Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting information

Synthesis of Lipase-Polymer Conjugates by Cu(0)-mediated Reversible Deactivation Radical Polymerization: Polymerization *vs* Degradation

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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*-Isopropylacrylamide (NIPAM, 97%, Aladdin) was recrystallized from hexane to remove the inhibitor before use. *N*-(2-hydroxyethyl) acrylamide (HEAA, 98%, Aladdin), *N-tert*-butylacrylamide (TBA, 97%, Aladdin), 2-Hydroxyethyl acrylate (HEA, 96%, Aladdin), poly (ethylene glycol) methyl ether acrylate ($PEGA_{480}$, average M_n 480, Aladdin) and methyl acrylate (MA, 99%, Aladdin) were de-inhibited by passing through a column of basic alumina prior to use. T*ris*(2-(dimethylamino)ethyl)amine (Me_6TREN), N-succinimidyl 2-bromo-2- methyl propionate (succinimidyl-Br) were synthesized according to literature procedure and stored in the freezer under a nitrogen atmosphere.[1] [2-4] 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 anhydrous acetonitrile (99.8%, Aladdin), *p*-nitrophenol palmitate (*p*-NPP, 98%, Aladdin), *p*-nitrophenol (pNP, 99%, Aladdin), Triton X-100 (BR), trichloroacetic acid (AR) and NaOH were obtained from Aladdin (China) and used without further purification.

Analytical techniques

Aqueous SEC (Waters 1515) was used for the characterization of protein-polymer conjugates and it was equipped with 2414 RI & 2489 UV detector, an Ultrahydrogel guard column and three Waters Ultrahydrogel columns (250, 500 & 1000 PKGD) and autosampler, using aqueous solution of HPLC grade water as the eluent at 25 °C with a flow rate of 1.00 mL min^{-1}. Hydrophilic poly(ethylene glycol) (1.0×10^2 – 2.2×10^4 $g\cdot mol^{-1}$) and dextran (5.0×10^3 - 7.5×10^5 $g\cdot mol^{-1}$) were used to calibrate the aqueous SEC system. All samples were passed through 0.45 μm PTFE filter before analysis. UV/Vis spectrophotometer (SHIMADZU UV-2600) was utilized to measure the lower critical solution temperature (LCST) of thermoresponsive polymers at the wavelength of 500 nm and the heating rate for the thermostatically controlled cuvette was 1 °C min^{-1}. The LCST was defined as the temperature corresponding to 50% decreases of transmittance. The enzyme activity of lipase was calculated according to the degradation of pNPP in defined buffer solution and temperature using SHIMADZU UV-2600 UV/ Vis spectrophotometer to measure the absorbance at 410 nm. ^{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 size and size distribution of polymer and conjugate particles were measured by dynamic light scattering (DLS) using a ZetaPALS variable temperature

analyzer (Brookhaven Instruments, UK). MALDI-ToF data was performed on a Bruker AutoFlexIII MALDI-ToF mass spectrometer. All samples were mixed with the saturated sinapinic acid (SA) solution as the matrix (0.1% TFA, 40% acetonitrile). Samples were analyzed in the positive ion linear mode. 3 μL of samples (1 mg/mL) was mixed with 3 μL the SA matrix and 1 μL was spotted directly on the target plate and allowed to dry at room temperature.

Conjugation of Succinimidyl-based initiator with CALB

To a vial equipped a magnetic stir bar and a rubber septum, CALB (0.6 g, 0.018 mmol) was dissolved in 10 mL phosphate buffer solution (200 mM, pH=8.0). N-succinimidyl 2-bromo-2-methylpropionate (14.52 mg, 0.055 mmol) was dissolved in 1 mL DMSO and added dropwise to protein solution and the mixture was stirred at 4 ^{o}C for one day. Subsequently the formed shallow yellow suspension was transferred to a dialysis tube (MWCO 1 KDa) and dialyzed against water for two days. The final product was recovered as white powder after lyophilisation. The product was named as CALB-Br.

Synthesis of protein-polymer conjugates by Cu(0)-RDRP

A typical polymerization of NIPAM was 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-Br (40 mg, 0.0006 mmol), NIPAM (40 mg, 0.35 mmol) and a drop DMF (used as internal standard for conversion calculation *via* ^{1}H NMR spectroscopy)

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 ^{1}H 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.

For the monomer of HEAA, the reaction procedure is the same as NIPAM. For the other water-soluble acrylamides or acrylates, the reaction procedure is the same with only initiator (all as 20 mg) changed.

For the polymerization of TBA (40 mg) or MA (40 mg), methanol was used as co-solvent in order to solubilize the hydrophobic monomers. In the second vial, a mixture of water /methanol (1 mL: 1 mL or 1.5 mL: 1 mL) is used for TBA or MA separately. After reaction, the reaction mixture is centrifuged at 10000 rpm for 15 min. After removing the supernatant, the sediments were washed by deionized water and centrifuged once more. The above process was repeated for at least three times before the sediments were freeze-dried.

Activity assays of CALB and conjugates

The enzyme activity of CALB was determined using *p*-nitrophenol palmitate (pNPP) as the substrate according to previously reported procedures[5] by UV/Vis spectroscopy, typically measuring the absorbance at 410 nm for a defined sample of *p*-nitrophenol (pNP) after defined period and the CALB activity was deduced according to the kinetic parameters.

$$x = \frac{c\,V}{t\,V'}\,[U \cdot mL^{-1}]$$

x was the enzyme activity of CALB. c represented the concentrate of pNP (μmol · mL^{-1}). V was the final solution volume of pH regulation (mL). V' was the volume of enzyme solution (mL). t was the reaction time (min). One enzyme unit was the amount of protein liberating 1 μmol of pNP per minute in these conditions. The enzyme activity was determined under 35 ℃.

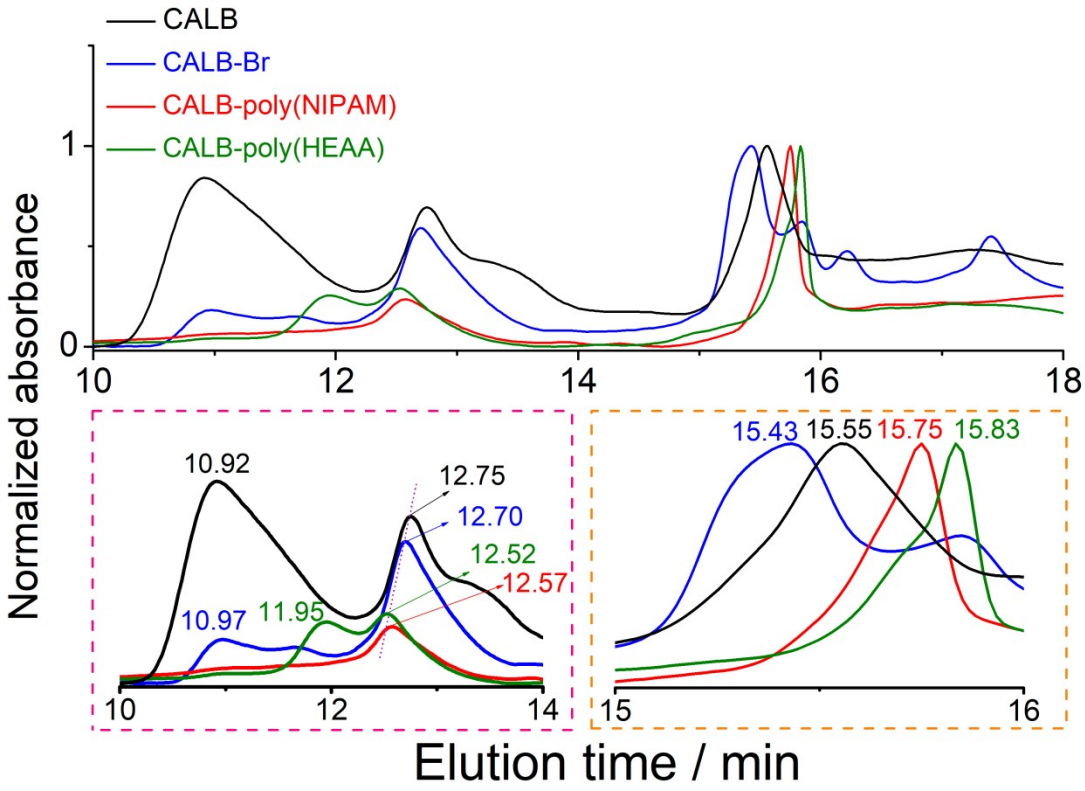

Figure. S1 SEC elution traces of CALB and CALB-polymer conjugates (the normalized UV absorbance were plotted versus elution time).

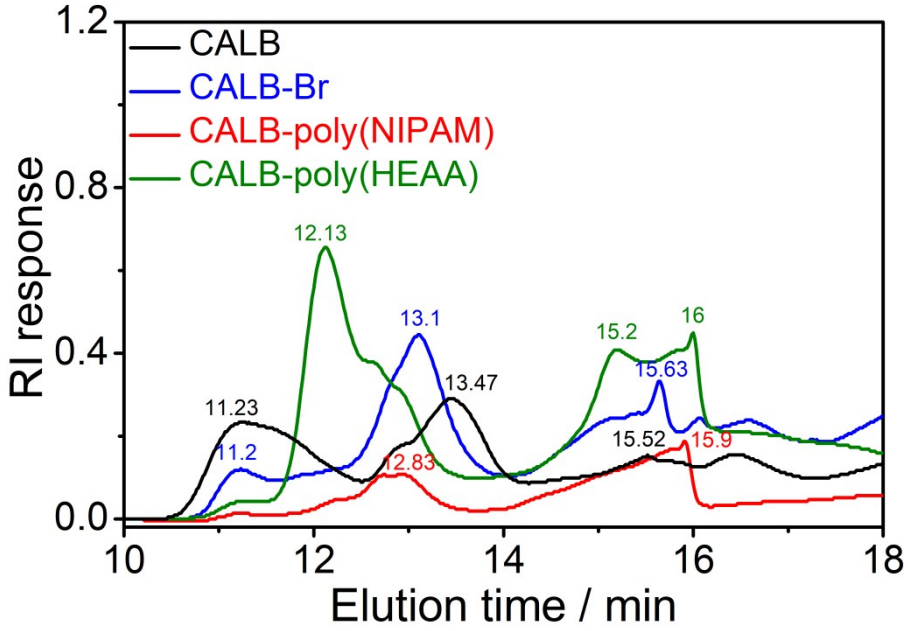

Figure. S2 SEC elution traces of CALB and CALB-polymer conjugates (the RI absorbance were plotted versus elution time).

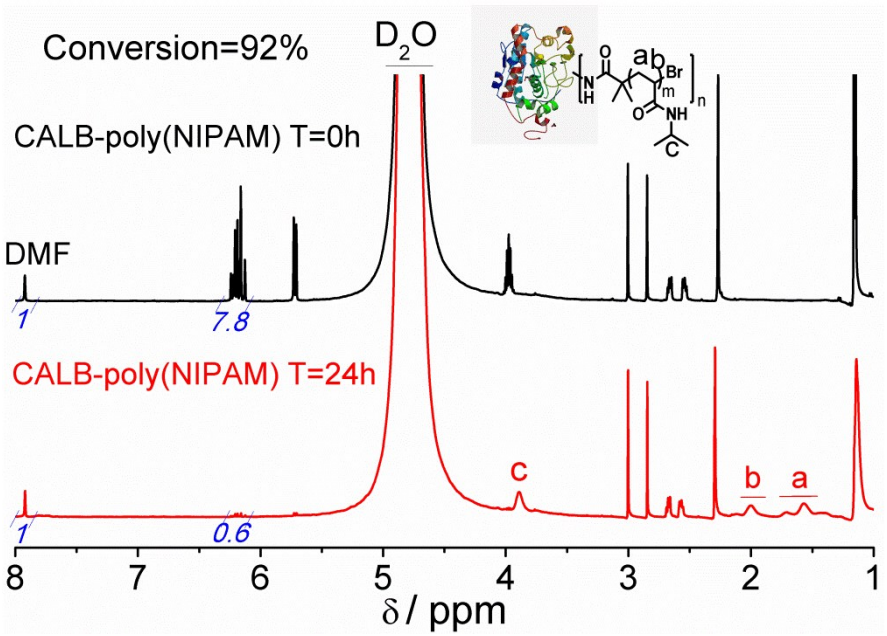

Figure. S3 ^{1}H NMR spectra of CALB-poly(NIPAM) in D_2O before (black line) and after polymerization (red line). Monomer conversions were determined *via* ^{1}H NMR spectroscopy, comparing the signal areas from the vinyl protons (δ ~ 6.40-6.10 ppm) 2H/mol to the signal area from the proton (δ ~ 7.90 ppm) of DMF internal standard.

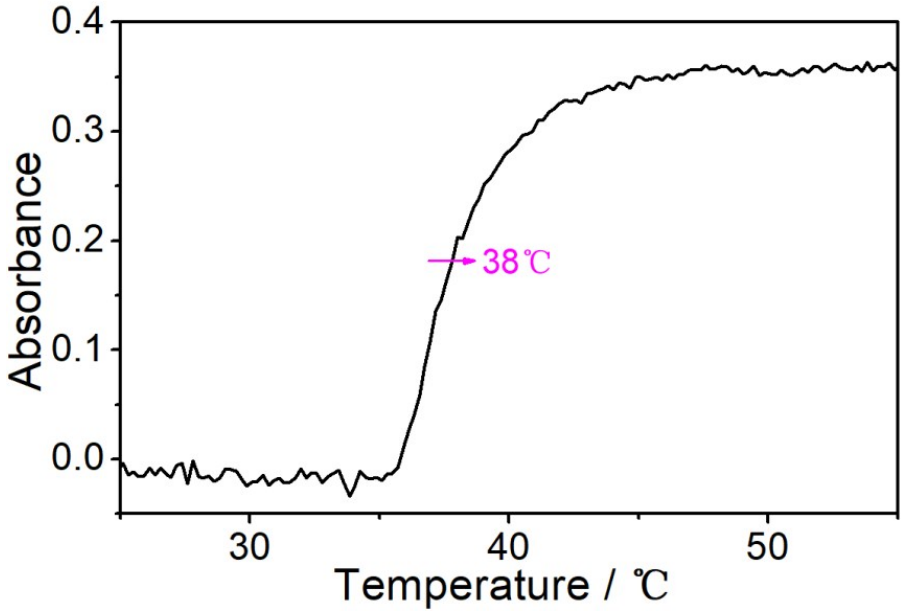

Figure. S4 Temperature dependence of the optical transmittance at 500 nm for CALB-poly(NIPAM) (1 mg mL^{-1}).

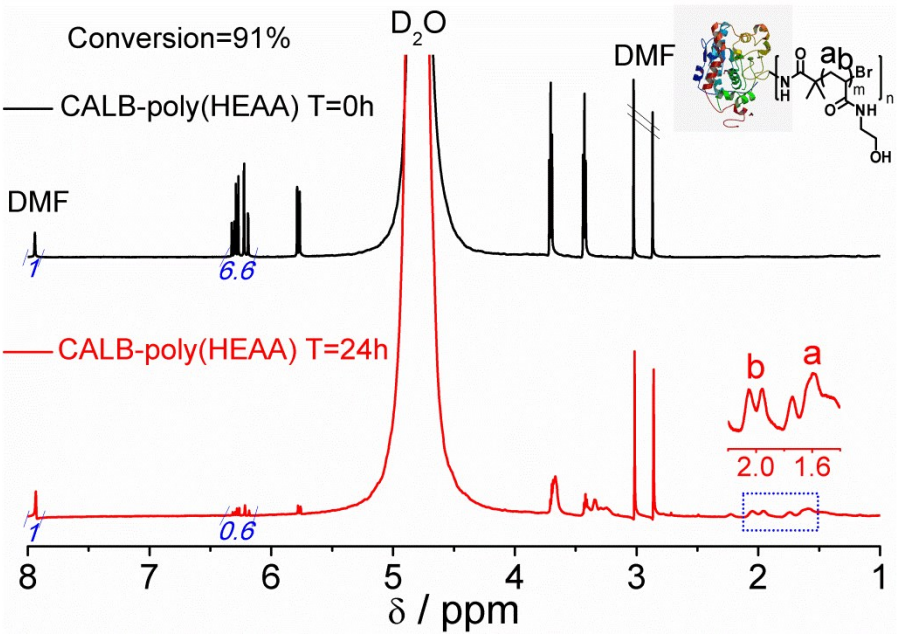

Figure. S5 ^{1}H NMR spectra of CALB-poly(HEAA) in D_2O before (black line) and after polymerization (red line). Monomer conversions were determined *via* ^{1}H NMR spectroscopy, comparing the signal areas from the vinyl protons (δ ~ 6.40-6.10 ppm) 2H/mol to the signal area from the proton (δ ~ 7.90 ppm) of DMF internal standard.

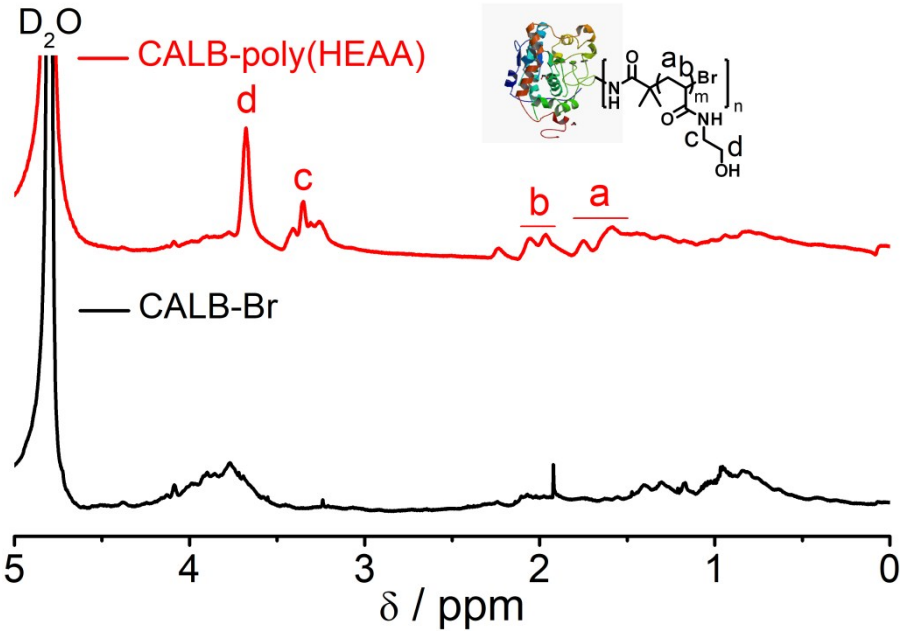

Figure S6 ^{1}H NMR spectra of CALB-Br, CALB-poly(HEAA).

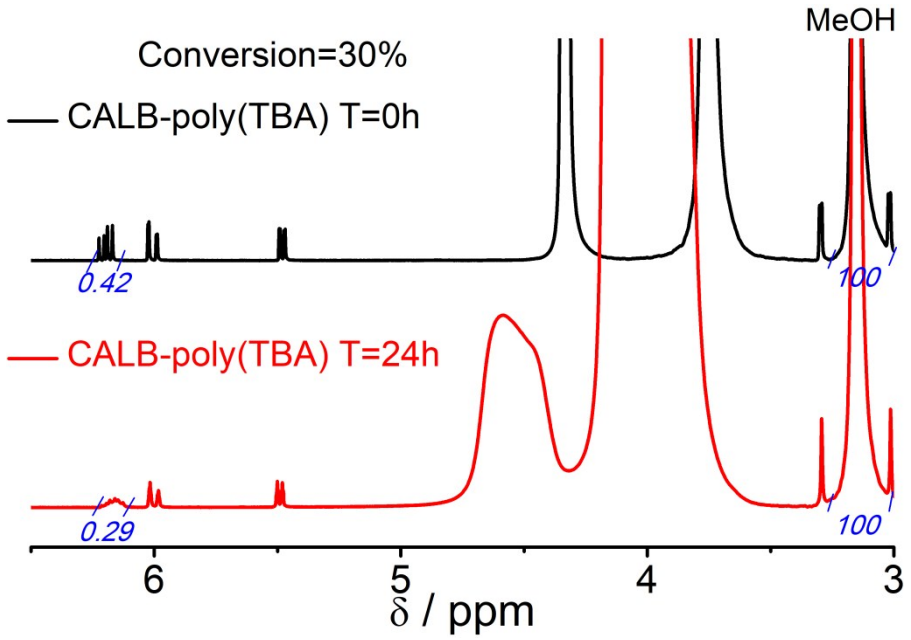

Figure. S7 ^{1}H NMR spectra of CALB-poly(TBA) in DMSO-d_6 before (black line) and after polymerization (red line).

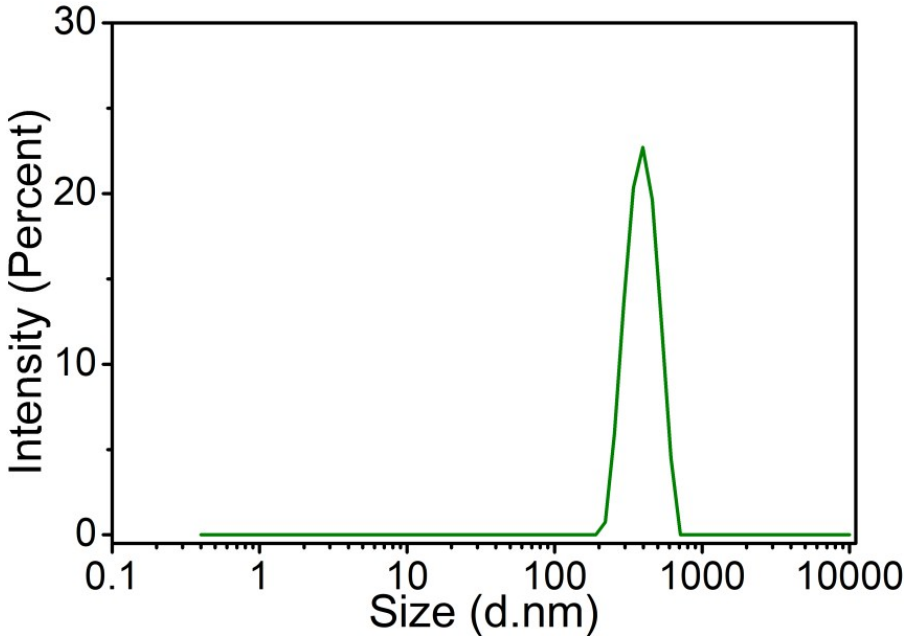

Figure. S8 Size and size distribution of CALB-poly(TBA) in water (1mg mL^{-1}) by DLS.

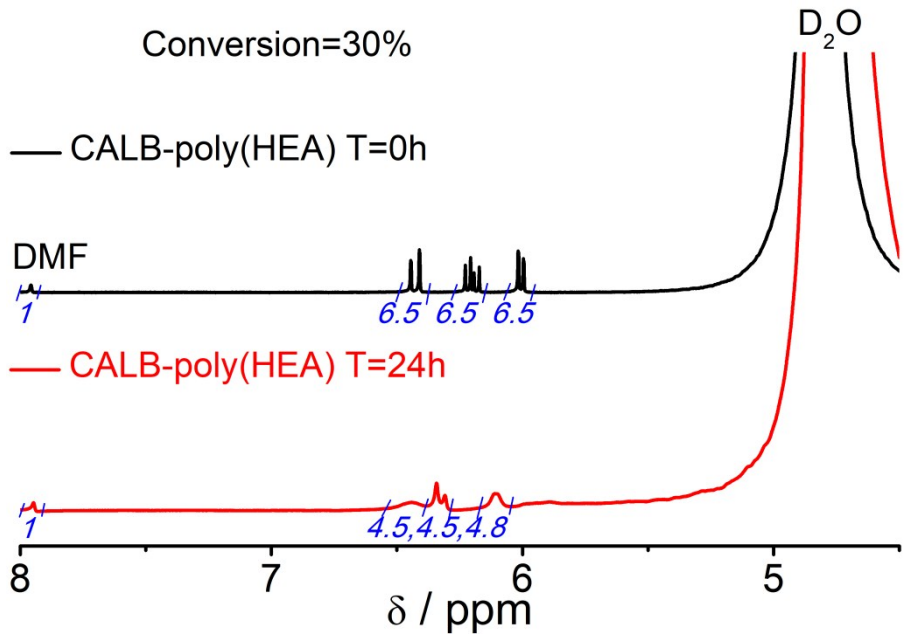

Figure. S9 ^{1}H NMR spectra of CALB-poly(HEA) in D_2O before (black line) and after polymerization (red line).

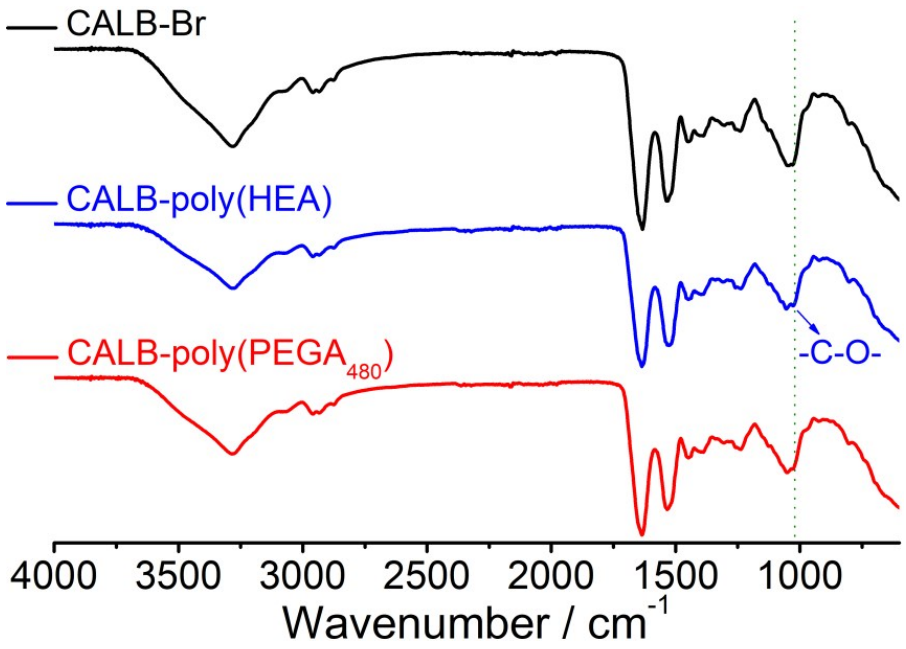

Figure. S10 FTIR spectra of CALB-Br, CALB-poly(HEA) and CALB-poly($PEGA_{480}$).

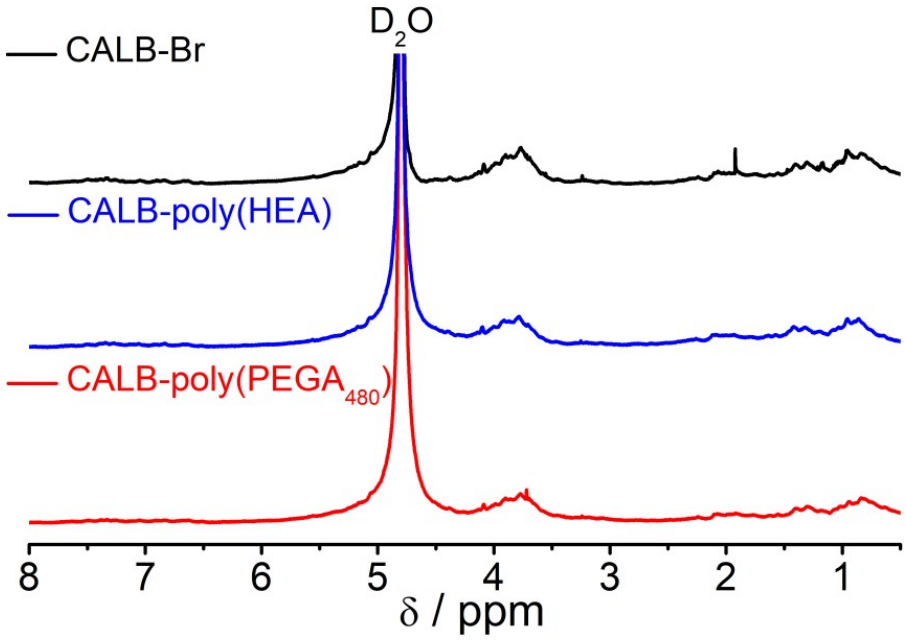

Figure. S11 ^{1}H NMR spectra of CALB-Br, CALB-poly(HEA) and CALB-poly($PEGA_{480}$) in D_2O.

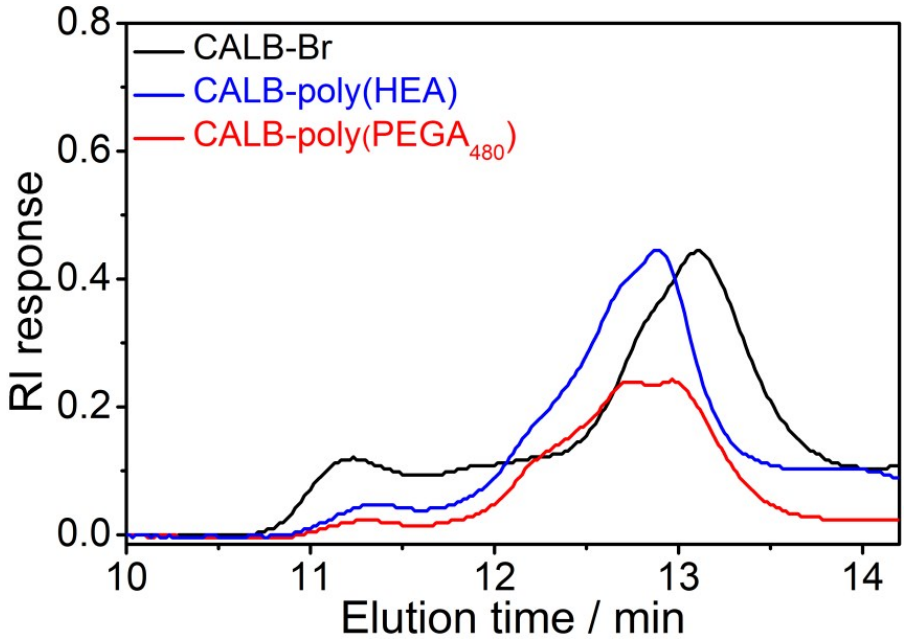

Figure. S12 Aqueous SEC elution traces of CALB-Br, CALB-poly(HEA) and CALB-poly($PEGA_{480}$) (the refractive index (RI) signals were plotted versus elution time).

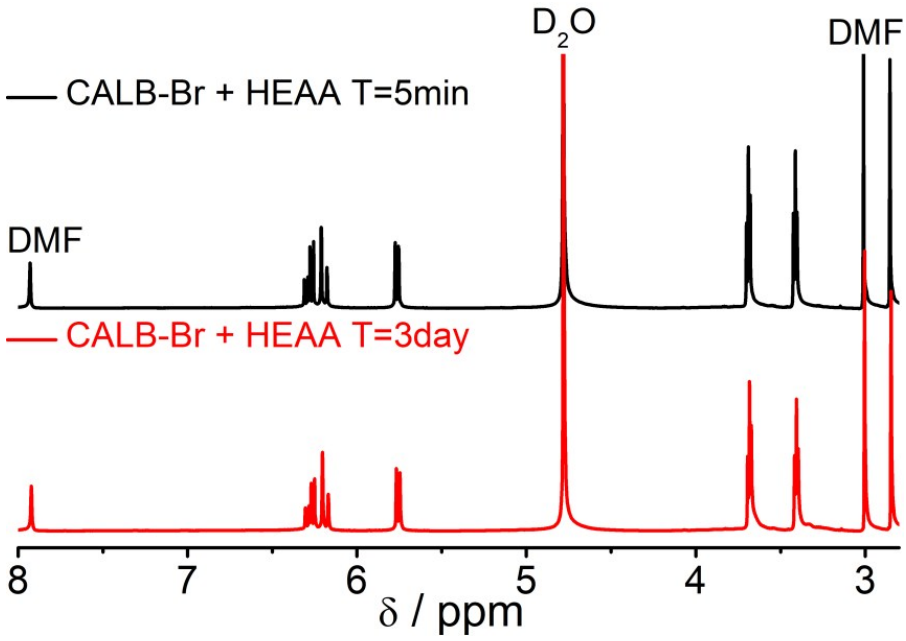

Figure. S13 ^{1}H NMR spectra of HEAA in the presence of CALB-Br in D_2O.

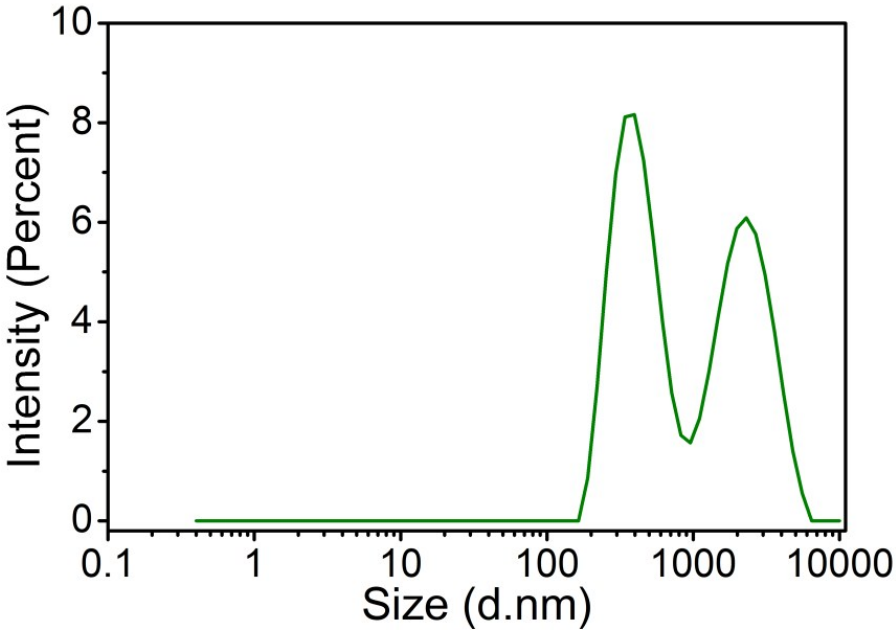

Figure. S14 Size and size distribution of CALB-poly(MA) in water (1mg mL^{-1}) by DLS.

Table S1 Conversion and yields of CALB-polymer conjugates by Cu(0)-LRP.

CALB-polymer conjugate	Conversion (by ^{1}H NMR, %)	Weight of initiator (mg)	Weight of monomer (mg)	Weight of product after lyophilization	Percentage of protein (%)
CALB-poly(NIPAM)	92	40	40	59	52
CALB-poly(HEAA)	91	40	40	57	52
CALB-poly(TBA)	30	20	40	23	63
CALB-poly(HEA)	30	20	40	19	63
CALB-poly($PEGA_{480}$)	33	20	40	14	60
CALB-poly(MA)	27	20	40	17	65

REFERENCES

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