Supplementary Information

ROS-responsive poly(ε-caprolactone) with pendent thioether and selenide motifs

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Syntheses of model compounds

Ethyl 2-(ethylthio)butanoate (T1) and ethyl 2-(phenylthio)butanoate (T2). Ethyl 2-bromobutyrate (585 mg, 3.0 mmol) and K_2CO_3 (830 mg, 6.0 mmol) were dissolved/dispersed in 10 mL of acetone, to which ethyl mercaptan (242 mg, 3.9 mmol) or thiophenol (430 mg, 3.9 mmol) was added under nitrogen atmosphere. The mixture was stirred for another 12 h at 60 °C. The precipitated salts were filtered off and the filtrate was evaporated to remove acetone. The residue was purified on a silica column with the mixed eluent of petroleum ether and ethyl acetate (8:1, v/v), affording **T1** or **T2** as colorless oil (~60% yields).

T1: ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 4.21 (m, 2H), 3.18 (dd, J_1 = 8.4 Hz, J_2 = 6.7 Hz, 1H), 2.62 (m, 2H), 1.88 (m, 1H), 1.71 (m, 1H), 1.25 (t, J = 6.8 Hz, 3H), 1.21 (t, J = 6.8 Hz, 3H), 1.00 (t, J = 7.4 Hz, 3H). ^{1}H NMR (400 MHz, CD_3CN, ppm): δ 4.14 (m, 2H), 3.21 (dd, J_1 = 8.4 Hz, J_2 = 6.7 Hz, 1H), 2.61 (m, 2H), 1.82 (m, 1H), 1.64 (m, 1H), 1.23 (t, J = 7.2 Hz, 3H), 1.21 (t, J = 7.2 Hz, 3H), 0.96 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 172.92, 60.92, 48.32, 25.35, 24.85, 14.51, 14.23, 11.98.

T2: ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.45 (m, 2H), 7.30-7.25 (m, 3H), 4.11 (m, 2H), 3.58 (dd, J_1 = 8.3 Hz, J_2 = 6.6 Hz, 1H), 1.97-1.75 (m, 2H), 1.17 (t, J = 7.1 Hz, 3H), 1.03 (t, J = 7.4 Hz, 3H). ^{1}H NMR (400 MHz, CD_3CN, ppm): δ 7.45 (m, 2H), 7.33 (m, 3H), 4.06 (m, 2H), 3.64 (dd, J_1 = 8.2 Hz, J_2 = 6.7 Hz, 1H), 1.89-1.71 (m, 2H), 1.13 (t, J = 7.1 Hz, 3H), 1.00 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 172.43, 133.67, 132.76, 128.88, 127.77, 61.04, 52.54, 25.13, 14.09, 11.85. FT-MS: m/z (M^+), Calcd. 224.09; Found: 225.09 [M + H^+], 247.08 [M + Na^+].

Ethyl 2-(phenylselanyl)butanoate (T3). Diphenyl diselenide (314 mg, 1.0 mmol) was dissolved in 2 mL of THF and the solution was cooled to 0 °C under nitrogen atmosphere. $NaBH_4$ (75 mg, 2.0 mmol) pre-dissolved in 2 mL of deionized water was added dropwise. The mixture was stirred until the solution became completely transparent. To this solution ethyl 2-bromobutyrate (390 mg, 2.0 mmol) was added at one time and stirred for 24 h at 50 °C. The precipitated salts were filtered off and the filtrate was evaporated to remove THF. The residue was extracted by DCM, the combined organic phases were concentrated and further purified on a silica column with the mixed eluent of petroleum ether and ethyl acetate (8:1, v/v), affording **T3** as light yellow oil in 52% yield. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.60 (m, 2H), 7.33-7.28 (m, 3H), 4.10 (q, J = 7.1 Hz, 2H), 3.53 (dd, J_1 = 8.7 Hz, J_2 = 6.5 Hz, 1H), 1.99-1.74 (m, 2H), 1.17 (t, J = 7.1 Hz, 3H), 1.00 (t, J = 7.3 Hz, 3H). ^{1}H NMR (400 MHz, CD_3CN, ppm): δ 7.60 (m, 2H), 7.37-7.31 (m, 3H), 4.03 (q, J = 7.1 Hz, 2H), 3.59 (dd, J_1 = 8.8 Hz, J_2 = 6.8 Hz, 1H), 1.89-1.71 (m, 2H), 1.12 (t, J = 7.1 Hz, 3H), 0.97 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 172.65, 135.57, 128.97, 128.37, 128.06, 60.77, 45.46, 25.26, 14.04, 12.53. FT-MS: m/z (M^+), Calcd. 272.03; Found: 273.04 [M + H^+], 295.02 [M + Na^+].

Ethyl 2-(ethylsulfinyl)butanoate (**S_x1**). Compound **T1** (176 mg, 1.0 mmol) was dissolved in 10 mL of DCM and the solution was cooled to 0 °C. 230 mg of mCPBA (75%, 1.0 mmol) was added in batches in 30 min under stirring. The mixture was stirred for another 12 h at 25 °C. The precipitated salts were filtered off and the filtrate was evaporated to remove DCM. The residue was purified on a silica column with the mixed eluent of petroleum ether and ethyl acetate (1:1, v/v), affording **S_x1** as a colorless oil in 62% yield. ^{1}H NMR (400 MHz, CD_3CN, ppm): δ 4.21 (m, 2H), 3.51 (ddd, J_1 = 12.8 Hz, J_2 = 9.8 Hz, J_3 = 4.8 Hz, 1H), 2.89-2.68 (m, 2H), 2.03-1.89 (m, 2H), 1.32-1.23 (m, 6H), 1.02 (m, 3H). ^{13}C NMR (100 MHz, CD_3CN, ppm): δ 166.51, 66.17, 61.97, 44.09, 20.01, 13.83, 10.96, 6.77.

Ethyl 2-(phenylsulfinyl)butanoate (**S_x2**). Model compound **S_x2** (white powder) was synthesized from **T2** following the same procedure as for **S_x1.** ^{1}H NMR (400 MHz, CD_3CN, ppm): δ 7.65-7.54 (m, 5H), 4.00-3.82 (m, 2H), 3.55-3.43 (m, 1H), 1.84 (m, 2H), 1.07-0.92 (m, 6H). ^{13}C NMR (100 MHz, CD_3CN, ppm): δ 166.32, 142.03, 132.11, 129.81, 125.23, 70.62, 61.60, 20.44, 13.82, 11.35. FT-MS: m/z (M^+), Calcd. 240.08; Found: 241.09 [M + H^+], 263.07 [M + Na^+].

Ethyl 2-(ethylsulfonyl)butanoate (**S_f1**). Model compound **S_f1** was also prepared from **T1** but using excess mCPBA (2.2 eq) as the oxidant. Briefly, **T1** (176 mg, 1.0 mmol) was dissolved in 10 mL of DCM and the solution was cooled to 0 °C. 500 mg of mCPBA (75%,

2.2 mmol) was added in batches under stirring in 30 min. The mixture was stirred for additional 12 h at 25 °C. The precipitated salts were filtered off and the filtrate was washed with 3% Na_2SO_3 aqueous solution to remove excess mCPBA. The organic phase was evaporated to remove DCM and the residue was further purified on a silica column with the mixed eluent of ethyl acetate and petroleum ether (2:1, v/v), affording **S_f1** as a colorless oil in 50% yield. ^{1}H NMR (400 MHz, CD_3CN, ppm): δ 4.25 (m, 2H), 3.87 (t, J = 7.2 Hz, 1H), 3.16 (m, 2H), 2.03 (m, 2H), 1.31 (t, J = 6.8 Hz, 3H), 1.27 (t, J = 6.8 Hz, 3H), 1.00 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, CD_3CN, ppm): δ 166.80, 69.16, 62.71, 46.11, 20.07, 13.85, 10.96, 5.71.

Ethyl 2-(phenylsulfonyl)butanoate (**S_f2**). **S_f2** (white powder) was synthesized from **T2** following the same procedure as for **S_f1**. ^{1}H NMR (400 MHz, CD_3CN, ppm): δ 7.85 (m, 2H), 7.63 (m, 3H), 4.05 (m, 2H), 3.99 (dd, J_1 = 10.8 Hz, J_2 = 4.0 Hz, 1H), 2.05-1.86 (m, 2H), 1.07 (t, J = 7.1 Hz, 3H), 0.94 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, CD_3CN, ppm): δ 166.27, 138.06, 134.70, 129.81, 129.59, 72.19, 62.31, 20.74, 13.85, 11.04. FT-MS: m/z (M^+), Calcd. 256.08; Found: 257.09 [M + H^+], 279.07 [M + Na^+].

Determination of critical aggregation concentration (CAC)

CACs of the amphiphilic block copolymers in 10 mM PB solution (pH 7.4) were measured by the fluorescence method using pyrene as a probe at 37 °C. The copolymer nanoparticle solution with a polymer concentration of 1.0 mg/mL was prepared by the same solvent evaporation procedure, and diluted to a series of samples with different polymer concentrations. To each (10 mL) of these polymer solutions, 100 μL of pyrene in acetone (0.06 mM) was added. The solutions were stirred overnight at 37 °C to evaporate acetone thoroughly. The final concentration of pyrene was 6.0×10^{-7} M. The excitation spectra were recorded from 300 to 360 nm on a Hitachi F-7000 fluorescence spectrometer equipped with a temperature controller. The emission wavelength and the scanning rate were 390 nm and 1200 nm/min, respectively. I_{338}/I_{333} denotes the ratio of the emission intensity at 338 nm to that at 333 nm.

Laser light scattering (LLS)

A commercial laser light scattering equipment (Brookhaven Inc., Holtsville, NY) with a BI-200SM goniometer, a BI-TurboCorr digital correlator for collecting and processing the data, and a vertically polarized He–Ne laser (R-30995, 633 nm, 17 mW, Newport, USA) as the light source was used for both dynamic light scattering (DLS) and static light scattering

(SLS) measurements. The z-averaged root-mean square radius of gyration (R_g) and hydrodynamic radius (R_h) were measured by SLS and DLS, respectively.[1] Prior to the LLS measurements, the stock copolymer nanoparticle solution in 10 mM PB (pH 7.4) was filtered into a dust free vial through a Millipore 0.45 μm PVDF membrane and incubated at 37 °C. For the H_2O_2-triggered disruption of the nanoparticles, the solution (2.0 mL) was first measured by LLS at 37 °C and the obtained data were used for 0 min time point. Afterwards, 0.2 mL of H_2O_2 solution was added to the solution and mixed thoroughly by shaking. The final H_2O_2 concentration was 5 mM. At desired time points, the LLS measurements were conducted from 90 ° to 30 °.

Loading and light-triggered release of Ce6 and DOX

The loading and release experiments were carried out following the published procedures.[2] DOX·HCl was dissolved in THF (2.0 mg/mL) with 4-fold excessive TEA. This acid-free DOX solution was stored at 4 °C and used as a stock solution. Ce6 dissolved in THF (1.0 mg/mL) was used as a stock solution. For drug encapsulation, 5 mg copolymer was dissolved in a mixture of 0.75 mL of Ce6 stock solution and 1.0 mL of DOX stock solution. The feed ratios of Ce6 and DOX to copolymer were 15 wt% and 40 wt%, respectively. To this solution 5 mL of PB solution (pH 7.4, 10 mM) was added and the mixture was stirred overnight at 37 °C to remove THF. After centrifugation, the supernatant was dialyzed against the same PB solution for 48 h at 37 °C in dark. During this process, the dialysis medium was changed 3 times. The final concentration of copolymer nanoparticles was tuned to 1.0 mg/mL. The loading content and efficiency of Ce6 were determined according to the fluorescence intensity at 675 nm, assuming that Ce6 in PB solution and in the nanoparticles possessed the same quantum yield. To determine the DOX loading content and efficiency, 50 mM H_2O_2 was added into 1.0 mL of the drug-loaded nanoparticle solution, which was stirred for 24 h to disrupt the nanoparticle. The DOX content was determined by the absorbance at 485 nm on the UV-vis spectrometer. All the measurements were conducted in triplicate under dark conditions.

The in vitro drug release experiments were performed by a dialysis method. Briefly, 1.0 mL of the drug-loaded nanoparticle solution was added in a dialysis tubing (MWCO: 50 kDa) which was immersed in 5.0 mL of PB solution (pH 7.4, 10 mM). The system was stirred gently at 37 °C for 1 h under red laser light irradiation (650 nm, 100 mW/cm^2 output) and for additional 23 h in dark. During this process, 0.5 mL of the dialysis medium was taken out for UV-vis analysis and replenished with the same volume of fresh medium at desired time points.

The amount of Ce6 was determined by the absorbance at 404 nm. DOX content was calculated based on the absorbance of 485 nm, after subtracting the weak absorption of Ce6 at this wavelength. All the measurements were conducted in triplicate in dark.

Table S1 Characterization of homopolymers **P1-P3**

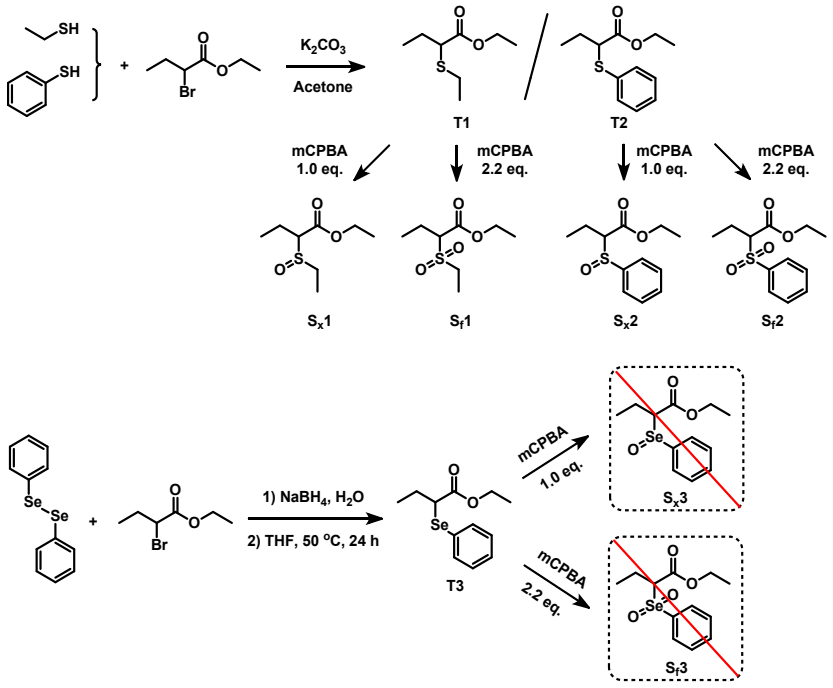
Polymer [a]	DP [b]	$M_{n,NMR}$ [b]	$M_{n,GPC}$ [c]	$Đ$ [c]	T_g (°C) [d]
P1	57	10500	16900	1.09	-27.9
P2	60	13400	20700	1.07	-12.1
P3	58	16200	23100	1.06	-17.1

[a] Reaction conditions: 130 °C, in toluene, catalyzed by $Sn(Oct)_2$ (0.5 wt % of monomer), 48 h, $[M]_0$ = 2.0 mol/L. [b] Determined by ^1H NMR. [c] Determined by GPC using polystyrene standards in THF. [d] Glass transition temperature determined by DSC, -50-150 °C (**Fig. S8**).

Table S2. Characterization of the drug-loaded nanoparticles

Polymer [a]	DOX (wt%)		Ce6 (wt%)		R_h [d] (nm)	R_h [e] (nm)
	LC [b]	LE [c]	LC [b]	LE [c]		
PS1	10.2 ± 2.3	25.5 ± 5.8	1.8 ± 0.3	12.0 ± 2.3	56.8	59.6
PS2	11.5 ± 1.8	28.8 ± 4.4	2.0 ± 0.2	13.3 ± 1.3	60.5	62.5
PSEc	11.1 ± 1.4	27.8 ± 3.6	2.1 ± 0.3	13.9 ± 2.0	58.2	60.5
PEG-*b*-PCl	10.8 ± 2.1	27.0 ± 5.2	1.7 ± 0.2	11.3 ± 1.3	55.3	57.7

[a] DOX/polymer in feed: 40 wt%; Ce6/polymer in feed: 15 wt%. [b] Loading content defined as drug in nanoparticles/polymer (× 100 %). [c] Loading efficiency defined as drug in nanoparticles/drug in feed (× 100 %). [d] R_h of the empty nanoparticles measured by DLS at 37 °C. [e] R_h of the drug-loaded nanoparticles.



Scheme S1 Synthesis of the small molecule model compounds.

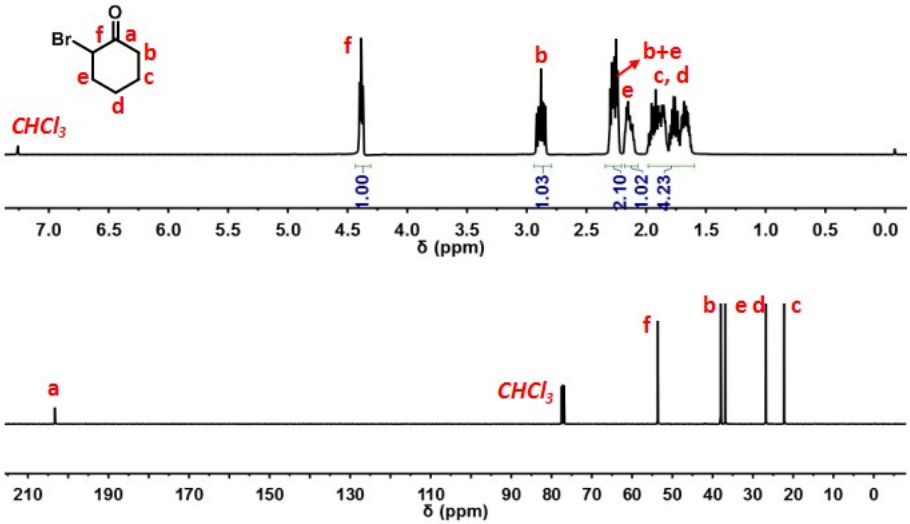


Fig. S1 ^{1}H NMR and ^{13}C NMR spectra of compound **1** in $CDCl_3$.

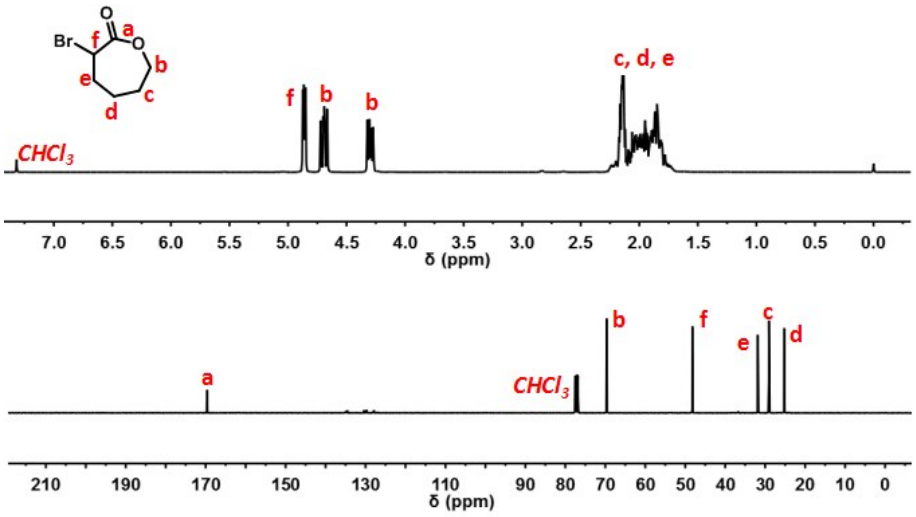


Fig. S2 ^{1}H NMR and ^{13}C NMR spectra of compound **2** in $CDCl_3$.

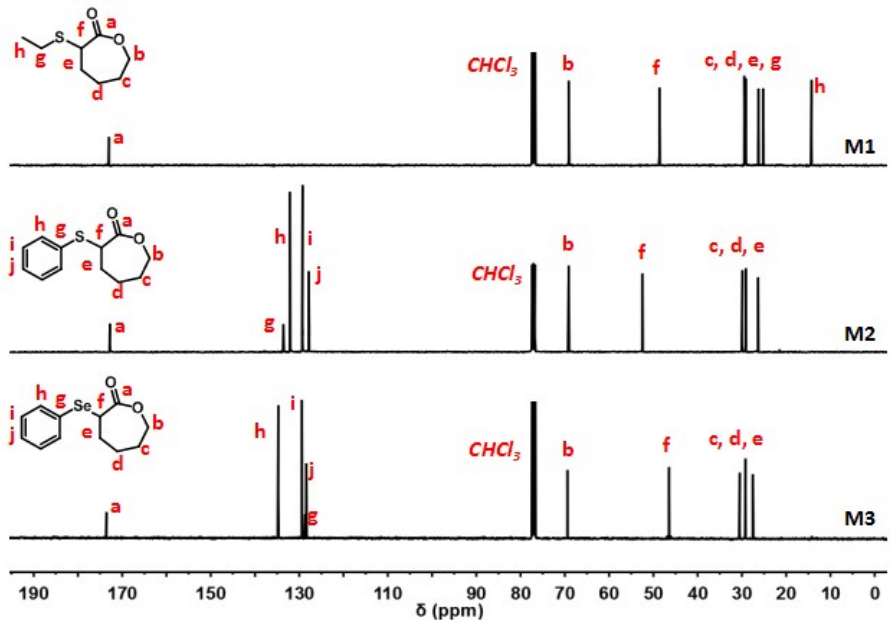


Fig. S3 ^{13}C NMR spectra of monomers **M1**, **M2**, and **M3** in $CDCl_3$.

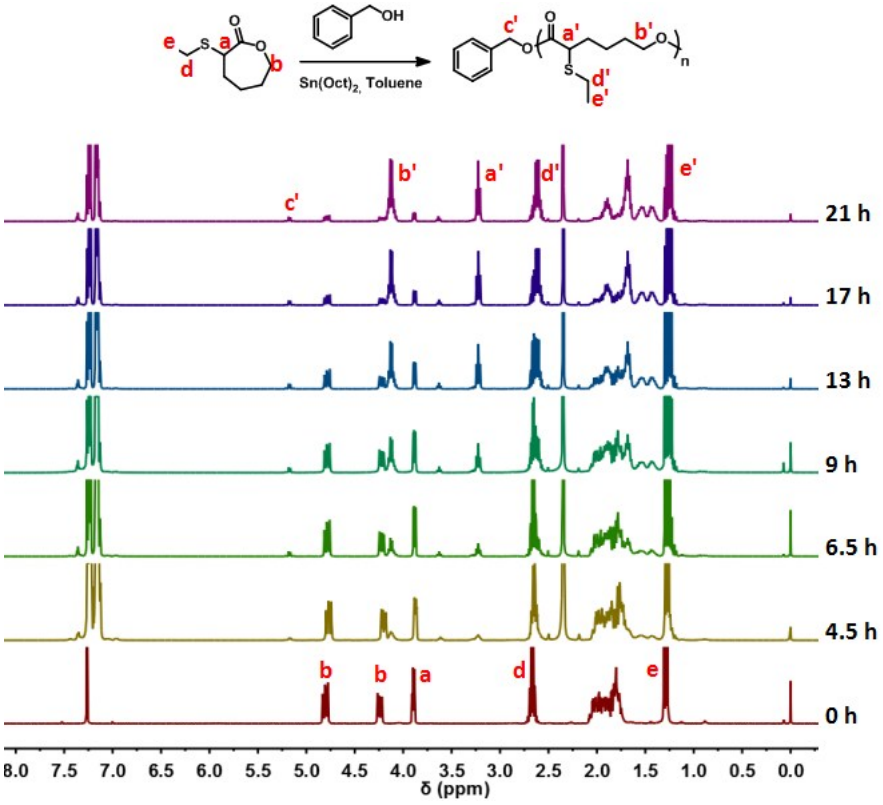


Fig. S4 Time-dependent ^{1}H NMR spectra of the samples taken out from the ROP mixture of **M1**. The conversion of monomer is determined by comparing intensity of the peak at 4.07-4.17 ppm (b') and that of the peaks in the range of 4.19-4.81 ppm (b). The degree of polymerization was calculated by comparing the peak intensities of proton b' (4.07-4.17 ppm) and c' (5.16-5.18 ppm).

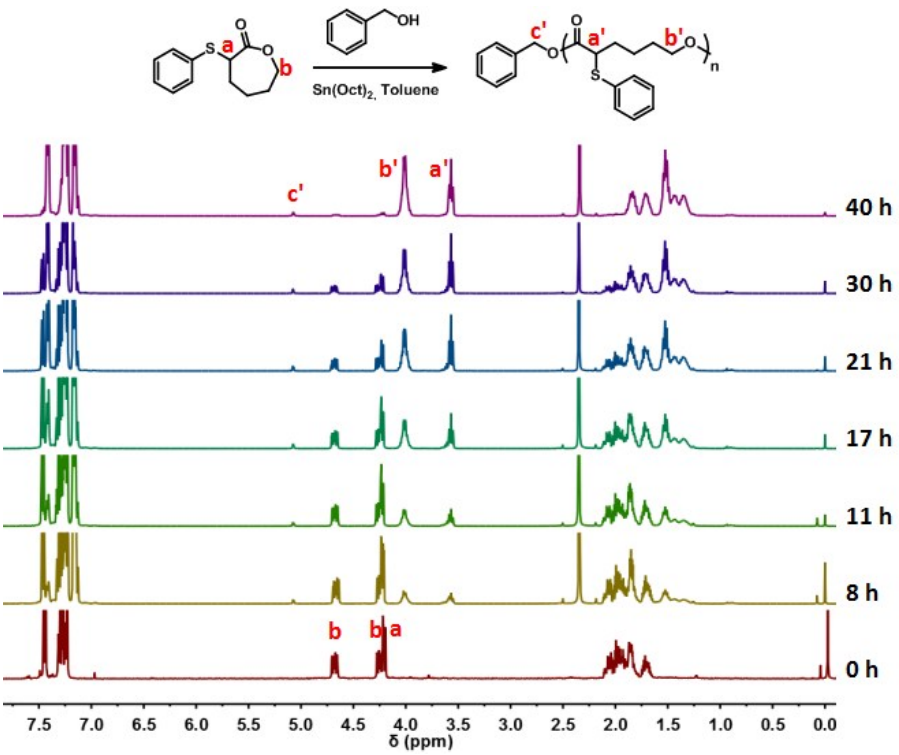


Fig. S5 Time-dependent ^{1}H NMR spectra of the samples taken out from the ROP mixture of **M2**. The conversion of monomer is determined by comparing intensity of the peak at 3.96-4.06 ppm (b') and that of the peaks in the range of 4.24-4.71 ppm (b). The degree of polymerization was calculated by comparing the peak intensities of proton b' (3.96-4.06 ppm) and c' (5.08 ppm).

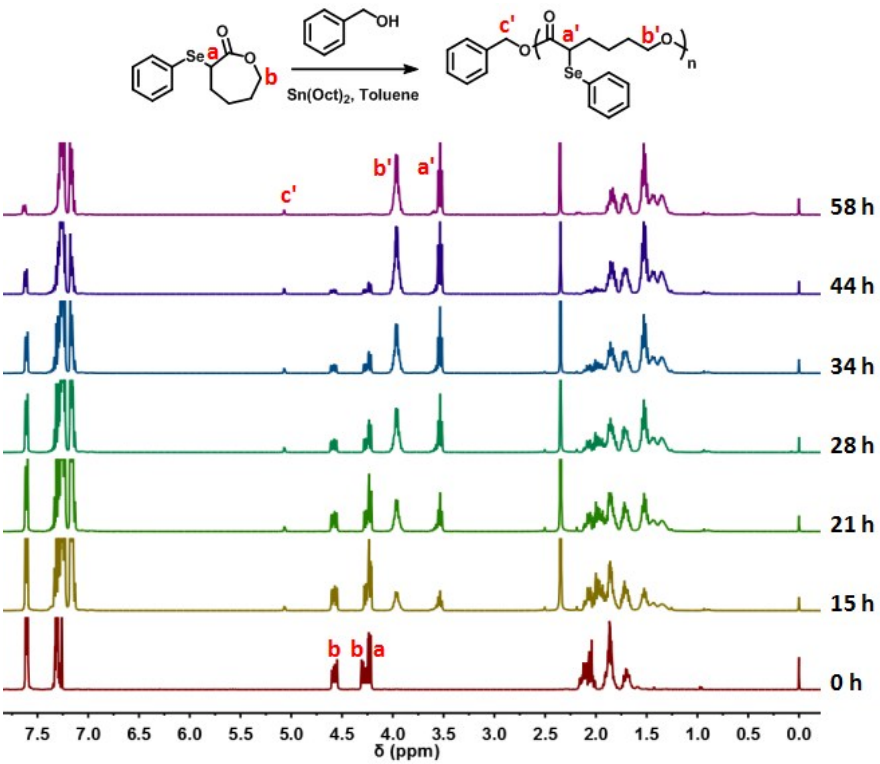


Fig. S6 Time-dependent ^{1}H NMR spectra of the samples taken out from the ROP mixture of **M3**. The conversion of monomer is determined by comparing intensity of the peak at 3.92-4.02 ppm (b') and that of the peaks in the range of 4.26-4.61 ppm (b). The degree of polymerization was calculated by comparing the peak intensities of proton b' (3.92-4.02 ppm) and c' (5.07 ppm).

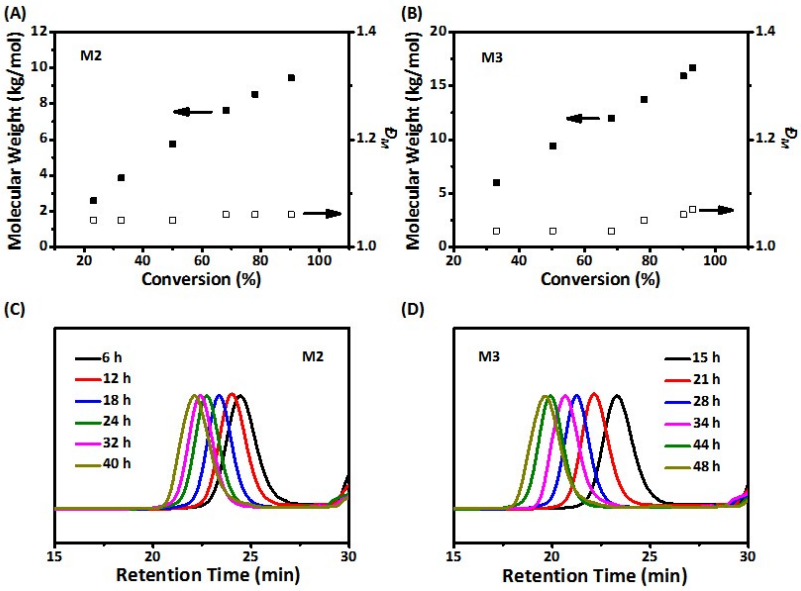


Fig. S7 (A, B) The plots of molecular weight and *Đ* vs monomer conversion of (A) **M2** and (B) **M3**; (C, D) GPC traces of (C) **M2** and (D) **M3** reaction mixtures at different times. The molecular weight was measured by GPC.

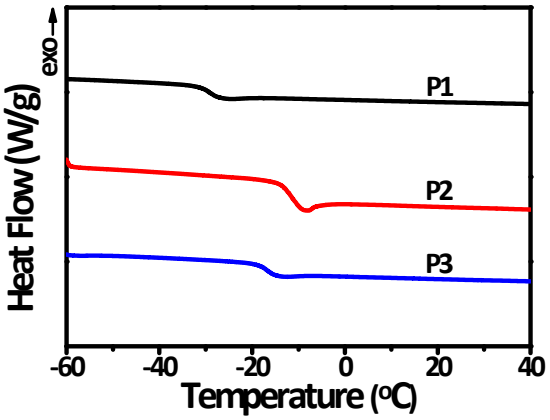


Fig. S8 DSC thermograms of **P1-P3**.

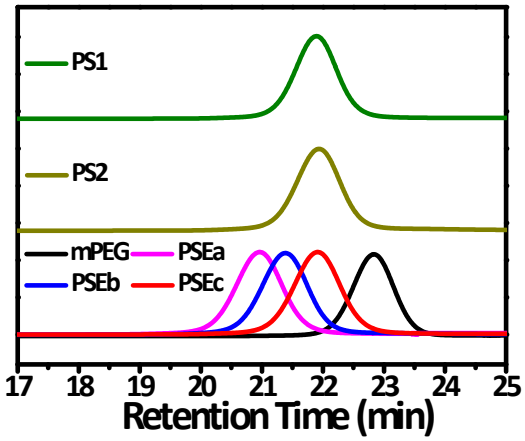


Fig. S9 GPC traces of **PS1**, **PS2**, and **PSEs** with varied block lengths using THF as the eluent.

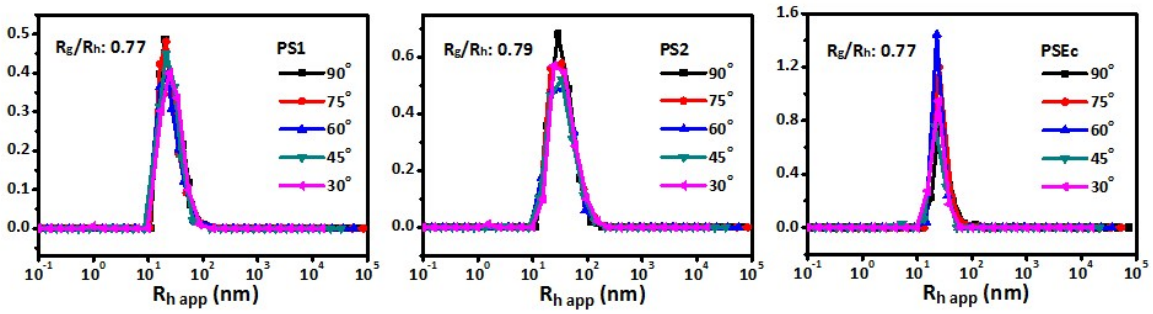


Fig. S10 Size distribution of nanoparticles (0.1 mg/mL) in 10 mM PB solution (pH 7.4) at 37 °C.

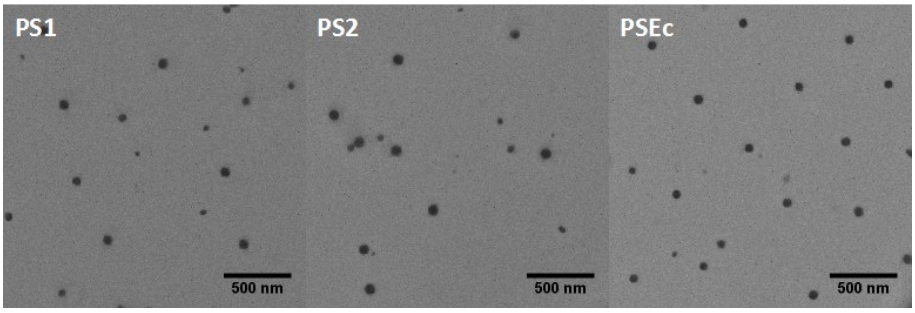


Fig. S11 TEM images of the nanoparticles prepared from **PS1**, **PS2**, and **PSEc**.

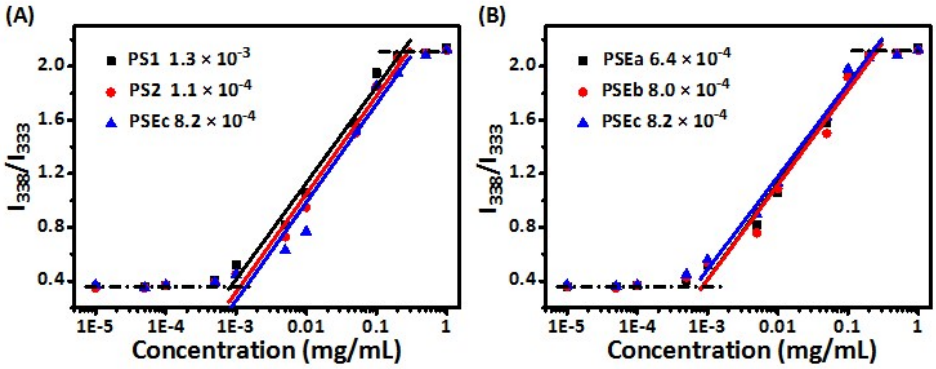


Fig. S12 Relationship between I_{338}/I_{333} ratio of pyrene and the concentration of block copolymers with (A) different side groups and (B) varied block lengths. Concentration of pyrene: 6.0×10^{-7} M.

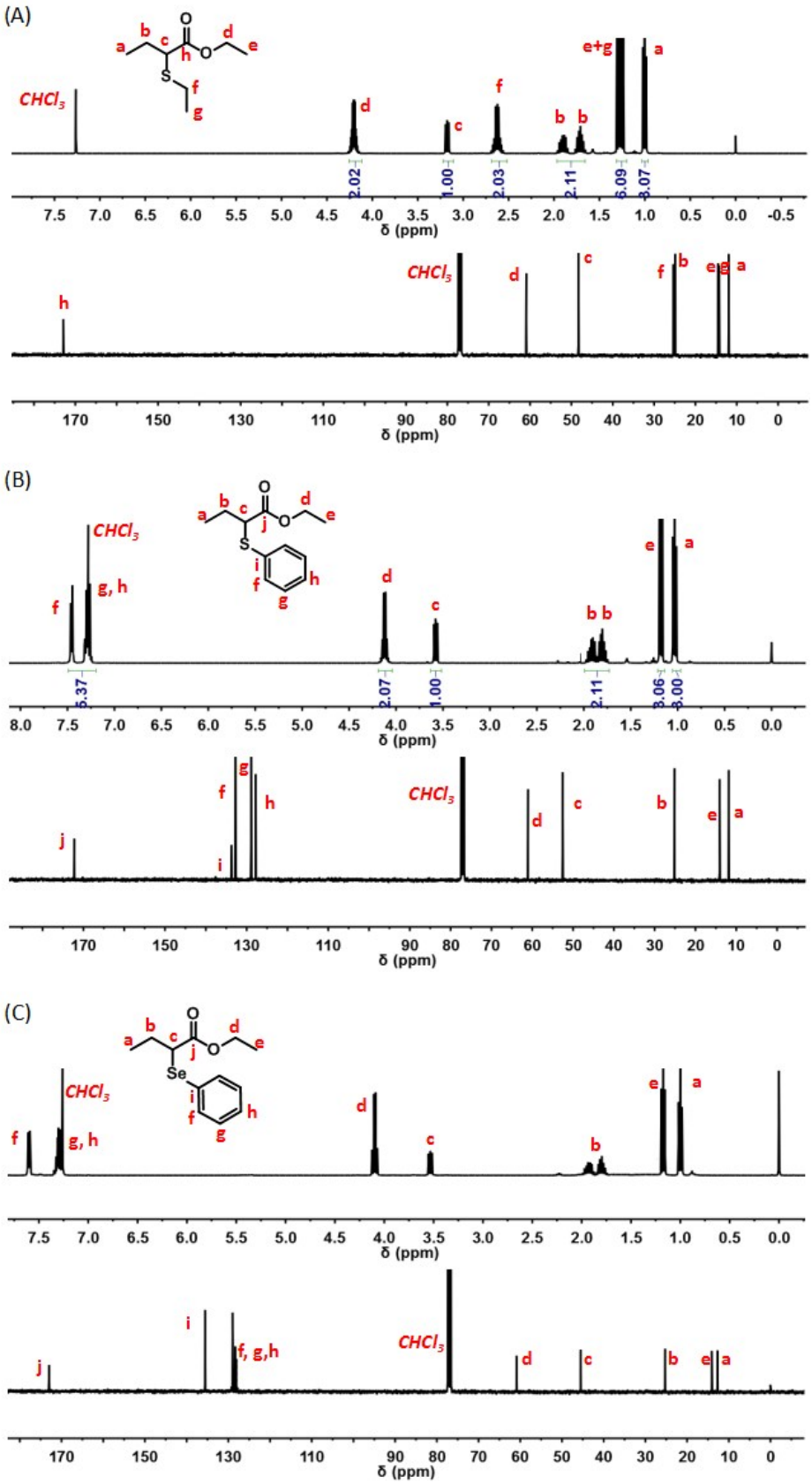


Fig. S13 ^{1}H NMR and ^{13}C NMR spectra of (A) **T1**, (B) **T2,** and (C) **T3** in $CDCl_3$.

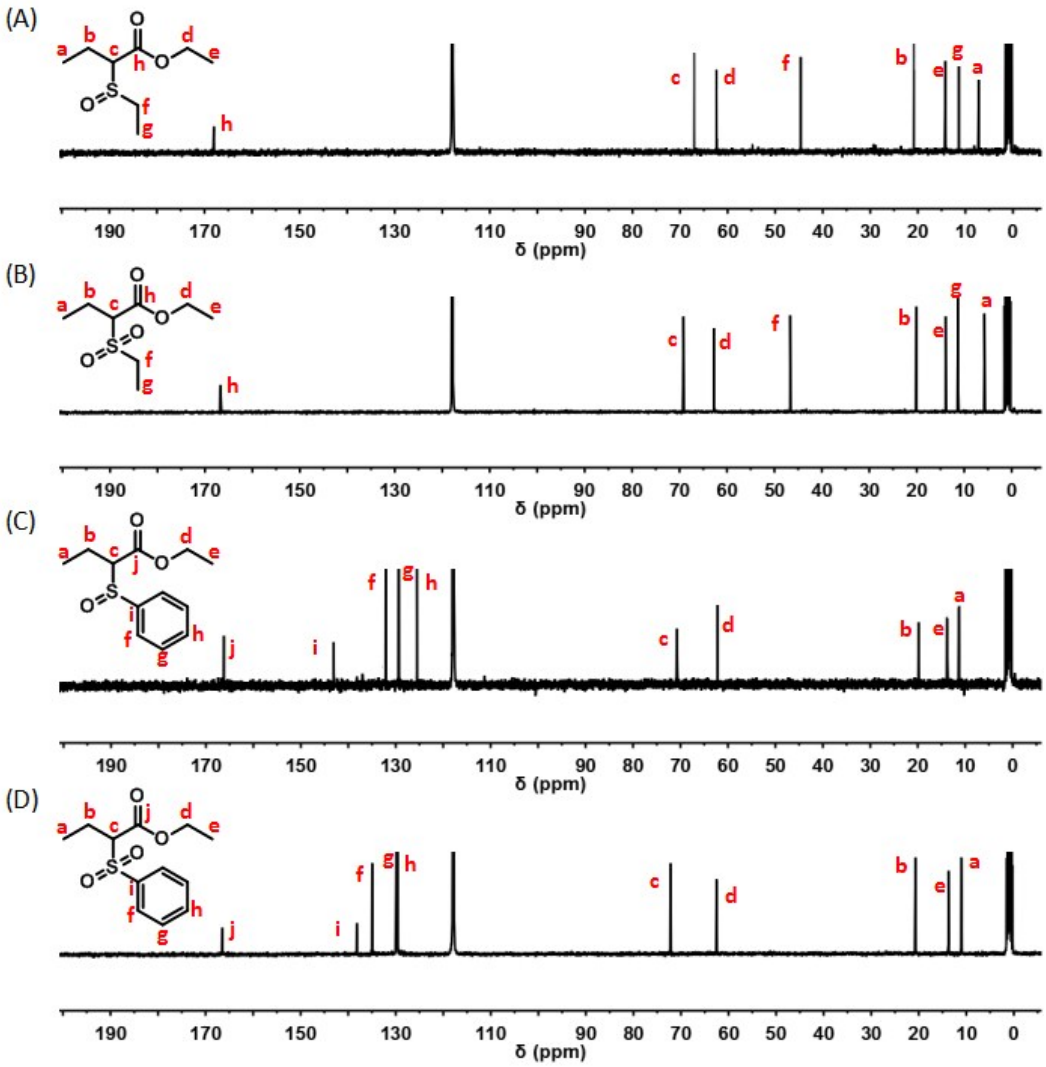


Fig. S14 ^{13}C NMR spectra of (A) $\mathbf{S_x1}$, (B) $\mathbf{S_f1}$, (C) $\mathbf{S_x2}$, and (D) $\mathbf{S_f2}$ in CD_3CN.

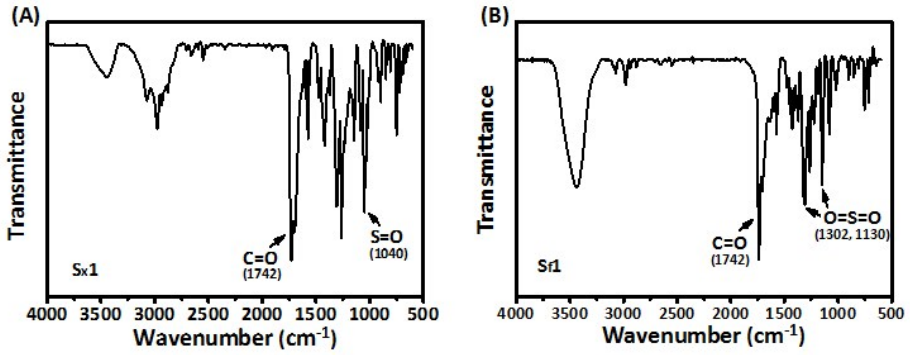


Fig. S15 FT-IR spectra of (A) $\mathbf{S_x1}$ and (B) $\mathbf{S_f1}$.

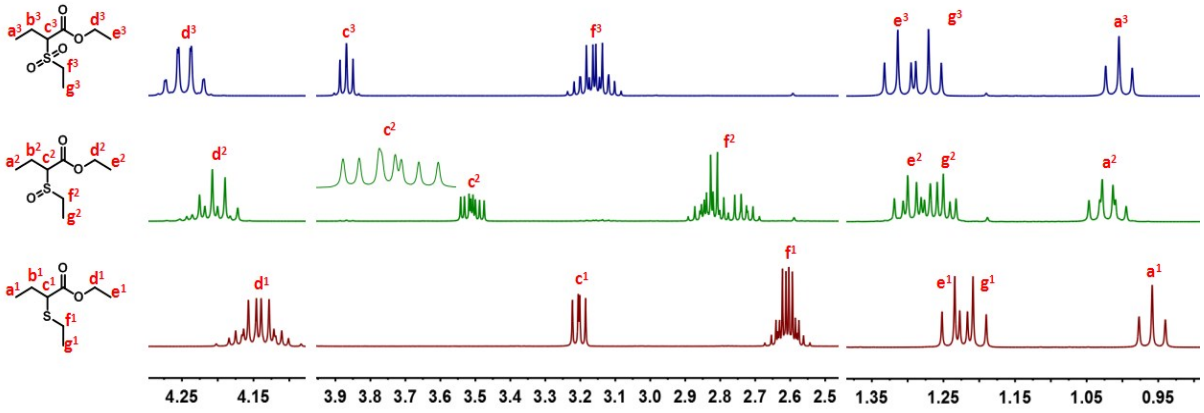


Fig. S16 The amplified ^{1}H NMR spectra in **Fig. 4B**.

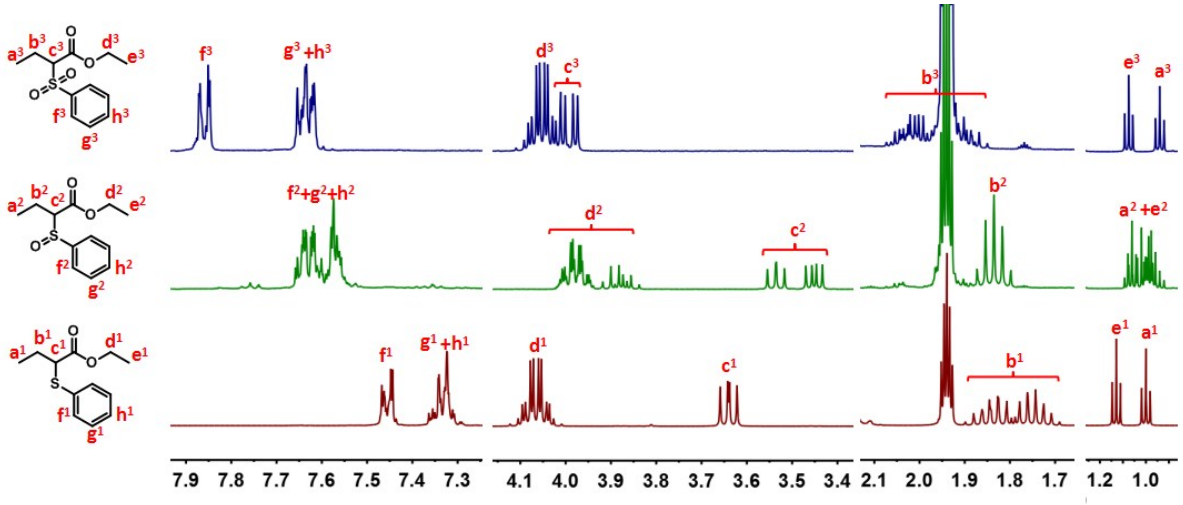


Fig. S17. The amplified ^{1}H NMR spectra in **Fig. 4C**.

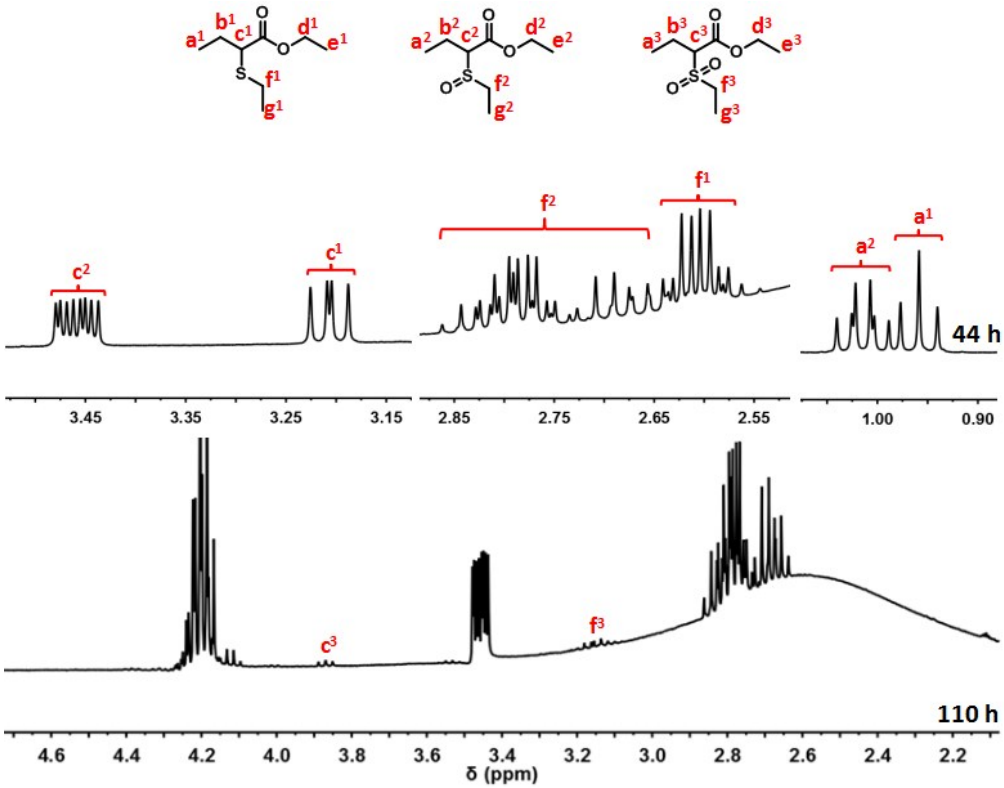


Fig. S18. The selectively amplified spectra (44 h and 110 h) of **Fig. 5**.

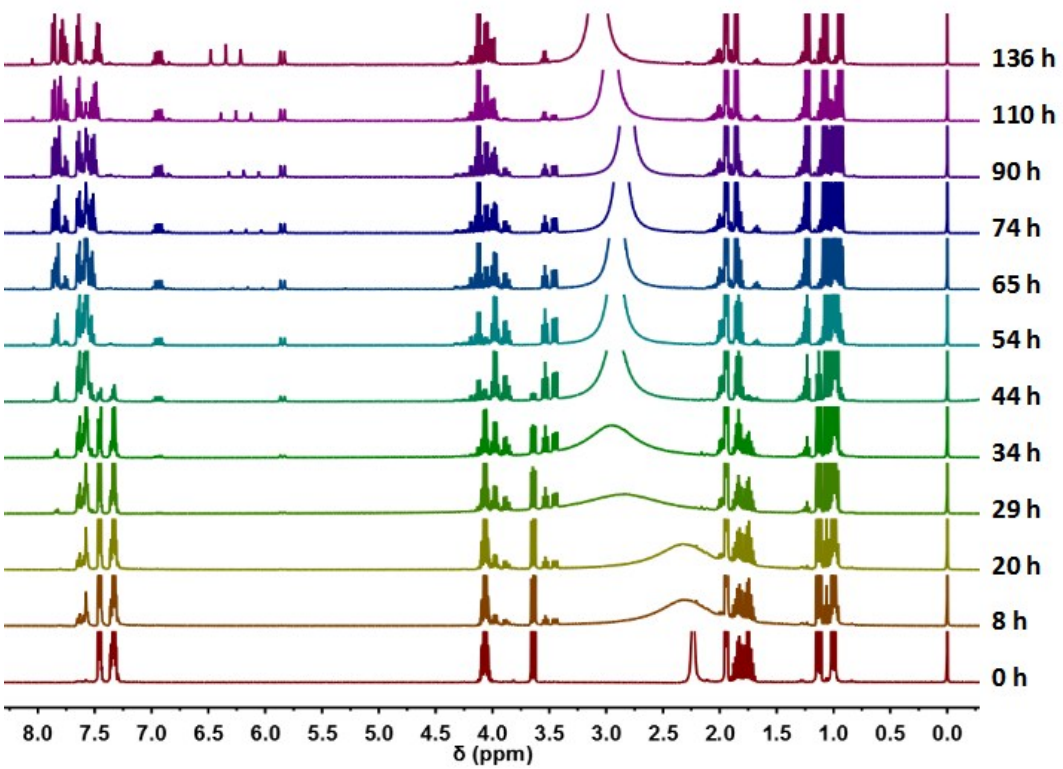


Fig. S19 Time-dependent ^{1}H NMR spectra of **T2** (5 mg/mL) in the mixed solution of CD_3CN and deuterated PB (pH 7.4, 50 mM) (9:1, v/v) with 45 mM H_2O_2 (2 eq), 60 °C.

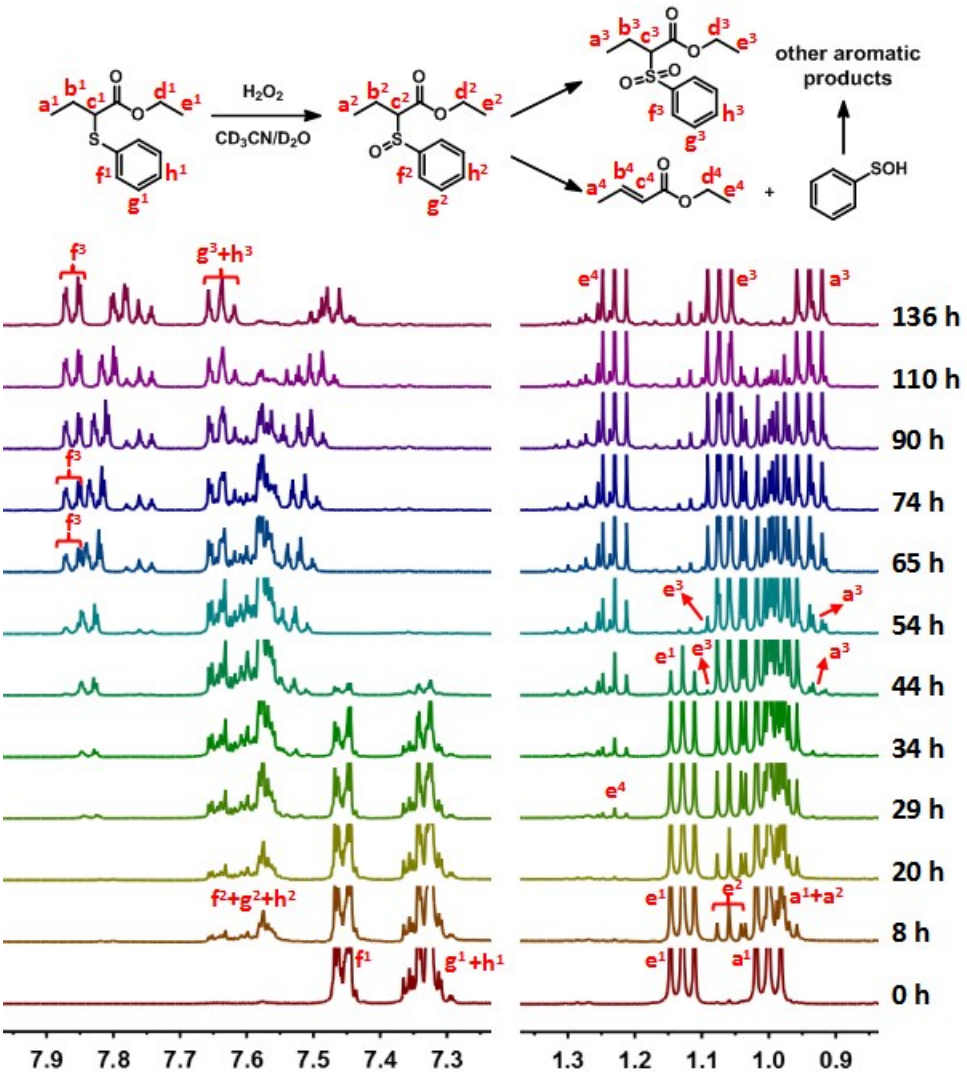


Fig. S20 The selectively amplified spectra of **Fig. S19**.

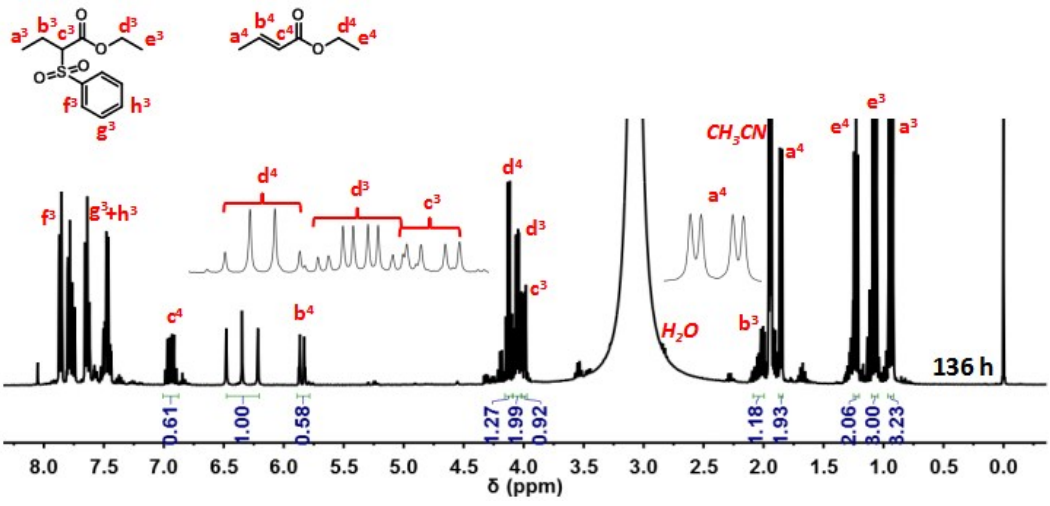


Fig. S21 The selectively amplified spectrum (136 h) in **Fig. S19**.

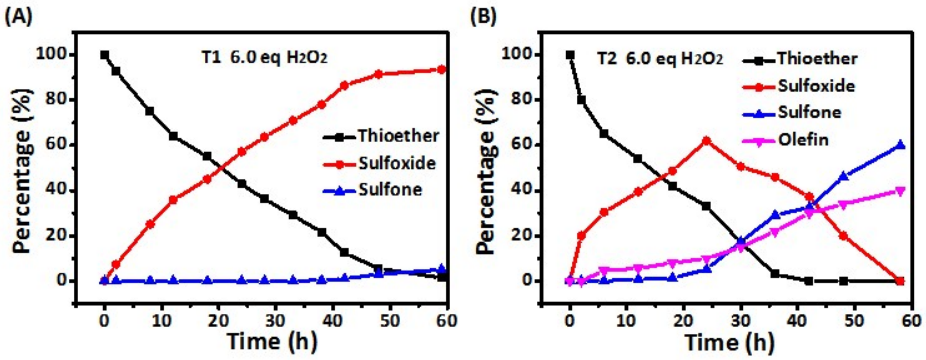


Fig. S22 Kinetic curves of oxidation of **T1** and **T2** by H_2O_2 (6 eq, 60 °C).

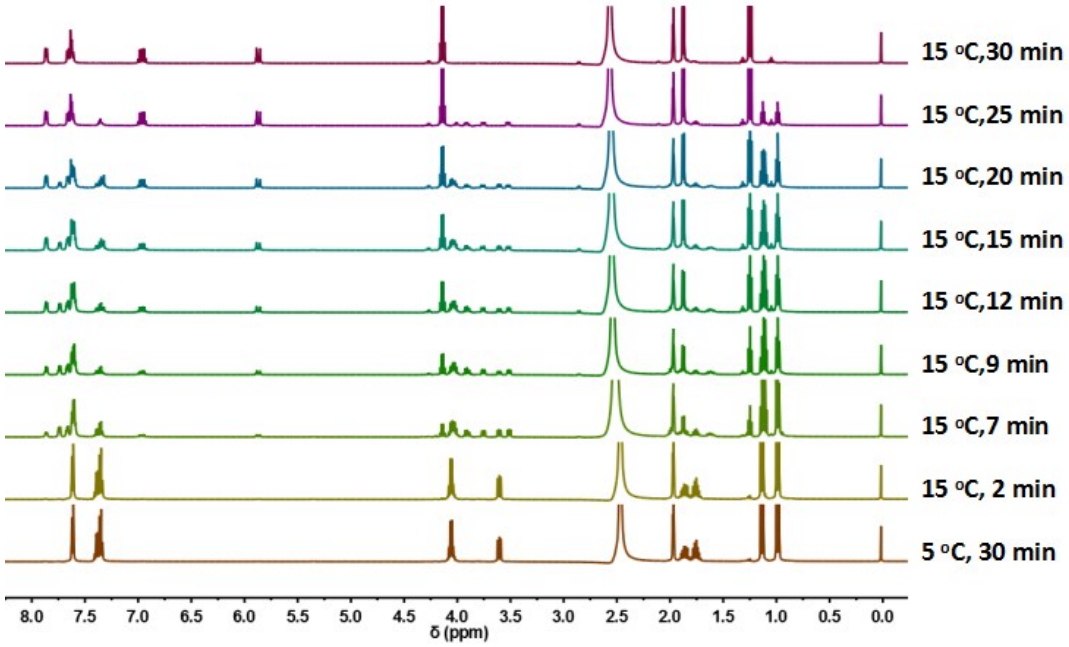


Fig. S23 Time-dependent ^{1}H NMR spectra of **T3** (5 mg/mL) in the mixed solution of CD_3CN and deuterated PB (pH 7.4, 50 mM) (9:1, v/v) with 36 mM H_2O_2 (2 eq) at 5 °C and 15 °C. The selectively amplified spectra are shown in **Fig. S24**.

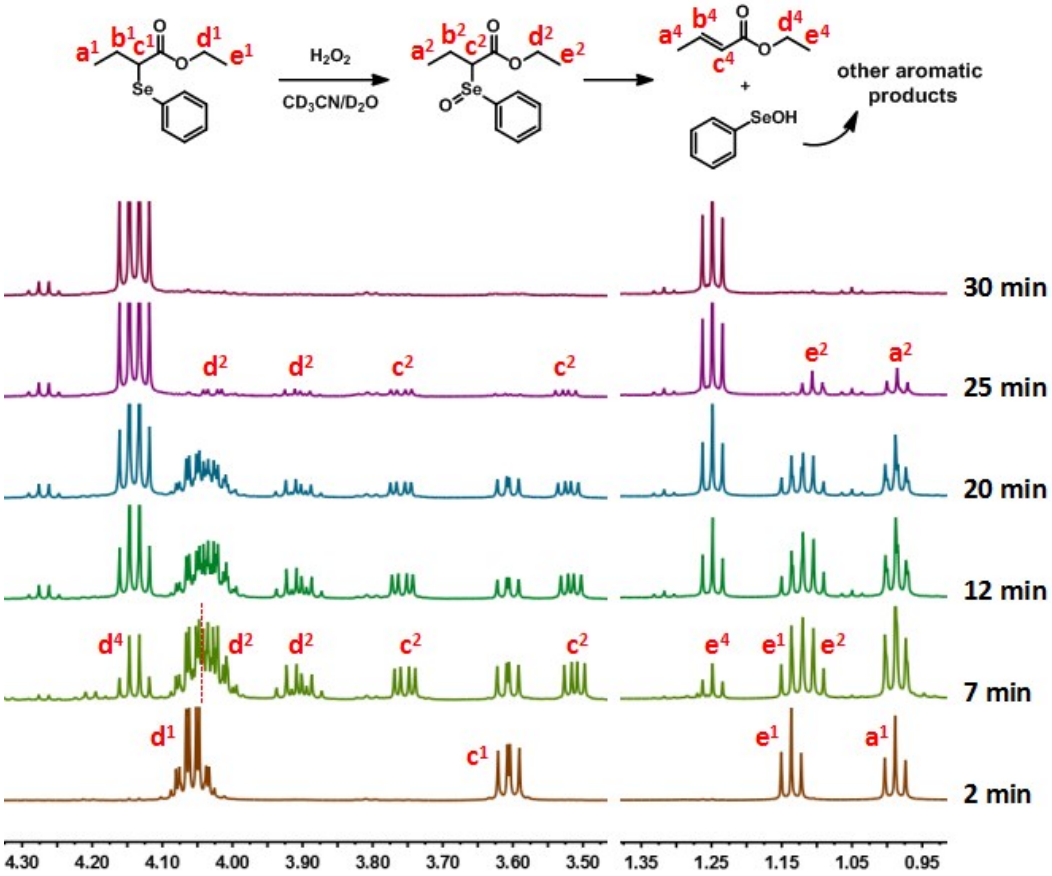


Fig. S24 The selectively amplified spectra of **Fig. S23** (15 °C).

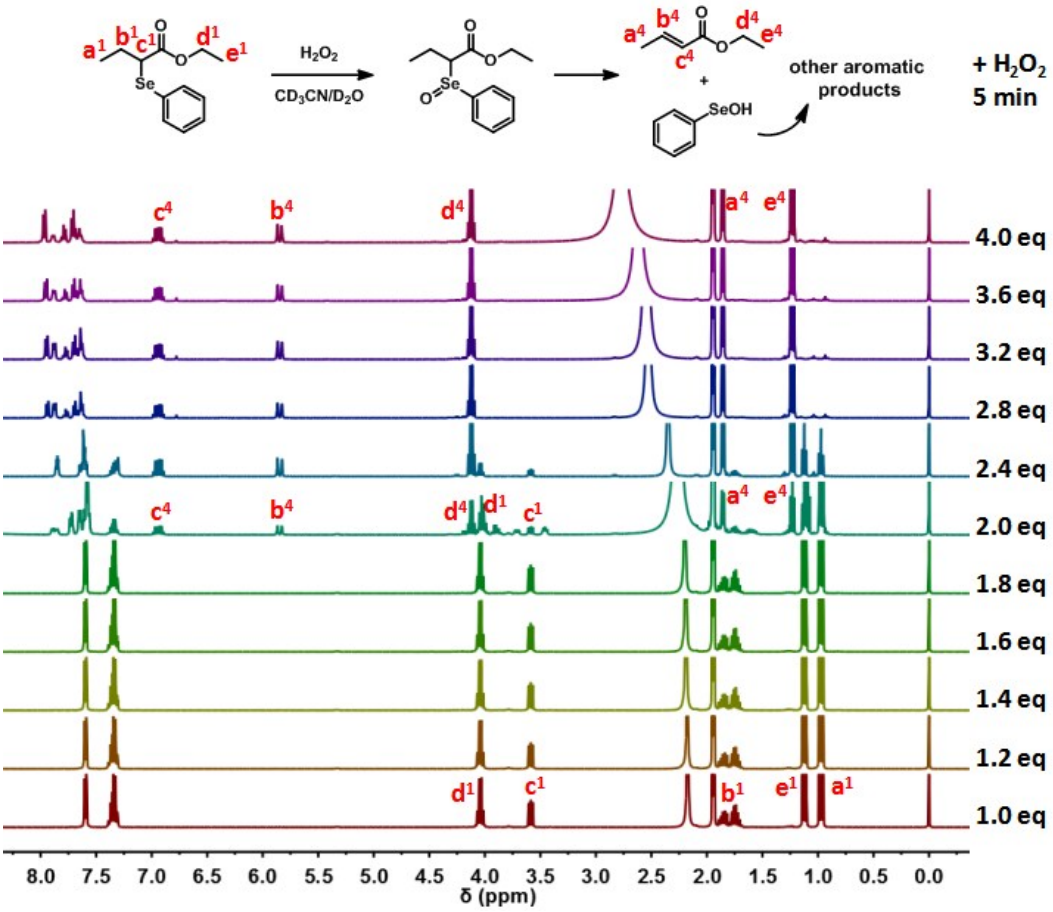


Fig. S25 The effect of H_2O_2/**T3** molar ratio on the oxidation reaction. Conditions: 5 mg **T3**/mL in the mixed solution of CD_3CN and deuterated PB (pH 7.4, 50 mM) (9:1, v/v), 25 °C. The spectra were recorded after 5 min of adding H_2O_2.

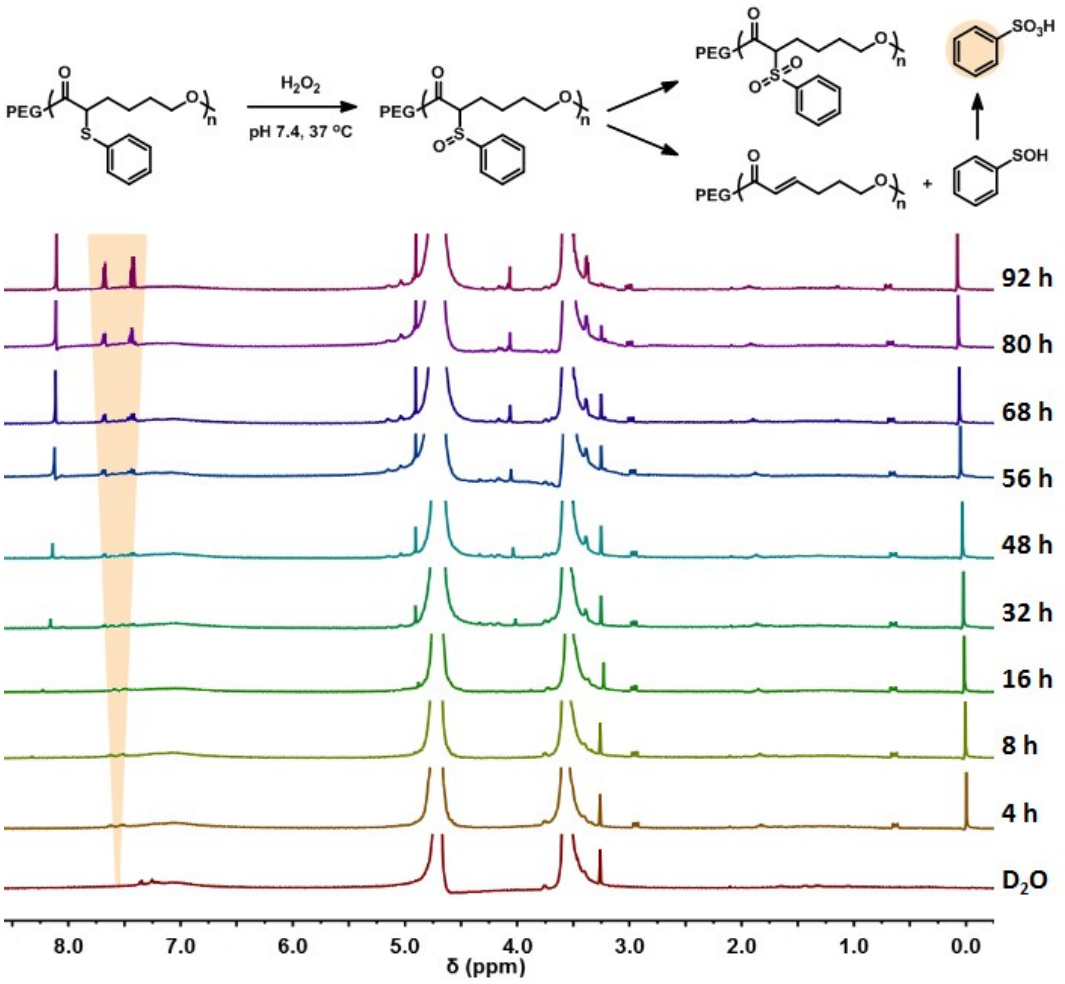


Fig. S26 Time-dependent ^{1}H NMR spectra of **PS2** nanoparticle (6 mg/mL) in deuterated PB (pH 7.4, 50 mM) with 50 mM H_2O_2 (5.3 eq) at 37 °C.

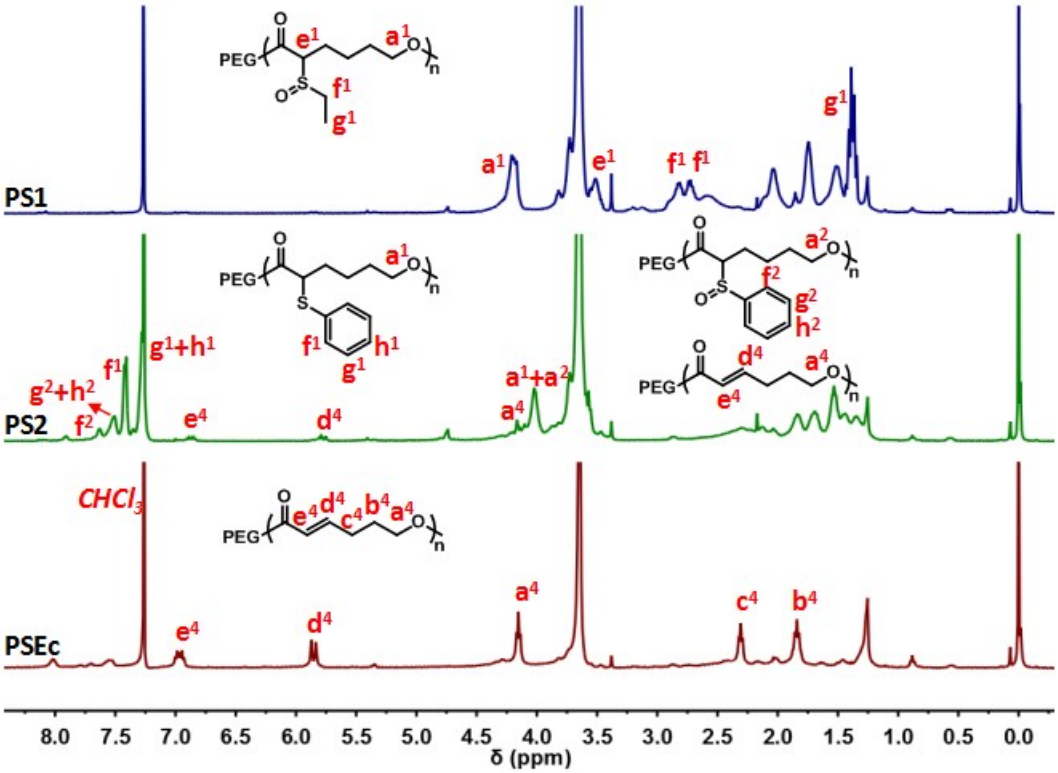


Fig. S27 ^{1}H NMR spectra of the oxidized products of the block copolymers in $CDCl_3$. The oxidation was carried out in PB (pH 7.4, 50 mM) for 92 h (**PS1** and **PS2**) and 24 h (**PSEc**), respectively, and the mixture solution was freeze-dried. The lyophilized powder was extracted with $CDCl_3$, and the extract was analyzed by ^{1}H NMR.

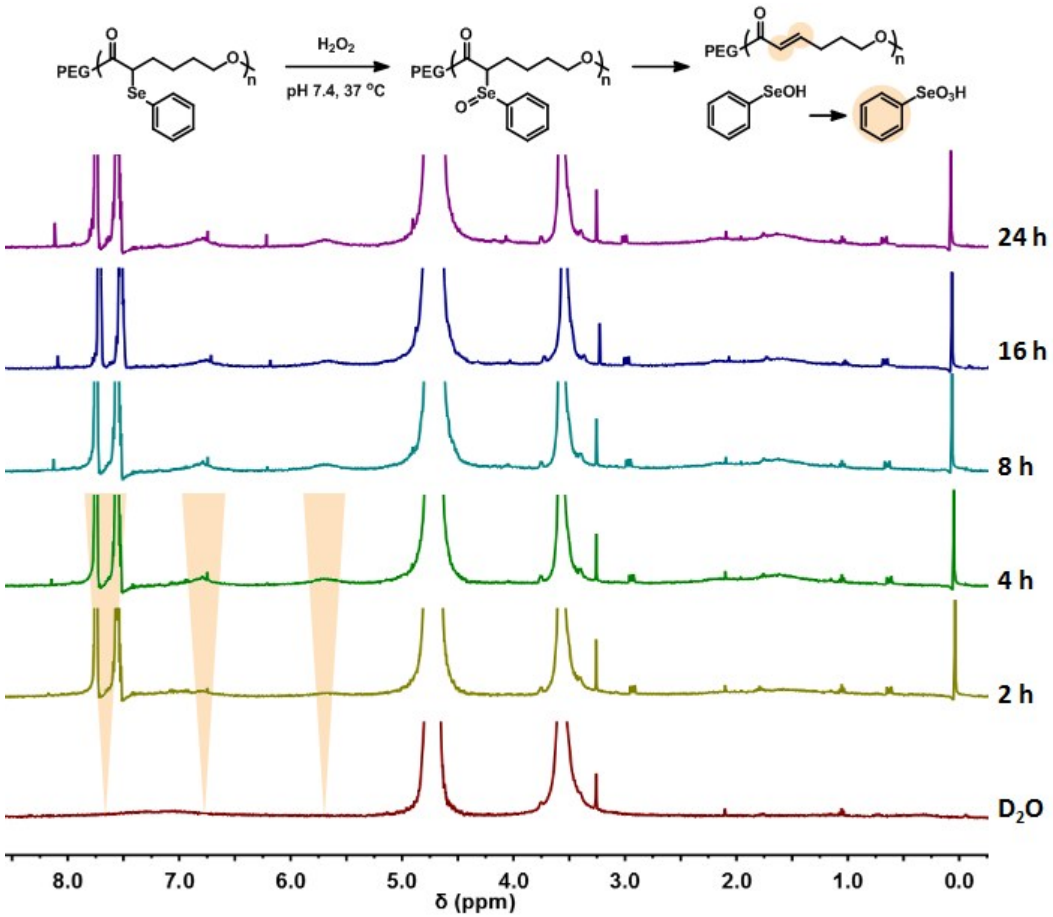


Fig. S28 Time-dependent ^{1}H NMR spectra of **PSEc** nanoparticle (6 mg/mL) in deuterated PB (pH 7.4, 50 mM) with 47 mM H_2O_2 (~6 eq) at 37 ^{o}C.

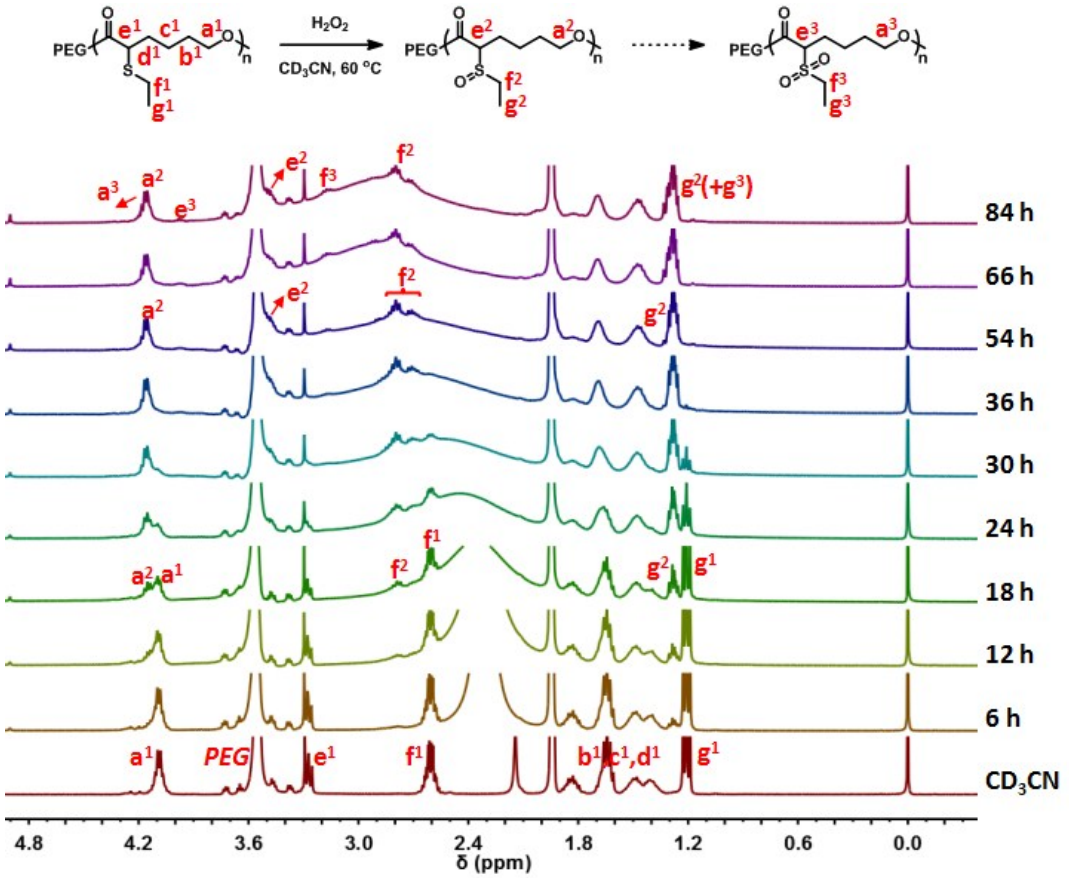


Fig. S29 Time-dependent ^{1}H NMR spectra of **PS1** (12 mg/mL) in CD_3CN with 106 mM H_2O_2 (5.5 eq) at 60 °C.

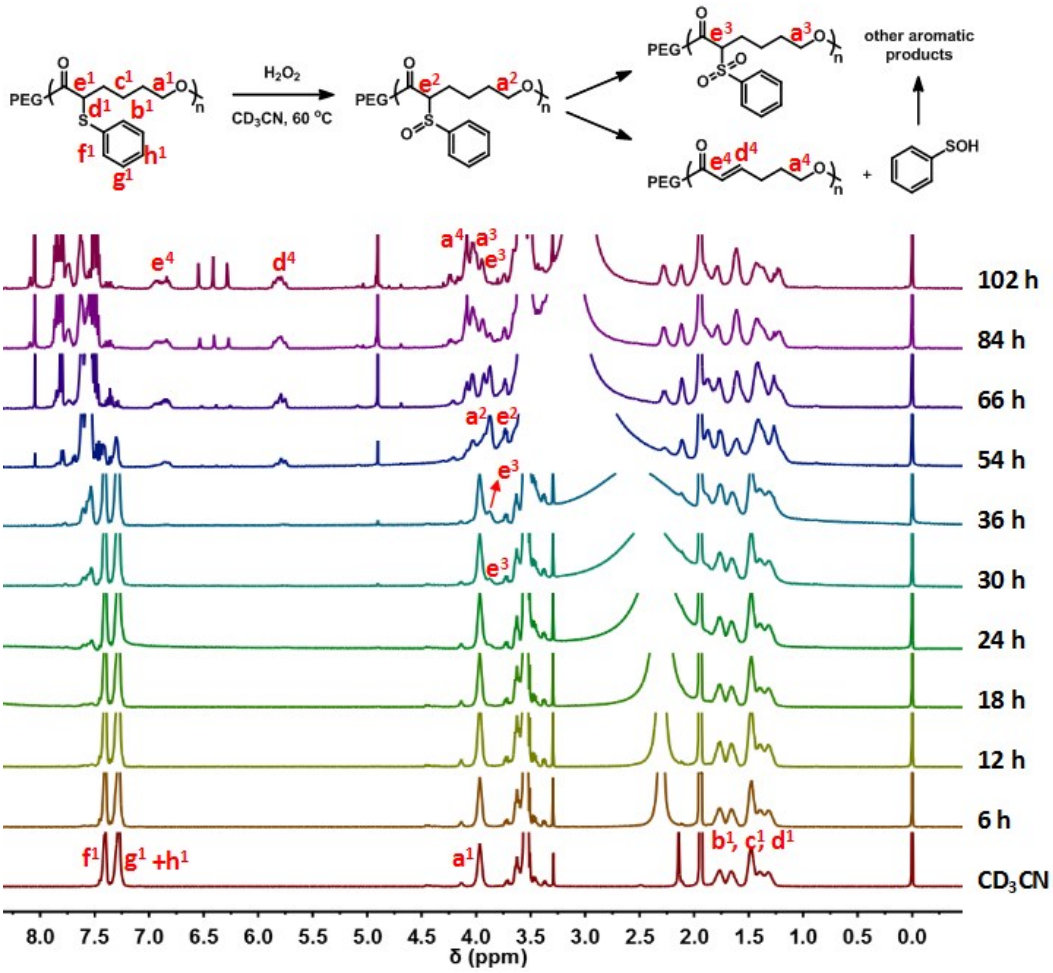


Fig. S30 Time-dependent ^{1}H NMR spectra of **PS2** (12 mg/mL) in CD_3CN with 100 mM H_2O_2 (5.5 eq) at 60 °C.

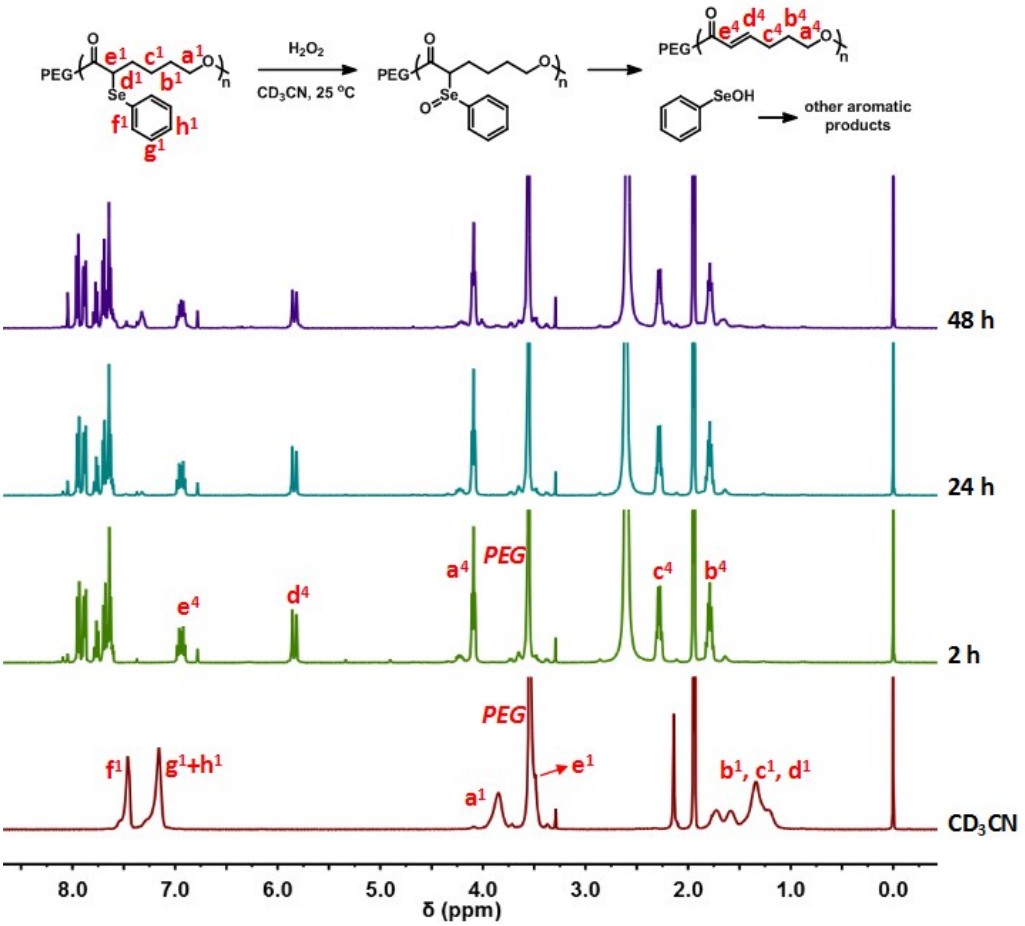


Fig. S31 Time-dependent ^{1}H NMR spectra of **PSEc** (12 mg/mL) in CD_3CN with 94 mM H_2O_2 at 25 °C.

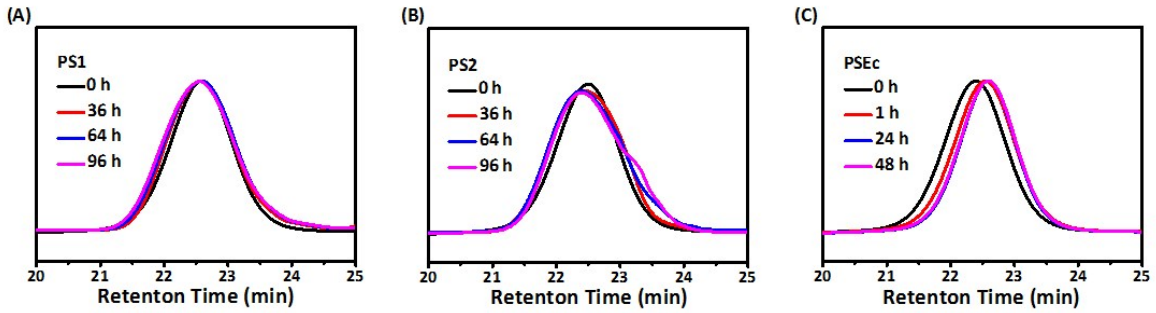


Fig. S32 GPC traces of the block copolymers (12 mg/mL) during oxidation. The oxidation was carried out in CH_3CN for 96 h (**PS1** and **PS2**) at 60 °C and 48 h (**PSEc**) at 25 °C with ~100 mM H_2O_2 (~5.5 eq). At the desired time point, the reaction mixture was quenched by adding MnO_2 and freeze-dried. The lyophilized powder was extracted with THF and analyzed by GPC.

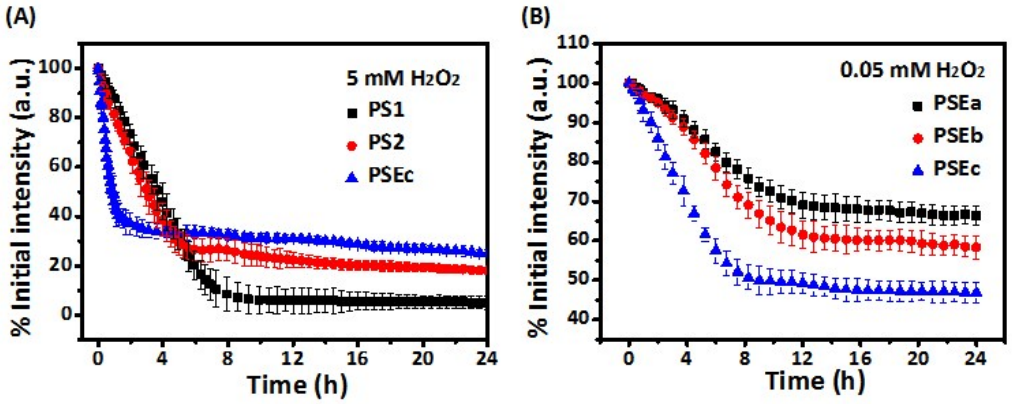


Fig. S33 Time-dependent change in the normalized intensity (at maximum wavelength) of NR fluorescence in various nanoparticles with (A) 5 mM H_2O_2 and (B) 50 μM H_2O_2. Polymer concentration: 0.1 mg/mL; NR concentration: 2.5 μg/mL (7.8 μM); 37 ^oC; pH = 7.4.

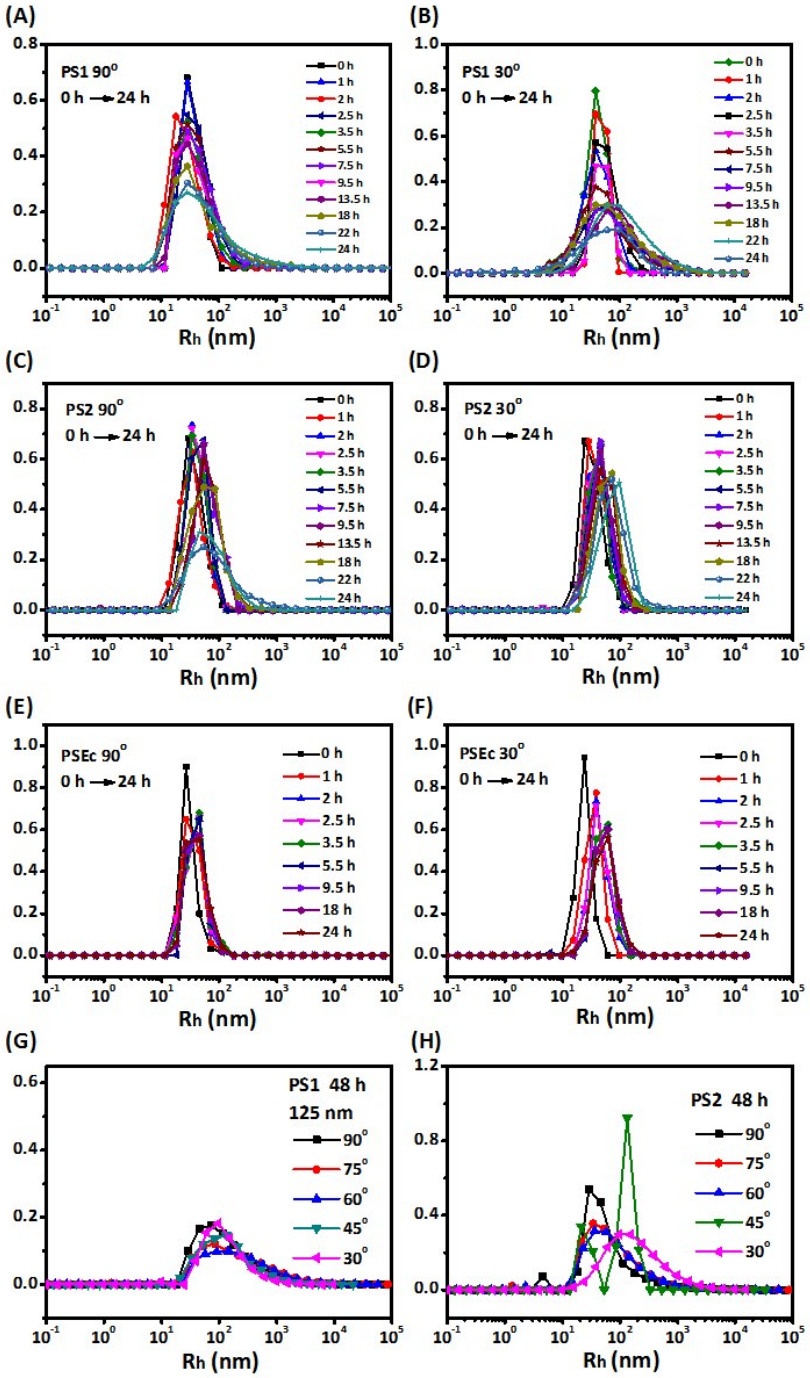


Fig. S34 CONTIN analyses of the nanoparticles (0.1 mg/mL) in PB solution (10 mM, pH 7.4) with 5 mM H_2O_2 at 37 °C.

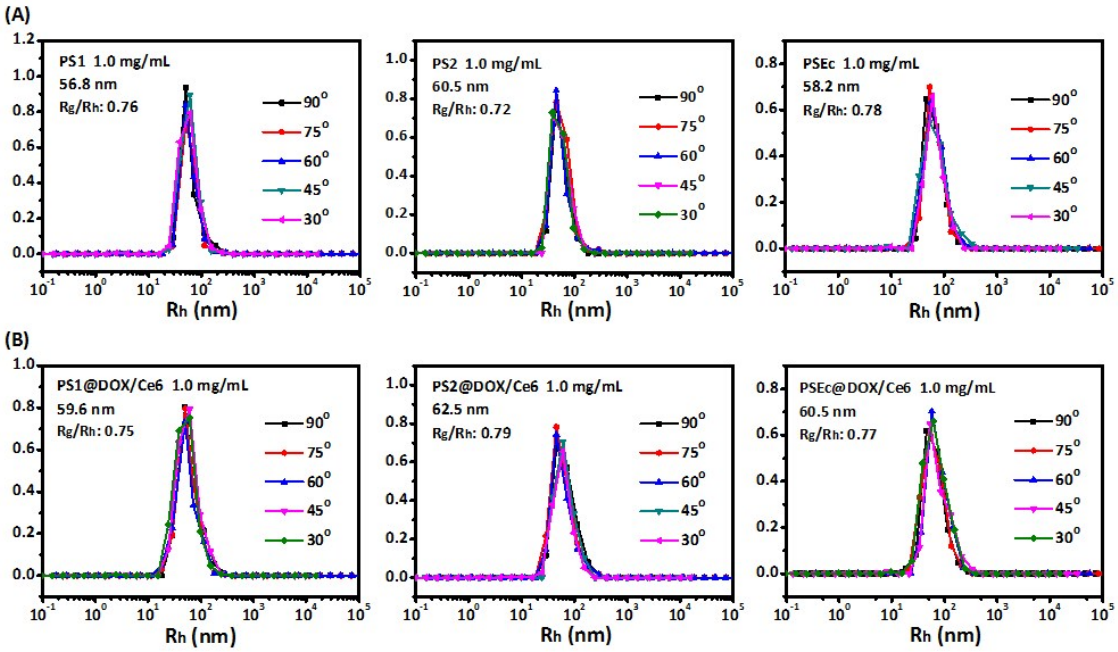


Fig. S35 Size distribution of the empty or drug-loaded nanoparticles (1.0 mg/mL) in 10 mM PB solution (pH 7.4) at 37 °C.

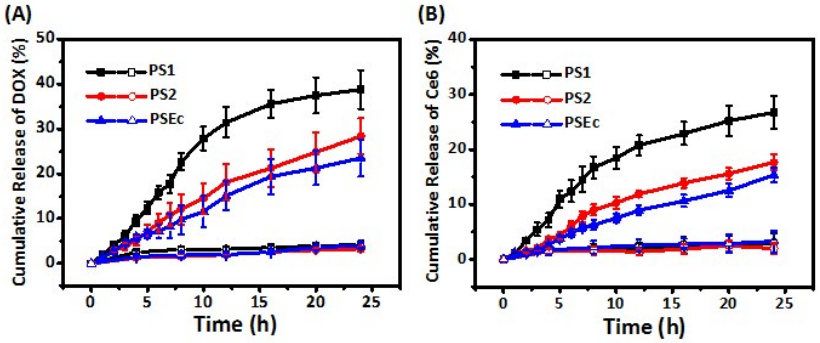


Fig. S36 Cumulative release of DOX (A) and Ce6 (B) from nanoparticles in PB solution (pH 7.4, 10 mM) irradiated with (solid symbols) or without (empty symbols) red light at 37 °C.

Reference

1. X. N. Huang, F. S. Du, J Cheng, Y. Q. Dong, D. H. Liang, S. P. Ji, S. S. Lin and Z. C. Li, *Macromolecules*, 2009, **42**, 783-790.

2. L. Yu, Y. Yang, F. S. Du and Z. C. Li, *Biomacromolecules*, 2018, **19**, DOI: 10.1021/acs.biomac.8b00271.