

Supporting information for:

Corona Charge Selective Micelle Degradation Catalyzed by *P. cepacia* Lipase Isoforms

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Table S1. Effective hydrodynamic diameters from DLS and Zeta potentials for diblock copolymer micelles.

Micelle Sample	Diameter (pH = 7.4, 37°C)	Zeta Potential (ζ , 25°C)
PCL ₅₁ - <i>b</i> -PEG ₃₂ -CH ₂ CH=CH ₂	32 nm	-13 mV
[PCL ₅₁ - <i>b</i> -PEG ₃₂ -RCO ₂] ⁻ Na ⁺	38 nm	-31 mV
[PCL ₅₁ - <i>b</i> -PEG ₃₂ -RSO ₃] ⁻ Na ⁺	43 nm	-27 mV
[PCL ₄₄ - <i>b</i> -PEG ₃₂ -RNH ₃] ⁺ Cl ⁻	27 nm	+ 20 mV

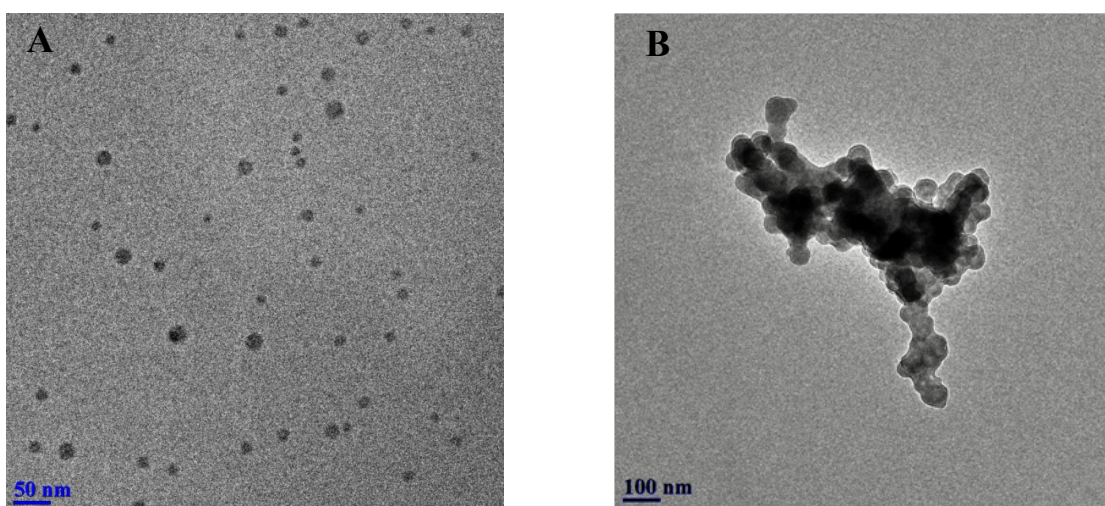


Figure S1. Cryo-TEM image for PCL₅₁-*b*-PEG₃₂-CH₂CH=CH₂ diblock copolymer micelles (**A**); TEM image for [PCL₅₁-*b*-PEG₃₂-RCO₂]⁻ Na⁺ diblock copolymer micelles (**B**).

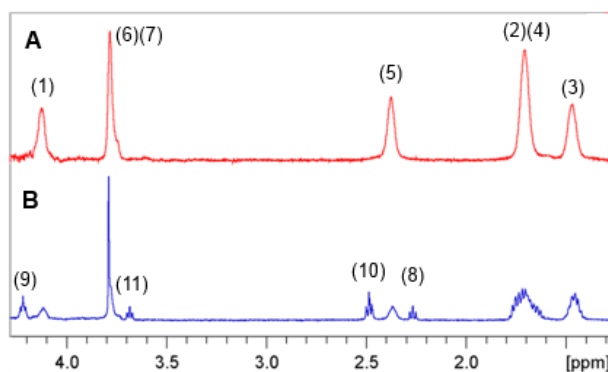


Figure S2. 500 MHz ¹H NMR of PCL₅₁-*b*-PEG₃₂-CH₂CH=CH₂ micelle in PBS buffer at 37 °C (**A**); micelle with *P. cepacia* lipase (1.0 U/mL) for 15 min (**B**). NMR peak assignments: (A): H-[O-CH₂(1)-CH₂(2)-CH₂(3)-CH₂(4)-CH₂(5)-C(O)]₅₁-*b*-[O-CH₂(6)-CH₂(7)]₃₂-O-CH₂CH=CH₂; (B): HOC(O)CH₂(8)(CH₂)₃CH₂(9)

O-[C(O)CH₂(10)(CH₂)₃CH₂(9)O]_mC(O)CH₂(10)(CH₂)₃CH₂(11)OH. Chemical shifts in ppm: $\delta(1)=4.12$, $\delta(2,4)=1.72$, $\delta(3)=1.45$, $\delta(5)=2.36$, $\delta(6,7)=3.78$, $\delta(8)\sim 2.27$, $\delta(9)\sim 4.22$, $\delta(10)\sim 2.48$, $\delta(11)\sim 3.70$.

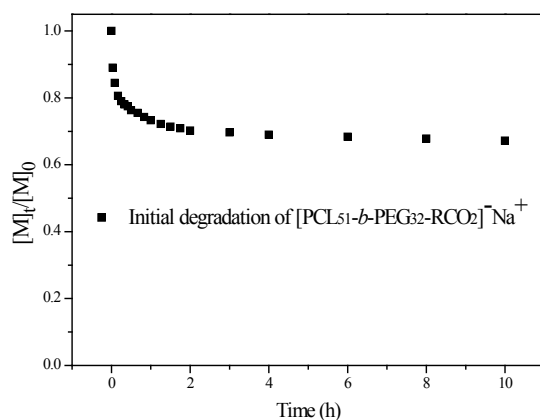


Figure S3. Kinetic plot of the degradation of [PCL₅₁-*b*-PEG₃₂-RCO₂]⁻ Na⁺ micelles catalyzed by commercial *P. cepacia* lipase at pH=7.4, T= 37 °C and E=1.0 U/mL for 10h.

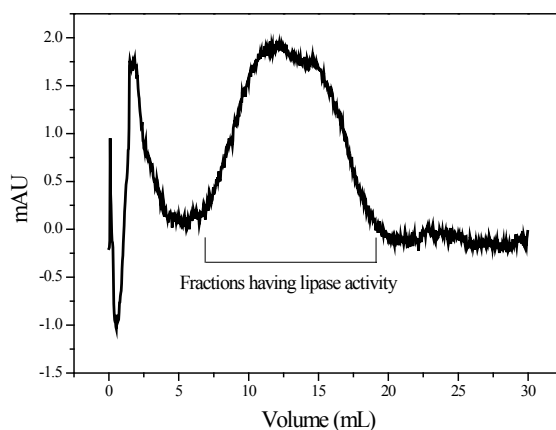


Figure S4. UV-vis trace for lipase fractions from the HiTrap Butyl-S Sepharose FF column.



Figure S5. Electrophoretic analysis of the commercial and purified lipase from *P. cepacia*. SDS-PAGE, lanes: (1) commercial *P. cepacia* solution, (2) solution from 1.5 M (NH₄)₂SO₄ trituration, (3) residue from trituration dissolved in 0.1 M (NH₄)₂SO₄ (material loaded on column), (4) undissolved material from lane

3, (5) molecular weight markers (97.4, 66.2, 45.0, 32.0, and 21.5 kDa), (6-10) column fractions at volume = 9, 11, 13, 15, 17 mL.

Experimental Section

Materials

Allyl alcohol ($\geq 99\%$, Sigma Aldrich) was dried by 3 Å molecular sieves prior to use. Ethylene Oxide (99%, PFALTZ & BAUER. INC) and ϵ -Caprolactone (CL) (99%, TCI America) were dried over CaH_2 and distilled prior to use. 18-Crown-6-ether (18C6, $\geq 99\%$), Stannous Octoate ($\text{Sn}(\text{Oct})_2$, $\sim 95\%$), 3-Mercaptopropionic acid ($\geq 99\%$), Sodium 2-mercaptoethanesulfonate (BioXtra, $\geq 98.0\%$), cysteamine hydrochloride (BioXtra), 2,2-Dimethoxy-2-phenylacetophenone (99%), Azobisisobutyronitrile (recrystallized, 99%), Phosphate buffered saline (PBS, tablet) and lipase from *P. cepacia* (37.8 U/mg, Lot # BCBD 7500V) were used as received from Sigma Aldrich. Buffer solutions from pH=4 (SB98-500, 0.05 M Potassium Acid Phthalate) and pH=10 (SB116-500, Potassium Carbonate/Potassium Tetraborate/Potassium Hydroxide) were purchased from Fisher Scientific. Potassium naphthalenide THF solution (1.0 M) was prepared by mixing of naphthalene (5.12 g), potassium (1.56 g) and THF (35 mL) in a flask. SnakeSkin Dialysis tubing (10K MWCO, 35mm dry I.D., 35 feet) was used for dialysis of aqueous media containing polymer micelles. Acetone, toluene, petroleum ether, dichloromethane and methanol were use as received from J.T. Baker and dimethylformamide (DMF, HPLC grade, 99.7% +, Alfa Aesar) was used as the GPC eluent.

Instrumentation

^1H NMR spectra were obtained using Bruker 500 MHz spectrometer with CDCl_3 and D_2O as NMR solvent media. ^1H NMR data were analyzed by using the Bruker Topspin 2.1 software.

Water suppressed ^1H NMR spectra of block copolymer micelles in buffer solutions containing 10% of D_2O were acquired using presaturation of water with 50 dB attenuation. An arrayed experiment was used to monitor the kinetics of lipase catalyzed degradation of micelles, collecting spectra every 5, 10, 15 or 30 min, with a preacquisition delay. The ^1H NMR covered a spectral window of -5.0 ppm to 15.0 ppm. The excitation relaxation delay was set to 5 s. Each NMR spectrum was collected from accumulation of 24 scans.

Gel permeation chromatography (GPC) measurements were carried out using a Shimadzu LC-20AV liquid chromatography system equipped with three linear PolarGel-M 300 $\times$ 7.5 mm columns, a RID-10A differential refractive index detector and a SPD-20AV UV/VIS detector. Data were analyzed by Shimadzu LCsolution software. DMF was used as the eluent at a flow rate of 1.0 mL/min at 50 °C. Molecular weight calibrations were based on narrow peak width polystyrene standards from Polymer Laboratories.

MALDI-TOF mass spectrum of HO-PEG- $\text{CH}_2\text{CH}=\text{CH}_2$: The HO-PEG- $\text{CH}_2\text{CH}=\text{CH}_2$ sample was dissolved in H_2O (5.0 g/L), 2,5-Dihydroxybenzoic acid dissolved in water: Ethanol (9:1 vol:vol) solution (10.0 g/L) was used as a matrix. 2.0 μL of the matrix solution was mixed with 0.5 μL of the PEG solution, placed on a steel sample holder, and air dry. MALDI-TOF mass spectra were acquired using the reflectron mode on an UltraflexIII TOF-TOF mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany). The serial instrument was equipped with a 337 nm, 50 Hz N_2 Laser used with a 33% attenuation level. The acceleration voltage was set to 19 kV, the pulsed extraction voltage to 17.15 kV, the extraction delay to 280 ns, and the reflector voltage to 20 kV.

The achieved peak resolution was comprised between 10,000 and 30,000, depending on the peaks.

Dynamic light scattering (DLS) measurements were conducted by by a Zetasizer Nano ZS (Malvern Instruments, Westborough, MA). Hydrodynamic diameter and size distribution of micelles in PBS buffer solution were measured at 37 °C, using 1 cm polystyrene cuvettes. The mean diameter was obtained from the instrument's DTS software using the volume reading. The Zeta potential (ζ) of the micelle samples in water was measured at 25 °C using the same instrument according to electrophoretic light scattering technology.

Transmission Electron Microscope (TEM). Micelle morphology was evaluated by JOEL JEM-1400 TEM operating at an acceleration voltage of 80 kV. One drop of the micelle solution (1.0 mg/mL) was placed on the ultrathin carbon type-A 400 mesh copper grid (Ted Pella Inc., Redding, CA), and the droplet was then blotted and allowed to evaporate under ambient conditions overnight before analysis. The average diameters of the spherical micelles were evaluated by DigitalMicrograph software (Gatan Inc.).

Cryogenic transmission electron microscopy (cryo-TEM). One drop of the micelle solution (1.0 mg/mL) was put on a quantifoil grid for Cryo-TEM (CF-MH-4C-25, Electron Microscopy Sciences). Most of the liquid was then removed with blotting paper, leaving aqueous thin film in the open holes of the grid. The aqueous thin film was instantly vitrified by rapid immersion into liquid ethane cooled by liquid nitrogen. The specimen was then transferred to the TEM instrument with a Gatan 626 cryo-transfer holder. The temperature was maintained at approximately -160 °C during cryo-TEM study. The transmission electron microscope was operated at an acceleration voltage of

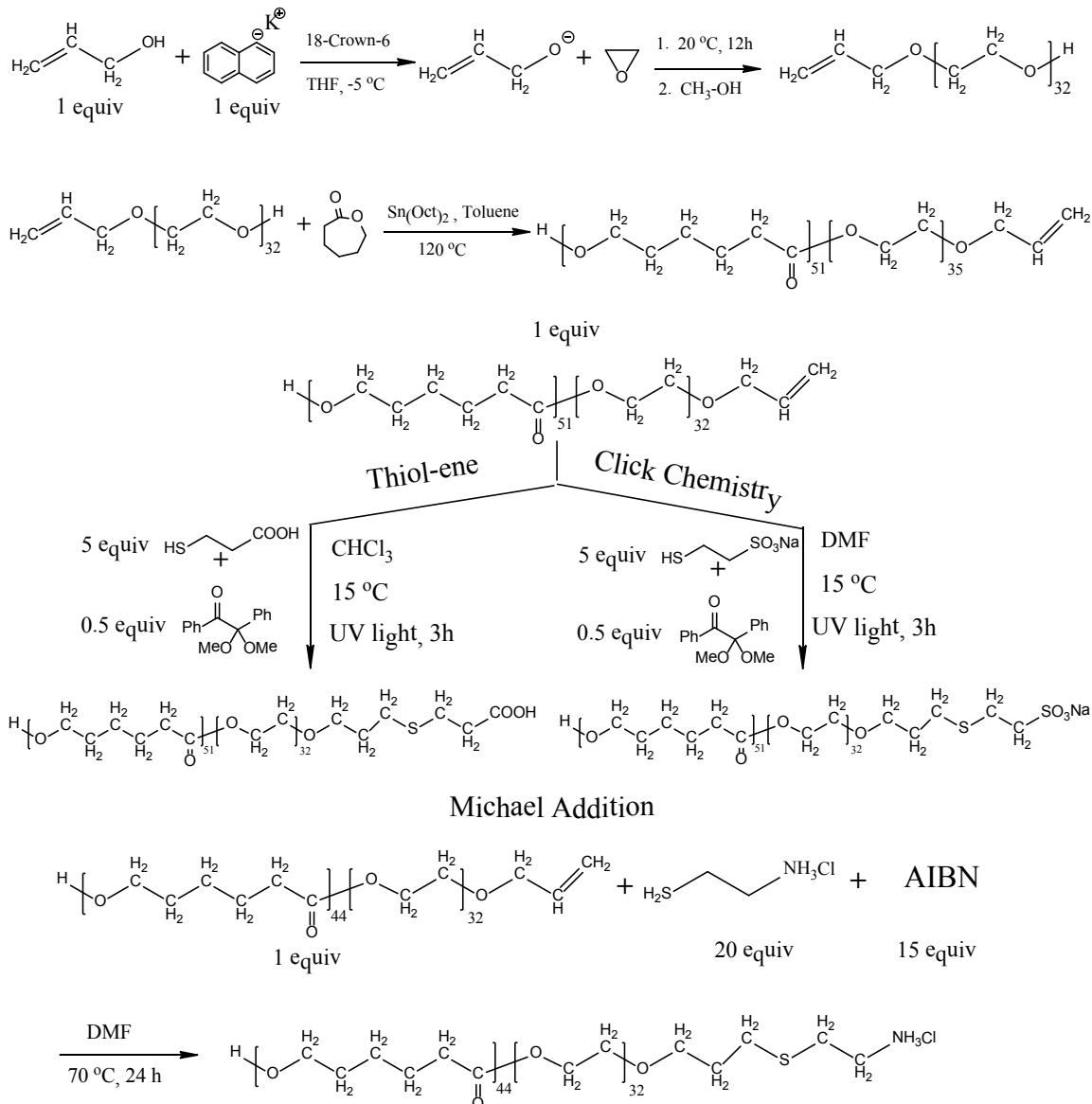
120 kV. All images were recorded by a bottom mounted Ultrascan-1000 CCD camera system (Gatan Inc.). The average diameters of the spherical micelles were evaluated by Digital Micrograph software.

Methods

Synthesis of HO-PEG-CH₂CH=CH₂ homopolymer

Dried Allyl alcohol (0.34 mL, 5 mmol), 18-crown-6-ether (1.98 g, 7.5 mmol) and THF (80 mL) were added into a vacuum dried 250 mL of two necked flask with rubber stopper sealed. The flask was placed into salt ice bath potassium naphthalenide THF solution (5.0 mL, 1.0 M) was added into the flask via syringe. Stir the solution for 15-20 min until the color of the solution became colorless. The CaH₂ dried EO (8.70 mL, 0.175 mol) was transfer into the flask through a dried cannula. The EO polymerization was first running at -5 °C for 30 min and then the temperature was increased to 15 °C. The reaction was terminated by adding excess of MeOH into the solution after 12 hours. THF and MeOH were evaporated and the white solid was dissolved into water. CH₂Cl₂ was used to extract out the HO-PEG-CH₂CH=CH₂ from aqueous solution. The CH₂Cl₂ was evaporated and THF was added back to dissolve the HO-PEG-CH₂CH=CH₂. Then the white HO-PEG-CH₂CH=CH₂ was precipitated out by adding the solution into excess petroleum ether. Redissolved the PEG in CH₂Cl₂ and precipitated it out from the petroleum ether again. The White HO-PEG-CH₂CH=CH₂ powder was dried under vacuum for 48 hours.

Scheme 1. Synthesis of HO-PEG₃₂-CH₂CH=CH₂ homopolymer and diblock copolymers from HO-PCL₅₁-*b*-PEG₃₂-CH₂CH=CH₂, HO-PCL₅₁-*b*-PEG₃₂-RX (X=CO₂H, -SO₃Na) and HO-PCL₄₄-*b*-PEG₃₂-RNH₃Cl



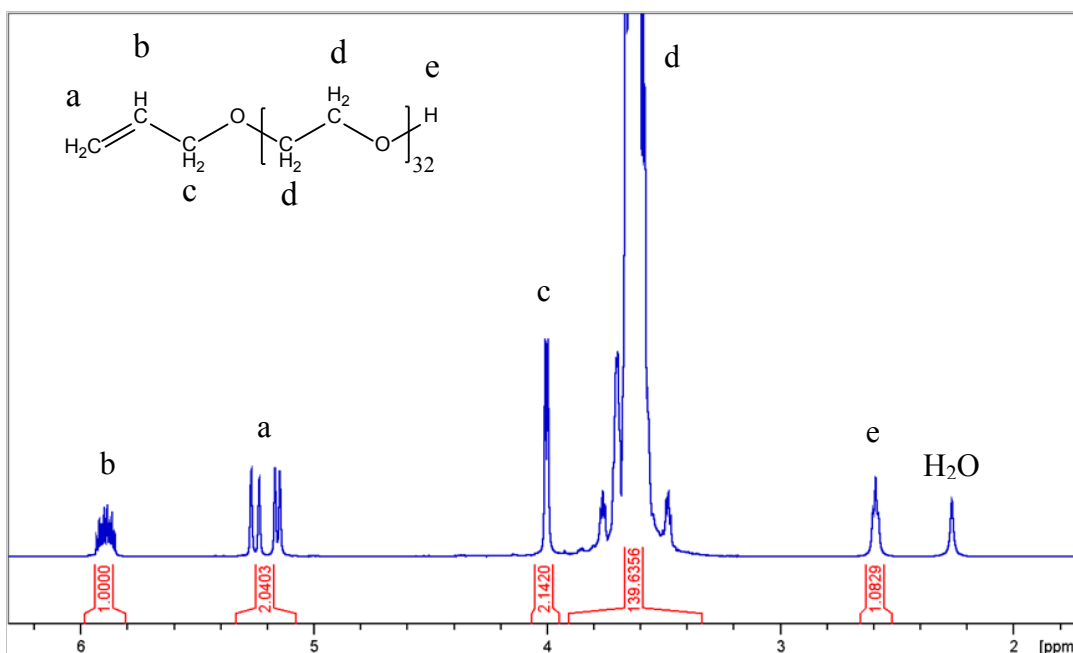


Figure 1. ^1H NMR for $\text{HO-PEG}_{32}\text{-CH}_2\text{CH=CH}_2$ in CDCl_3 and peak assignments.

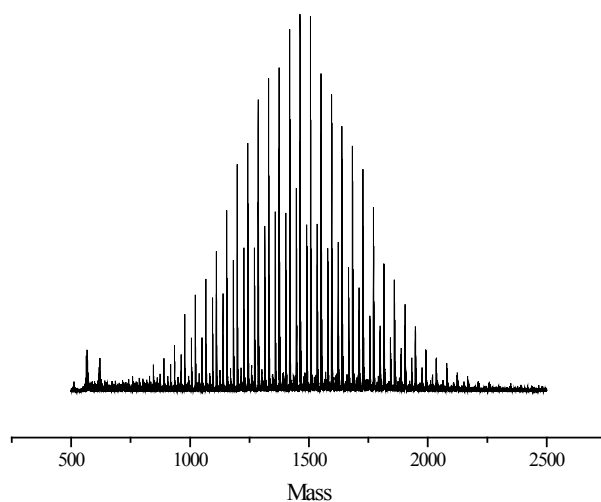


Figure 2. MALDI mass spectrum for the $\text{HO-PEG}_{32}\text{-CH}_2\text{CH=CH}_2$

Synthesis of $\text{HO-PCL}_{51}\text{-}b\text{-PEG}_{32}\text{-CH}_2\text{CH=CH}_2$ diblock copolymer

Dried $\text{HO-PEG}_{32}\text{-CH}_2\text{CH=CH}_2$ (0.73 g, 0.50 mmol) was dissolved in 22 mL of dry toluene in a 50 mL dry flask under a nitrogen inert atmosphere. A mixture of ϵ -caprolactone (CL) (9 mL, 0.080 mol) and $\text{Sn}(\text{Oct})_2$ (0.03 g) was added into the PEG

solution via a syringe. The reaction mixture was gently refluxed under nitrogen atmosphere at 120 °C. After stirring the solution for 3 hours, excess cold methanol was poured into the solution to terminate the reaction and precipitate the polymer product. The white solid precipitate (HO-PCL₅₁-*b*-PEG₃₂-CH₂CH=CH₂) was collected by filtration, redissolved in dichloromethane and precipitated by methanol. The precipitate was collected and dried under vacuum at 40 °C for 48 h.

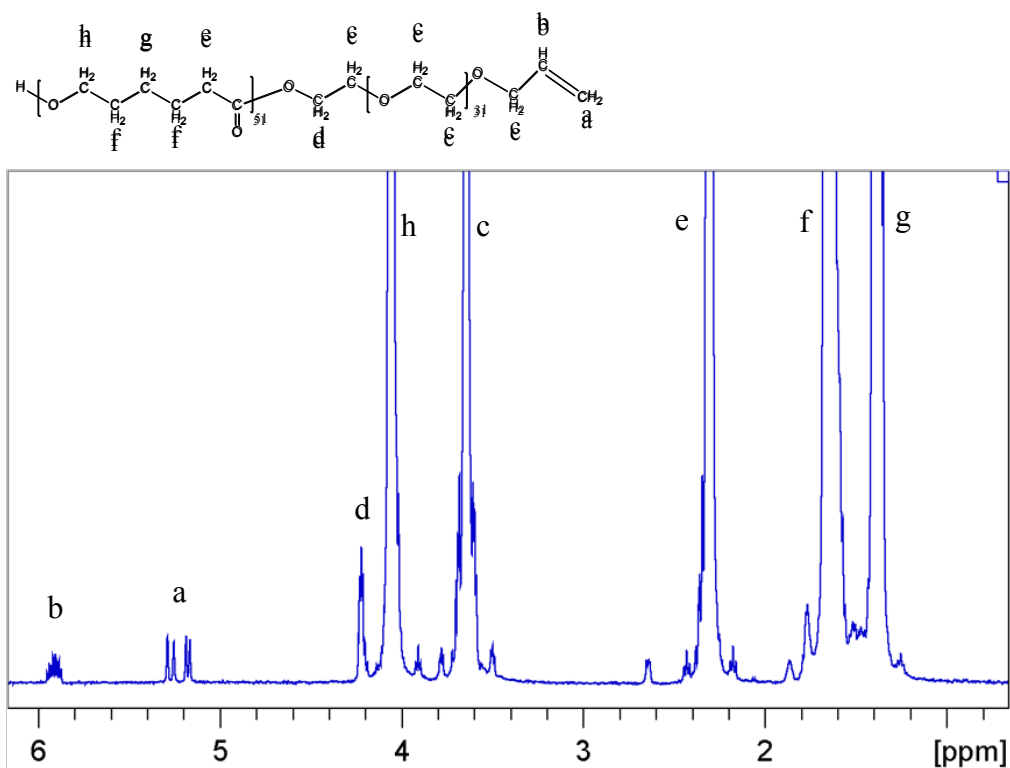


Figure 3. ¹H NMR of HO-PCL₅₁-*b*-PEG₃₂-CH₂CH=CH₂ diblock copolymer in CDCl₃ and peak assignments.

Synthesis of HO-PCL₅₁-*b*-PEG₃₂-RCO₂H and HO-PCL₅₁-*b*-PEG₃₂-RSO₃Na diblock copolymers via “thiol-ene” click chemistry

Diblock copolymer of HO-PCL₅₁-*b*-PEG₃₂-CH₂CH=CH₂ (0.36 g, 0.050 mmol), 3-Mercaptopropionic acid (0.0218 mL, 0.25 mmol) and 2,2-Dimethoxy-2-

phenylacetophenone (6.4 mg 0.025 mmol) were dissolved in 5 mL of CH₃Cl in a 25 mL flask. The solution was bubbled with N₂ for 15 min. The reaction solution was stirred in a UV reactor (3500 Å) at 15 °C for 3h. The product was precipitated out by pouring the reaction solution into excess cold methanol. The white precipitate (HO-PCL₅₁-*b*-PEG₃₂-RCO₂H) was collected by gravity filtration, washed by cold methanol several times and dried at 40 °C under vacuum for 48 hours.

Diblock copolymer of HO-PCL₅₁-*b*-PEG₃₂-CH₂CH=CH₂ (0.36 g, 0.050 mmol), Sodium 2-mercaptoethanesulfonate (41.0 mg, 0.25 mmol) and 2,2-Dimethoxy-2-phenylacetophenone (6.4 mg, 0.025 mmol) were dissolved in 5 mL of DMF in a 25 mL flask. The solution was bubbled with N₂ for 15 min. The reaction solution was stirred in a UV reactor (3500 Å) at 15 °C for 3h. The product was precipitated out by pouring the reaction solution into excess cold methanol. The white precipitate (HO-PCL₅₁-*b*-PEG₃₂-RSO₃Na) was collected by gravity filtration, washed by cold methanol several times and dried at 40 °C under vacuum for 48 hours.

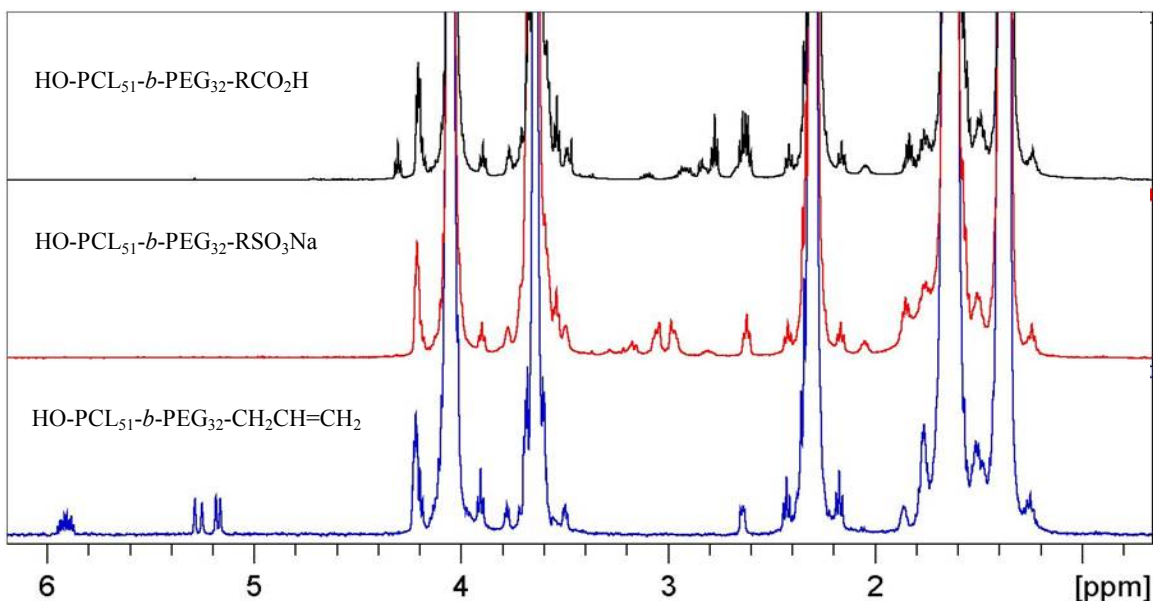


Figure 4. ¹H NMR for diblock copolymers from HO-PCL₅₁-*b*-PEG₃₂-CH₂CH=CH₂ and HO-PCL₅₁-*b*-PEG₃₂-RX (X= -CO₂H, -SO₃Na) in CDCl₃.

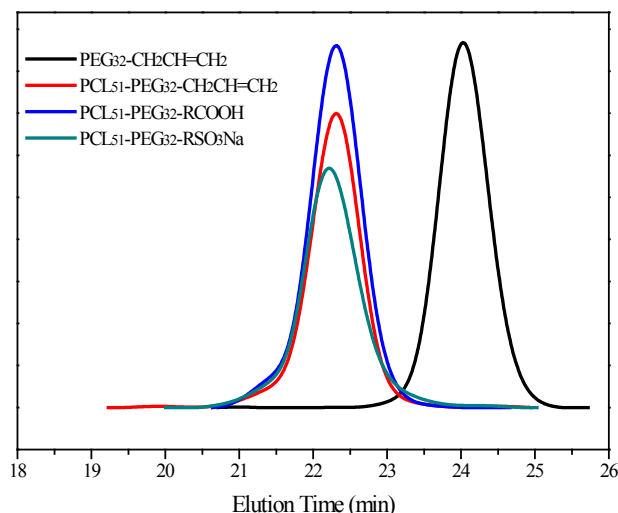


Figure 5. GPC traces for HO-PEG₃₂-CH₂CH=CH₂ homopolymer and di block copolymers HO-PCL₅₁-*b*-PEG₃₂-CH₂CH=CH₂ and HO-PCL₅₁-*b*-PEG₃₂-RX (X = -CO₂H, -SO₃Na).

Synthesis of HO-PCL₄₄-*b*-PEG₃₂-RNH₃Cl via Michael addition

Diblock copolymer of HO-PCL₄₄-*b*-PEG₃₂-CH₂CH=CH₂ (0.37 g, 0.057 mmol), cysteamine hydrochloride (129.5 mg, 1.14 mmol) and Azobisisobutyronitrile (AIBN, 140.4 mg 0.855 mmol) were dissolved in 5 mL of DMF in a 25 mL flask. The solution was bubbled with N₂ for 15 min. The reaction solution was stirred in oil bath at 70 °C for 24h. The product was precipitated out by pouring the reaction solution into excess cold methanol. The white precipitate (HO-PCL₄₄-*b*-PEG₃₂-RNH₃Cl) was collected by gravity filtration, washed by cold methanol several times and dried at 40 °C under vacuum for 48 hours.

Formation of PCL₅₁-*b*-PEG₃₂-CH₂CH=CH₂ diblock copolymer micelles via nano-precipitation method

A stock solution of the HO-PCL₅₁-*b*-PEG₃₂-CH₂CH=CH₂ diblock copolymer (10 mg/mL) was prepared in acetone. An aliquot of the polymer stock solution (0.80 mL) was added to stirring HPLC grade water (8.0 mL) at the rate of 0.2 mL/min which resulted in

formation of the block copolymer micelles (1.0 mg/mL). The micelle suspension was then transferred into dialysis bags (MWCO: 10,000 Da) and dialyzed against HPLC grade water for 24 hours. ^1H NMR was used to confirm the acetone was completely removed after the dialysis.

^1H NMR for lipase catalyzed degradation of block copolymer micelles at pH = 7.4 and T = 37 °C and lipase activity of 1.0 U/mL:

The *P. cepacia* lipase catalyzed degradation of block copolymer micelles were studied at pH=7.4, T=37 °C and lipase activities of 1.0 U/mL. PBS (0.70 mL, pH = 7.4) was evaporated to dryness by air in a vial. A micelle suspension (0.50 mL, 1.0 mg/mL) and D_2O (0.10 mL) was added into the vial to redissolve the PBS salts. A solution of micelle with dissolved PBS salt was transferred into a NMR tube and the solution was heated to 37 °C in the NMR instrument. *P. cepacia* lipase water solution (0.10 mL, 0.185 mg/mL) was added into the NMR tube quickly to catalyze the hydrolysis of micelle. The degradation of micelle was followed by ^1H NMR at 37 °C. Water solvent suppressed NMR was used in this experiment.

Enrichment of the commercial *P. cepacia* lipase by hydrophobic column

A 30 mg portion of *P. cepacia* lipase (Sigma 62309, lot # BCBG7500) was suspended in 1.5 mL of 1.5 M $(\text{NH}_4)_2\text{SO}_4$ in 10 mM phosphate buffer (pH 7). The resulting cloudy suspension was centrifuged at 16000 g for 10 min. The supernatant was removed and the pellet was dissolved in 1.5 mL of 0.1 M $(\text{NH}_4)_2\text{SO}_4$ in 10 mM phosphate buffer (pH 7). The latter solution was centrifuged at 16000 g for 10 min; a small pellet was visible. The supernatant was loaded onto a 1 mL HiTrap Butyl-S Sepharose FF column (GE

Heathcare Life Sciences) equilibrated with 0.1 M $(\text{NH}_4)_2\text{SO}_4$ in 10 mM phosphate buffer (pH 7) on an ÄKTA prime chromatography system (Amersham Biosciences). The column was washed with 5 mL of this buffer and protein was eluted with a 20 mL linear gradient from 0.1 M to 0 M of $(\text{NH}_4)_2\text{SO}_4$ in 10 mM phosphate buffer (pH 7) at a flow rate of 1.0 mL/min. Finally, the column was washed with 5 mL of 10 mM phosphate buffer (pH 7). The eluted fractions were collected every 1.0 mL.

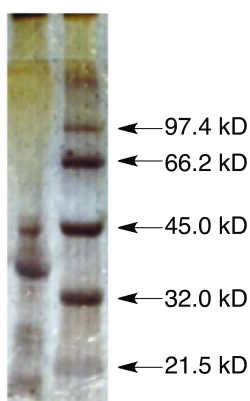


Figure 6. SDS-PAGE for the commercial *P. cepacia* lipase, lane 1: lipase sample, lane 2: protein markers.