Supporting Information For

Sulfur Dioxide Signaling Molecule-Responsive Polymeric Nanoparticles

Ruru Li,[a,b] *Xiaoyu Huang*,[a,b,*] *Guolin Lu*,[a] *Chun Feng*[a,*]

[a] Key Laboratory of Synthetic and Self-Assembly Chemistry for Organic Functional Molecules, Center for Excellence in Molecular Synthesis, Shanghai Institute of Organic Chemistry, University of Chinese Academy of Sciences, Chinese Academy of Sciences, 345 Lingling Road, Shanghai 200032, People's Republic of China

[b] School of Physical Science & Technology, ShanghaiTech University, 100 Haike Road, Shanghai 201210, People's Republic of China

* To whom correspondence should be addressed, E-mail: cfeng@mail.sioc.ac.cn (Tel: +86-21-54925606, Fax: +86-21-64166128); xyhuang@mail.sioc.ac.cn (Tel: +86-21-54925310, Fax: +86-21-64166128).

Materials

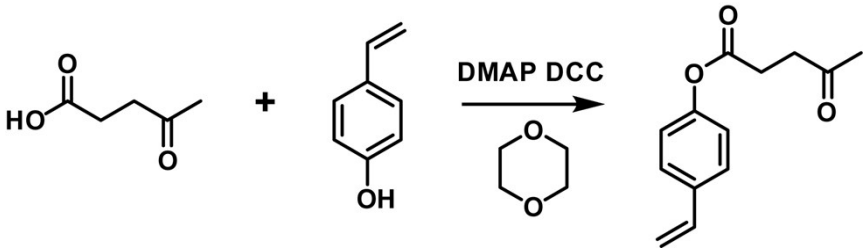
2,2'-Azobisisobutyronitrile (AIBN, Aladdin, 99.0%) was recrystallized from anhydrous ethanol. 4-Cyano-4-(dodecylsulfanylthiocarbonyl)sulfanylpentanoic acid (TCI, >97.0%), 4-hydroxystyrene (J&K, 95.0%), 4-oxopentanoic acid (TCI, 97.0%), poly(ethylene glycol) monomethyl ether (PEG-OH, M_n = 2000 g/mol, Aldrich, 99.0%), 7-ethyl-10-hydroxycamptothecin (SN-38, Bidepharm, 98.0%), Rhodamine 6G (R6G, Macklin, 95%), *L*-cysteine (Cys, Acros, 99%), *L*-glutathione (GSH, Acros, 99%) and *DL*--homocysteine (Hcy, TCI, 90%) were used as received. Other reagents not specially mentioned were purchased from Aladdin and used as received without further purification.

Instrumentation

All ^{1}H and ^{13}C NMR analyses were performed on a JEOL resonance ECZ 400S (400 MHz) in CD_2Cl_2 and DMSO-d_6, tetramethylsilicone (TMS) was used as internal standard. Electron impact ionization mass spectrometry (EI-MS) and high resolution mass spectrometry (HR-MS) were performed by an Agilent Technologies 5973N system and a Thermo Fisher Scientific LTQ FT Ultra system, respectively. Relative molecular weights and molecular weight distributions were measured by a conventional gel permeation chromatography (GPC) system equipped with a Waters 515 Isocratic HPLC pump, a Waters 2414 refractive index detector, a Waters 2487 dual λ absorbance detector and a set of Waters Styragel columns (HR3 (500-30,000), HR4 (5,000-600,000) and HR5 (50,000-4000,000), 7.8×300 mm, particle size: 5 μm).

GPC measurements were carried out at 35°C using THF as eluent with a flow rate of 1.0 mL/min. The system was calibrated with linear polystyrene standards. Fluorescence spectra were measured at room temperature on a Hitachi F-2700 fluorescence spectrophotometer with a bandwidth of 10/10 nm. Transmission electron microscopy (TEM) images were obtained by a JEOL JEM-2100 instrument operated at 80 kV. Dynamic light scattering (DLS) measurements were performed at room temperature (23°C) on a Malvern Nano-ZS90 Zetasizer at a scattering angle of 173°.

Synthesis of 4-vinylphenyl-4-oxopentanoate

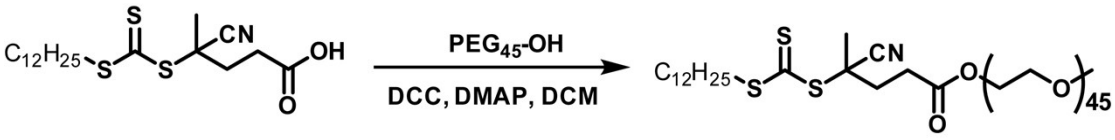


Scheme S1. Synthetic route of VPOP.

4-Oxopentanoic acid (2.32 g, 20.0 mmol), 4-hydroxystyrene (1.22 g, 10.0 mmol), 4-dimethylaminopyridine (0.10 g, 0.8 mmol) and 1,4-dioxane (20 mL) were added into a three-neck flask (flame-dried under vacuum prior to use) equipped with a magnetic stirrer under N_2. Subsequently, 1,3-dicyclohexylcarbodiimide (4.12 g, 20.0 mmol) in 1,4-dioxane (20 mL) was added dropwise. The mixture was stirred overnight at room temperature under N_2. After the removal of solid, the filtrate was concentrated and purified by silica column chromatography (eluent: *n*-hexane/ EtOAC, v:v = 8/1) to give 4-vinylphenyl-4-oxopentanoate (VPOP, 2.15 g, 97%) as a white liquid. ^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm): 7.49 (d, 2H, ArH), 7.06 (d,

2H, ArH), 6.73 (m, 1H, CH_2=C*H*), 5.80 (d, 1H, C*H*$_2$=CH), 5.25 (d, 1H, C*H*$_2$=CH), 2.83 (t, 2H, $CH_3COCH_2CH_2CO_2$), 2.70 (t, 2H, $CH_3COCH_2CH_2CO_2$), 2.13 (s, 3H, $CH_3COCH_2CH_2CO_2$). ^{13}C NMR (101 MHz, DMSO-d_6): δ (ppm): 206.72 ($CH_3COCH_2CH_2CO_2$), 171.20 ($CH_3COCH_2CH_2CO_2$), 150.06 (CO_2C), 135.68 (CH_2=CH), 134.71 (CH_2=CH*C*), 127.09, 121.77 (C_6H_4), 114.34 (CH_2=CH), 37.47 ($CH_3COCH_2CH_2CO_2$), 29.46 ($CH_3COCH_2CH_2CO_2$), 27.79 ($CH_3COCH_2CH_2CO_2$). HR-MS (DART$^+$): *m/z* Calcd for $C_{13}H_{15}O_3$ ([M+1]$^+$): 219.1016, found: 219.1014.

Synthesis of PEG-CTA

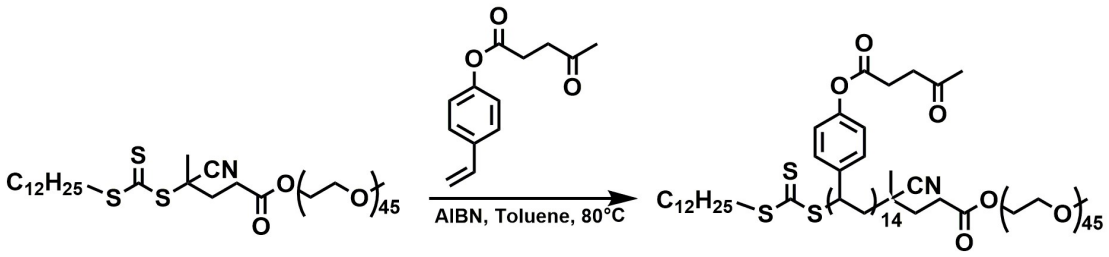


Scheme S2. Synthetic route of PEG-CAT.

PEG-OH (12.40 g, 6.2 mmol), 4-dimethylaminopyridine (0.15 g, 1.24 mmol), 4-cyano-4-(dodecylsulfanylthiocarbonyl)sulfanylpentanoic acid (5.00 g, 12.4 mmol) and dry CH_2Cl_2 (150 mL) were added into a three-neck flask (flame-dried under vacuum prior to use) equipped with a magnetic stirrer under N_2. Subsequently, 1,3-dicyclohexylcarbodiimide (2.56 g, 12.4 mmol) in CH_2Cl_2 (50 mL) was added dropwise. The mixture was slowly warmed to room temperature and stirred overnight under N_2. After the removal of solid, the filtrate was washed with CH_2Cl_2. The crude product was purified by repeated dissolution in CH_2Cl_2 and precipitation in cold diethyl ether, followed by drying *in vacuo* overnight to provide PEG-CTA (11.10 g, 75%) as a yellow powder. GPC: M_n = 3,200 g/mol, M_w/M_n =1.10. ^{1}H NMR (400 MHz,

CD_2Cl_2): δ (ppm): 4.22 (t, 2H, $CO_2CH_2CH_2$), 3.77 (t, 2H, $COOCH_2CH_2$), 3.59 (m, 176H, OCH_2CH_2), 3.32-3.36 (m, 3H, OCH_3, SCH_2CH_2), 2.63 (t, 2H, $CCH_2CH_2CO_2$), 2.38-2.52 (m, 2H, $CCH_2CH_2CO_2$), 1.85 (s, 3H, $SC(CN)CH_3$), 1.69 (m, 2H, SCH_2CH_2), 1.26 (m, 18H, CH_2), 0.87 (t, 3H, CH_3).

Synthesis of PEG_{45}-*b*-$PVPOP_{14}$



Scheme S3. Synthetic route of PEG_{45}-*b*-$PVPOP_{14}$.

AIBN (27 mg, 0.17 mmol) and PEG-CTA (0.50 g, 0.21 mmol) were added into a 25 mL Schlenk flask (flame-dried under vacuum prior to use). 4-Vinylphenyl-4-oxopentanoate (0.85 g, 3.9 mmol) and dry toluene (2.0 mL) were then added via a gastight syringe. The flask was degassed by three cycles of freezing-pumping-thawing followed by stirring at 80°C for 15 hours under N_2. The polymerization was terminated by putting the flask into liquid N_2. The reaction mixture was precipitated in cold diethyl ether. The crude product was purified by repeated dissolution in CH_2Cl_2 and precipitation in diethyl ether. The crude product was further purified by ultrafiltration using a membrane (MW_{cutoff} = 50 KDa) in water, followed by freezing-dry to give PEG_{45}-*b*-$PVPOP_{14}$ as a white solid. GPC: M_n = 5,000 g/mol, M_w/M_n =1.15. ^1H NMR (400 MHz, CD_2Cl_2): δ (ppm): 6.31-7.00 (m, 56H, ArH), 4.15 (t, 2H, $CO_2CH_2CH_2$), 3.77 (t, 2H, $COOCH_2CH_2$), 3.60 (m, 176H, OCH_2CH_2), 3.33 (s, 3H,

OCH_3), 3.24 (t, 2H, SCH_2CH$_2$), 2.77-2.84 (m, 56H, CH$_3$COCH_2CH_2CO$_2$), 2.60 (t, 2H, CCH$_2$CH_2CO$_2$), 2.34 (t, 2H, CCH_2CH$_2$CO$_2$), 2.18 (s, 42H, CH_3COCH$_2$CH$_2$CO$_2$), 1.25-2.02 (m, 65H, CCH_3, SCH$_2$CH_2, ArCHCH$_2$, ArCHCH_2, CH_2), 0.87 (t, 3H, CH_3).

Preparation of PEG$_{45}$-*b*-PVPOP$_{14}$ micellar solution encapsulated with SN-38 and R6G

To prepare PEG$_{45}$-*b*-PVPOP$_{14}$ micellar solution, PEG$_{45}$-*b*-PVPOP$_{14}$ (28 mg) was dissolved in THF (25 mL), followed by the adding water (20 mL) dropwise. The mixture was stirred overnight and dialyzed against water for 48 h to remove THF.

To prepare PEG$_{45}$-*b*-PVPOP$_{14}$ micelles encapsulated with SN-38, a DMF solution of SN-38 (770 μL, 10 mg/mL) and a THF solution of PEG$_{45}$-*b*-PVPOP$_{14}$ (39 mL, 2.8 mg/mL) were added dropwise into water (54 mL) under stirring. After the removal of THF by stirring for 24 h at room temperature, the mixture was dialyzed by using a dialysis tube (MW$_{cutoff}$ = 3.5 KDa) in water for two days. Next, 5.0 mL of dialysate and 0.5 mL of micellar solution were frozen-dry, followed by dissolving in DMSO (2 mL). 50 μL of micellar solution was diluted with DMSO (2 mL) for fluorescence measurement.

To prepare PEG$_{45}$-*b*-PVPOP$_{14}$ micelles encapsulated with R6G, a THF solution of PEG$_{45}$-*b*-PVPOP$_{14}$ (56 mL, 2 mg/mL) was added dropwise into water (35 mL). Next, an aqueous solution of R6G (21 mL, 0.56 mg/mL) was added to the THF/water solution of PEG$_{45}$-*b*-PVPOP$_{14}$. After the removal of THF by stirring for 24 h at room temperature, the mixture was dialyzed by using a dialysis tube (MW$_{cutoff}$ = 3.5 KDa)

in water for two days. Finally, 5.0 mL of dialysate and 3.0 mL of micellar solution were frozen-drying, followed by dissolving in H_2O (3 mL) for fluorescence and UV/vis measurements.

SO_2-responsiveness of VPOP monitored by ^1H NMR and HPLC

A D_2O solution of stimuli-species (Na_2SO_3, Na_2SO_4, Hcy, Cys or GSH, 500 μL, 0.17 M) was charged into a NMR tube followed by adding a DMSO-d_6 solution of VPOP (50 μL, 0.035 M). ^1H NMR spectra were recorded at different intervals (Figure S1A). Similarly, after the mixture was stirred at room temperature for 20 min, all of the reaction solution were transferred into sample tubes (1.5 mL) and frozen with liquid N_2 immediately for further HPLC detection.

Kinetic study of PEG_{45}-*b*-$PVPOP_{14}$ micelles treated with Na_2SO_3 by DLS

Four aliquots of PEG_{45}-*b*-$PVPOP_{14}$ micellar solution (2.00 mL for each) were transferred into dialysis bags (MW_{cutoff} = 3.5 KDa), respectively, followed by putting into Na_2SO_3 aqueous solution (1000 mL, 0.05 M) in a shaker (150 rpm, 37°C). The sample in each dialysis bag was taken out gradually at preset intervals for DLS measurement (Figure S3).

DLS and TEM detection of PEG_{45}-*b*-$PVPOP_{14}$ micelles with different stimuli

A PEG_{45}-*b*-$PVPOP_{14}$ micellar solution (1.50 mL) was transferred into a dialysis bag (MW_{cutoff} = 3.5 KDa) followed by putting into different stimuli-species solution

(30 mL, 0.05 M) in a shaker (190 rpm, 37°C). After 41 h, the micellar solution in dialysis bag was transferred to a sample bottle for further DLS and TEM measurements. For TEM, 10.00 μL of micellar solution was placed on a Formvar and carbon-coated copper grid for 1 min and then a filter paper touched the edge of drop to absorb most of liquid on the grid. The grid was allowed to dry *in vacuo*.

Release of SN-38 from PEG_{45}-*b*-$PVPOP_{14}$ micelles with different stimuli

A PEG_{45}-*b*-$PVPOP_{14}$ micellar solution (4.0 mL) loaded with SN-38 was transferred into a dialysis bag (MW_{cutoff} = 3.5 KDa). The dialysis bag with same volume (4.0 mL) of micellar solution was dialyzed against water, aqueous solutions of Na_2SO_3 (0.05 M) and Na_2SO_4 (0.05 M) at 37°C, respectively. At preset intervals, 4.0 mL of release medium was taken out and 4.0 mL of fresh medium was replenished. All the solution were lyophilized and dissolved in 2.0 mL of DMSO. The amount of released SN-38 was determined by fluorescence based on the standard curve shown in Figure S4B.

SO_2-responsiveness of PEG_{45}-*b*-$PVPOP_{14}$ monitored by ^1H NMR and GPC

A THF solution of PEG_{45}-*b*-$PVPOP_{14}$ (0.2 mL, 15 mg/mL) was added into Na_2SO_3 (2.0 mL, 0.05 M) and Na_2SO_4 (2.0 mL, 0.05 M) aqueous solutions, respectively. After the mixtures were placed in a shaker (150 rpm, 37°C) for 24 h, GPC and ^1H NMR measurements were carried out after lyophilization.

SUPPORTING FIGURES

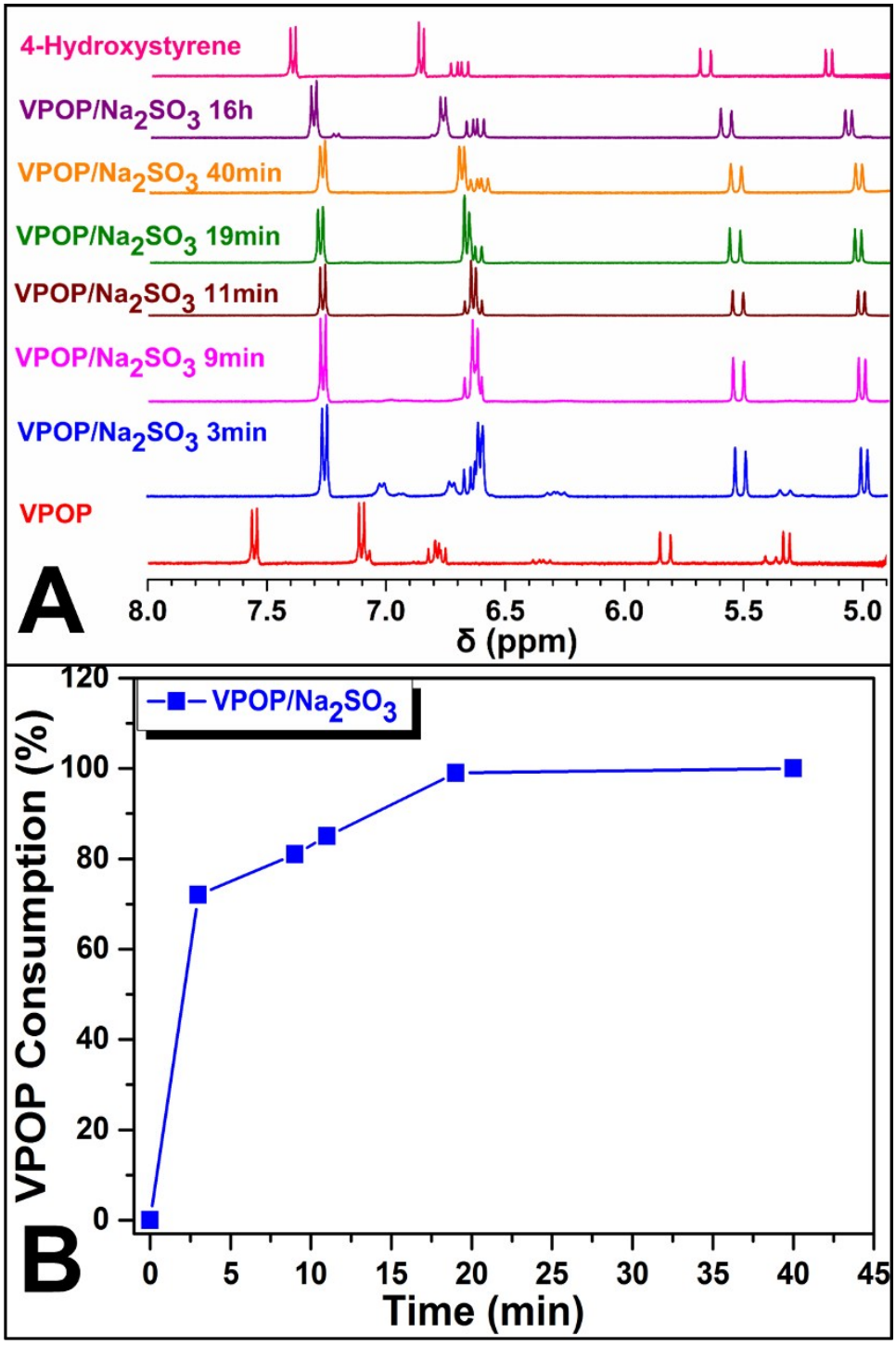


Figure S1. (A) ^{1}H NMR spectra of VPOP, 4-hydroxystyrene and VPOP treated with Na_2SO_3 for different time in D_2O/DMSO-d_6 ($V_{D2O}/V_{DMSO\text{-}d6}$ = 10/1). (B) Conversion of VPOP upon the treatment with Na_2SO_3 obtained on the basis of ^{1}H NMR results as shown in panel (A).

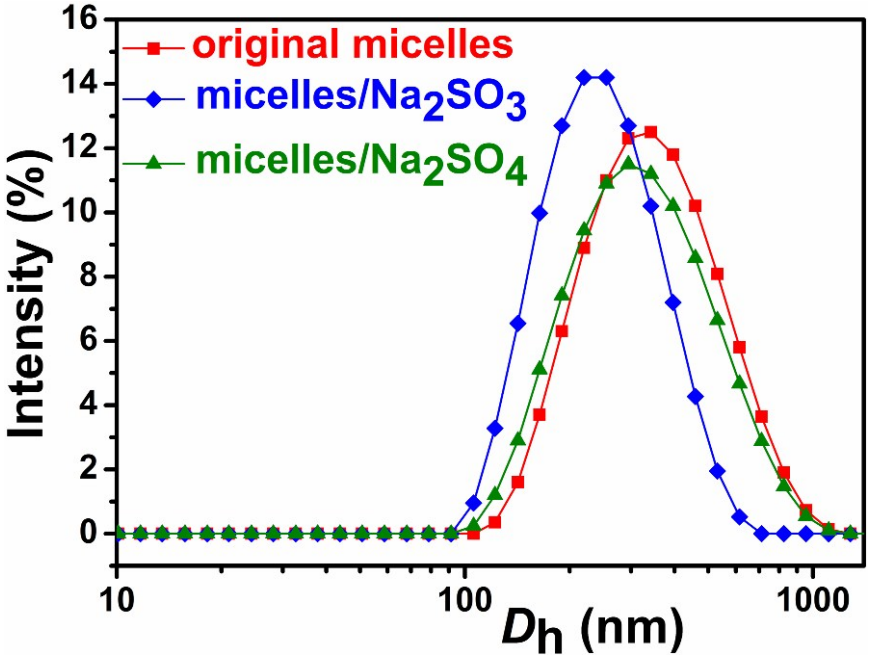


Figure S2. Hydrodynamic diameter distribution of pristine micellar solution of PEG_{45}-*b*-$PVPOP_{14}$ and micellar solutions after the treatment with Na_2SO_3 and Na_2SO_4, respectively.

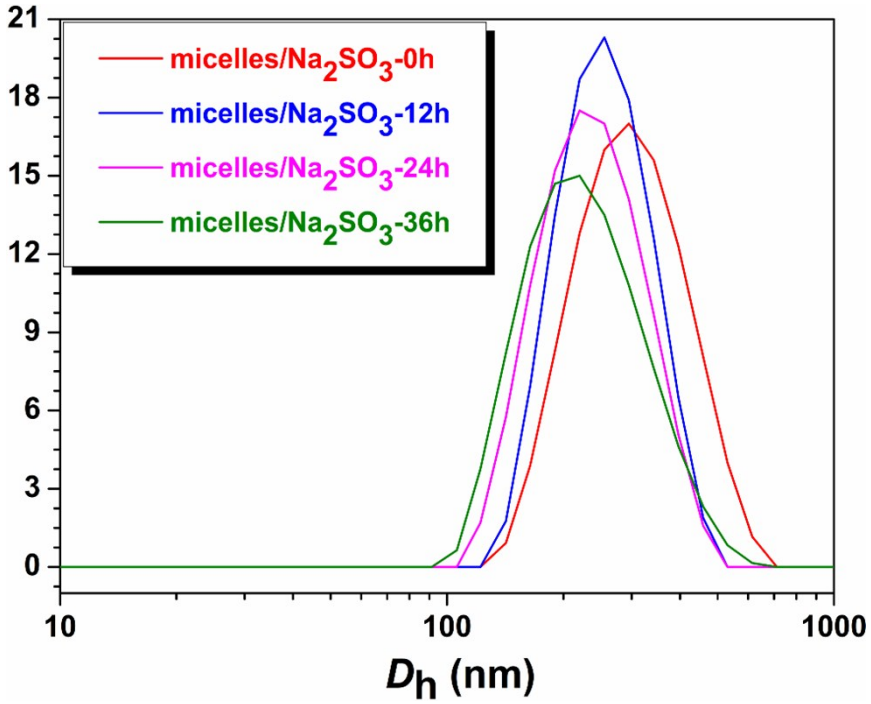


Figure S3. Hydrodynamic diameter distribution of micelles treated with SO_3^{2-} for different time.

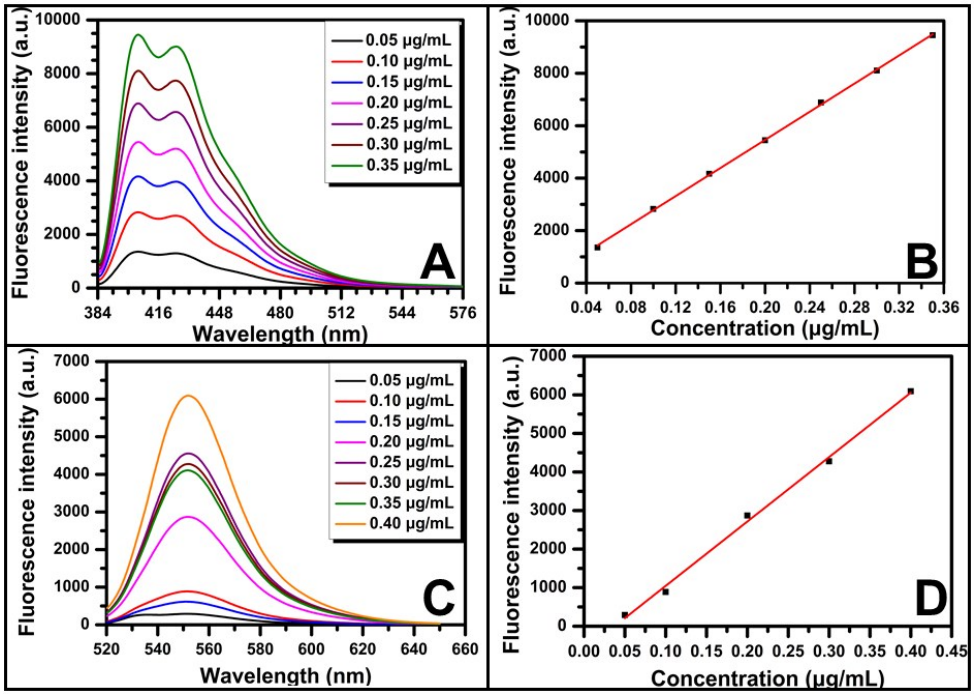


Figure S4. (A) Fluorescence spectra (λ_{ex} = 365 nm) of SN-38 in DMSO with different concentrations and (B) fluorescence intensity of SN-38 in DMSO at 405 nm as a function of concentration of SN-38. (C) Fluorescence spectra of R6G in H_2O with different concentrations and (D) fluorescence intensity of R6G as a function of concentration of R6G.

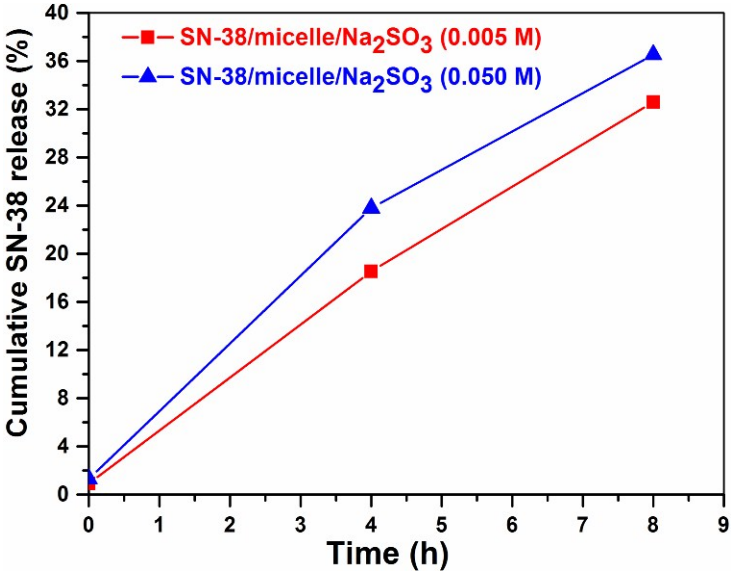


Figure S5. Cumulative SN-38 release of micelles upon the treatment with Na_2SO_3 (0.05 and 0.005 M) at different time intervals.

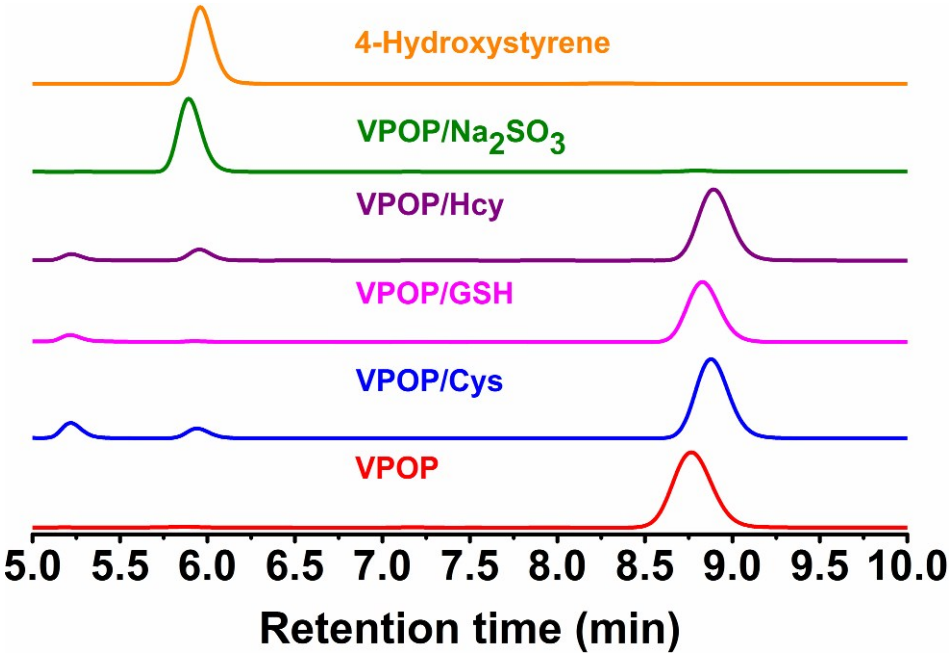


Figure S6. HPLC curves of VPOP, 4-hydroxystyrene and VPOP treated with Na_2SO_3, Cys, Hcy and GSH for 20 min, respectively.

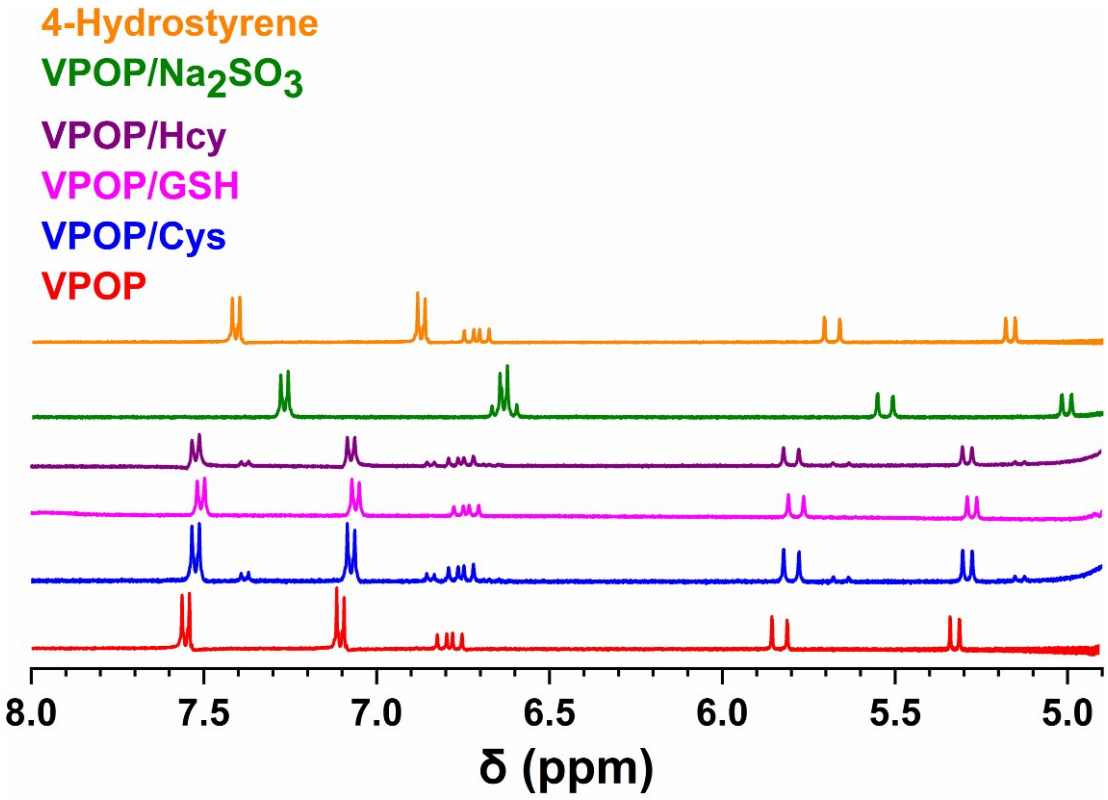


Figure S7. ^1H NMR spectra of VPOP, 4-hydroxystyrene and VPOP treated with Na_2SO_3, Cys, Hcy and GSH for 20 min in D_2O/DMSO-d_6 ($V_{D2O}/V_{DMSO\text{-}d6}$ = 10/1), respectively.