Supplementary Information

Synthesis of water-soluble poly(α-hydroxy acids) from living ring-opening polymerization of *O*-benzyl-L-serine carboxyanhydrides

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General

Materials

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as received unless otherwise specified. Dichloromethane (DCM) and tetrahydrofuran (THF) were dried by passing the solvent through an alumina column and then stored in a glovebox. Anhydrous $CDCl_3$ was prepared by treating commercially available $CDCl_3$ (Sigma, St. Louis, MO, USA) with $CaSO_4$ overnight followed by distillation under nitrogen. The purified $CDCl_3$ was stored in the presence of 4Å molecular sieves. *O*-Benzyl-L-serine was purchased from Chem-Impex International (Des Plaines, IL, USA) and used as received.

Instrumentation

NMR spectra were recorded on a Varian Unity-400 or a VXR-500 spectrometer. Gel permeation chromatography (GPC) was performed on a system equipped with an isocratic pump (Model 1100, Agilent Technology, Santa Clara, CA, USA), a DAWN HELEOS 18-angle laser light scattering detector (Wyatt Technology, Santa Barbara, CA, USA) and an Optilab rEX refractive

index detector (Wyatt Technology). Separations were performed using serially connected size-exclusion columns (100, 500, 10^3 and 10^4Å Phenogel columns, 5 μm, 300 × 7.8 mm, Phenomenex, Torrance, CA, USA) at 60°C using *N*,*N*-dimethylformamide containing 0.1 M LiBr as the eluent. Low-resolution electrospray ionization mass spectrometry was performed on a Waters Quattro II mass spectrometer. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was performed on an Applied Biosystems Voyager-DE STR system. Infrared spectra were recorded on a Perkin Elmer 100 serial FTIR spectrophotometer calibrated with polystyrene film.

Synthesis of Ser(Bn)-OCA

(*S*)-3-(Benzyloxy)-2-hydroxypropanoic acid was obtained from *O*-benzyl-L-serine by following the literature reported procedure.[1] To a solution of (*S*)-3-(benzyloxy)-2-hydroxypropanoic acid (10.40 g, 53.3 mmol) in anhydrous THF (120 mL), phosgene in toluene (106.6 mmol) was added in one portion via syringe. The resulting mixture was stirred at room temperature for 16 h. The solvent was removed under vacuum. The crude OCA was purified by flash column chromatography with DCM as the eluent to yield a light-yellow oil, which was further purified by crystallization from diisopropyl ether/DCM to give clean OCA monomer in crystalline form (6.22 g, 53%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.28 (m, 5H, Ph), 5.11 (d, 1H, C*H*), 4.60 (s, 2H, OCH_2Ph), 3.91 (d, 2H, OCH_2CH). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 165.7, 148.5, 136.4, 128.7–127.9, 79.7, 74.0, 66.3. Crystal data for Ser(Bn)-OCA. $C_{11}H_{10}O_5$, M = 222.19, orthorhombic, space group $P2_12_12_1$, a = 7.2088(2), b = 11.4242(3), c = 12.8867(4) Å, V = 1061.28(5) Å 3, T = 173(2) K, Z = 4, 8283 reflections collected (1880 independent, R_{int} = 0.0297), 146 parameters, $R_1[I > 2\sigma(I)]$ = 0.0261, $wR_2[I > 2\sigma(I)]$ = 0.0634; R_1(all data) = 0.0263, wR_2 (all data) = 0.0636.

Polymerization of Ser(Bn)-OCA

In a glovebox, Ser(Bn)-OCA (22.0 mg, 0.1 mmol) in DCM (1 mL) was added to a DCM solution

(0.2 mL) of DMAP (0.12 mg, 1 μmol) and pyrenemethanol (0.23 mg, 1 μmol) at room temperature. The real-time concentration of Ser(Bn)-OCA was quantified by measuring the intensity of the anhydride peak of OCA at 1810 cm^{-1} by FTIR. The conversion of OCA was determined by comparing the OCA concentration in the polymerization solution with the OCA concentration at t = 0. When the polymerization was complete, Ser(Bn)-PAHA was precipitated with ether, and the precipitate was dried under vacuum (13.2 mg, 74% isolated yield). ^{1}H NMR ($CDCl_3$, 500MHz): δ 8.10 (m, 9H, Py), 7.27 (m, 495H, Ph), 5.90 (2H, Py-CH_2), 5.46 (d, 98H, CH), 4.50 (d, 197H, OCH_2Ph), 3.91 (d, 203H, OCH_2CH).

Removal of the benzyl group from Ser(Bn)-PAHA

Ser(Bn)-PAHA (0.70 g, 0.08 mmol) was dissolved in a THF/methanol (2:1, 60 mL). Degussa E101 Pd/C catalyst (0.21 g, 30 wt% of PAHA) was added to the flask, which was purged with nitrogen for 5 min, sealed and filled with hydrogen via a balloon. The mixture was stirred for 24 h under hydrogen and then filtered through celite. The filter cake was washed with THF and methanol. The filtrate and wash solutions were combined, and the solvent was removed under vacuum. The resulting Ser-PAHA was dissolved in THF/methanol (v/v 1/1) and then precipitated with ether. A white power was obtained (0.31 g, 90% yield). ^{1}H NMR (DMSO-d_6, 500MHz): δ 5.35(d, H, CH), 3.98(d, 2H, CHCH_2OH).

Cell proliferation assay

Cover slips coated with Ser-PAHA were prepared by adding 20 μL of the polymer solution to coverslips (16 mm in diameter) followed by spin-coating. The coated cover slips were put in a 6-well plate, onto which HeLa cells were seeded at a density of 2 × 10^5 cells/well. After incubation for 24 h at 37 ^{o}C, cell adhesion and proliferation on the coated coverslips were analyzed using a Zeiss Axiovert 40 CFL fluorescence microscope equipped with a 20 × objective.

MTT assay

HeLa cells were seeded on a 96-well plate at 1×10^4 cells/well and incubated for 24 h at 37 ^oC in 5% CO_2. Ser-PAHA was diluted with fresh medium (Dulbecco's Modified Eagle Medium supplemented with 10% (v/v) fetal bovine serum) to desired concentrations (2-1000 μg/10 μL) and added to the corresponding well. After a further incubation for 4 h or 24 h, cell viability was assessed using the MTT assay.[2] Briefly, MTT solution (5 mg/mL, 20 μL) was added to each well. Cells were allowed to incubate for 4 h in the presence of MTT reagent. The solution in each well was aspirated. Dimethyl sulfoxide (100 μL) was added to each well. OD_{560} was monitored using the microplate reader, and cell viability was expressed as the percentage of control cells without treatment by Ser-PAHA.

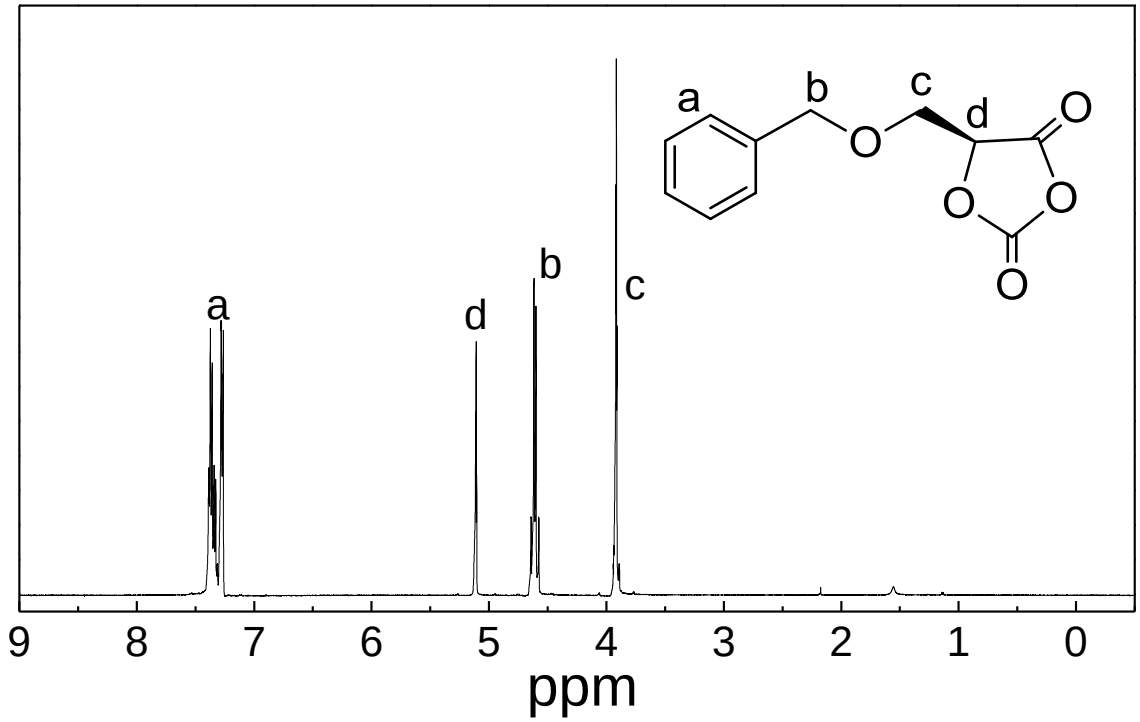


Fig. S1 ^{1}H NMR spectrum of the Ser(Bn)-OCA monomer in $CDCl_3$.

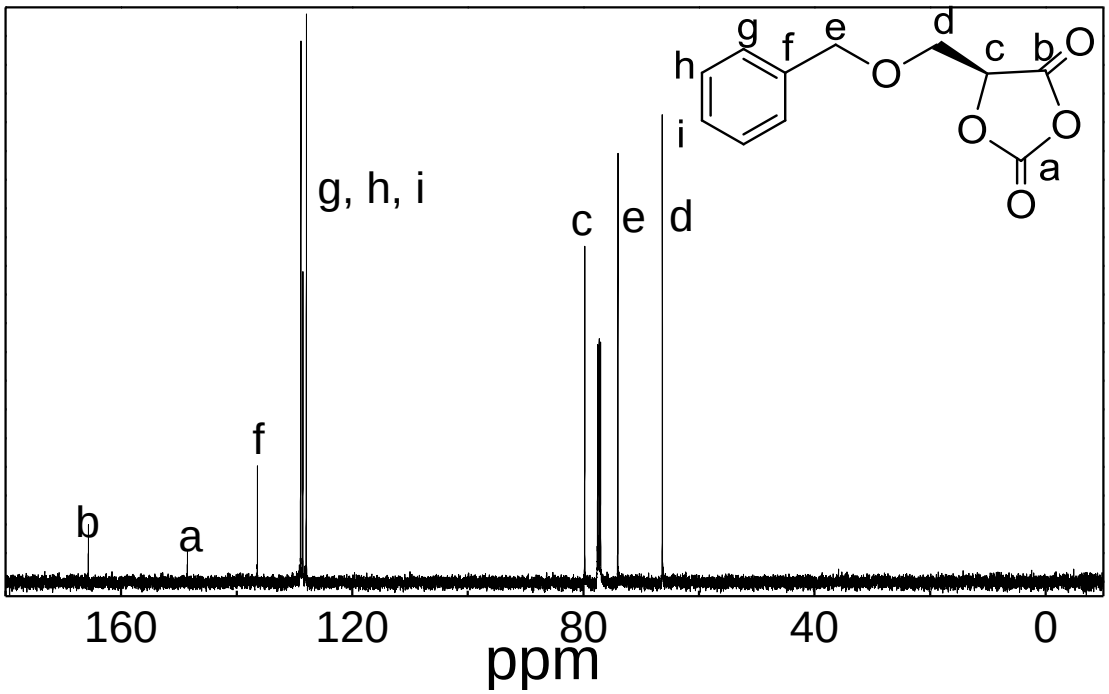


Fig. S2 ^{13}C NMR spectrum of the Ser(Bn)-OCA monomer in $CDCl_3$.

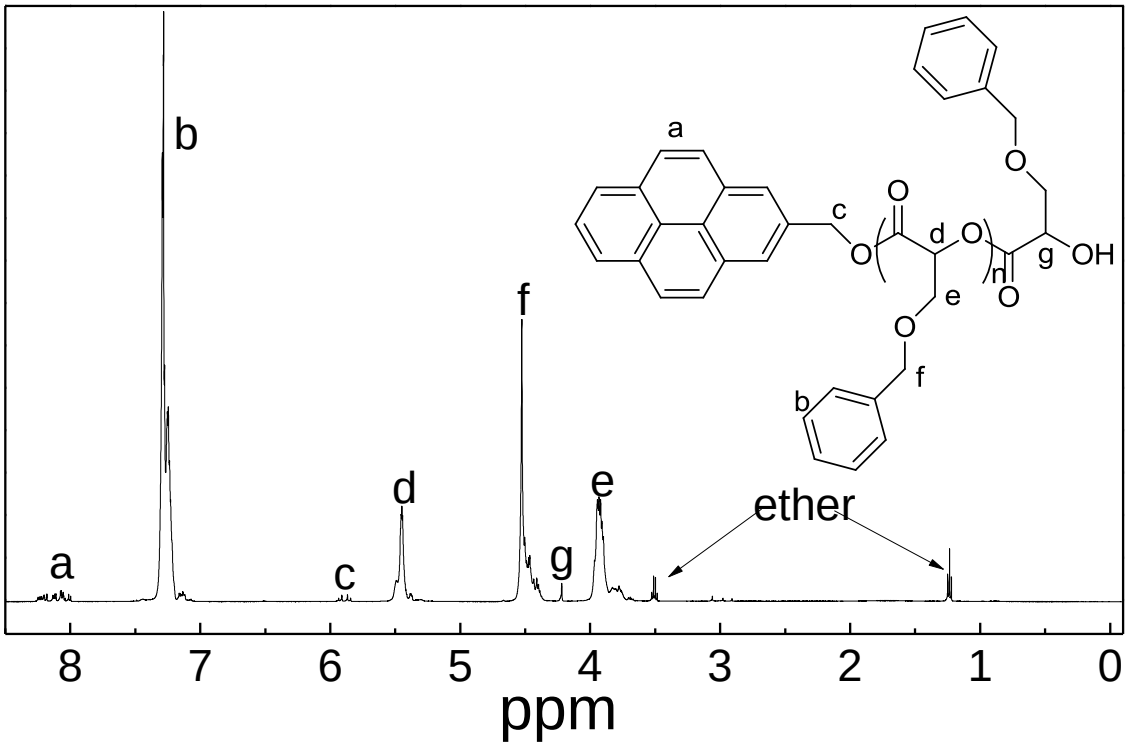


Fig. S3 ^{1}H NMR spectrum of the Ser(Bn)-PAHA polymer with a pyrene terminal group.

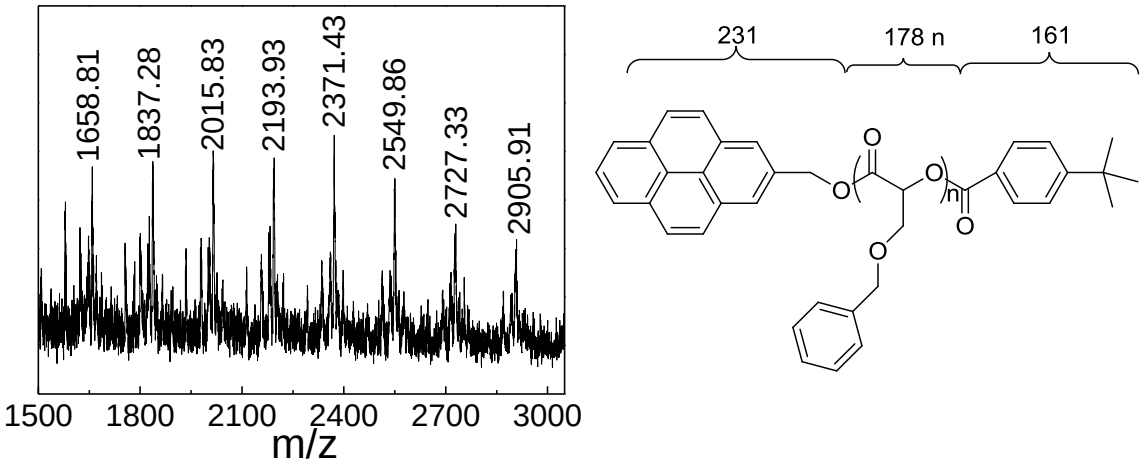
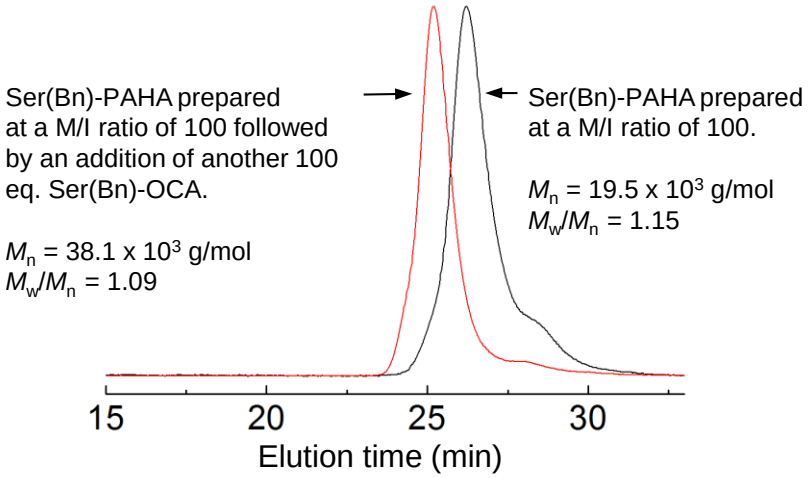
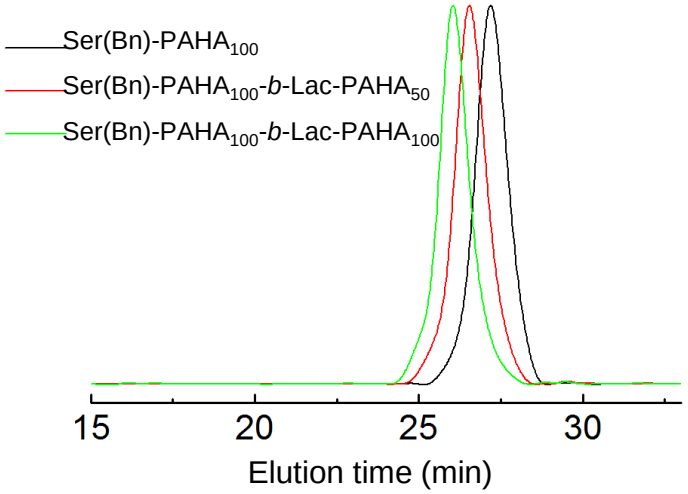


Fig. S4 MALDI-TOF mass spectrum of Ser(Bn)-PAHA with a pyrene terminal group obtained from polymerization of Ser(Bn)-OCA at an M/I ratio of 20 in the presence of DMAP.

(A)



(B)



(C)

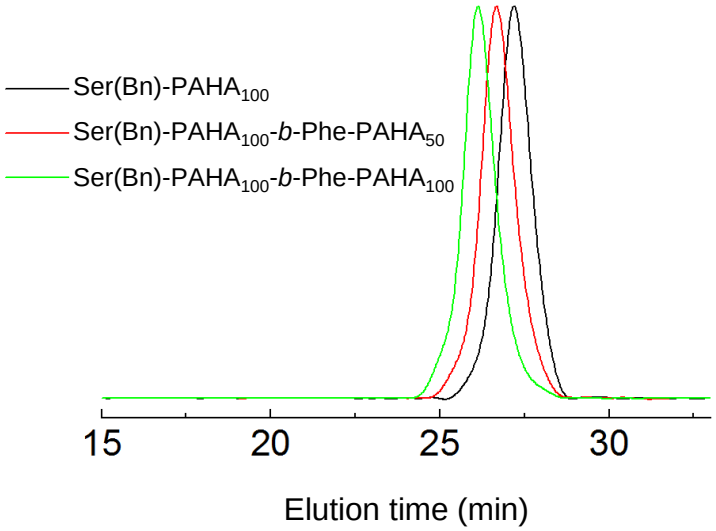


Fig. S5 (A) GPC curves of Ser(Bn)-PAHA prepared at a M/I ratio of 100 and after addition of a second portion of 100 equivalent Ser(Bn)-OCA. (B) GPC curve overlay of Ser(Bn)-PAHA and Ser(Bn)-PAHA-*b*-Lac- PAHAs. (C) GPC curves of Ser(Bn)-PAHA and Ser(Bn)-PAHA-*b*-Phe-PAHAs.

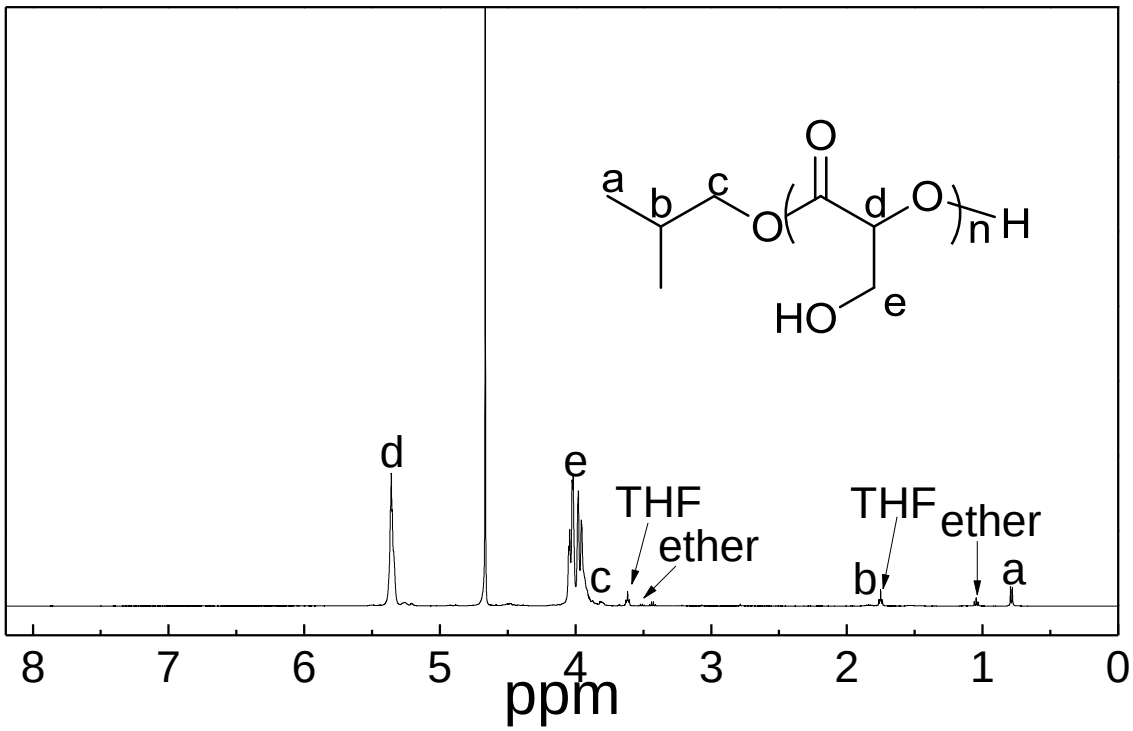


Fig. S6 ^{1}H NMR spectrum of Ser-PAHA in DMSO-d_6.

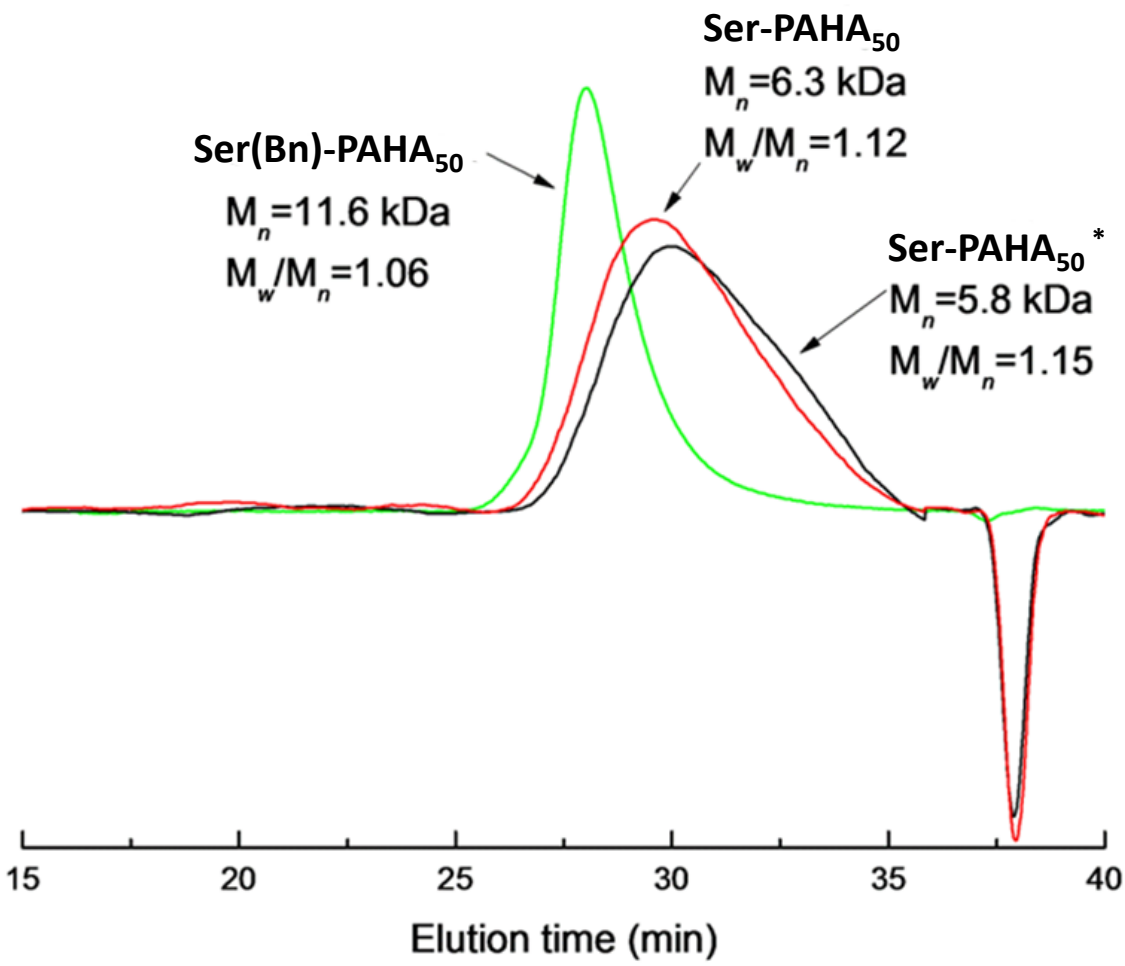


Fig. S7. The overlay of GPC spectrum of Ser(Bn)-PAHA$_{50}$ (green), Ser-PAHA$_{50}$ derived from the de-protection of the benzyl group of Ser(Bn)-PAHA$_{50}$ in THF/methanol (2/1, v/v) under H_2 in the presence of Degussa-type Pd/C catalyst (30% wt) for 24 h (red), and the Ser-PAHA$_{50}$ (asterisked) derived from a further treatment of Ser-PAHA$_{50}$ under the same de-protection condition (solvent, catalyst, H_2) for an additional 24 h (black).

References:

(1) Leemhuis, M.; van Nostrum, C. F.; Kruijtzer, J. A. W.; Zhong, Z. Y.; ten Breteler, M. R.; Dijkstra, P. J.; Feijen, J.; Hennink, W. E. *Macromolecules* **2006**, *39*, 3500-3508.

(2) Romijn, J. C.; Verkoelen, C. F.; Schroeder, F. H. *Prostate* **1988**, *12*, 99-110.