Supplementary Information

Accelerated and controlled polymerization of *N*-carboxyanhydrides in the presence of tertiary amines with minimized activated monomer mechanism

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Materials

All commercial reagents were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China) and used as received unless otherwise specified. Amino acids were purchased from Tokyo Chemical Industry (Shanghai, China). Triphosgene, *N*,*N*-diisopropylethylamine (DIPEA), and 4-dimethylaminopyridine (DMAP) was purchased from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China). The initiator *n*-hexylamine (Hex-NH_2), propargylamine, and benzylamine was purchased from Millipore Sigma Chemical Co. (St. Louis, USA). Deuterated solvents were purchased from Cambridge Isotope Laboratories, Inc. (Tewksbury, USA). Monomers including γ-benzyl-L-glutamate *N*-carboxyanhydride (BLG-NCA), γ-ethyl-L-glutamate *N*-carboxyanhydride (ELG-NCA), γ-(4-propargyloxy)benzyl-L-glutamate NCA (POB-NCA), and N^{ε}-carboxybenzyl-L-lysine NCA (ZLL-NCA) were prepared according to literature procedures.[1-3]

Instrumentations

^1H nuclear magnetic resonance (^1H NMR) spectra were recorded on a 600 MHz-Solution NMR Spectrometer, Westlake University. Chemical shifts (δ) were reported in ppm and referenced to the residual protons in deuterated solvents. MestReNova software (version 14.0.0, Mestrelab Research, Escondido, CA, USA) was used for all NMR analysis. Gel permeation chromatography (GPC) experiments were performed on a system equipped with an isocratic pump (1260 Infinity II, Agilent, Santa Clara, USA), a multi-angle static light scattering (MALS) detector (DAWN HELEOS-II, Wyatt Technology, Santa Barbara, USA), and a differential refractometer (dRI) detector (Optilab, Wyatt Technology, Santa Barbara, USA). The detection wavelength of HELEOS was set at 658 nm. Separations were performed using serially connected size exclusion columns (three Shodex™ packed columns KD-803, KD-804, and KD-805, 10 μm, 8 × 300 mm, Yokohama, Japan) using *N*,*N*-dimethylformamide (DMF) containing LiBr (0.1 mol/L) as the mobile phase at a flow rate of 1.0 mL/min at 50 °C. The MALS detector was calibrated using pure toluene and can be used for the determination of the absolute molecular weights (MWs). The MWs of polymers were determined based on the d*n*/d*c* value of each polymer sample using the internal calibration system processed by the ASTRA software (version 8.12, Wyatt Technology, Santa Barbara, USA). Fourier transform infrared (FTIR) spectra were recorded on a Perkin Elmer 100 serial FTIR spectrophotometer (PerkinElmer, Santa Clara, CA, USA) calibrated with polystyrene film. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectra were collected on a Bruker Rapiflex in the mass spectrometry laboratory, Westlake University, with *trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene] malononitrile (DCTB) as the matrix. Circular dichroism (CD) data was collected using a chirascan spectrometer V100 (Applied Photophysics, Leatherhead, UK). The pathlength of the CD cuvette was 0.5 mm.

Polymerization setup and polypeptide characterization

Accelerated polymerization of NCA in the presence of AcOH with the addition of TEA at 5 min was carried out under ambient conditions. Typically, AcOH (2.17 μL, 0.038 mmol) was mixed with the DCM solution of BLG-NCA (10 mg, 0.038 mmol), into which the DCM solution of Hex-NH_2 (0.076 M, 5 μL, 0.38 μmol) was added to start the polymerization ($[M]_0 = [AcOH]_0 = 0.1$ M, $[M]_0/[Hex\text{-}NH_2]_0 = 100$). At 5 min, TEA (1.32 μL, 0.0095 mmol) was added into the mixture. After > 95% conversion of NCA as monitored by FTIR, the resulting polymers were purified by precipitation in hexane/ether (1:1, v/v) and dried under vacuum. The obtained polypeptides were dissolved in DMF containing 0.1 M LiBr, filtered through a 0.45 μm PTFE membrane (Thermo Fisher Scientific, Waltham, USA), and analyzed by GPC.

Polymerization in other solvents, with other monomers, initiators, and bases were conducted in a similar way.

In order to check the MWs at different monomer conversions, the polymerization mixture was stopped at different time intervals through the addition of trifluoroacetic acid (TFA, 2.5 vol%). The polypeptides were then purified by precipitation, dried, and dissolved in DMF containing LiBr (0.1 mol/L) for GPC analysis.

The secondary structure analysis of polypeptides was conducted in a similar way, but diluted by 40 times with DCM after quenching. Only the CD spectra at $\lambda > 220$ nm were measured owing to the absorbance of DCM at low-wavelength region.

Polymerization kinetics

For the polymerization kinetic experiment, the consumption of NCA was monitored through FTIR. In a typical FTIR experiment, the polymerization mixture was transferred into a liquid FTIR cell after the mixing of monomer, initiator, and acid. The FTIR spectra were monitored at different time intervals until the disappearance of anhydride peaks from NCA at 1860 cm^{-1} and 1790 cm^{-1}. The concentration of NCA monomer was then quantified through the standard curve based on the absorbance at 1790 cm^{-1}.

NMR titration

NMR titration experiments were conducted to probe the molecular interactions between the reactants during NCA polymerization, including BLG-NCA, Hex-NH_2, AcOH, and TEA. Taking the experiments to elucidate the NCA/AcOH/TEA interactions as an example, BLG-NCA (10.00 mg, 0.038 mmol) was dissolved in CD_2Cl_2 (380 μL), into which AcOH (2.17 μL) were added. Then, various amounts of TEA were added (from 0 to 1.32 μL), so that the $[TEA]_0/[NCA]_0$ ratio was 0 to 0.25. The chemical shifts of ring N-H at different $[TEA]_0/[NCA]_0$ ratios were recorded.

Other NMR titration experiments like Hex-NH_2/AcOH/TEA interactions were conducted in a similar manner. For Hex-NH_2/AcOH/TEA experiments, the fraction of Hex-NH_2 protonation was quantified by the chemical shift of α-H of Hex-NH_2, which was calculated according to the following equation:

$$\delta_m = \delta_b + (\delta_s - \delta_b) \cdot X_s$$

Where δ_b and δ_s are the standard chemical shifts of α-H in the amine (-NH_2) and ammonium (-NH_3^+) forms of Hex-NH_2, respectively, and δ_m is the chemical shift of α-H under certain conditions. The mole fraction of protonated amine, X_s, was then calculated from the equation.[4]

Supporting Figures

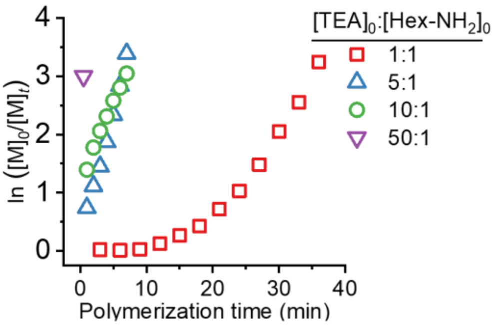


Figure S1. Semilogarithmic kinetic plots of polymerization of BLG-NCA in DCM in the presence of Hex-NH$_2$ and TEA at different [TEA]$_0$/[Hex-NH$_2$]$_0$ ratios. [M]$_0$ = 0.1 M, [M]$_0$/[Hex-NH$_2$]$_0$ = 100.

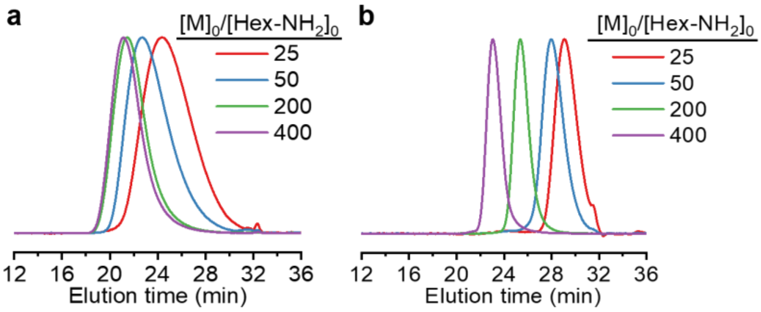


Figure S2. Impact of organic acid on the MW control of polymerization of BLG-NCA in the presence of Hex-NH$_2$ and TEA. Normalized GPC-LS traces of the obtained PBLG from the polymerization at different [M]$_0$/[Hex-NH$_2$]$_0$ ratio in the absence (a) or presence (b) of AcOH. [M]$_0$ = 0.1 M, [M]$_0$/[AcOH]$_0$/[TEA]$_0$ = 100:100:25.

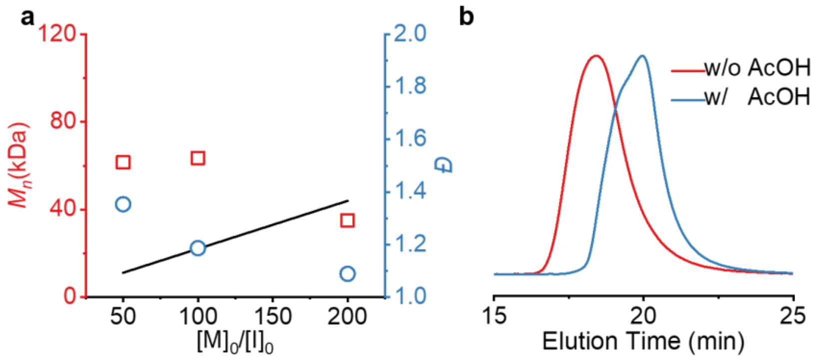


Figure S3. The polymerization results with TEA initiation. (a) The obtained MWs and dispersity of resulting PBLG from the polymerization at different $[M]_0/[I]_0$ ratios. (b) Normalized GPC-LS traces of obtained PBLG from the polymerization initiated by TEA at $[M]_0/[I]_0 = 100$ with or without the addition of AcOH. $[M]_0 = 0.1$ M, $[M]_0/[AcOH]_0 = 1$.

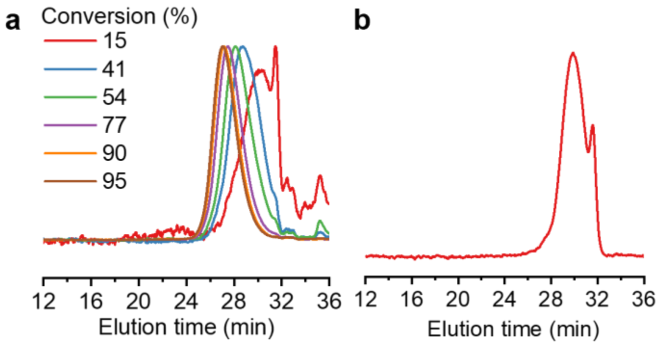


Figure S4. Poor MW control of polymerization of BLG-NCA in the presence of Hex-NH_2, TEA, and AcOH. (a) Normalized GPC-LS traces of the obtained PBLG from polymerization at different monomer conversions. (b) GPC-LS trace of obtained PBLG at $[M]_0/[Hex\text{-}NH_2]_0 = 10$. $[M]_0 = 0.1$ M, $[M]_0/[AcOH]_0/[TEA]_0 = 100:100:25$.

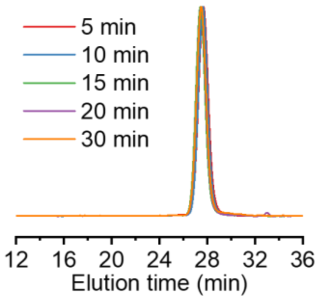
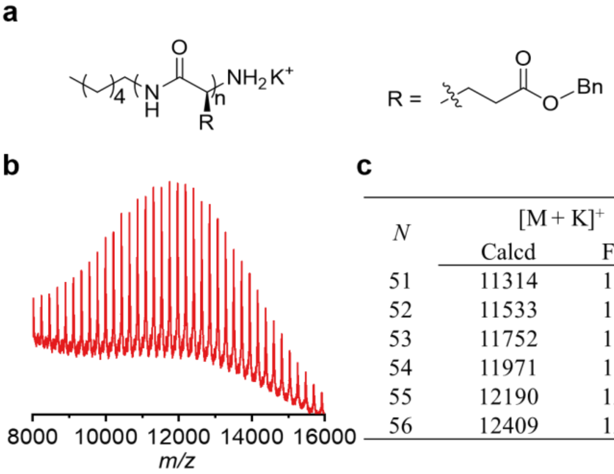


Figure S5. Normalized GPC-LS traces of the resulting polypeptides obtained from the polymerization with sequential addition of AcOH and TEA at different time intervals. $[M]_0 = 0.1$ M, $[M]_0/[AcOH]_0/[TEA]_0 = 100:100:25$.



N	$[M+K]^+$	
	Calcd	Found
51	11314	11313
52	11533	11532
53	11752	11752
54	11971	11970
55	12190	12188
56	12409	12409

Figure S6. MALDI-TOF characterization of PBLG obtained from polymerization with sequential addition of AcOH and TEA. (a) Chemical structure of polymeric species detected by MALDI-TOF. (b) MALDI-TOF spectrum of PBLG. (c) Comparison of representative *m*/*z* signals between calculated values from molecular formula and obtained values from MALDI-TOF spectrum. The obtained *m*/*z* signals agree well with the calculated values of (140.08 + 219.09*n*) ($[M + K]^+$), indicating initiation from Hex-NH_2 with minimized AMM initiation from TEA.

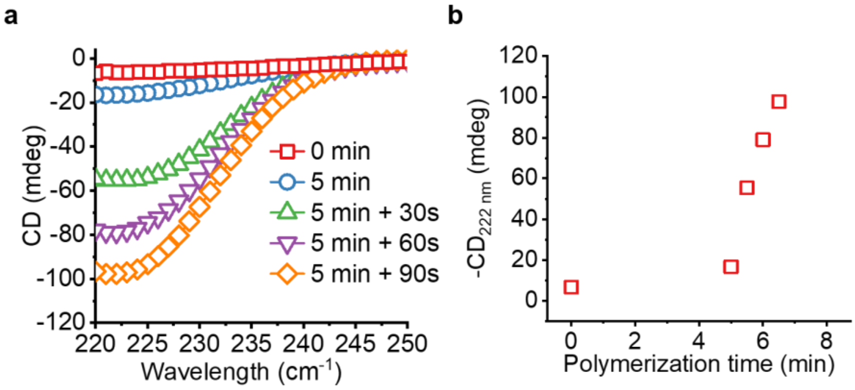


Figure S7. Secondary structure transition of polypeptides during the polymerization with sequential addition of AcOH and TEA (5 min interval). (a) Overlaid CD spectra showing the change in secondary structure. (b) The change in CD signal at 222 nm over time during the polymerization. $[M]_0 = 0.1$ M, $[M]_0/[Hex\text{-}NH_2]_0/[AcOH]_0/[TEA]_0 = 100:1:100:25$.

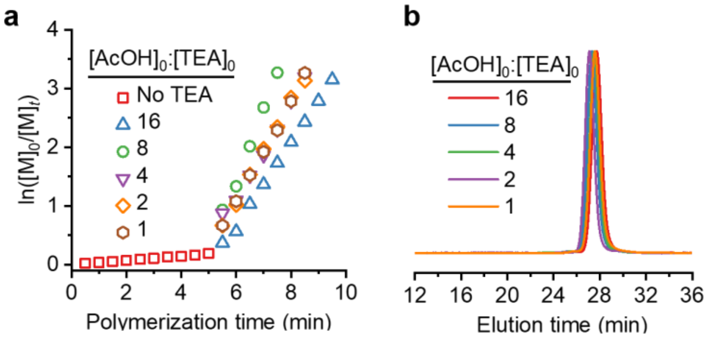


Figure S8. Polymerization of BLG-NCA in DCM initiated by Hex-NH_2 with sequential addition of AcOH and TEA at various $[AcOH]_0/[TEA]_0$ ratios. (a) Semilogarithmic kinetic plots of the polymerization. (b) Normalized GPC-LS traces of the resulting PBLG.

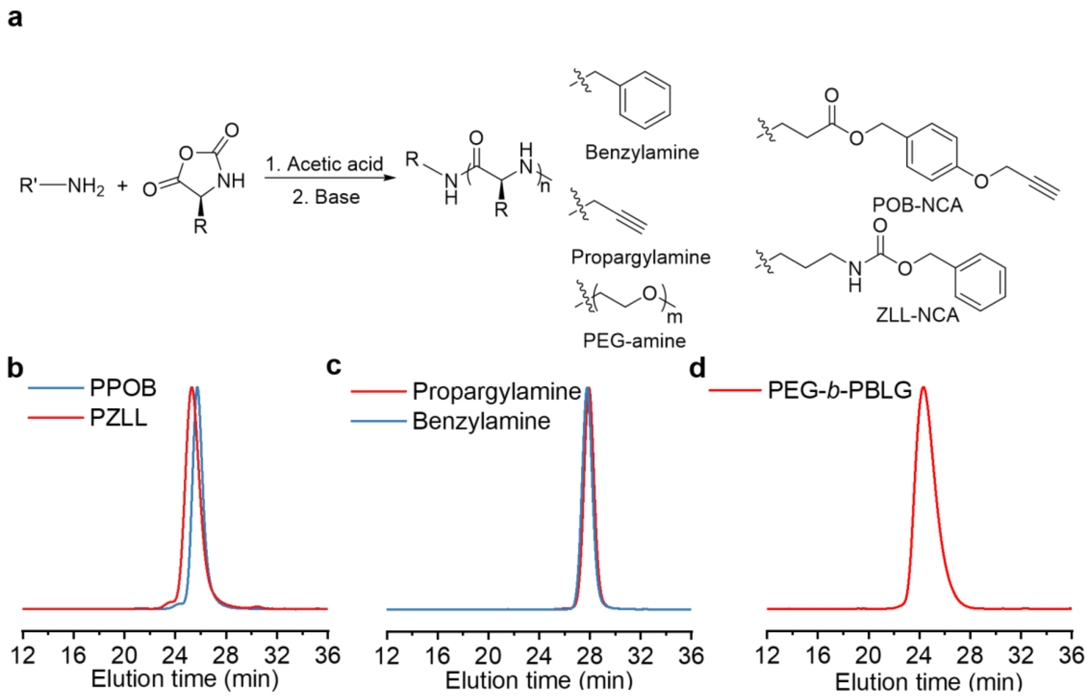


Figure S9. Polymerization of various NCA monomers in the presence of different initiators. (a) Chemical structures of various initiators and NCA monomers. (b) Normalized GPC-LS traces of the resulting polypeptides obtained from the polymerization with sequential addition of AcOH and TEA, with ZLL-NCA and POB-NCA as the monomer. (c, d) Normalized GPC-LS traces of the resulting polypeptides initiated by benzylamine, propargylamine (c) and PEG-amine (5.0 kDa, d) with sequential addition of AcOH and TEA. $[M]_0 = 0.1$ M, $[M]_0/[I]_0/[AcOH]_0/[TEA]_0 = 100:1:100:25$.

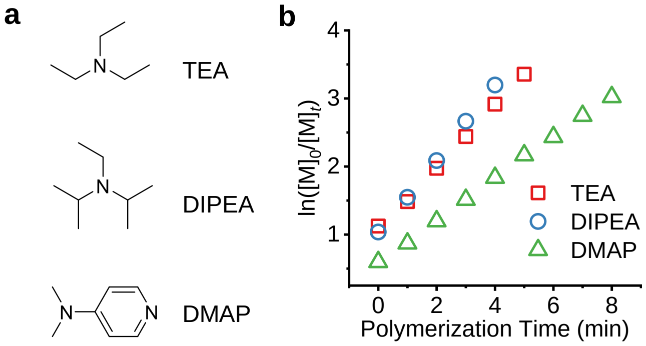


Figure S10. Polymerization of BLG-NCA in DCM initiated by Hex-NH_2 with sequential addition of AcOH and various tertiary amines. (a) Chemical structures of different tertiary amines. (b) Semilogarithmic kinetic plots of the polymerization. $[M]_0 = 0.1$ M, $[M]_0/[I]_0/[AcOH]_0/[\text{tertiary amine}]_0 = 100:1:100:25$.

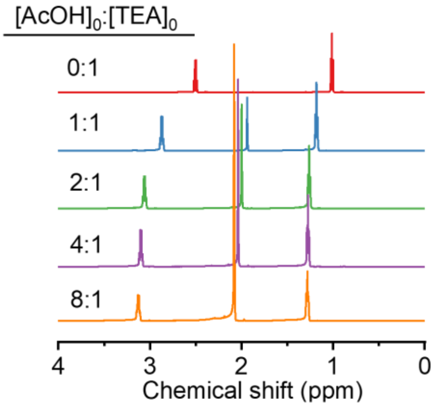


Figure S11. Overlaid ^{1}H NMR spectra of the mixture of TEA and AcOH at different molar ratios.

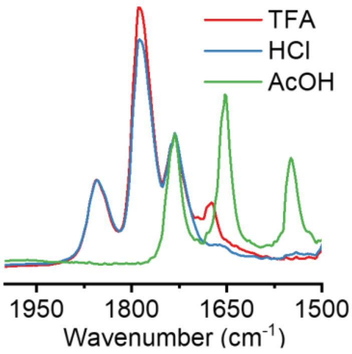


Figure S12. Overlaid FTIR spectra showing the polymerization progress of BLG-NCA initiated by Hex-NH$_2$ with sequential addition of acids and TEA. Acids with different acidity was added to study its impact on polymerization behavior. $[M]_0 = 0.1$ M, $[M]_0/[\text{Hex-NH}_2]_0/[\text{acid}]_0/[\text{TEA}]_0 = 100:1:100:25$.

Supporting Tables

Table S1. Polymerization of BLG-NCA at various $[M]_0/[Hex\text{-}NH_2]_0$ ratios in the presence of Hex-NH_2 and TEA.[a]

Entry	$[M]_0/[Hex\text{-}NH_2]_0$	t (min)[b]	$M_{n,\ GPC}$ (kDa)[c]	$M_{n,\ theo.}$ (kDa)	$Đ$[c]
1	25	1	26.0	5.48	1.59
2	50	1	37.9	11.0	1.83
3	100	1	68.8	21.9	1.61
4	200	3	91.9	43.8	1.59
5	400	3	104	87.6	1.55

[a] All polymerization were conducted at room temperature in DCM using BLG-NCA as the monomer. $[M]_0$ = 0.1 M, $[M]_0/[TEA]_0$ = 4. [b] Polymerization time reaching 95% monomer conversion. [c] Determined by GPC; dn/dc = 0.104.

Table S2. Polymerization of BLG-NCA at various $[M]_0/[\text{Hex-NH}_2]_0$ ratios in the presence of Hex-NH_2, TEA, and AcOH.[a]

Entry	$[M]_0/[\text{Hex-NH}_2]_0$	t (min)[b]	$M_{\text{n, GPC}}$ (kDa)[c]	$M_{\text{n, theo.}}$ (kDa)	$Đ$[c]
1	25	3	6.59	5.48	1.45
2	50	3	13.0	11.0	1.25
3	100	4	20.4	21.9	1.11
4	200	5	40.9	43.8	1.05
5	400	10	84.4	87.6	1.10

[a] All polymerization were conducted at room temperature in DCM using BLG-NCA as the monomer. $[M]_0$ = 0.1 M, $[M]_0/[\text{AcOH}]_0/[\text{TEA}]_0$ = 100:100:25. [b] Polymerization time reaching 95% monomer conversion. [c] Determined by GPC; dn/dc = 0.104.

Table S3. Polymerization of BLG-NCA initiated by TEA at different $[M]_0/[TEA]_0$.[a]

Entry	$[M]_0/[TEA]_0$	$M_{n,\ GPC}$ (kDa)[c]	$M_{n,\ theo.}$ (kDa)	$Đ$[c]
1	50	61.5	11.0	1.42
2	100	63.5	21.9	1.20
3	200	35.0	43.8	1.19
4[b]	100	33.9	21.9	1.33

[a] All polymerization were conducted at room temperature in DCM using BLG-NCA as the monomer. $[M]_0 = 0.1$ M. [b] Polymerization in the presence of AcOH at $[M]_0/[AcOH]_0 = 1$. [c] Determined by GPC; $dn/dc = 0.104$.

Table S4. Polymerization of BLG-NCAs initiated by Hex-NH_2 with sequential addition of AcOH and TEA with different time intervals.[a.]

Entry	Time interval (min)	t (min)[b]	$M_{n,\ GPC}$ (kDa)[c]	$Đ$[c]
1	5	3	22.1	1.05
2	10	3	22.4	1.05
3	15	3	23.9	1.05
4	20	3	23.7	1.05
5	30	3	23.6	1.05

[a] All polymerization were conducted at room temperature in DCM using Hex-NH_2 as the initiator and BLG-NCA as the monomer. $[M]_0$ = 0.1 M, $[M]_0/[AcOH]_0/[TEA]_0$ = 100:100:25. $M_{n,theo}$ = 21.9 kDa [b] Polymerization time reaching 95% monomer conversion after the addition of TEA. [c] Determined by GPC; dn/dc = 0.104.

Table S5. Characterization of resulting polypeptides from polymerization of BLG-NCAs with sequential addition of AcOH and TEA in different solvents.[a]

Entry	Solvent	$M_{\mathrm{n,\,GPC}}$ (kDa)[b]	$Đ$[b]
1	DMF	54.4	1.60
2	Ethyl acetate	24.9	1.21
3	1,4-Dioxane	29.9	1.12
4	Chloroform	20.6	1.05

[a] All polymerization were conducted at room temperature using Hex-NH_2 as the initiator and BLG-NCA as the monomer. $[M]_0/[\text{Hex-NH}_2]_0/[\text{AcOH}]_0/[\text{TEA}]_0 = 100{:}1{:}100{:}25$, $[M]_0 = 0.1$ M. $M_{\mathrm{n,\,theo}} = 21.9$ kDa. [b] Determined by GPC; $dn/dc = 0.104$.

Reference:

1. Tian, Z. Y.; Zhang, Z.; Wang, S.; Lu, H., A moisture-tolerant route to unprotected α/β-amino acid *N*-carboxyanhydrides and facile synthesis of hyperbranched polypeptides. *Nat. Commun.* **2021,** *12* (1), 5810.

2. Xia, Y.; Song, Z.; Tan, Z.; Xue, T.; Wei, S.; Zhu, L.; Yang, Y.; Fu, H.; Jiang, Y.; Lin, Y.; Lu, Y.; Ferguson, A. L.; Cheng, J., Accelerated polymerization of *N*-carboxyanhydrides catalyzed by crown ether. *Nat. Commun.* **2021,** *12* (1), 732.

3. Zhang, R.; Zheng, N.; Song, Z.; Yin, L.; Cheng, J., The effect of side-chain functionality and hydrophobicity on the gene delivery capabilities of cationic helical polypeptides. *Biomaterials* **2014,** *35* (10), 3443-54.

4. Liu, Q.; Zhu, M., Determination of molar ratio of primary secondary and tertiary amines in polymers by applying derivatization and NMR spectroscopy. *Polym. Test.* **2016,** *56*, 174-179.