Supporting Information

Photobase generators for amino acid *N*-carboxyanhydride ring-opening photopolymerization: Rapid access to degradable polypeptide-based networks

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1. Experimental

1.1 Materials.

All chemicals were obtained from Sigma Aldrich unless otherwise noted. γ-benzyl-L-glutamate, Z-L-lysine, Boc-di-L-cysteine, and triphosgene were purchased from Doug Discovery (Fluorochem).

1.2 Methods.

All NMR spectra were recorded on a Bruker Avance 400 (400 MHz) spectrometer at room temperature using DMSO-d6, $CDCl_3$, TFA-d as solvents. Attenuated total reflection (ATR) FTIR was recorded using a Thermo Scientific iS10 spectrometer in the region of 4000–600 cm^{-1}. Initially, a background measurement was performed before analysing the sample, with 16 scans completed using a resolution of 2 cm^{-1}. Size exclusion chromatography (SEC) was performed in hexafluoroisopropanol (HFIP) using an PSS SECurity GPC system equipped with a PFG 7 µm 8 × 50 mm pre-column, a PSS 100 Å, 7 µm 8 × 300 mm and a PSS 1000 Å, 7 µm 8 × 300 mm column in series and a differential refractive index (RI) detector at a flow rate of 1.0 mL min^{-1}.The system was calibrated against Agilent Easi-Vial linear poly(methyl methacrylate) (PMMA) standards and analysed by the software package PSS winGPC UniChrom.

1.3 Rheology.

Rheological measurements were carried out on an MCR 301 digital rheometer (Anton Paar). All experiments were conducted at room temperature (20 °C) using a parallel plate (PP25, Anton Paar) with a 25 mm diameter geometry and a gap length of 0.095 mm. For photorheology, a 365 nm LED (M365L3-C1, Thorlabs) was installed to conduct photocrosslinking experiments, with the irradiance measured using an optical power meter (PM100D, Thorlabs), fixing the intensity at 8.0 mW/cm^2 at the sample surface. 190 uL of NCA formulation was used, prepared as described below (section 2.9), and a protective hood was employed to prevent evaporation.

1.4 3D photostructuring of polypeptides.

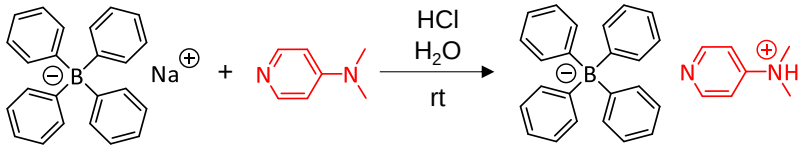
DBU-$HBPh_4$ (3.58 mg, 7.60×10^{-3} mmol, 1 equiv.) and ITX (1.78 mg, 7.05×10^{-3} mmol, 0.93 equiv.) were first dissolved in 380 µL of DMF. A single portion of DLC NCA (111.02 mg, $3.8\cdot10^{-1}$ mmol, 50 equiv.) was added into the vial containing the PBG solution, followed by a brief vortexing step for homogezination. Then, the solution was immediately transferred into silicone moulds and irradiated using a 365 nm LED (8.0 mW/cm^2) at room temperature for 2 h.

1.5 Degradation of crosslinked polypeptides.

Tris(2-carboxyethyl)phosphine.hydrochloride **(**TCEP·HCl) (203 mg, 0.704 mmol, 5 equiv.) was dissolved in 563 µL of 1:1 DMF/H_2O (v/v). The solution was then added into a vial containing the moulded PDLC network (0.14 mmol of S-S bonds, 1 equiv.), which was synthesized from 28.70 mg of PDLC, swollen with 140 µL of DMF, resulting in a final concentration of repeat units or S-S bonds of 0.2 M. After 24 h stirring at 60°C, another 760 µL of DMF and 300 µL of water were added to dilute the system and the degradation was carried out for further 2 h. The resulting product was precipitated in 20 ml of acetone, centrifuged at 9000 RPM for 3 min, and then dissolved into 1 mL of DMF before being precipitated a second time using the same procedure. After the second precipitation, the degradation product was dried for 1 h under vacuum (8.74 mg, 30%).

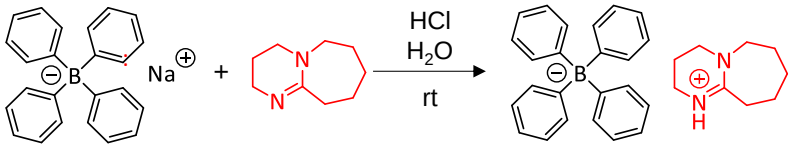
2. Synthetic protocols

2.1 Synthesis of 4-Dimethylaminopyridine tetraphenylborate (DMAP·$HBPh_4$).



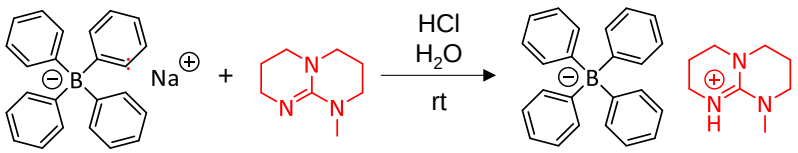
4-Dimethylaminopyridine (DMAP) (1.22 g, 10 mmol, 1 eq.) was dissolved in 10% HCl aqueous solution and dropwise added to a sodium tetraphenylborate (3.76 g, 11 mmol, 1.1 eq.) aqueous solution. The subsequent mixture was filtered, and washed thoroughly with water and chilled MeOH, before drying in vacuo to afford a white solid (3.45 g, 81%).

2.2 Synthesis of 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU·$HBPh_4$).



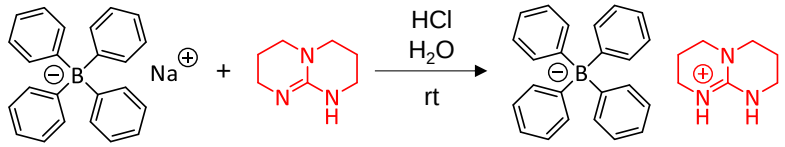
1,8-Diazabicyclo[5.4.0]undec-7-ene (1.52 g, 10.00 mmol, 1 eq.) was dissolved in 10% HCl aqueous solution and dropwise added to a sodium tetraphenylborate (3.76 g, 11.00 mmol, 1.1 eq.) aqueous solution. The subsequent mixture was filtered, and washed thoroughly with water, chilled MeOH and ethyl acetate, before drying in vacuo to afford a white solid. The white solid was then recrystallized from a mixture of 4:1 MeOH/$CHCl_3$ (3.33 g, 71%).

2.3 Synthesis of 7-methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene (MTBD·$HBPh_4$).



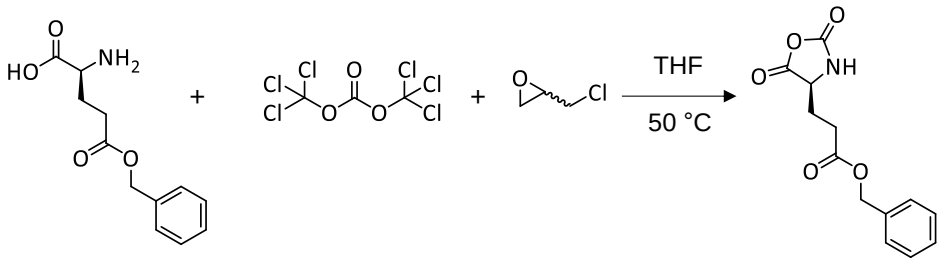
7-methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene (1.54 g, 10.00 mmol, 1 eq.) was dissolved in 10% HCl aqueous solution and dropwise added to a sodium tetraphenylborate (3.76 g, 11 mmol, 1.1 eq.) aqueous solution. The subsequent mixture was filtered, and washed thoroughly with water and chilled MeOH, before drying in vacuo to afford a white solid (3.54 g, 75 %).

2.4 Synthesis of 1,5,7-Triazabicyclo[4.4.0]dec-5-ene (TBD·HBPh$_4$).



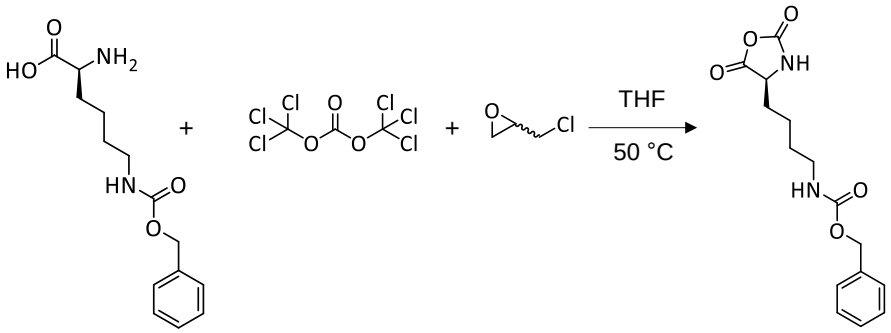
1,5,7-Triazabicyclo[4.4.0]dec-5-ene (1.39 g, 10.00 mmol, 1 eq.) was dissolved in 10% HCl aqueous solution and dropwise added to a sodium tetraphenylborate (3.76 g, 11 mmol, 1.1 eq.) aqueous solution. The subsequent mixture was filtered, and washed thoroughly with water and chilled MeOH, before drying in vacuo to afford a white solid (3.63 g, 79 %).

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



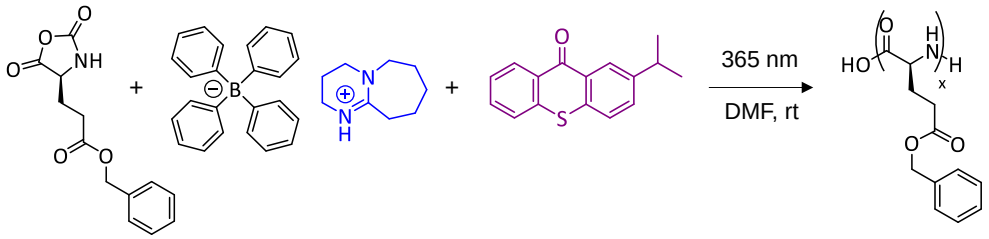
Triphosgene (18.76 g, 63.22 mmol, 0.5 equiv.) and (±)-epichlorohydrin (46.80 g, 505.80 mmol, 4 equiv.) were initially dissolved in 400 mL of THF in a round-bottomed flask with stirring. γ-benzyl-L-glutamate (30.00 g, 126.45 mmol, 1 equiv.) was then added in one portion and the reaction suspension was heated under reflux (70 °C). The reaction was continued until all solids disappeared and the solution became clear (1.5 h). The solution was then cooled using N_2, any unreacted γ-benzyl-L-glutamate was filtered off and the solution was reduced to 1/3 of its original volume in vacuo. The NCA was precipitated by addition of 500 mL of hexane and stored overnight at -18 °C to fully precipitate. The solid NCA was dried in vacuo and then re-dissolved in 200 mL ethyl acetate. This solution was then precipitated into excess hexane (900 mL), filtered and dried (this was completed two to three times). It was then dried in vacuo to afford a white fluffy solid (yield: 28.40 g, 85%).

2.6 Synthesis of ε-carbobenzyloxy-L-lysine *N*-carboxyanhydride (ZLL NCA).



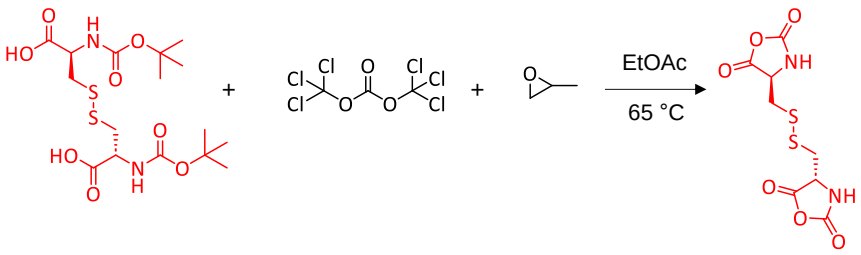
Triphosgene (7.94 g, 26.76 mmol, 0.5 equiv.) and (±)-epichlorohydrin (19.80 g, 214.04 mmol, 4.0 equiv.) were initially dissolved in 150 mL of THF in a round-bottomed flask with stirring. ε-carbobenzyloxy-L-lysine (Z-L-lysine) (15.00 g, 53.51 mmol, 1 equiv.) was then added in one portion and the reaction suspension was heated under reflux (70 °C). The reaction was continued until all solids disappeared and the solution became clear (2-3 h). The solution was then cooled using N_2, any unreacted Z-L-lysine was filtered off and the solution was reduced to 1/3 of its original volume in vacuo. The NCA was precipitated by addition of 350 mL of hexane and stored overnight at -18 °C to fully precipitate. The solid NCA was dried in vacuo and then re-dissolved in 100 mL ethyl acetate. This solution was then precipitated into excess hexane (500 mL), filtered and dried (this was completed two to three times). It was then dried in vacuo to afford a white powder (yield: 13.60 g, 83%).

2.7 Synthesis of poly(benzyl-L-glutamate) (PBLG) via NCA photo-ROP (0.4 M).



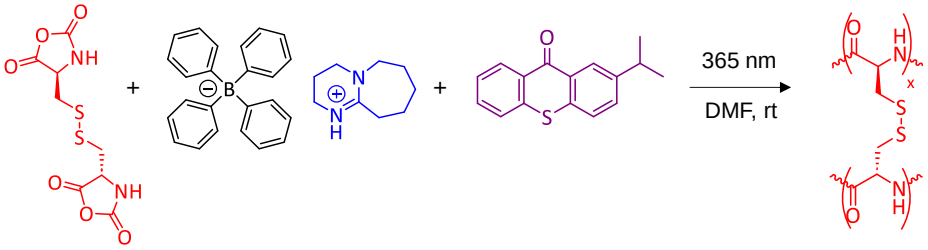
$DBU{\cdot}HBPh_4$ (7.17 mg, 1.52×10^{-2} mmol, 1 equiv.) and ITX (3.59 mg, 1.41×10^{-2} mmol, 0.93 equiv.) were first dissolved in 380 µL of DMF. BLG NCA (200.00 mg, 7.59×10^{-1} mmol, 50 equiv.) was then added in one portion to the PBG solution, followed by a brief vortexing step for homogezination, then immediate irradiation using a 365 nm LED (8.0 mW/cm^2) (no stirring). NCA conversion was tracked by quantifying the reduction of anhydride peaks via FTIR spectroscopy at different timepoints. The homopolypeptide was then precipitated into excess diethyl ether (2 mL) and dried in vacuo (yield: 136.50 mg, 82%).

2.8 Synthesis of di-L-cysteine *N*-carboxyanhydride (DLC NCA).



Triphosgene (4.55 g, 15.32 mmol, 1.35 equiv.) and (±)-propylene oxide (1.97 g, 34.05 mmol, 3 equiv.) were initially dissolved in 100 mL of EtOAc in a round-bottomed flask with stirring. Boc-di-L-cysteine (5.00 g, 11.35 mmol, 1 equiv.) was then added in one portion and the reaction suspension was heated (70 °C) with stirring. The reaction was continued until all solids disappeared and the solution became clear (5 h). The solution was then cooled using an N_2 balloon, and it was then filtered into 400 mL hexane, and stored overnight at -18 °C to fully precipitate. The hexane was decanted, and the NCA residue was then redissolved in 50 mL EtOAc, filtered again into 300 mL cold hexane to precipitate the NCA. The solid was then filtered and dried in vacuo to yield an off-white powder (yield: 2.10 g, 63%).

2.9 Synthesis of crosslinked poly(di-L-cysteine) PDLC via NCA photo-ROP (1 M).



$DBU{\cdot}HBPh_4$ (1.79 mg, 3.79×10^{-3} mmol, 1 equiv.) and ITX (0.89 mg, 3.50×10^{-3} mmol 0.93 equiv.) were first dissolved in 190 µL of DMF. DLC NCA (55.51 mg, 1.90×10^{-1} mmol, 50 equiv.) was then added in one portion to the PBG solution, followed by a brief vortexing step for homogezination, and then immediate irradiation using a 365 nm LED (no stirring) (8.0 mW/cm^2). NCA conversion was tracked by quantifying the reduction of anhydride peaks via FTIR spectroscopy at different timepoints. The polypeptide was then placed in acetone (1 mL) to remove DMF, which was repeated twice, followed by drying (yield: 38.20 mg, 81%).

3. Additional figures

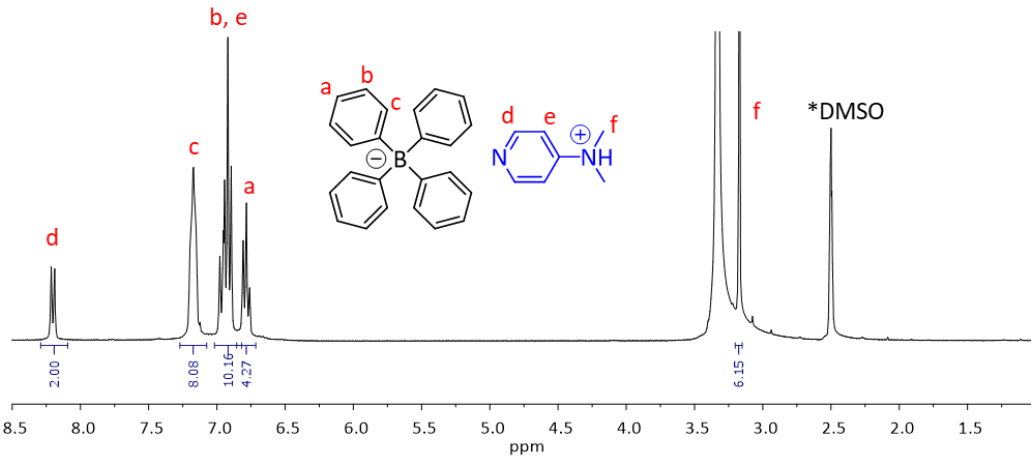


Figure S1. ^{1}H NMR spectrum of DMAP·HBPh$_4$ (DMSO-d6).

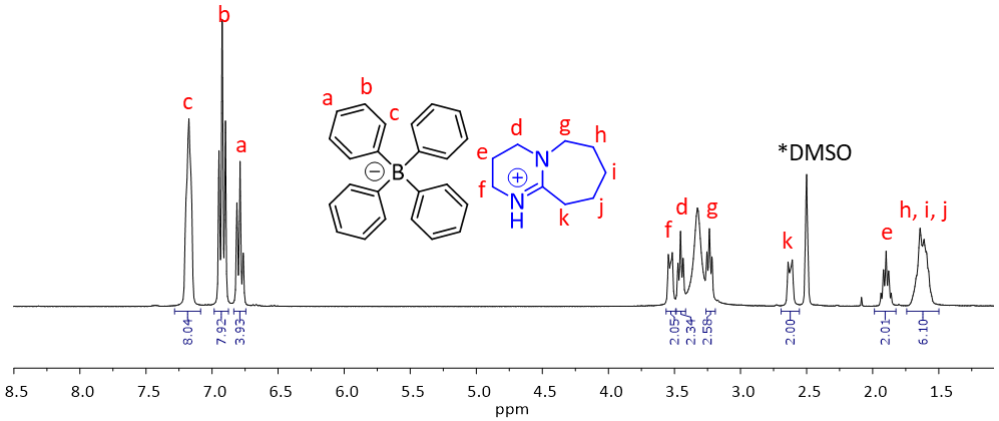


Figure S2. ^{1}H NMR spectrum of DBU·HBPh$_4$ (DMSO-d6).

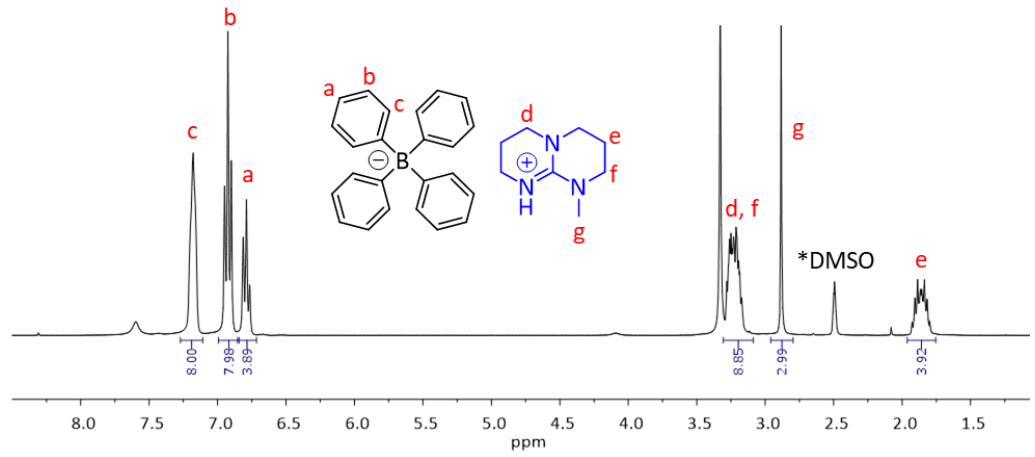


Figure S3. ^{1}H NMR spectrum of MTBD·HBPh$_4$ (DMSO-d6).

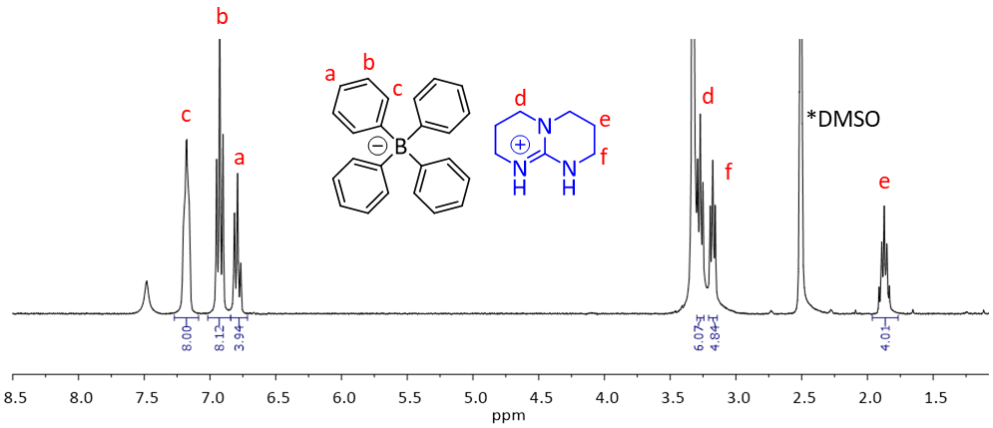


Figure S4. ^{1}H NMR spectrum of TBD·HBPh$_4$ (DMSO-d6).

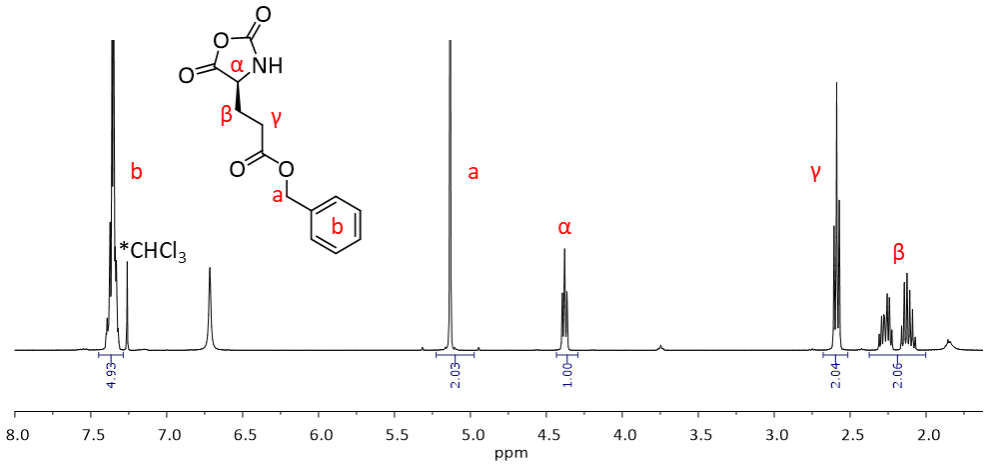


Figure S5. ^{1}H NMR spectrum of BLG NCA (CDCl$_3$/TFA-d).

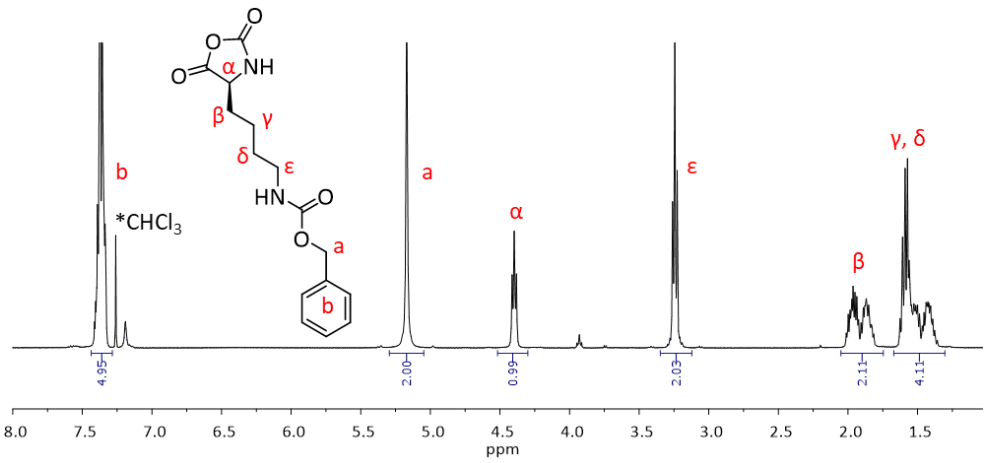


Figure S6. ^{1}H NMR spectrum of ZLL NCA (CDCl$_3$/TFA-d).

Table S1. Experimental conditions for polymerization of BLG NCA and characteristics of synthesized PBLGs.

Entry	$[M]_0$[a]	Solvent	Base	$[M]_0/[I]_0$	λ (nm)[b]	Time (min)[c]	M_n[d]	M_n[e]	$Đ$[f]
1	0.4	DMF	DMAP·$HBPh_4$	50	365	105	11.0	8.8	1.14
2	0.4	DMF	MTBD·$HBPh_4$	50	365	30	11.0	7.5	1.21
3	0.4	DMF	TBD·$HBPh_4$	50	365	30	11.0	6.2	1.25
4	0.4	DMF	DBU·$HBPh_4$	50	365	30	11.0	6.5	1.18
5	1	DMF	DBU·$HBPh_4$	50	365	12	11.0	9.3	1.27
6	2	DMF	DBU·$HBPh_4$	50	365	8	11.0	9.8	1.41
7	4	DMF	DBU·$HBPh_4$	50	365	8	11.0	12.1	1.38
8	2	DMF	DBU·$HBPh_4$	50	405	12	11.0	12.0	1.24
9	2	DMF	DBU·$HBPh_4$	50	n/a[f]	396	11.0	11.0	1.37
10	2	DMAc	DBU·$HBPh_4$	50	365	10	11.0	11.1	1.49
11	2	DMSO	DBU·$HBPh_4$	50	365	6	11.0	7.1	1.42
12	2	NMP	DBU·$HBPh_4$	50	365	15	11.0	10.4	1.26
13	2	DMF	DBU·$HBPh_4$	25	365	3	5.5	5.7	1.39
14	2	DMF	DBU·$HBPh_4$	10	365	1.5	2.2	5.2	1.26
15	2	NMP	DBU·$HBPh_4$	25	365	9	5.5	6.1	1.15
16	2	NMP	DBU·$HBPh_4$	10	365	1.5	2.2	4.9	1.19

[a]Monomer concentration (M). [b]Wavelength of LED. [c]Time to reach >90% conversion. [d]Theoretical number average molecular weight (kg/mol). [e]Number average molecular weight as measured by SEC (kg/mol). [f]Dispersity as measured by SEC. [f]Left in darkness.

Figure S7. Image of set up for NCA photo-ROP experiments.

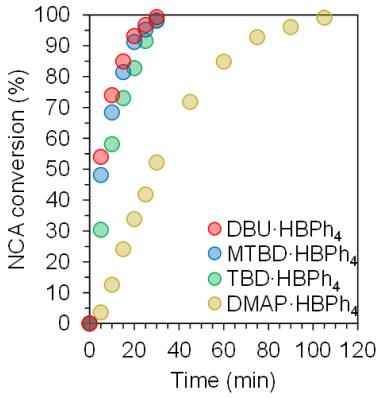


Figure S8. Comparison of conversion of BLG NCA over time with different PBGs ([M] = 0.4 M, $[M]_0/[I]_0 = 50$) in DMF.

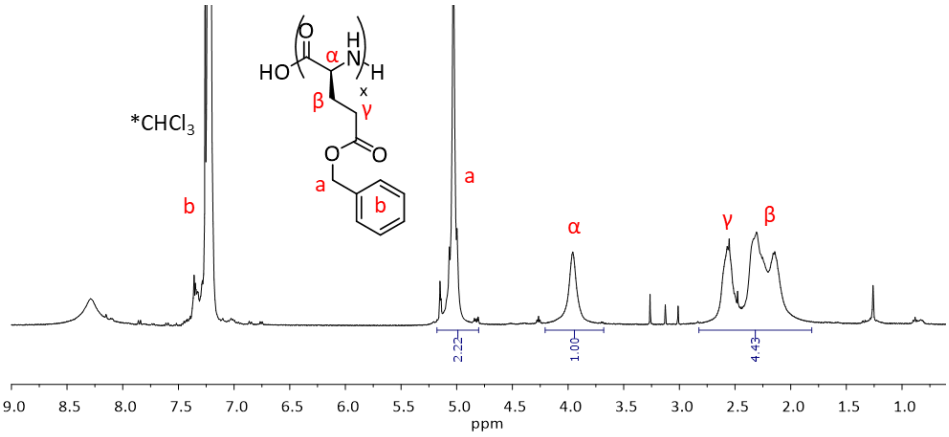


Figure S9. ^{1}H NMR spectrum of $PBLG_{50}$ from DMAP·$HBPh_4$ NCA ROP ($CDCl_3$/TFA-d).

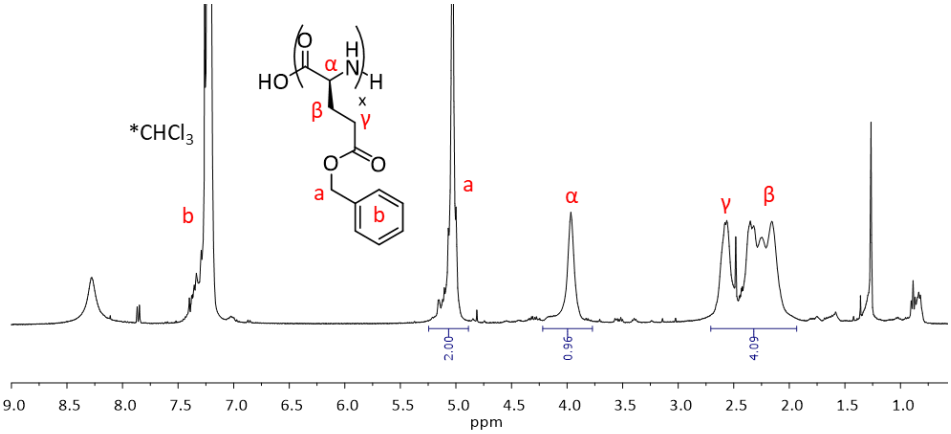


Figure S10. ^{1}H NMR spectrum of $PBLG_{50}$ from DBU·$HBPh_4$ NCA ROP ($CDCl_3$/TFA-d).

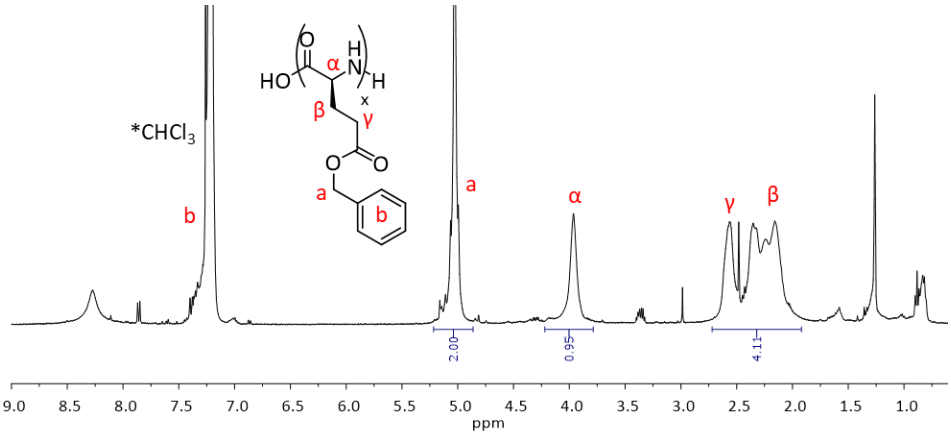


Figure S11. ^{1}H NMR spectrum of $PBLG_{50}$ from MTBD·$HBPh_4$ NCA ROP ($CDCl_3$/TFA-d).

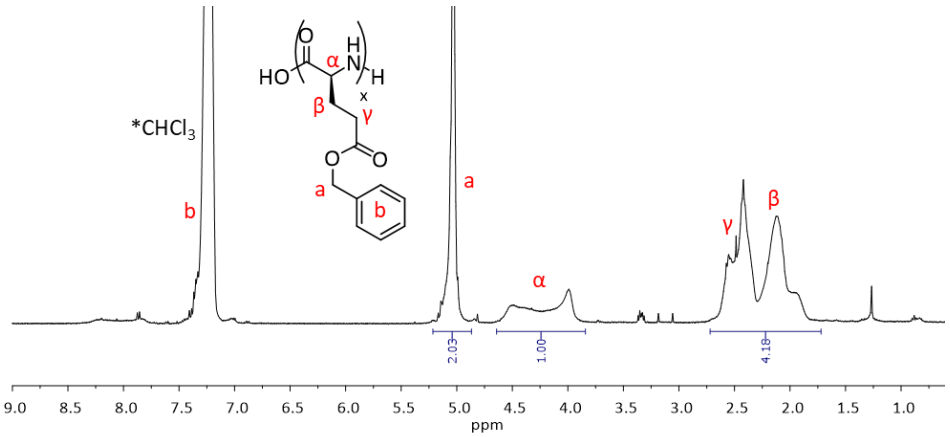


Figure S12. ^{1}H NMR spectrum of $PBLG_{50}$ TBD·$HBPh_4$ NCA ROP ($CDCl_3$/TFA-d).

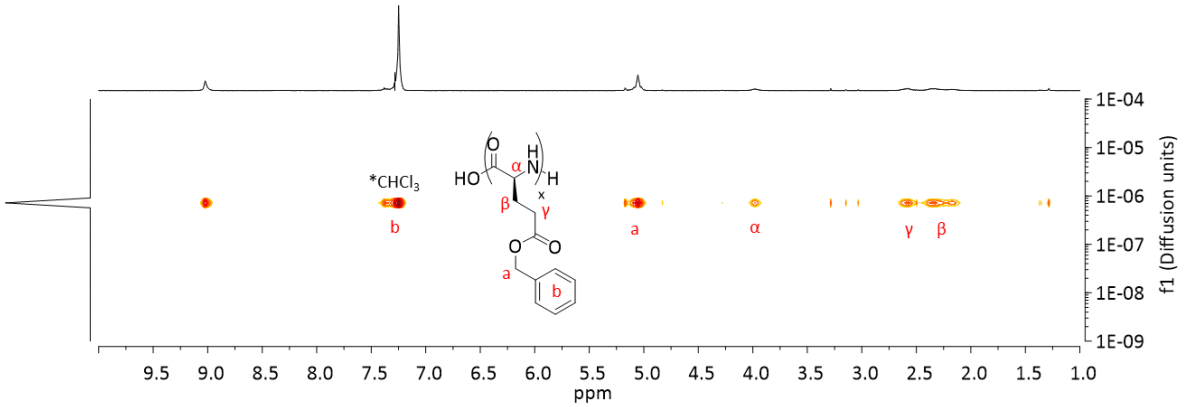


Figure S13. DOSY NMR spectrum of $PBLG_{50}$ from DMAP·$HBPh_4$ NCA ROP ($CDCl_3$/TFA-d).

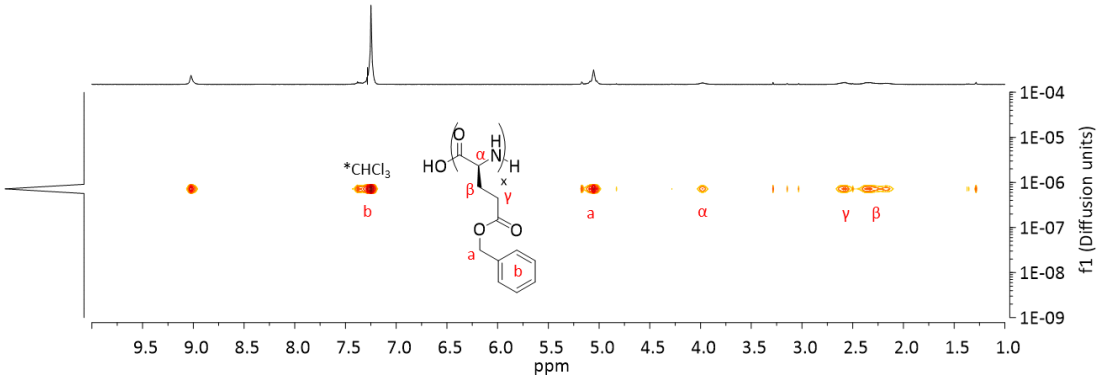


Figure S14. DOSY NMR spectrum of $PBLG_{50}$ from DBU·$HBPh_4$ NCA ROP ($CDCl_3$/TFA-d).

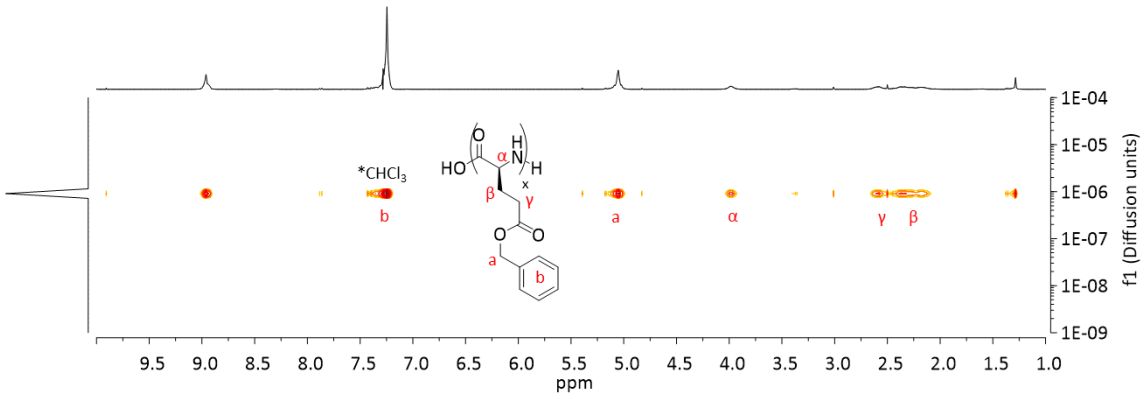


Figure S15. DOSY NMR spectrum of $PBLG_{50}$ from TBD·$HBPh_4$ NCA ROP ($CDCl_3$/TFA-d).

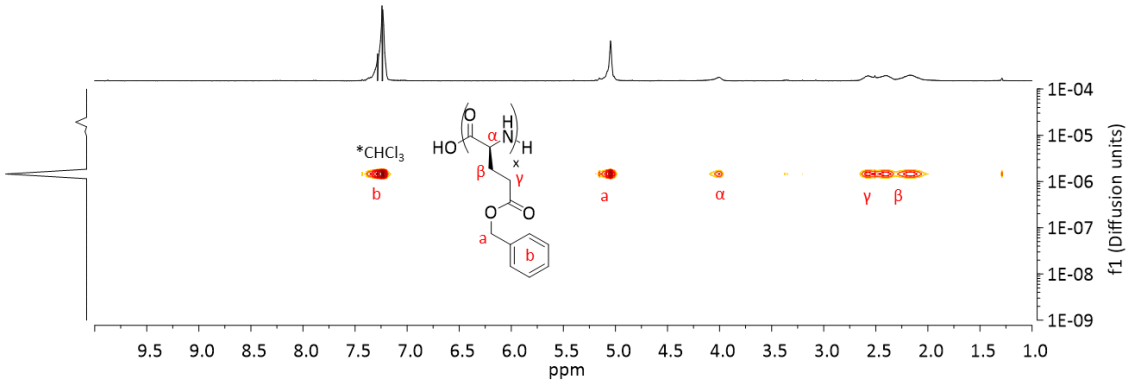


Figure S16. DOSY NMR spectrum of $PBLG_{50}$ from TBD·$HBPh_4$ NCA ROP ($CDCl_3$/TFA-d).

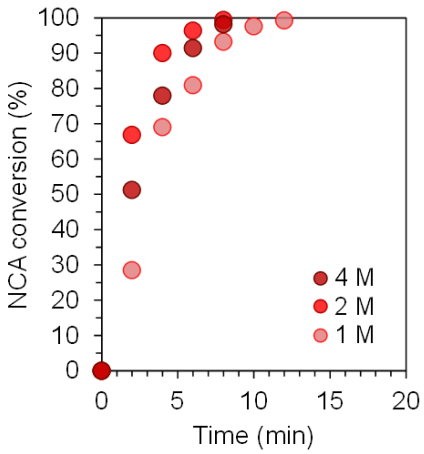


Figure S17. (A) Comparison of conversion of BLG NCA using DBU·$HBPh_4$/ITX PBG at different molarities in DMF ([M] = 1, 2, 4 M, and $[M]_0/[I]_0 = 50$).

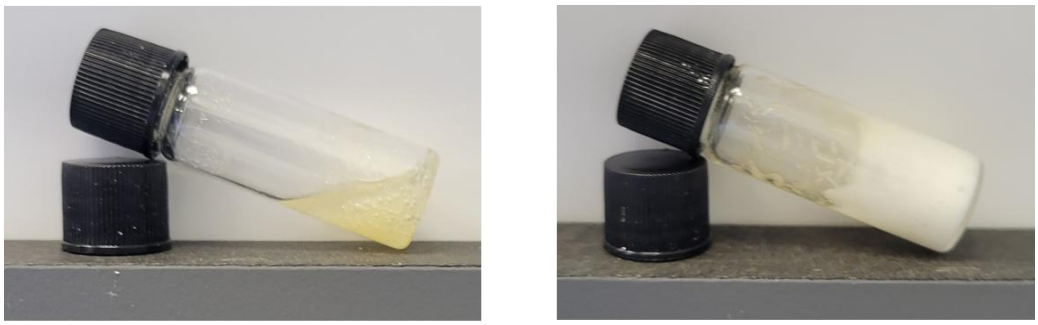


Figure S18. Images of BLG NCA photopolymerization at concentrations of 2 M and 4 M in DMF ($[M]_0/[I]_0 = 50$), showing polymer precipitation in 4 M.

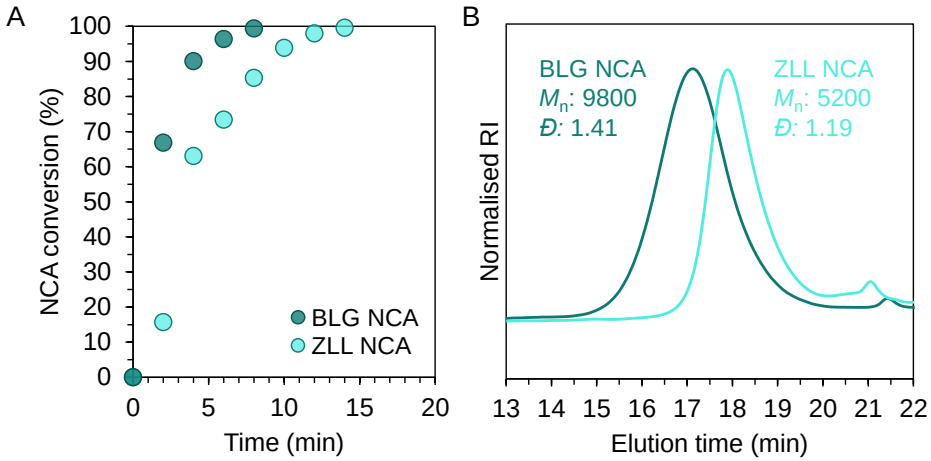


Figure S19. (A) Comparison of conversion of ZLL NCA and BLG NCA over time, and (B) normalized SEC traces of corresponding polypeptides ([M] = 2 M, $[M]_0/[I]_0 = 50$).

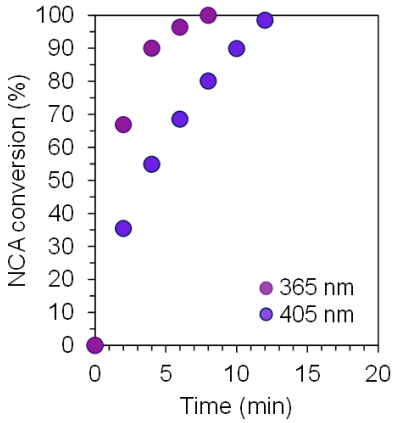


Figure S20. (A) Comparison of conversion of BLG NCA photopolymerization using DBU·$HBPh_4$/ITX at different wavelengths (365 nm, 405 nm) in DMF ([M] = 2 M, $[M]_0/[I]_0 = 50$).

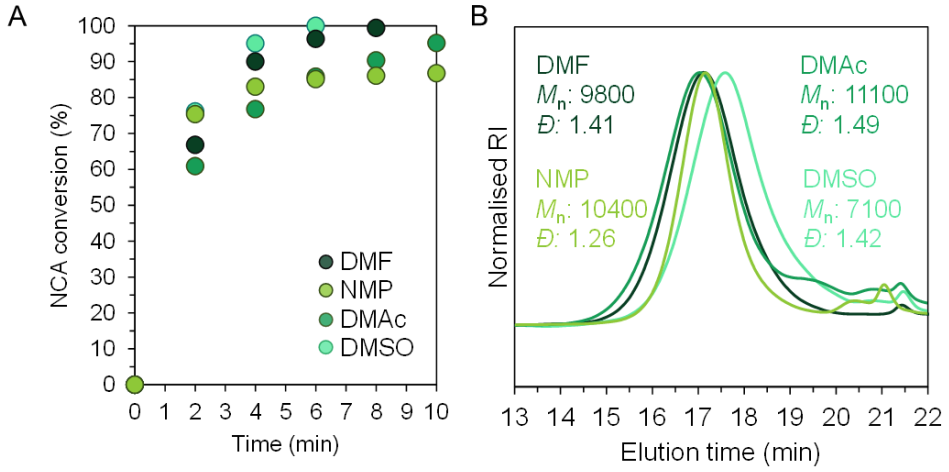


Figure S21. (A) Conversion of BLG NCA over time using different solvents (DMF, NMP DMAc, DMSO), and (B) normalized SEC traces of corresponding polypeptides ([M] = 2 M, $[M]_0/[I]_0 = 50$).

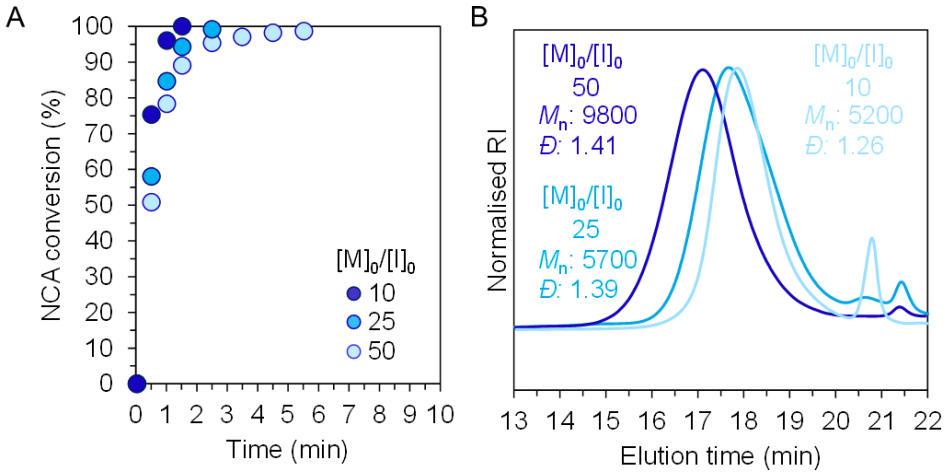


Figure S22. (A) Conversion of BLG NCA over time using DBU·HBPh4/ITX PBG at different monomer/initiator ratios ($[M]_0/[I]_0$ = 50, 25, 10) in DMF, and (B) normalized SEC traces of corresponding polypeptides ([M] = 2 M).

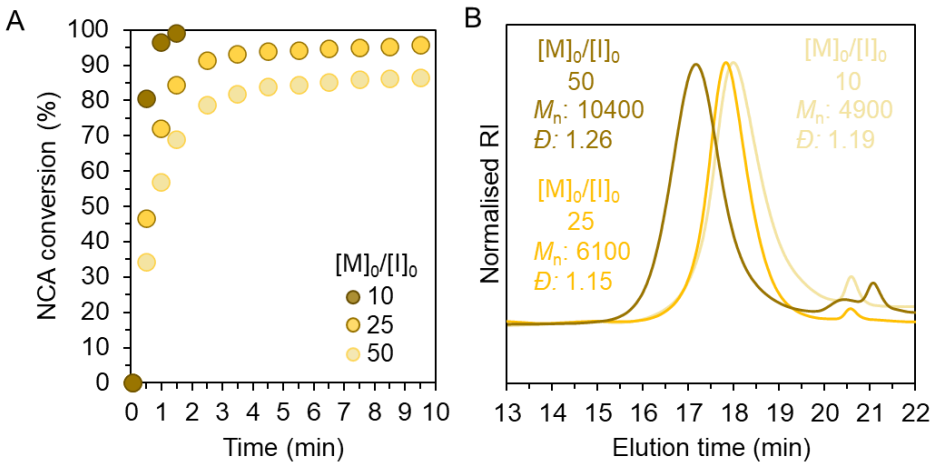


Figure S23. (A) Conversion of BLG NCA over time using DBU·HBPh4/ITX PBG at different monomer/initiator ratios ($[M]_0/[I]_0$ = 50, 25, 10) in NMP, and (B) normalized SEC traces of corresponding polypeptides ([M] = 2 M).

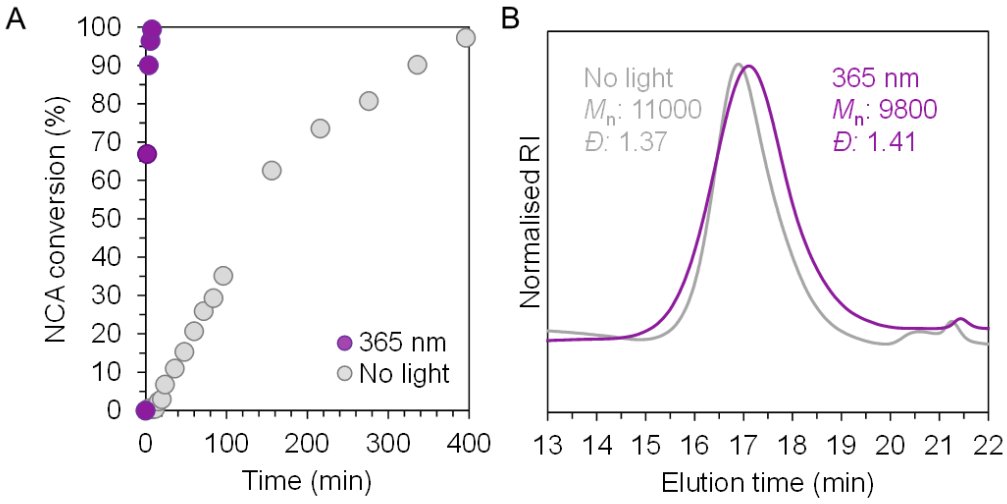


Figure S24. (A) Conversion of BLG NCA over time using 365 nm light and in darkness in DMF, and (B) normalized SEC traces of corresponding polypeptides ([M] = 2 M, $[M]_0/[I]_0$ = 50).

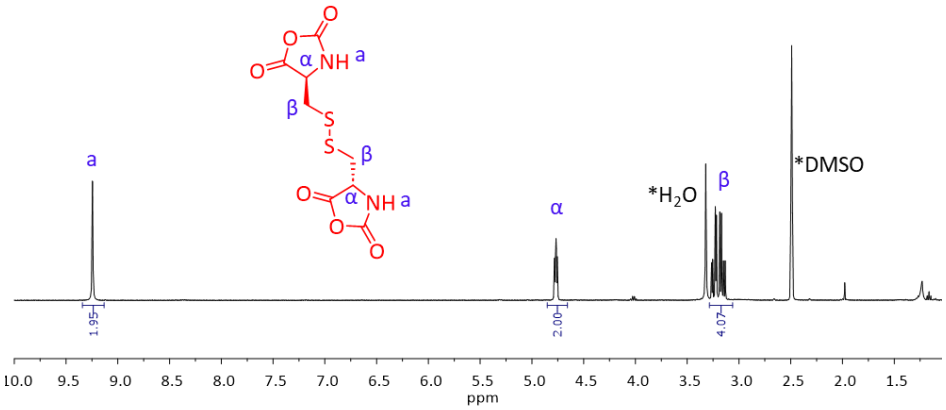


Figure S25. ^{1}H NMR spectrum of DLC NCA (DMSO-d).

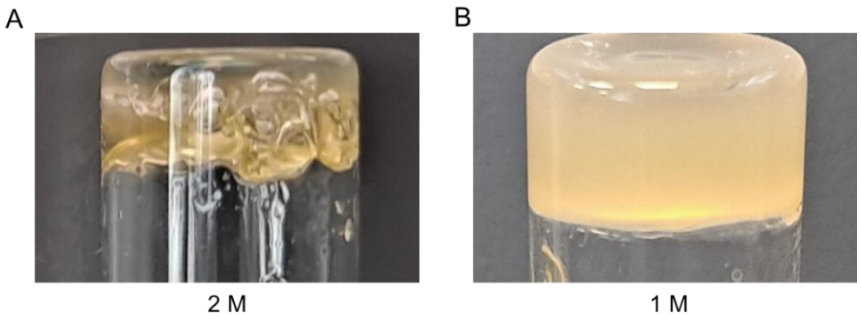


Figure S26. (A) Image of CO_2 bubbles from photo-ROP of DLC NCA ([M] = 2 M, $[M]_0/[I]_0 = 50$), and (B) image of gel without bubbles from photo-ROP of DLC NCA ([M] = 1 M, $[M]_0/[I]_0 = 50$).

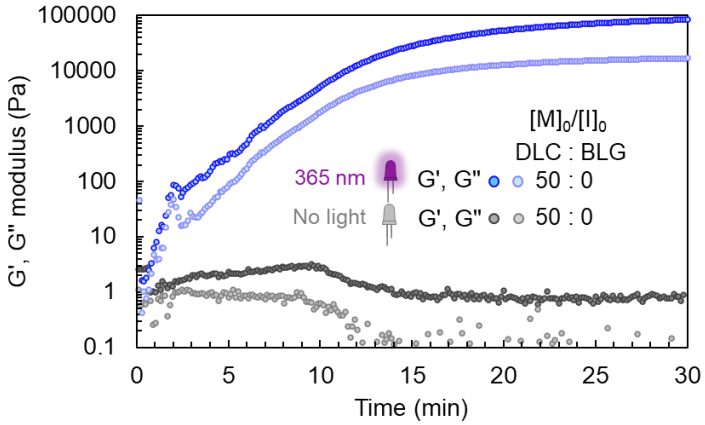


Figure S27. Photo-rheology measurements in the presence (365 nm, 8.0 mW/cm^2) and absence of light irradiation for DLC NCA resin ([M] = 1 M, [M]0/[I]0 = 50).

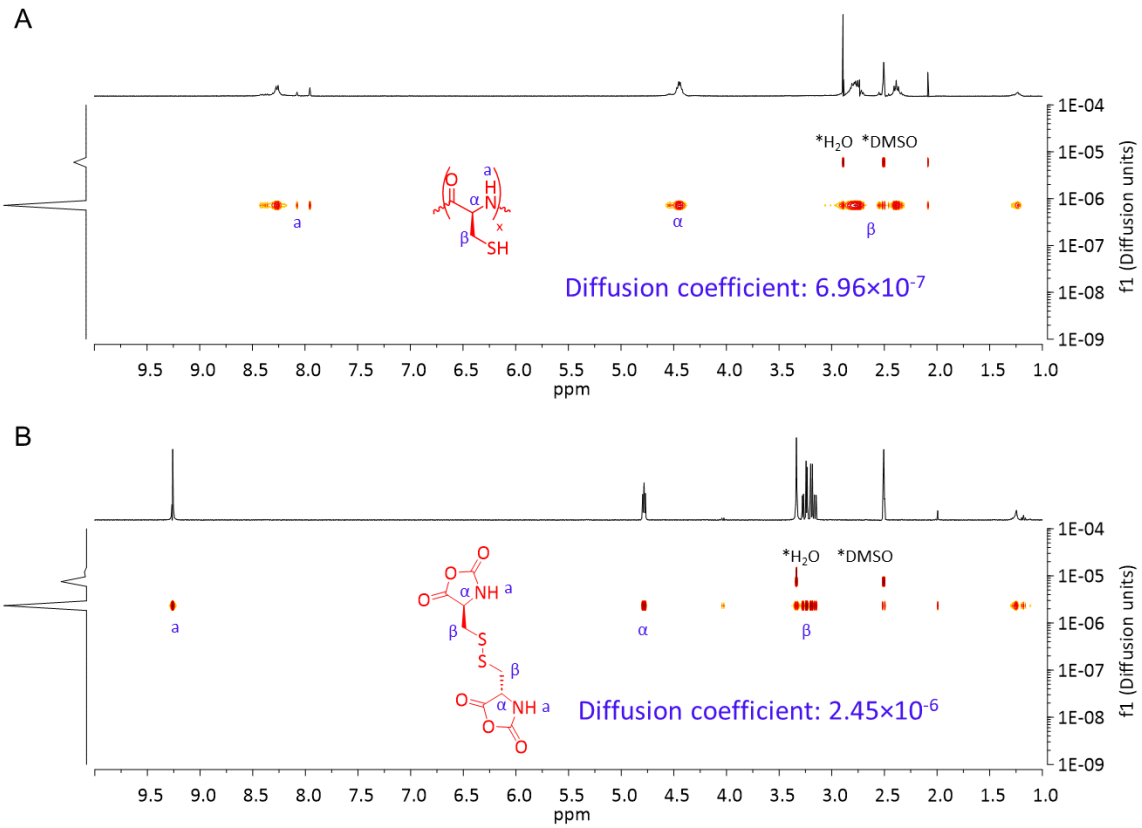


Figure S28. (A) DOSY NMR spectrum of PLC after reduction of PDLC network (DMSO-d), and (B) DOSY NMR spectrum of DLC NCA (DMSO-d).