 494H), 3.45 (t, $J = 4$ Hz, 2H), 3.36 (s, 3H), 3.31 (t, $J = 8$ Hz, 2H), 2.64 (t, $J = 8$ Hz, 2H), 2.51 (m, 1H), 2.37 (m, 1H), 1.86 (s, 3H), 1.68 (t, $J = 8$ Hz, 2H), 1.42-1.18 (m, 18H), 0.87 (t, $J = 4$ Hz, 3H).

**Synthesis of poly(FBEMA)**

PEG-$b$-poly(FBEMA) was prepared according to a previously reported procedure:² A typical polymerization procedure is as follows: To an oven-dried Schlenk tube equipped with a magnetic stir bar was added **CTA** (13 mg, 0.032 mmol), **FBEMA** (1.00 g, 3.81 mmol), and 5 mL of anhydrous DMF. A solution of AIBN was prepared by dissolving 2.61 mg (15.9 μmol) in 1 mL of anhydrous DMF in a vial. 100 μL of this solution was added to the Schlenk tube. The tube
was deoxygenated by subjecting the contents to three freeze–pump–thaw cycles. The Schlenk tube was then backfilled with N₂ and submerged in an oil bath maintained at 75 °C. Samples were removed periodically by N₂-purged syringe to monitor molar mass evolution by SEC and conversion by ¹H NMR spectroscopy. The polymerization was quenched by submerging the tube into liquid N₂ and exposing the reaction mixture to air. The resulting poly(FBEMA) was isolated via precipitation from diethyl ether. If necessary, further precipitations from CH₂Cl₂ into diethyl ether were preformed to remove residual monomer.

**Synthesis of PEG-b-poly(FBEMA)**

PEG-b-poly(FBEMA) was prepared according to a previously reported procedure:⁶ A typical polymerization procedure is as follows: To an oven-dried Schlenk tube equipped with a magnetic stir bar was added macroCTA (127 mg, 25.0 µmol), FBEMA (1.00 g, 3.81 mmol), and 5 mL of anhydrous DMF. A solution of AIBN was prepared by dissolving 4.2 mg (2.54 µmol) in 1 mL of anhydrous DMF in a vial. 100 µL of this solution was added to the Schlenk tube. The tube was deoxygenated by subjecting the contents to three freeze–pump–thaw cycles. The Schlenk tube was then backfilled with N₂ and submerged in an oil bath maintained at 75 °C. Samples were removed periodically by N₂-purged syringe to monitor molar mass evolution by SEC and conversion by ¹H NMR spectroscopy. The polymerization was quenched by submerging the tube into liquid N₂ and exposing the reaction mixture to air. The resulting PEG-b-poly(FBEMA) was isolated via precipitation from a diethyl ether. If necessary, further precipitations from CH₂Cl₂ into diethyl ether were preformed to remove residual monomer.

**Removal of Dithioester End Group**
A round-bottom flask was charged with PEG-\textit{b}-poly(FBEMA) (0.50 g, 14.0 µmol), AIBN (47.0 mg, 284 µmol), and 1,4-dioxane (3 mL). The flask was outfitted with a condenser, and the reaction mixture was heated in an oil bath maintained at 80 °C for 1.5 h. The reaction mixture was allowed to cool to rt, and the polymer was recovered via precipitation from diethyl ether.

**Addition of SATHA to PEG-\textit{b}-poly(FBEMA)**

A general procedure for the SATO formation reaction is as follows: Following end group removal, PEG-\textit{b}-poly(FBEMA) (100 mg, 3.21 µmol) was dissolved in CH\textsubscript{2}Cl\textsubscript{2} (3 mL) in a 1 dram vial. To the vial was added SATHA (147 mg, 962 µmol) in one portion. Approximately 10 µL of trifluoroacetic acid (TFA) was added as a catalyst. The reaction mixture was left to stand at rt for 16 h over molecular sieves. \textsuperscript{1}H NMR spectroscopy was conducted after this time to determine the conversion of aldehyde to SATO. The modified polymer was recovered via precipitation from diethyl ether.

**Thiooxime micelle synthesis**

A scintillation vial equipped with a stir bar was charged with polymer (10 mg) and THF (5 mL). Distilled H\textsubscript{2}O (5 mL) was rapidly added to the vial, and the solution was allowed to stir for 15 min. The solution was transferred to dialysis tubing (6-8 kD MWCO) and dialyzed against distilled water for 48 h, changing the water every 12 h.

**Comparing H\textsubscript{2}S release from SATO-containing small molecules, copolymers and micelles**

Reactions for kinetic studies were run in quadruplicate, with each reaction vial containing 20 µL of phosphate buffer (1 M in H\textsubscript{2}O, pH = 7), SATO solution (either small molecule SATO\textsubscript{1}, linear copolymer, or micelles to make an overall concentration of SATO groups of 250 µM), 100 µL of Zn(OAc)\textsubscript{2} solution (40 mM in H\textsubscript{2}O), 20 µL of cysteine solution (100 mM in H\textsubscript{2}O), and DI H\textsubscript{2}O to
2 mL total volume. Final concentrations were 250 µM SATO functional groups, 2 mM Zn(OAc)$_2$, and 1 mM cysteine. A blank vial was run for each experiment containing DI H$_2$O instead of SATO solution. At predetermined timepoints, 100 µL aliquots were taken from each vial and diluted with 100 µL of FeCl$_3$ solution (30 mM in 1.2 M HCl) and 100 µL of $p$-phenylene diamine solution (20 mM in 7.2 M HCl). Each aliquot solution remained sealed in a microcentrifuge tube for a minimum of 24 h prior to addition of 250 µL to a 96-well plate. The absorbance for each aliquot was measured at 750 nm using a plate reader.

**H$_2$S Release from small molecule SATO as a function of EtOH concentration**

Reactions for kinetic studies were run in quadruplicate, with each reaction vial containing 20 µL of phosphate buffer (1 M in H$_2$O, pH = 7), 200 µL SATO$_1$ solution (2.5mM in THF), 400 µL THF, 100 µL of Zn(OAc)$_2$ solution (40 mM in H$_2$O), 20 µL of cysteine solution (100 mM in H$_2$O), 0-600 uL of EtOH, and DI H$_2$O to 2 mL total volume. Final concentrations were 250 µM SATO functional groups, 2 mM Zn(OAc)$_2$, 0-30% EtOH, and 1 mM cysteine. A blank vial was run for each experiment containing DI H$_2$O instead of SATO solution. At predetermined timepoints, 100 µL aliquots were taken from each vial and diluted with 100 µL of FeCl$_3$ solution (30 mM in 1.2 M HCl) and 100 µL of $p$-phenylene diamine solution (20 mM in 7.2 M HCl). Each aliquot solution remained sealed in a microcentrifuge tube for a minimum of 2 h prior to addition of 250 µL to a 96-well plate. The absorbance for each aliquot was measured at 750 nm using a plate reader.

**H$_2$S Release from micelles as a function of EtOH concentration.**

Reactions for kinetic studies were run in quadruplicate, with each reaction vial containing 20 µL of phosphate buffer (1 M in H$_2$O, pH = 7), micelle solution (final concentration of SATO
functional groups 250 µM), 100 µL of Zn(OAc)$_2$ solution (40 mM in H$_2$O), 20 µL of cysteine solution (100 mM in H$_2$O), 0-600 µL of EtOH, and DI H$_2$O to 2 mL total volume. Final concentrations were 250 µM SATO functional groups, 2 mM Zn(OAc)$_2$, 0-30% EtOH, and 1 mM cysteine. A blank vial was run for each experiment containing no micelle solution. At predetermined timepoints, 100 µL aliquots were taken from each vial and diluted with 100 µL of FeCl$_3$ solution (30 mM in 1.2M HCl) and 100 µL of p-phenylene diamine solution (20 mM in 7.2M HCl). Each aliquot solution remained sealed in a microcentrifuge tube for a minimum of 24 h prior to addition of 250 µL to a 96-well plate. The absorbance for each aliquot was measured at 750 nm using a plate reader.

**Evaluation of Synthesis of SATO-co-Oxime Copolymers**

To determine the reactivity of oxime and SATHA nucleophiles towards the pendant aldehydes of poly(FEBMA), a series of NMR tube experiments were conducted. In each, 10 mg of polymer 3 was dissolved in CDCl$_3$ in a vial. To the vial was added a predetermined amount of either SATHA or alkyl oxime, both according to Table S6. 20 µL of TFA and molecular sieves were then added to the vials, and the reactions were allowed to proceed at rt for 16 h. $^1$H NMR spectroscopy was then utilized to compare the relative incorporation of these nucleophiles via integration of resonances from the oxime, SATO, and/or aldehyde groups. These results are summarized in Table S6.

In addition, a 2-step method was also evaluated wherein poly(FEBMA) was first functionalized with either oxime or SATO groups, isolated via precipitation, and then treated with an excess of the other nucleophile type. NMR spectroscopic analysis was then conducted as described above. If the oxime was installed first, addition of excess SATHA and catalytic TFA did not alter the proportion of oxime that was present prior to this reaction (ca. 70 mol%). In contrast, oxime
addition to a polymer pre-functionalized with SATO groups (ca. 45 mol%) led to a polymer that was completely functionalized with oximes.

Based on these results, we concluded that to achieve a desired sidechain composition, a post-functionalization reaction could be conducted in one step wherein the desired amount of oxime nucleophile would be added in addition to excess SATHA nucleophile. This procedure was employed to prepare polymers 4a-e and 5a-e.

**Fluorescein release from micelles as a function of alkyl content**

PEG-\(\text{b-poly(FBEMA)}\) (1.0 mg, 0.059 \(\mu\)mol) was added to a scintillation vial and dissolved in 0.9 mL of THF. To the vial was added 100 \(\mu\)L of a 1 mg/mL solution of fluorescein in THF, after which 1 mL of \(\text{H}_2\text{O}\) was rapidly added. The solution was stirred for 5 min, then diluted with phosphate buffer (100 mM in \(\text{H}_2\text{O}\), pH = 7.0) to 10 mL of total volume, transferred to dialysis tubing (MWCO 6-8 kD), and placed in a beaker containing 200 mL of phosphate buffer (100 mM in \(\text{H}_2\text{O}\), pH =7.0). At predetermined times points, aliquots (2 mL each) were removed from the external dialysate solution and analyzed by fluorescence to quantify fluorescein content.
**H$_2$S Release from micelles as a function of alkyl content**

Reactions for kinetic studies were run in quadruplicate, with each reaction vial containing 20 µL of phosphate buffer (1M in H$_2$O, pH = 7), micelle solution (final concentration of SATO functional groups 250 µM), 100 µL of Zn(OAc)$_2$ solution (40 mM in H$_2$O), 20 µL of cysteine solution (100 mM in H$_2$O), and DI H$_2$O to 2 mL total volume. Final concentrations were 250 µM SATO functional groups, 2 mM Zn(OAc)$_2$, and 1 mM cysteine. A blank vial was run for each experiment containing no micelle solution. At predetermined timepoints, 100 µL aliquots were taken from each vial and diluted with 100 µL of FeCl$_3$ solution (30 mM in 1.2M HCl) and 100 µL of p-phenylene diamine solution (20 mM in 7.2M HCl). Each aliquot solution remained sealed in a microcentrifuge tube for a minimum of 24 h prior to addition of 250 µL to a 96-well plate. The absorbance for each aliquot was measured at 750 nm using a plate reader.
**Figure S1.** $^1$H NMR spectrum of dodecyl hydroxylamine.

**Figure S2.** $^{13}$C NMR spectrum of dodecyl hydroxylamine.
Figure S3. $^1$H NMR spectrum of 4-cyano-4-(dodecylsulfanylthiocarbonyl)sulfanyl pentanoic acid (CTA).

Figure S4. $^{13}$C NMR spectrum of 4-cyano-4-(dodecylsulfanylthiocarbonyl)sulfanyl pentanoic acid (CTA).
Figure S5. $^1$H NMR spectrum of PEG-(4-cyano-4-(dodecylsulfanylthiocarbonyl)sulfanyl pentanoic acid) (macroCTA).

Figure S6. $^1$H NMR spectrum of polymer 3a (PEG-$b$-(SATO-F)).
Figure S7. $^1$H NMR spectrum of polymer 3b (PEG-$b$-(SATO)).

Figure S8. $^1$H NMR spectrum of polymer 3c (PEG-$b$-(SATO-OMe)).
Figure S9. $^1$H NMR spectrum of polymer 4a (poly(SATO)).

Figure S10. $^1$H NMR spectrum of polymer 4b (poly(SATO-co-alkyl)).
Figure S11. $^1$H NMR spectrum of polymer 4c (poly(SATO-co-alkyl)).

Figure S12. $^1$H NMR spectrum of Polymer 4d (poly(SATO-co-alkyl)).
Figure S13. $^1$H NMR spectrum of polymer 4e (poly(alkyl)).

Figure S14. Stacked $^1$H NMR spectra of polymers 4a-e highlighting differences in monomer loading.
Figure S15. $^1$H NMR spectrum of polymer 5a (PEG-b-(SATO)).

Figure S16. $^1$H NMR spectrum of polymer 5b (PEG-b-(SATO-co-alkyl)).
Figure S17. $^1$H NMR spectrum of polymer 5c (PEG-$b$-(SATO-$co$-alkyl)).

Figure S18. $^1$H NMR spectrum of polymer 5d (PEG-$b$-(SATO-$co$-alkyl)).
Figure S19. $^1$H NMR spectrum of polymer 5e (PEG-$b$-(alkyl)).

Figure S20. Stacked $^1$H NMR spectra of polymers 5a-e highlighting differences in monomer loading.
Figure S21. SEC trace of polymer 1 (PEG-\textit{b}-poly(FBEMA)).
Figure S22. SEC trace of polymer 2 (PEG-b-poly(FBEMA) after end-group removal).
Figure S23. SEC trace of polymer 3a (PEG-b-(SATO-F)).
Figure S24. SEC trace of polymer 3b (PEG-b-(SATO)).
**Figure S25.** SEC trace of polymer 3c (PEG-\textit{b}-(SATO-OMe)).

**Table S1.** H$_2$S Release from SATO-functionalized small molecules and copolymers

<table>
<thead>
<tr>
<th>Architecture</th>
<th>$t_{1/2}$ H$_2$S Release (min)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small molecule (\textbf{SATO1}, \textit{C}_6\textit{H}_5\textit{COSN}═\textit{CH}_2\textit{H}_5\textit{COOH})</td>
<td>19 ± 1</td>
</tr>
<tr>
<td>Poly(\textit{FBEMA}-SATO-\textit{co}-\textit{OEGMA})</td>
<td>52 ± 1</td>
</tr>
<tr>
<td>Block copolymer (micelles)</td>
<td>206 ± 6</td>
</tr>
</tbody>
</table>

$^a$H$_2$S half-lives measured using the methylene blue assay.

**Table S2.** Characterization of polymers 3a-c.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>para-Substituent</th>
<th>$M_{n, SEC}$ (kg mol$^{-1}$)$^a$</th>
<th>$M_{n, theo}$ (kg mol$^{-1}$)$^b$</th>
<th>$D^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>F</td>
<td>16</td>
<td>15.7</td>
<td>1.29</td>
</tr>
<tr>
<td>3b</td>
<td>H</td>
<td>16</td>
<td>15.3</td>
<td>1.21</td>
</tr>
<tr>
<td>3c</td>
<td>OMe</td>
<td>18</td>
<td>16.1</td>
<td>1.13</td>
</tr>
</tbody>
</table>

$^a$Absolute molar mass measured by light scattering. $^b$Calculated from conversions determined via $^1$H NMR spectroscopy.
Figure S25. Effect of $p$-substituent on number average micelle size prepared from polymers 3a-c.
**Figure S26.** Effect of $p$-substituent on volume average micelle size prepared from polymers 3a-c.
Figure S27. Effect of \( p \)-substituent on intensity average micelle size prepared from polymers 3a-c.
Figure S28. Effect of $p$-substituent on the rate of H$_2$S release from polymers 3a-c. Rates were determined using the methylene blue assay. A SATO concentration of 250 μM was utilized for each experiment, and 1 mM Cys was applied to trigger H$_2$S release. Release reactions were conducted in phosphate buffer (pH = 7.0) at room temperature. The error bars represent the standard deviation in conversion of n=3 replicates.

Table S3. Effect of $p$-substituent on the rate of H$_2$S release.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>para-Substituent</th>
<th>$t_{1/2}$ H$_2$S release (h)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>F</td>
<td>0.83 ± 0.2</td>
</tr>
<tr>
<td>3b</td>
<td>H</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td>3c</td>
<td>OMe</td>
<td>2.5 ± 0.5</td>
</tr>
</tbody>
</table>

$^a$H$_2$S half-lives measured using the methylene blue assay.
Figure S29. Effect of EtOH concentration on the rate of H$_2$S release from a small molecule SATO1 (C$_6$H$_5$COSN=CHC$_6$H$_5$-COOH). Rates were measured using the methylene blue assay. A SATO1 concentration of 250 μM was utilized for each experiment, and 1 mM Cys was applied to trigger H$_2$S release. Release reactions were conducted in 30% THF in phosphate buffer (pH = 7.0) at room temperature. The error bars represent the standard deviation in conversion of n=4 replicates.

Table S4. Summary of H$_2$S release half-lives from small molecules SATOs based on EtOH concentration. Release half-lives were determined based on pseudo-first-order kinetics fits to the data in Figure S29.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>$t_{1/2}$ (min)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% EtOH</td>
<td>19 ± 1</td>
</tr>
<tr>
<td>10% EtOH</td>
<td>19 ± 1</td>
</tr>
<tr>
<td>20% EtOH</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>30% EtOH</td>
<td>20 ± 1</td>
</tr>
</tbody>
</table>

$^a$H$_2$S half-lives measured using the methylene blue assay.
Figure S30. Effect of EtOH concentration on number average micelle size prepared using polymer 3b (PEG-b-(SATO)).
Figure S31. Effect of EtOH concentration on volume average micelle size prepared using polymer 3b (PEG-b-(SATO)).
Figure S32. Effect of EtOH concentration on intensity average micelle size prepared using polymer 3b (PEG-b-(SATO)).
Figure S33. Effect of EtOH concentration on H₂S release from SATO micelles prepared using polymer 3b. Rates were determined using the methylene blue assay. 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 room temperature. The error bars represent the standard deviation in conversion of n=3 replicates.

Table S5. Summary of H₂S release half-lives from SATO micelles based on EtOH concentration.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>t₁/₂ H₂S Release (h) (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% EtOH</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td>10% EtOH</td>
<td>3.7 ± 0.5</td>
</tr>
<tr>
<td>20% EtOH</td>
<td>2.0 ± 0.9</td>
</tr>
<tr>
<td>30% EtOH</td>
<td>1.8 ± 0.5</td>
</tr>
</tbody>
</table>

\(^a\)H₂S half-lives measured using the methylene blue assay.
Table S6. Evaluation of post-polymerization modification of poly(FBEMA) homopolymers using oxime and SATHA nucleophiles.

<table>
<thead>
<tr>
<th>Eq Oxime(^a)</th>
<th>Eq SATHA(^a)</th>
<th>Mol% Oxime(^b)</th>
<th>Mol% SATO(^b)</th>
<th>Mol% Aldehyde(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>5</td>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>2.5</td>
<td>2.5</td>
<td>13</td>
<td>61</td>
<td>26</td>
</tr>
</tbody>
</table>

\(^a\)Equivalents of nucleophile relative to the concentration of pendant aldehydes of poly(FBEMA).  
\(^b\)Determined via relative integration of peaks corresponding to oxime, SATO, and aldehyde protons.

Equation S1.

\[
Wt.\%\ SATO = \frac{(thiooxime\ peak\ area)(397.45\ g/mol)}{[(thiooxime\ peak\ area)(397.45\ g/mol)] + [(oxime\ peak\ area)(445.60\ g/mol)]}
\]

Table S7. Summary of critical micelle concentrations for polymers 5a-e.

<table>
<thead>
<tr>
<th>Sample</th>
<th>CMC (µg/mL)(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>51 ± 4</td>
</tr>
<tr>
<td>5b</td>
<td>59 ± 2</td>
</tr>
<tr>
<td>5c</td>
<td>51 ± 6</td>
</tr>
<tr>
<td>5d</td>
<td>87 ± 3</td>
</tr>
<tr>
<td>5e</td>
<td>120 ± 2</td>
</tr>
</tbody>
</table>

\(^a\)Critical micelle concentrations determined using the Nile red assay.
**Fig S34.** Critical micelle concentration plot for polymer 5a (PEG-\textit{b}-(SATO)) determined using the Nile Red assay.

**Fig S35.** Critical micelle concentration plot for polymer 5b (PEG-\textit{b}-(SATO-\textit{co}-alkyl)) determined using the Nile Red assay.
Fig S36. Critical micelle concentration plot for polymer 5c (PEG-\(b\)-(SATO-\(co\)-alkyl)) determined using the Nile Red assay.

Fig S37. Critical micelle concentration plot for polymer 5d (PEG-\(b\)-(SATO-\(co\)-alkyl)) determined using the Nile Red assay.
Fig S38. Critical micelle concentration plot for polymer 5e (PEG-b-(alkyl)) determined using the Nile Red assay.
Figure S39. Effect of alkyl content on number average micelle size using polymers 5a-e.
Figure S40. Effect of alkyl content on volume average micelle size using polymers 5a-e.
Figure S41. Effect of alkyl content on intensity average micelle size using polymers 5a-e.

Scheme S1. \( \text{H}_2\text{S} \) release from SATOs.\(^a\)

\[\text{Proposed mechanism of } \text{H}_2\text{S} \text{ release from SATOs determined via product analysis.}^1\]
References


