Supplementary Information for

Unimolecular Polypeptide Micelles *via* Ultra-fast Polymerization of *N*-Carboxyanhydrides

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Materials and Methods

Materials

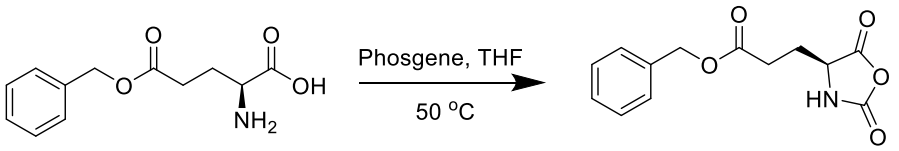
N^{ε}-benzyloxycarbonyl-L-lysine-*N*-carboxyanhydride (Lys(Z)-NCA), γ-(4-propargyloxybenzyl)-L-glutamate (POBLG), γ-(4-allyloxylbenzyl)-L-glutamate (AOBLG) were synthesized according to the previous work.[1] PAMAM dendrimers were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used as received. Anhydrous dichloromethane (DCM) and chloroform ($CHCl_3$) were purchased from Sigma-Aldrich and stored in the glove box. Dimethylformamide (DMF) was dried by a column packed with 4Å molecular sieves and stored in the glove box. Tetrahydrofuran (THF) and hexane were dried by a column packed with alumina and stored in the glove box. All other chemicals were purchased from Sigma-Aldrich and used as received unless otherwise specified.

Characterization

The nuclear magnetic resonance (NMR) spectra were recorded on a Varian UI400 MHz, a UI500NB MHz or a VXR-500 MHz spectrometer. MestReNova 8.1.1 was used to analyze all spectra. Fourier transform infrared (FT-IR) spectra were performed using a Spectrum 100 spectrometer (Perkin Elmer) in a 0.1 mm KBr permanent sealed liquid cell (Buck Scientific). Molecular weight (MW) and polydispersity index ($Đ = M_w/M_n$) of the polymers were determined by gel permeation chromatography (GPC). GPC was performed on a system equipped with a Model1200 isocratic pump (Agilent Technology) in series with a 717 Autosampler (Waters) and size exclusion columns

(10^2 Å, 10^3 Å, 10^4 Å, 10^5 Å, 10^6 Å Phenogel columns, 5 μm, 300 × 7.8 mm, Phenomenex) which were maintained at a temperature of 60 °C. A DAWN HELEOS (Wyatt Technology) multiangle laser light scattering (MALLS) operating at a wavelength of 658 nm and an Optilab rEX refractive index detector (Wyatt Technology) operating at a wavelength of 658 nm were used as detectors. The mobile phase consisted of DMF containing 0.1 M LiBr at a flow rate of 1 mL min^{-1}. Samples were filtered through a 0.45 μm PTFE filter before analysis. Absolute molecular weights of polymers were determined using ASTRA 6.1.1.17 software (Wyatt Technology) and calculated from *dn/dc* values assuming 100% mass recovery. Particle size and polydispersity were measured with a ZetaPlus dynamic light scattering (DLS) detector (15 mW laser, incident beam = 676 nm, detecting angle = 90 °, CONTIN algorithm, Brookhaven Instruments, Holtsville, NY, USA).

Synthesis of γ-benzyl-L-glutamate-*N*-carboxyanhydride (BLG-NCA)



To an oven dried 250 mL round bottom flask, γ-benzyl-L-glutamic acid (10.0 g, 42.2 mmol) was added and dried under high vacuum for 2 h. The flask was filled with nitrogen and 100 mL of dry THF was added to create a suspension. The flask was cooled on ice, then phosgene (15 wt% in toluene, 63.0 mmol) was added in one portion. (**Caution:** phosgene is toxic and should be handled with care. All glasswares

needed to be quenched with saturated solution of $NaHCO_3$ after use.) The flask was then placed into a prewarmed oil bath at 50 °C, heated for 2 h under nitrogen. The clear, colorless solution was cooled and evaporated under vacuum. The crude product was recrystallized by dry THF and hexanes. The recrystallization was repeated three times to yield a white crystalline solid (9.1 g, 82%). The pure BLG-NCA was stored in the glove box at -30 ^oC before use. ^1H NMR (500 MHz, $CDCl_3$): δ 7.41-7.29 (m, 5H, Ar*H*), 6.94 (s, 1H, N*H*), 5.12 (s, 2H, ArCH_2), 4.38 (t, 1H, α-*H*), 2.57 (t, 2H, -CH_2CH_2COO-), 2.11-2.25 (m, 2H, -CH_2CH_2COO-).

The enantiomer γ-benzyl-$_D$-glutamate-*N*-carboxyanhydride (BDG-NCA) was prepared by the same procedure.

Synthesis of γ-(4-propargyloxybenzyl)-$_L$-glutamate *N*-carboxyanhydride (POBLG-NCA)

In brief, POBLG (1.15 g, 4.0 mmol) was dissolved in dry THF (25 mL) followed by addition of the phosgene solution (15 wt% in toluene, 4.0 mL, 5.6 mmol). The mixture was refluxed at 50 ^oC for 2 h to obtain a clear solution. The solvent was removed under vacuum, and the crude product was recrystallized three times (THF/hexane, 1:5, v/v) to give POBLG-NCA as faint yellow crystals (770 mg, 61%). POBLG-NCA was stored in the glove box at -30 ^oC before use. ^1H NMR ($CDCl_3$, 500 MHz): δ 7.29 (d, 2H, Ar*H*), 6.98 (d, 2H, Ar*H*), 6.3 (s,1H, N*H*), 5.08 (s, 2H, ArCH_2-), 4.70 (d, 2H, ArOCH_2-), 4.36 (t,1H, α-*H*), 2.55 (m, 3H, -COCH_2CH_2-, *H*CC-), 2.04 (m, 2H, -CH_2CH_2COO-), 2.29-2.10 (m, 2H, -CH_2CH_2COO-).

Synthesis of γ-(4-allyloxylbenzyl)-L-glutamate *N*-carboxyanhydride (AOBLG-NCA)

In brief, AOBLG (2.0 g, 6.8 mmol) was dried under vacuum for 1 h. Anhydrous THF (20 mL) was added under nitrogen followed by the addition of phosgene (15% in toluene, 7.1 mL, 10 mmol). The suspension was stirred at 50 °C for about 2 h until a clear solution was obtained. The solvent was removed under vacuum. The crude NCA was recrystallized three times with anhydrous THF/hexane (1:5, v/v) in a glove box to yield AOBLG-NCA in needle crystalline form (1.5 g, 70%). AOBLG-NCA was stored in the glove box at -30 °C before use. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.24 (d, 2H, Ar*H*), 6.88 (d, 2H, Ar*H*), 6.01 (m, 1H, $PhOCH_2CH{=}CH_2$), 5.39 (dd, 1H, $PhOCH_2CH{=}CH_2$), 5.26 (dd, 1H, $PhCH{=}CH_2$), 5.03 (s, 2H, $PhCH_2$), 4.51 (d, 2H, $PhOCH_2CH{=}CH_2$), 4.37 (t, 1H, α-*H*), 2.52 (t, 2H, $CH_2CH_2COOCH_2$), 2.14 (m, 2H, $CH_2CH_2COOCH_2$).

Typical NCA polymerization

In a glovebox, BLG-NCA was weighed into an oven-dried vial and dissolved in DCM. A proper volume of initiator (diluted by DCM to ~0.02 M in terms of primary amines) was added at the desired monomer/initiator ($[M]_0/[I]_0$) ratio such that the final concentration of monomer was 0.05 M. FT-IR was utilized to monitor the conversion of NCA. For analysis of resulting polymers, polymerization solutions were analyzed after drying in vacuum and dissolving in DMF containing 0.1 M LiBr for GPC

analysis.

Polymerizations of other NCA monomers including BDG-NCA, BDLG-NCA, Lys(Z)-NCA, POBLG-NCA, and AOBLG-NCA by the PAMAM initiators in DCM, $CHCl_3$ or DMF were performed under the similar reaction condition.

For in situ polymerization study, the solution was transferred into a 0.1 mm amalgamated KBr liquid transmission cell, then placed into the FT-IR instrument.

Circular dichroism (CD) measurement

CD measurements were carried out on a JASCO J-815 CD spectrometer (JASCO, Easton, MD, USA). The polymerization solution of BLG-NCA by PAMAM G5 ($[M]_0$ = 50 mM) was diluted by 10 times DCM before transferred into the quartz cuvette, and the CD spectrum was collected every minute until full conversion of NCA monomers. Due to the light absorbance of DCM solvent, we were only able to collect the CD spectra between 220 and 250 nm.

Deprotection study of spherical PBLG polymers by trimethylsilyl iodide

The deprotection of spherical PBLG unimolecular micelles was performed according to the previous procedure.[2] To G3-*g*-PBLG or G5-*g*-PBLG solutions (ca. 50 mg in 4 mL of DCM), trimethylsilyl iodide (195 μL, 1.37 mmol, 6 equiv. per benzyl group) was added by a syringe forming a slightly yellow solution. The reaction was stirred at room temperature for 24 hours, and the solvent was removed under vacuum. Saturated $NaHCO_3$ solution (4 mL) and DI water (4 mL) were added to dissolve the

resulting residue and a minimal amount of NaS_2O_3 was added to create a colorless solution. After the residue was completely dissolved, the aqueous phase was washed by 3 × 10 mL ether to remove benzyl iodide. The resulting colorless water solution was transferred to dialysis tubing (50k MWCO) and dialyzed against DI water for 72 hours. The deprotected polymers were obtained as white powder after lyophilization. ^{1}H NMR test was utilized to analyze the deprotection efficiency of the polymers using D_2O as the solvent.

Cryogenic transmission electron microscopy investigation

Cryogenic transmission electron microscopy (cryo TEM) was performed using JEOL 2100 Cryo-TEM at 200kV to investigate the morphology of the micelles in its native solution state. In the case of protected polymeric micelles, samples were prepared on a Quantifoil grid (200#; 2μM GOLD 2 μm hole 2 um distance, EMS Acquisition Corp, Hatfield, PA, USA) using a semi-automated Vitrobot (Vitrobot Mark II, FEI). Briefly, 3 μL of samples (5 mg mL^{-1} in deionized water) was casted on top of a Quantifoil grid loaded in the chamber of the Vitrobot. Because the vapor was deionized water, it was rather important to control blotting condition than humidity control. Successful thickness of sample was achieved at 100 % relative humidity and 25 ^oC. After blotting excess solution 3 times with 2 seconds blotting time and -2 mm blot offset, rapid immersion of the grid into liquid ethane effectively vitrifies the sample. In the case of deprotected samples in water, same procedure was used except it was blotted by 2 times with 2 seconds blot time and at -2 mm blot offset. Prepared

samples then transferred to Cryo TEM without increase of temperature above -170 ^{o}C to prevent any kind of crystallization formation. Images were all obtained at an underfocus of ~4000 nm.

Table S1. PBLG polymers initiated by PAMAM initiators.

Entry	Polymers	M_n[a] (M_n[*]) MDa	M_w/ M_n[a]
1	G2-*g*-PBLG$_{25}$	0.091 (0.091)	1.13
2	G3-*g*-PBLG$_{25}$	0.182 (0.182)	1.06
3	G4-*g*-PBLG$_{25}$	0.367 (0.365)	1.06
4	G5-*g*-PBLG$_{25}$	0.684 (0.729)	1.09
5	G2-*g*-PBLG$_{50}$	0.178 (0.178)	1.10
6	G3-*g*-PBLG$_{50}$	0.362 (0.357)	1.05
7	G4-*g*-PBLG$_{50}$	0.757 (0.715)	1.04
8	G5-*g*-PBLG$_{50}$	1.41 (1.43)	1.08
9	G2-*g*-PBLG$_{75}$	0.264 (0.266)	1.09
10	G3-*g*-PBLG$_{75}$	0.543 (0.532)	1.05
11	G4-*g*-PBLG$_{75}$	1.19 (1.06)	1.05
12	G5-*g*-PBLG$_{75}$	1.99 (2.13)	1.07
13	G2-*g*-PBLG$_{100}$	0.380 (0.353)	1.08
14	G3-*g*-PBLG$_{100}$	0.659 (0.707)	1.04
15	G4-*g*-PBLG$_{100}$	1.38 (1.42)	1.03
16	G5-*g*-PBLG$_{100}$	2.66 (2.83)	1.07

Polymerizations were performed in DCM. Monomer conversion for all polymerizations was > 99% as measured by FT-IR. [a] Determined by GPC, *theoretical molecular weight.

Table S2. Polymers initiated by G3 or G5 at higher designed DPs.

Entry	Polymers	M_n[a] (M_n[*]) MDa	M_w/ M_n[a]
1	G3-*g*-PBLG$_{200}$	1.24 (1.40)	1.03
2	G3-*g*-PBLG$_{400}$	2.43 (2.80)	1.02
3	G3-*g*-PBLG$_{800}$	4.46 (5.60)	1.01
4	G3-*g*-PBLG$_{1600}$	12.6 (11.2)	1.03
5	G3-*g*-PBLG$_{3200}$	22.6 (22.4)	1.03
6	G5-*g*-PBLG$_{200}$	5.62 (5.61)	1.03
7	G5-*g*-PBLG$_{400}$	10.7 (11.2)	1.04
8	G5-*g*-PBLG$_{800}$	22.9 (22.4)	1.03
9	G5-*g*-PBLG$_{1600}$	46.2 (44.8)	1.04
10	G5-*g*-PBLG$_{3200}$	85.1 (89.6)	1.01

Polymerizations were performed in DCM. Monomer conversion for all polymerizations was > 99% as measured by FT-IR. [a] Determined by GPC, *theoretical molecular weight.

Table S3. Polymers of PBDG initiated by G5 in DCM.

Entry	Polymers	M_n[a] (M_n[*]) MDa	M_w/ M_n[a]
1	G5-g-PBDG$_{25}$	0.731 (0.729)	1.09
2	G5-g-PBDG$_{50}$	1.47 (1.43)	1.08
3	G5-g-PBDG$_{100}$	2.72 (2.83)	1.06
4	G5-g-PBDG$_{200}$	5.29 (5.61)	1.02
5	G5-g-PBDG$_{400}$	11.4 (11.2)	1.03
6	G5-g-PBDG$_{800}$	29.8 (22.4)	1.03
7	G5-g-PBDG$_{1600}$	45.4 (44.8)	1.03

Polymerizations performed in DCM with $[M]_0$ = 0.05 M. Monomer conversion for all polymerizations was > 99% as measured by FT-IR. [a] Determined by GPC, *theoretical molecular weight.

Table S4. Polymers initiated by G3 or G5 in $CHCl_3$.

Entry	Polymers	M_n[a] (M_n[*]) MDa	M_w/ M_n[a]
1	G3-*g*-PBLG$_{50}$	0.344 (0.353)	1.04
2	G3-*g*-PBLG$_{100}$	0.647 (0.707)	1.04
3	G3-*g*-PBLG$_{200}$	1.22 (1.41)	1.03
4	G3-*g*-PBLG$_{400}$	2.24 (2.81)	1.02
5	G5-*g*-PBLG$_{50}$	1.38 (1.43)	1.06
6	G5-*g*-PBLG$_{100}$	2.68 (2.83)	1.04
7	G5-*g*-PBLG$_{200}$	5.33 (5.63)	1.03
8	G5-*g*-PBLG$_{400}$	10.8 (11.2)	1.04

Polymerizations performed in $CHCl_3$ with $[M]_0$ = 0.05 M. Monomer conversion for all polymerizations was > 99% as measured by FT-IR. [a] Determined by GPC, *theoretical molecular weight.

Table S5. Polymerization of POBLG-NCA initiated by G5 initiator.

Entry	Polymers	M_n[a] (M_n[*]) MDa	M_w/ M_n[a]
1	G5-*g*-PPOBLG$_{25}$	0.991 (0.902)	1.16
2	G5-*g*-PPOBLG$_{50}$	2.02 (1.78)	1.12
3	G5-*g*-PPOBLG$_{100}$	3.93 (3.52)	1.10
4	G5-*g*-PPOBLG$_{200}$	7.96 (7.02)	1.07

Polymerizations performed in DCM. Monomer conversion for all polymerizations was > 99% as measured by FT-IR. [a] Determined by GPC, *theoretical molecular weight.

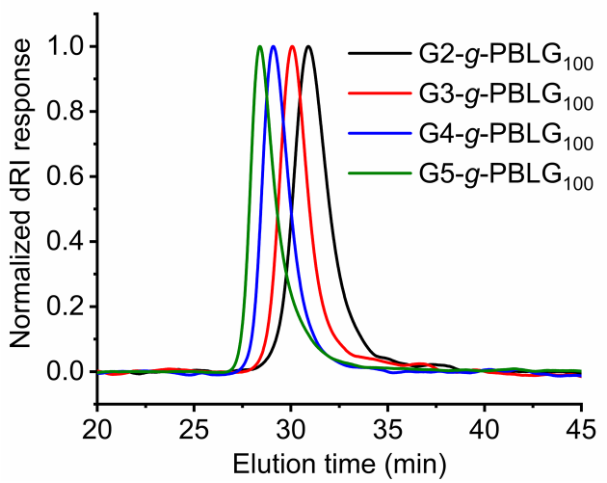


Figure S1. GPC dRI traces of the polymers by PAMAM initiators at $[M]_0/[I]_0$ ratio of 100 (polymerization solvent : DCM).

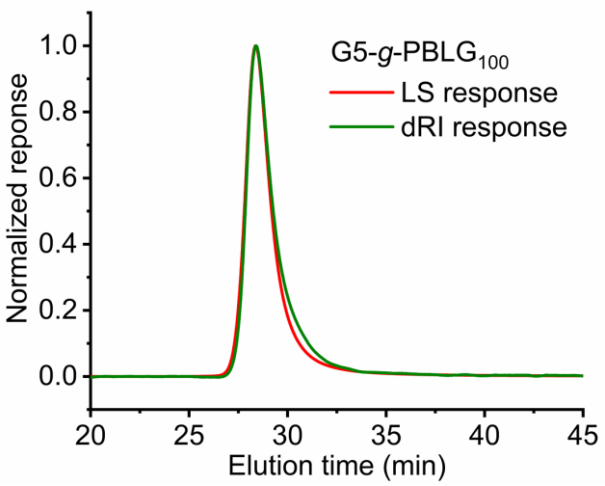


Figure S2. dRI and LS reponses of G5-*g*-$PBLG_{100}$ (polymerization solvent : DCM).

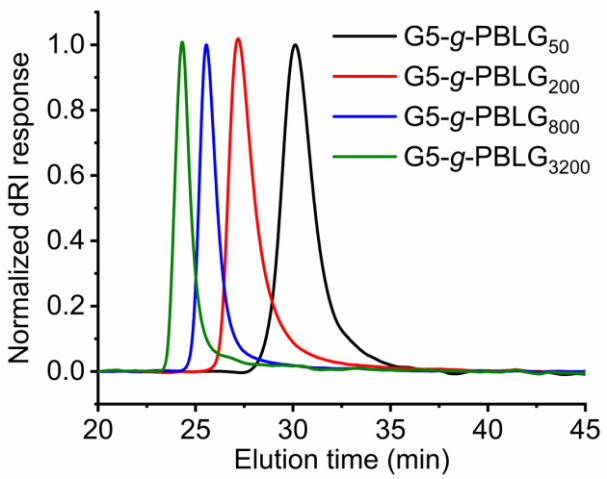


Figure S3. GPC dRI traces of the polymers of varying DP from G5 initiator (polymerization solvent : DCM).

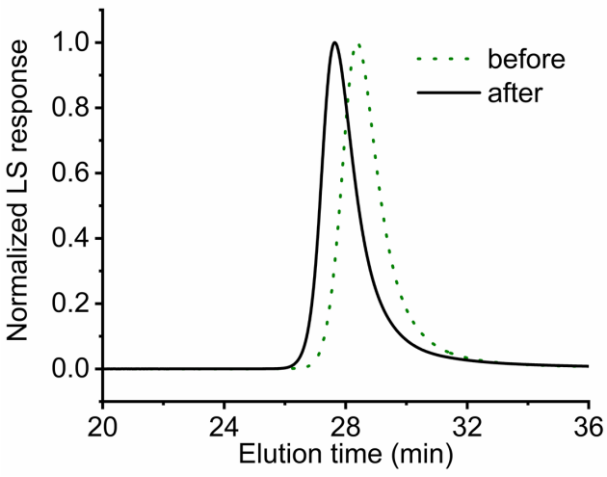


Figure S4. Normalized GPC LS traces of G5-*g*-PBLG$_{100}$ before and after the addition of an additional 100 equiv. of BLG-NCA to the reaction solution.

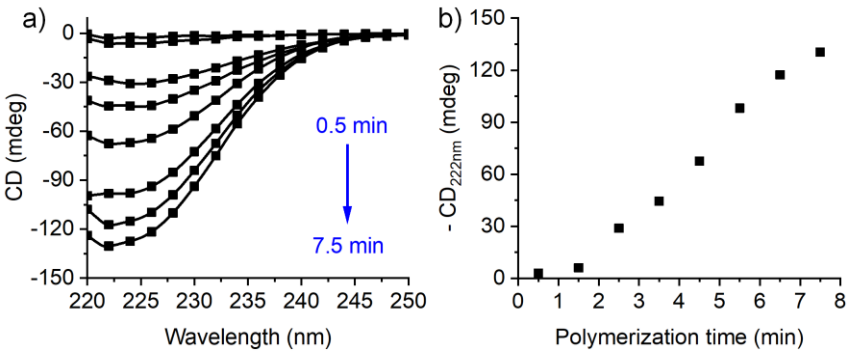


Figure S5. (a), CD measurement of polymerization of BLG-NCA initiated by G5 PAMAM in DCM at the $[M]_0/[I]_0$ ratio of 100. (b), the change in ellipticity as monitored by CD at a wavelength of 222 nm.

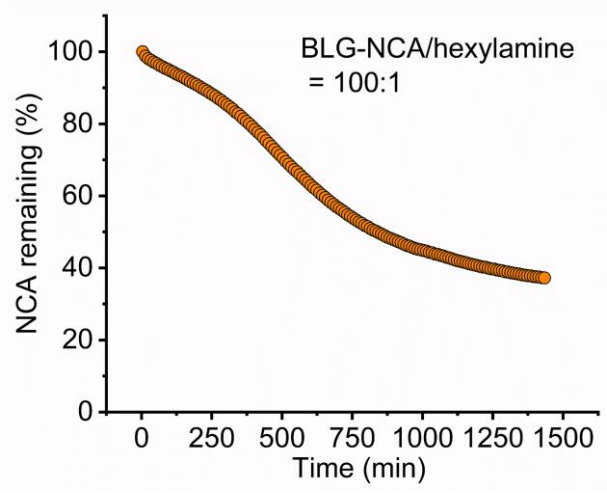


Figure S6. Conversion of BLG-NCA in DCM as measured by FT-IR after initiation with hexylamine ($[M]_0$ = 50 mM).

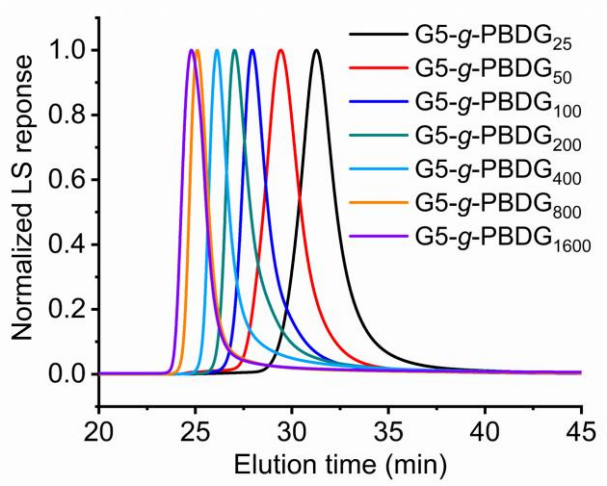


Figure S7. GPC traces of the PBDG polymers from G5 initiator.

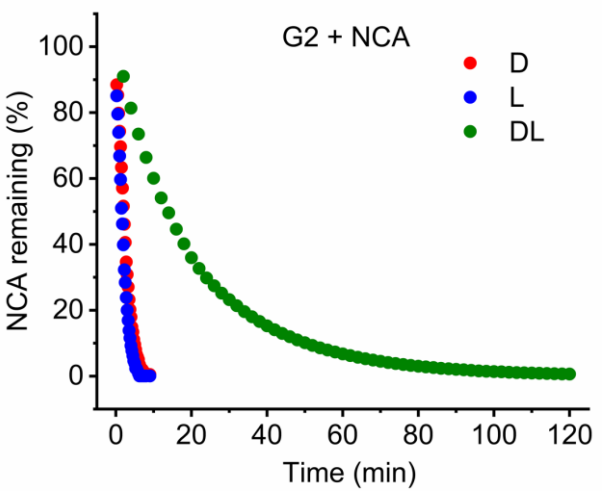


Figure S8. Polymerization of L (BLG-NCA), D (BDG-NCA), and DL (BDLG-NCA) in DCM initiated by G2 initiator ($[M]_0$ = 50 mM).

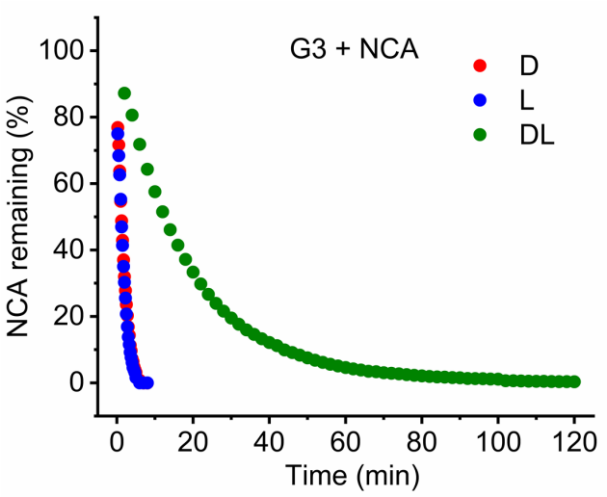


Figure S9. Polymerization of L (BLG-NCA), D (BDG-NCA), and DL (BDLG-NCA) in DCM initiated by G3 initiator ($[M]_0$ = 50 mM).

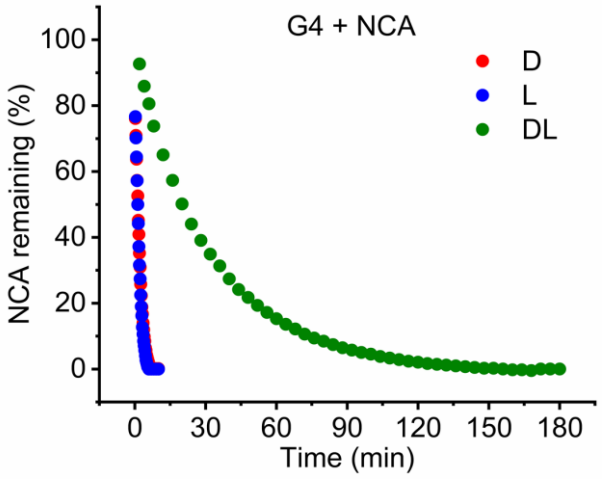


Figure S10. Polymerization of L (BLG-NCA), D (BDG-NCA), and DL (BDLG-NCA) in DCM initiated by G4 initiator ($[M]_0$ = 50 mM).

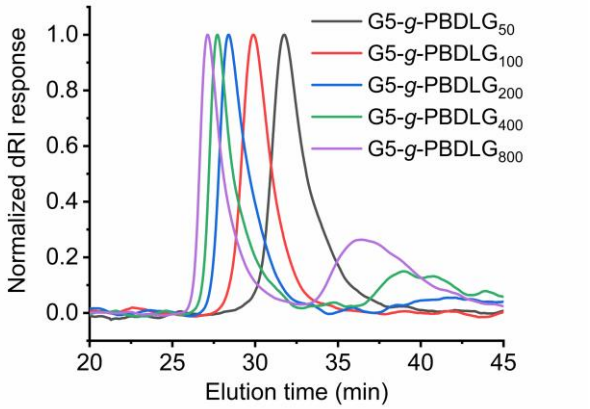


Figure S11. Polymerization of BDLG-NCA initiated by G5 (polymerization solvent : DCM).

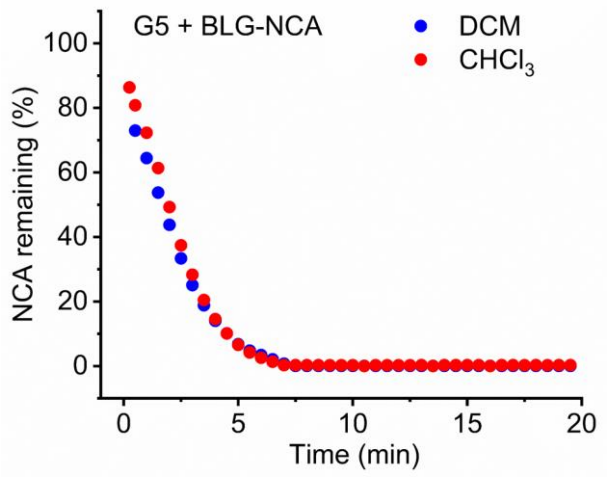


Figure S12. Conversion of BLG-NCA by G5 initiator in DCM or $CHCl_3$ ($[M]_0$ = 50 mM).

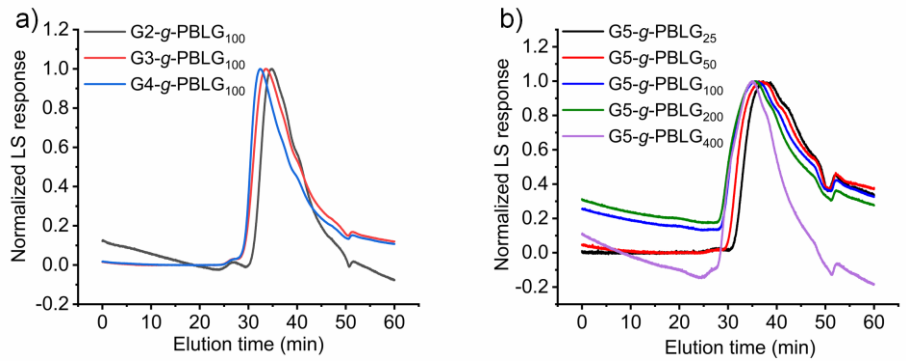


Figure S13. GPC traces of the polymers by various PAMAM initiators at varying DP using DMF as the polymerization solvent.

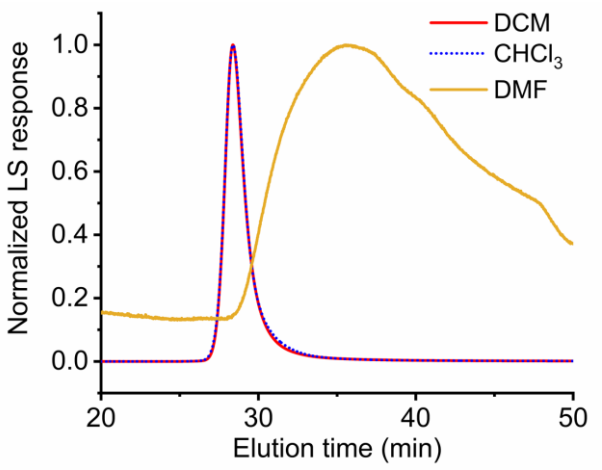


Figure S14. Comparison of LS GPC traces of PBLG polymers initiated by G5 at $[M]_0/[I]_0$ = 100 in different polymerization solvents.

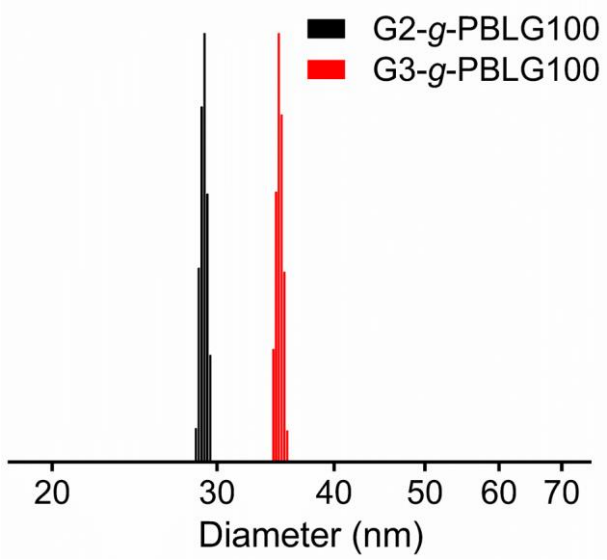


Figure S15. Size distribution of G2/G3-g-PBLG$_{100}$ in DMF by DLS.

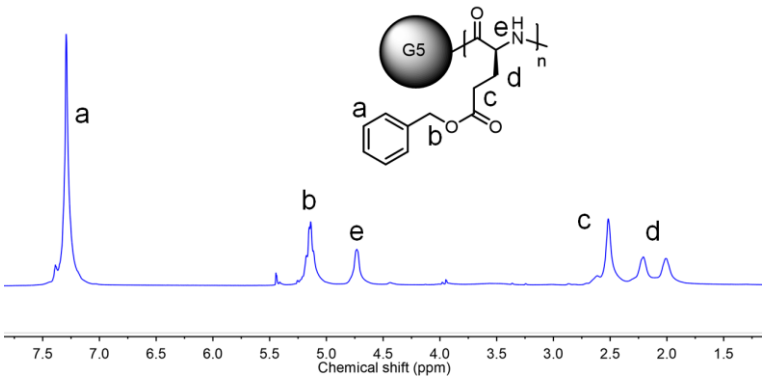


Figure S16. ^{1}H NMR spectrum of G5-*g*-PBLG$_{100}$ in CF_3COOD.

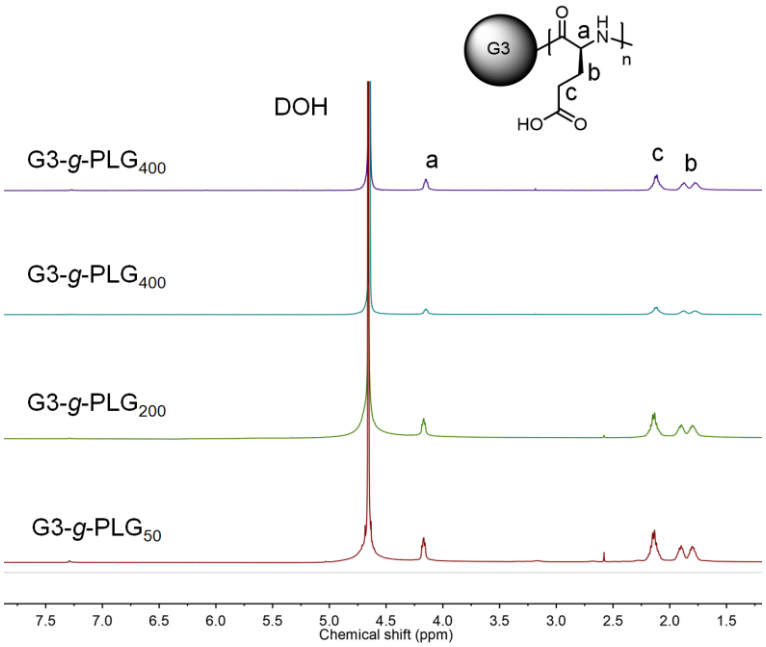


Figure S17. ^{1}H NMR spectra of deprotected G3-*g*-PLG polymers in D_2O.

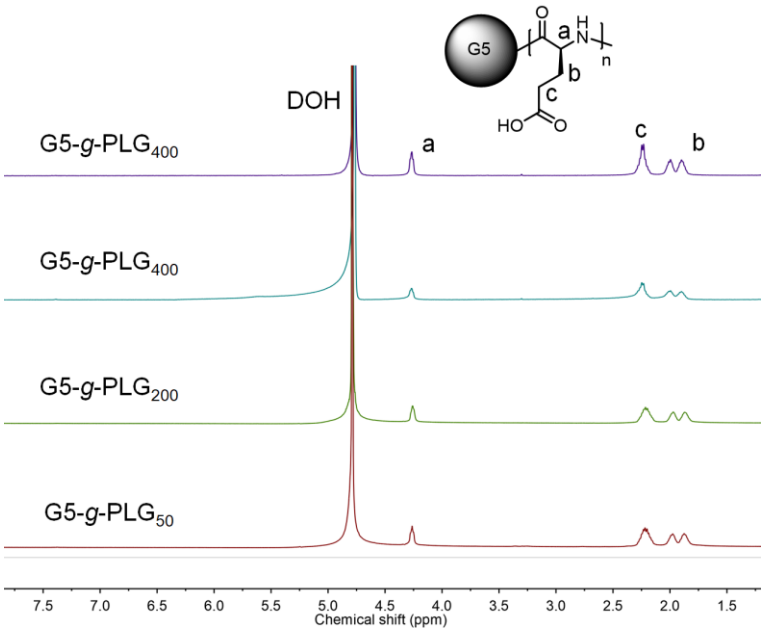


Figure S18. ^{1}H NMR spectra of deprotected G5-g-PLG polymers in D_2O.

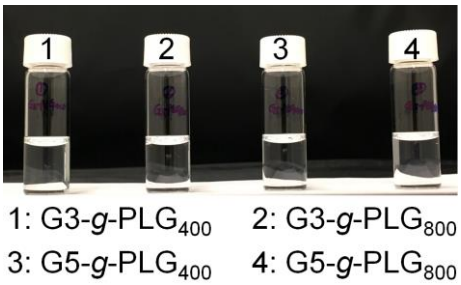


Figure S19. Deprotected polymers in DI water (10 mg mL^{-1}).

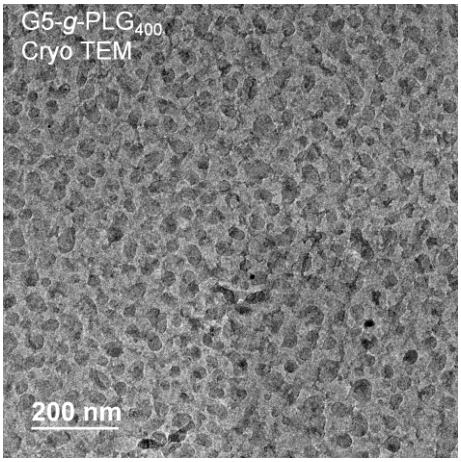


Figure S20. Cryo-TEM image of G5-*g*-PLG_{400} in DI water at 5 mg mL^{-1}.

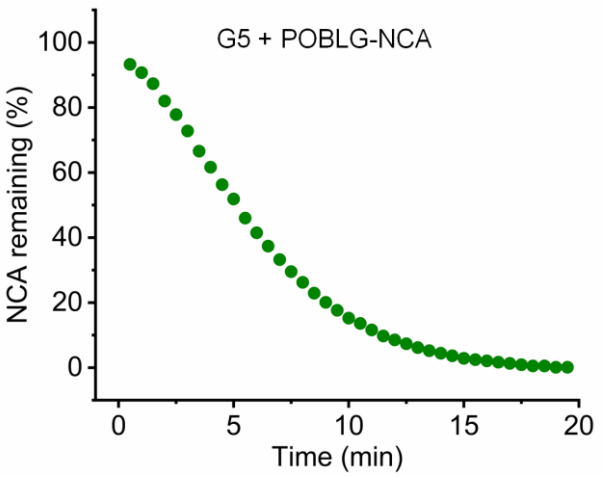


Figure S21. Conversion of POBLG-NCA by G5 initiator in DCM ($[M]_0$ = 50 mM).

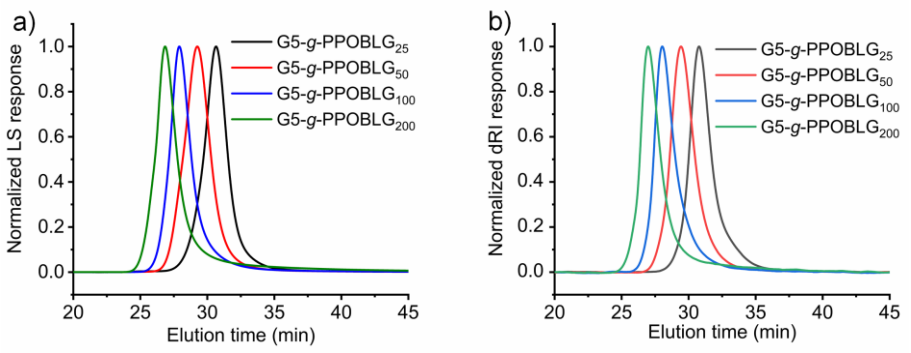


Figure S22. Normalized LS (a) and dRI (b) traces of PPOBLG polymers from G5 at varying DP (polymerization solvent = DCM).

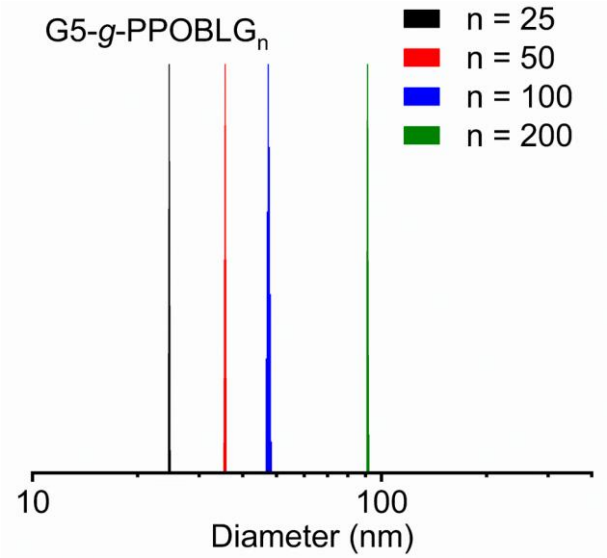


Figure S23. Size distributions of PPOBLG unimolecular micelles in DMF.

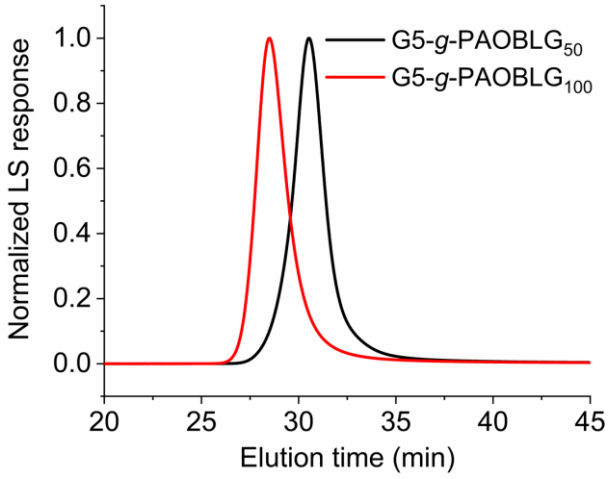


Figure S24. GPC traces of PAOBLG polymers initiated by G5 (polymerization solvent = DCM).

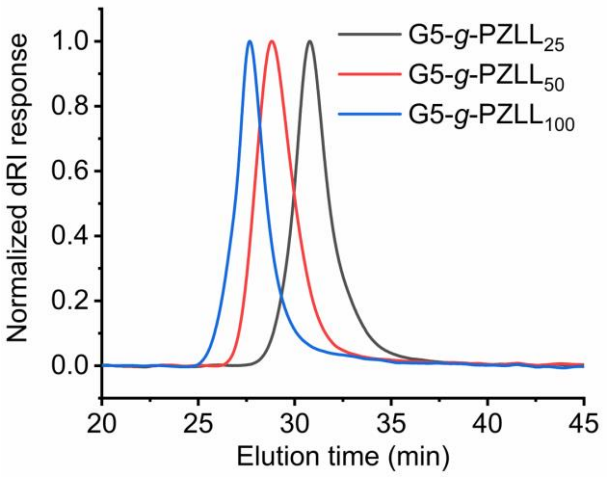


Figure S25. GPC traces of PZLL polymers initiated by G5 (polymerization solvent : DCM).

Reference

(1) (a) Lv, S.; Tang, Z.; Li, M.; Lin, J.; Song, W.; Liu, H.; Huang, Y.; Zhang, Y.; Chen, X. *Biomaterials* **2014**, *35*, 6118-6129. (b) Zhang, Y.; Lu, H.; Lin, Y.; Cheng, J. *Macromolecules* **2011**, *44*, 6641-6644. (c) Zhang, R.; Zheng, N.; Song, Z.; Yin, L.; Cheng, J. *Biomaterials* **2014**, *35*, 3443-3454.

(2) Baumgartner, R.; Kuai, D.; Cheng, J. *Biomater. Sci.* **2017**, *5*, 1836-1844.