Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

Synthesis of Biodegradable Protein-Poly(ε-caprolactone) Conjugates *via* Enzymatic Ring Opening Polymerization

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Experimental Section

Materials

Candida antarctica lipase B (CALB) was purchased from Novozymes (Suzhou Hongda Enzyme Co Ltd., China), purified by dialysis against water for one day following lyophilization. *N*-(2-hydroxyethyl) acrylamide (HEAA, 98%, Aladdin) was de-inhibited by passing through a column of basic alumina prior to use. ε–Caprolactone (ε-CL, 98%, Aladdin) was distilled under reduced pressure before use. T*ris*(2-(dimethylamino)ethyl)amine (Me_6TREN) was synthesized according to literature procedure and stored in the freezer under a nitrogen atmosphere.[1, 2] CALB-poly(HEAA) conjugate was synthesized according to a previous report (Synthesis of Lipase-Polymer Conjugates by Cu(0)-mediated Reversible Deactivation Radical Polymerization: Polymerization vs Degradation, PY-ART-09-2019-001462.R1). Copper (Ⅰ) bromide (CuBr, 98%, Aladdin) was washed sequentially with acetic acid and ethanol and dried under vacuum. Membrane dialysis (1K MWCO) was obtained from Spectrum Laboratories. All other reagents and solvents such as, ethyl α-bromoisobutyrate (EBiB, 98%) and anhydrous acetonitrile (99.8%, Aladdin) were obtained from Aladdin (China) and used without further purification.

Analytical techniques

The MW and the MW distribution (M_w/M_n) of linear polymers were determined by Waters 1515 size exclusion chromatography (SEC) in *N*, *N*-dimethylbenzamide (DMF) at 40 °C with a flow rate of 1.00 mL min^{-1}, which was equipped with 2414

refractive index (RI) and 2489 UV detectors, a 20 μm guard column (4.6 mm ×30 mm, 100 - 10K) followed by three Waters Styragel columns (HR1, HR3 & HR4) and autosampler. Narrow linear polystyrene standards in range of 540 to 7.4×10^5 g·mol^{-1} were used to calibrate the system. ^{1}H NMR spectra were recorded at 25 °C with a Bruker AV 500M spectrometer using deuterated solvents obtained from Aladdin. Fourier transform infrared (FTIR) spectra were recorded on a Nicolet iS5 FTIR spectrometer using an iD7 diamond attenuated total reflectance optical base. Transition electron microscopy (TEM) images were acquired by FEI TECNAI G2 20 TEM microscope equipped with LaB6 filament. The turbidity of the nanoparticles was detected by WGZ-2000 turbidity meter (Beijing Warwick Industrial Science and technology, China). Thermal gravimetric analysis (TGA, Mettler Toledo, Switzerland) was performed at a heating rate of 10 ℃ min^{-1} from 50 ℃ to 600 ℃ under nitrogen protection. The turbidity of the nanoparticles was detected by WGZ-2000 turbidity meter (Beijing Warwick Industrial Science and technology, China). Differential scanning calorimeter (DSC) was used to determine the thermal transitions that occur during cooling and heating scans under the purge gas of dry nitrogen. The sample was held at 70 ℃ for a certain time, then cooled at 10 ℃ min^{-1} to -70 ℃, and finally heated at 10 ℃ min^{-1} to 200 ℃. MALDI-ToF MS data was performed on a Bruker AutoFlex spectrometer operating at the following conditions: Nitrogen laser (337 nm), accelerating potential (20 kV) in positive linear ion mode. Tran-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB) was employed as the matrix. An analyte solution and matrix solution with a concentration of 10 g L^{-1} in

THF (1:4 v/v analyte-to-matrix solution) were mixed with 1 μL of potassium trifluoroacetate (10 g L^{-1}).

Synthesis of poly(HEAA) by Cu(0)-LRP

The procedure was similar with the synthesis of conjugates. The first vial containing the catalyst suspension (H_2O, 1 mL; ME_6TREN, 26 μL, 0.1 mmol; CuBr, 14.3 mg, 0.1 mmol) was bubbled with nitrogen using a stainless steel needle for 10 min. Meanwhile, the second vial fitted with a magnetic stir bar and a rubber stopper, H_2O (1 mL), methanol (0.5 ml), EBiB (50 mg, 0.25 mmol) and HEAA (2.3 g, 20 mmol) were charged and the solution was bubbled with nitrogen for 15 min. After that, the degassed monomer/initiator aqueous solution was carefully transferred to the mixture with Cu(0)/$CuBr_2$/ME_6TREN catalyst under nitrogen protection. The vial was sealed and the mixed solution was allowed to polymerize under ice/water bath for defined reaction time. After reaction, the samples were taken for ^1H NMR and SEC analysis.The residue products were directly transferred to a dialysis tube (MWCO 1 KDa) for dialysis against water for two days to remove the residual monomer and catalyst. The final conjugate could be recovered as white solid after lyophilization.

Synthesis of CALB-poly(HEAA) conjugate by Cu(0)-LRP

The polymerization of HEAA was performed according to a previous report (Synthesis of Lipase-Polymer Conjugates by Cu(0)-mediated Reversible Deactivation Radical Polymerization: Polymerization *vs* Degradation, PY-ART-09-2019-001462.R1) and the procedures of a typical polymerization are shown below. The first

vial containing the catalyst suspension (H_2O, 1 mL; ME_6TREN, 3.8 μL, 0.0139 mmol; CuBr, 2mg, 0.0139 mmol) was bubbled with nitrogen using a stainless steel needle for 10 min. Meanwhile, the second vial fitted with a magnetic stir bar and a rubber stopper, H_2O (2.5 mL), CALB-based macroinitiator (CALB-Br, 40 mg), HEAA (40 mg, 0.35 mmol) was charged and the solution was bubbled with nitrogen for 15 min. After that, the degassed monomer/initiator aqueous solution was carefully transferred to the mixture with Cu(0)/$CuBr_2$/ME_6TREN catalyst under nitrogen protection. The vial was sealed and the mixed solution was allowed to polymerize under ice/water bath for defined reaction time. The residue products were directly transferred to a dialysis tubing (MWCO 1 KDa) for dialysis against water for two days to remove the residual monomer and catalyst. The final conjugate could be recovered as white solid after lyophilization.

Ring opening polymerization (ROP) of ε-CL catalyzed by CALB or CALB-poly(HEAA) conjugates

The ROP of ε-CL was conducted according to previous report.[3] To prevent water initiation, the ROP of the lactones was carried out in "dry" conditions. To do so, CALB, poly(HEAA) and CALB-poly(HEAA) were freeze-dried for one day before polymerization.

For the synthesis of linear PCL, the reaction mixture was prepared under nitrogen protection by adding ε-CL (570 mg, 5 mmol), 15 mg of CALB and anhydrous acetonitrile (2ml, 0.02mmol) into the reaction vial. The vial was then placed into a

constant-temperature (60 ℃) oil bath with stirring for 24 h under nitrogen protection. After reaction, the mixture was processed by either dialysis in water or centrifugation in acetonitrile at 10000 rpm for 15 min. After removing the sediments, the supernatant were dispersed in acetonitrile *via* sonication for centrifugation again. The above procedures were repeated for three times in order to remove unreacted monomers or solvents. Finally, the sediments were recovered as yellow solids after drying.

For the synthesis of poly(HEAA)-*g*-PCL, which is a mixture of grafting copolymer and linear PCL, 40 mg poly(HEAA) and 10 mg of CALB were added into the vial containing ε-CL (270 mg, 2.37 mmol). The vial was then placed into a constant-temperature (60 ℃) oil bath with stirring for 6 h under nitrogen protection. After reaction, the mixture was directly processed by centrifugation. After separating the supernatant, the sediments were dispersed in acetonitrile *via* sonication for centrifugation again. The above procedures were repeated for three times in order to remove unreacted monomers and soluble linear PCL. Finally, the sediments (poly(HEAA)-*g*-PCL) were recovered as white powders after drying under vacuum at room temperature. Meanwhile, the obtained supernatants were recovered as yellow solids after drying under vacuum at 60 ℃.

For the synthesis of CALB-poly(HEAA)-*g*-PCL, which is a mixture of grafting copolymer conjugate and linear PCL, 40 mg CALB-poly(HEAA) were added into the vial containing ε-CL (270 mg, 2.37 mmol). The vial was then placed into a constant-temperature (60 ℃) oil bath with stirring under nitrogen protection. After

polymerization, the mixture was transported into a centrifugation tube directly for centrifugation. After removing the supernatant, the sediments were dispersed in acetonitrile *via* sonication for centrifugation again. The above procedures were repeated for three times in order to remove unreacted monomers and soluble linear PCL. Finally, the sediments (CALB-poly(HEAA)-*g*-PCL) were recovered as white powders after drying under vacuum at room temperature. Meanwhile, the both obtained supernatants were recovered as yellow solids after drying under vacuum at 60 ℃.

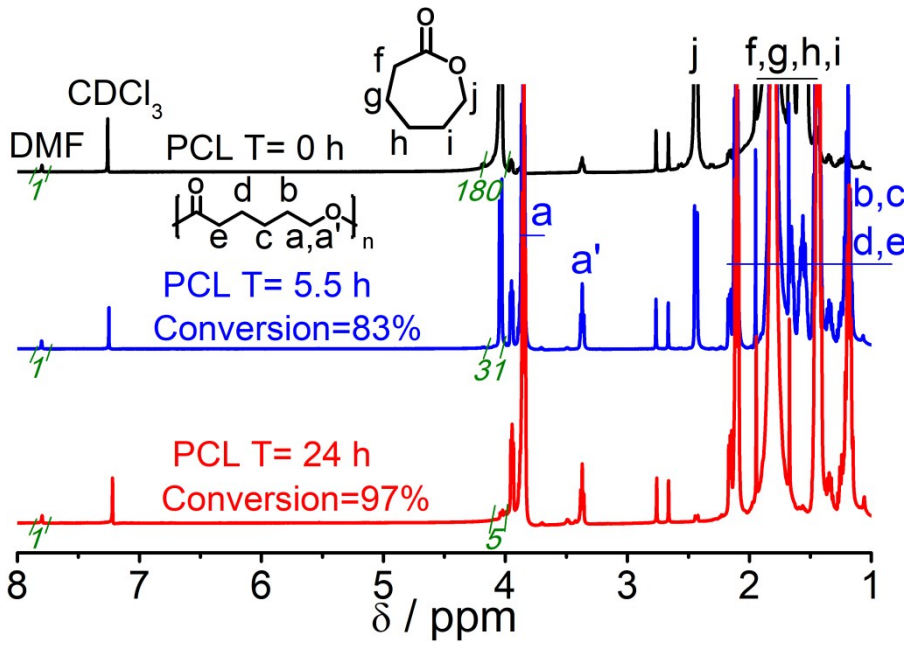

Figure. S1 ^{1}H NMR spectra of samples taken from the synthesis of PCL catalyzed by free CALB.

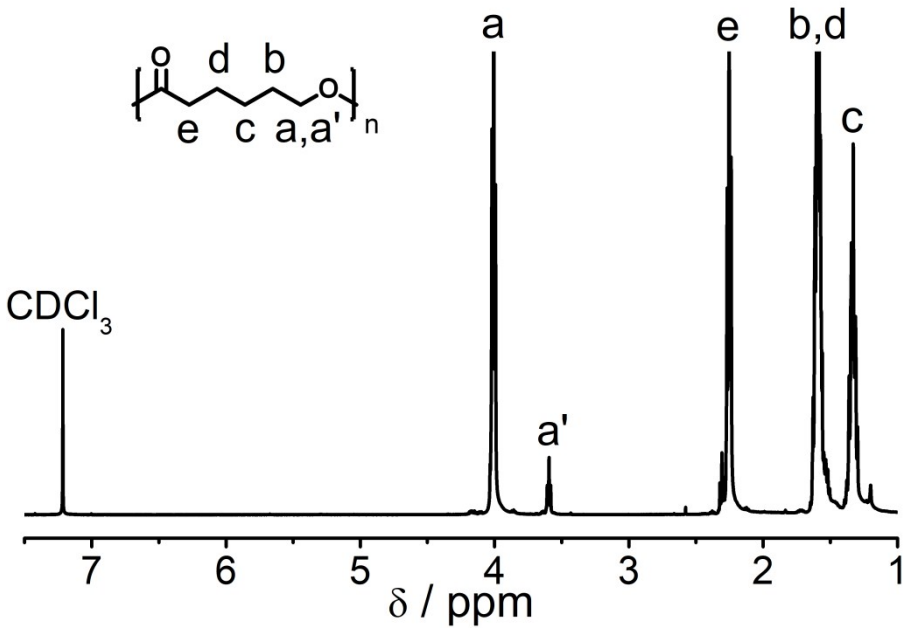

Figure. S2 ^{1}H NMR spectra of PCL catalyzed by free CALB after centrifugation.

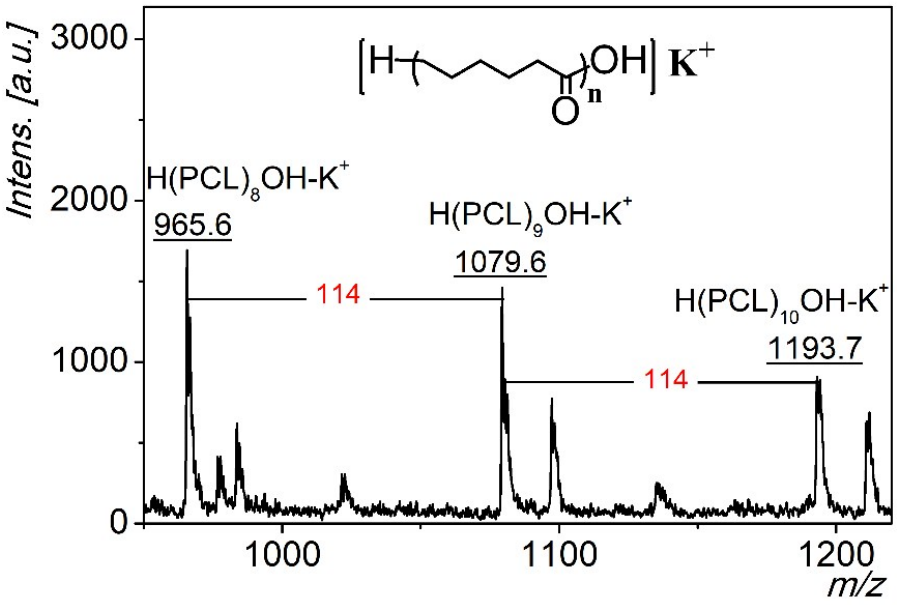

Figure. S3 MALDI-ToF MS spectra of PCL synthesized by eROP in MeCN.

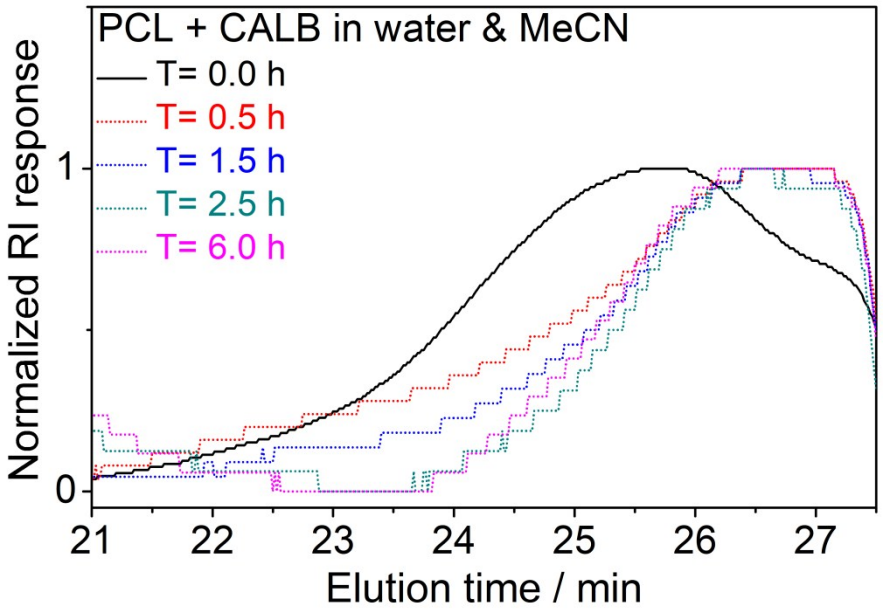

Figure. S4 SEC elution traces of PCL in water/acetonitrile (1: 1, v/v) for different time in the presence of CALB.

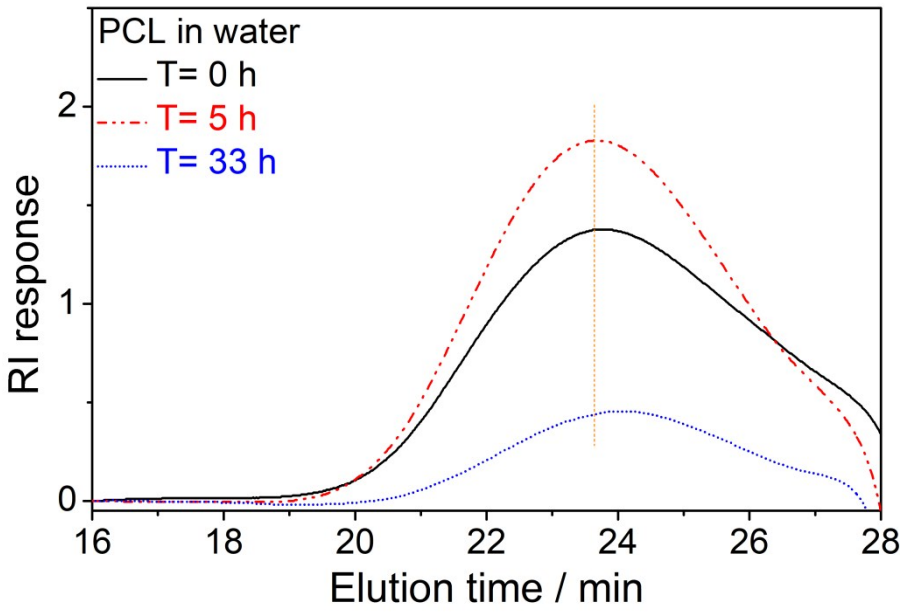

Figure. S5 SEC elution traces of PCL after dispersed in water for different time in the absence of CALB.

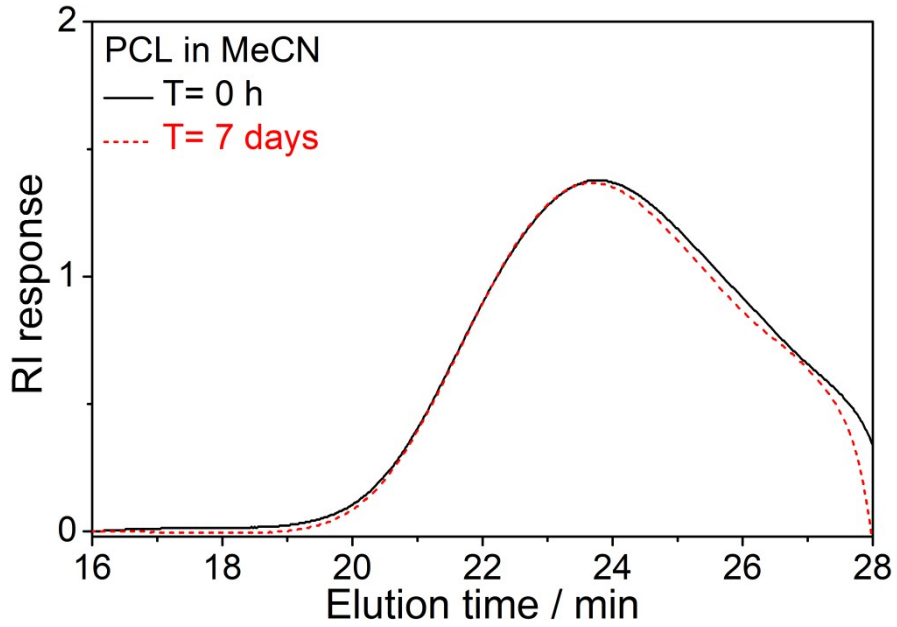

Figure. S6. SEC elution traces of PCL after solubilized in acetonitrile for different time in the absence of CALB.

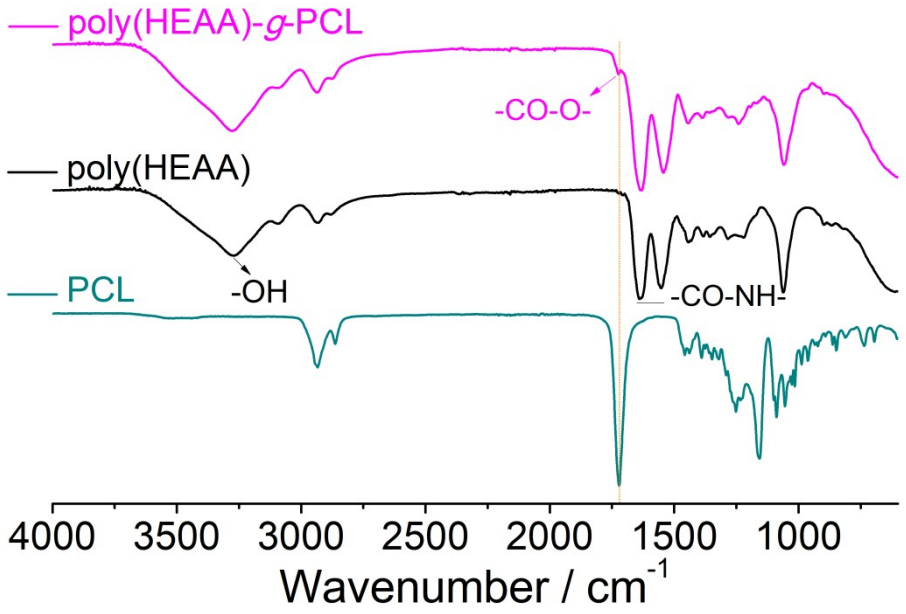

Figure. S7 FTIR spectra of poly(HEAA)-*g*-PCL, poly(HEAA) and PCL.

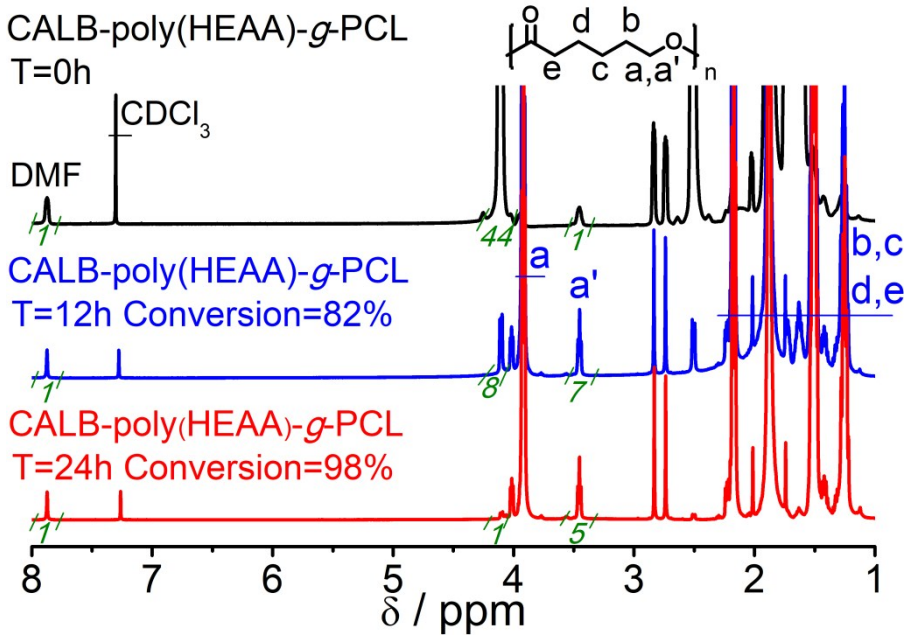

Figure. S8 ^{1}H NMR spectra of samples taken during the synthesis of CALB-poly(HEAA)-*g*-PCL in $CDCl_3$.

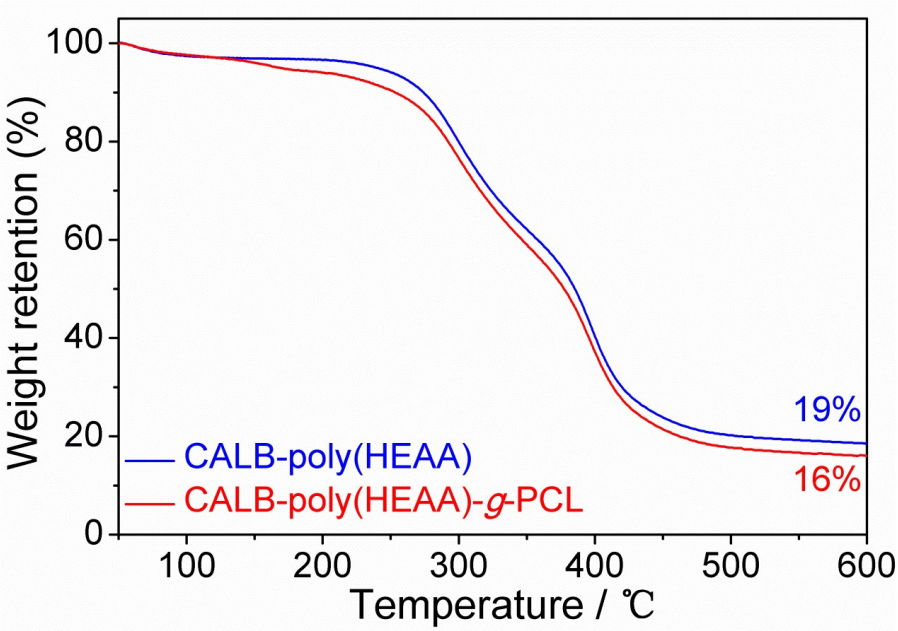

Figure. S9 TGA of CALB-poly(HEAA) and CALB-poly(HEAA)-*g*-PCL.

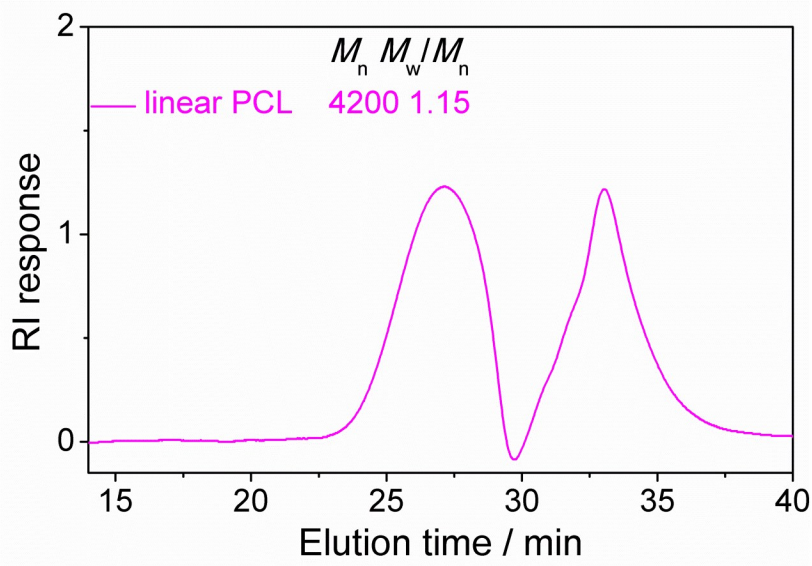

Figure. S10 SEC elution traces (the RI signals were plotted versus elution time) of PCL in acetonitrile catalyzed by CALB-poly(HEAA).

Table S1. Conversion and yields of PCL and CALB-poly(HEAA)-*g*-PCL catalyzed by CALB and CALB-poly(HEAA) *via* eROP.

Product	Reaction time (h)	Conversion (by ^{1}H NMR, %)	Weight of initiator (mg)	Weight of monomer (mg)	Weight of product after dialysis (mg)	Weight of precipiatates after centrifugation (mg)	Weight of linear PCL in acetonitrile after drying under vacuum (mg)
PCL [a]	24	97	15	570	10	/	221
CALB-poly(HEAA)-*g*-PCL [b]	6	80	40	270	/	36	83

[a] CALB was used as the catalyst.

[b] CALB-poly(HEAA) conjugate was used as the catalyst and initiator.

REFERENCES

1. M. Ciampolini and N. Nardi, *Inorg. Chem.*, 1966, 5, 41-44.
2. W. K. Storms-Miller and C. Pugh, *Macromolecules*, 2015, 48, 3803-3810.
3. S. Namekawa, S. Suda, H. Uyama and S. Kobayashi, *Int. J. Biol. Macromol.*, 1999, 25, 145-151.