Supporting information

From competition to cooperation: a highly efficient strategy towards well-defined (co)polypeptides

Wei Zhao,[a] Yves Gnanou[b] and Nikos Hadjichristidis*[,a]

[a] Physical Sciences and Engineering Division, KAUST Catalysis Center, Polymer Synthesis Laboratory,

[b] Physical Sciences and Engineering Division, King Abdullah University of Science and Technology (KAUST), Thuwal 23955, Saudi Arabia

E-mail: nikolaos.hadjichristidis@kaust.edu.sa.

General Methods. All reactions were carried out under a dry and oxygen-free argon atmosphere by using Schlenk techniques or under an argon atmosphere in an MBraun glovebox. Solvents were purified using a MBraun SPS system. Anhydrous dimethylformamide (DMF) was further dried by passing through an aluminum column. Anhydrous DMSO-d_6 was further dried over calcium hydride at 70°C under Ar overnight followed by distillation under reduced pressure. All other liquids were dried over activated 4 Å molecular sieves for a week and distilled before use, and solid materials were used as received. All purified reagents were stored over 4Å molecular sieves in a glove box. H-Glu(OBn)-OH and H-Lys(Z)-OH were purchased from Sigma-Aldrich and used as received. Glu-NCA and Lys-NCA were prepared and recrystallized four times according to published procedures.[1]

Instruments and Measurements. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV400 (FT, 400 MHz for ^{1}H; 100 MHz for ^{13}C) spectrometer. NMR assignments were confirmed by ^{1}H-^{1}H (COSY), ^{1}H-^{13}C (HMQC), and ^{13}C NMR (DEPT) experiments when necessary. *Operando* IR study of NCA polymerization was carried out by using ReactIR 45m with MCT Detector from METTLER TOLEDO

AutoChem. DiComp (Diamond) probe was connected via AgX 9.5mm x 1.5m Fiber (Silver Halide). Sampling is from 2800cm^{-1} to 650cm^{-1} at 8 wavenumber resolution and the automatic sampling interval is one minute. The real-time concentration of NCA was quantified by measuring the intensity of NCA's anhydride peak at 1787 cm^{-1} by FT-IR. The conversion of NCA was determined by comparing the NCA concentration during polymerization with the NCA concentration at t = 0. Polymer characterizations were carried out by combining an Agilent 1260 infinity SEC instrument with a multiangle laser light scattering (MALLS) apparatus at 60 °C. The system equipped with three Styragel® columns, a 1200 HPLC pump, an Optilab T-rEX RI detector, a ViscoStar-II viscometer and a DAWN HELEOS-II multiangle laser-light scattering (MALLS) detector at a laser wavelength of 690 nm (from Wyatt Technology). One guard column and three 7.8×300 mm columns (Styragel® HT 2 DMF, Styragel® HT 3 DMF and Styragel® HT 4 DMF) were used for polymer fractionation. HPLC-grade DMF (containing 0.1 M LiBr) was used as the mobile phase at a flow rate of 1.0 mL/min. The whole system, including columns and detectors, was maintained at 60 °C. Polymer solutions with concentrations between 5.0 and 10.0 mg/mL were injected at an injection volume of 200 μL. ASTRA software from Wyatt Technology was used to collect and analyze the data from the detectors.

Polymerization procedure. A typical procedure for polymerization of NCA was performed in a 10 mL ampule in a Braun Labmaster glovebox. Taking the polymerization of entry 4 in Table 1 as an example, to a vigorously stirred solution of TREN in 2mL of DMF (2.2mg, 0.015mmol) was added NCA monomer (0.2g, 0.759mmol) in 2 mL of DMF. The reaction mixture was stirred for a specific time at room temperature. After the measured time interval, a small amount of aliquot (several drops) was taken from the reaction mixture via a syringe for the determination of monomer conversion via FT-IR. At the same time, 0.2ml of the reaction mixture was taken out from the system and diluted to 10 mg (PBLG) /mL using DMF (containing 0.1 M LiBr). The solution was then analyzed by SEC to determine the molecular weight and PDI of PBLG. The remaining final reaction mixture was precipitated with methanol, sonicated and centrifuged to remove the solvent. The obtained PBLG was collected and dried under vacuum overnight after two repetitions of sonication-centrifugation procedure.

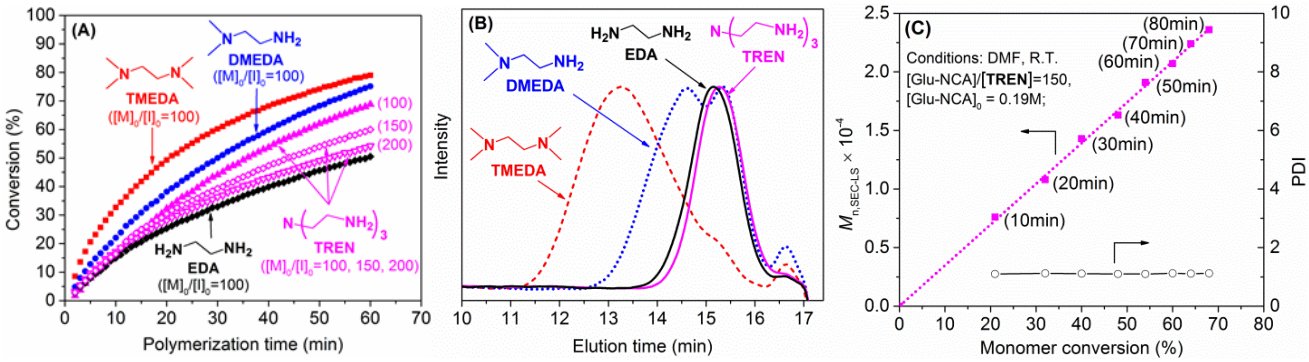

Figure S1. A) Conversion *vs* time for ROPs of Glu-NCA obtained from *operando* IR using different initiators (**TMEDA**, **DMEDA**, **EDA**, and **TREN**), conditions: $[NCA]_0$ = 0.19 M, DMF, 25 °C and the sampling interval of *operando* IR is 1 minute. B) SEC profiles of PBLG (RI signals) obtained from ROP of Glu-NCA initiated by different initiators (**TMEDA**, **DMEDA**, **EDA**, and **TREN**) with the same monomer/initiator ratio (M/I=100) and >99% monomer conversion. C) Plots of $M_{n,SEC\text{-}LS}$ *vs* conversion (polymerization time indicated in parentheses) for the ROP of Glu-NCA.

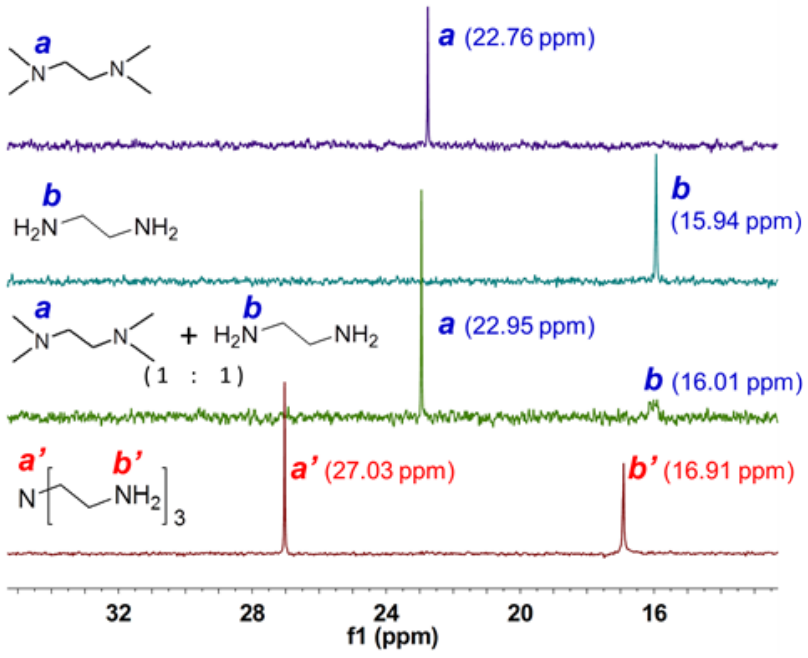

Figure S2. ^{15}N NMR spectra of **TMEDA**, **EDA**, **TMEDA/EDA** (1:1) mixture and **TREN** ($CDCl_3$, 298K).

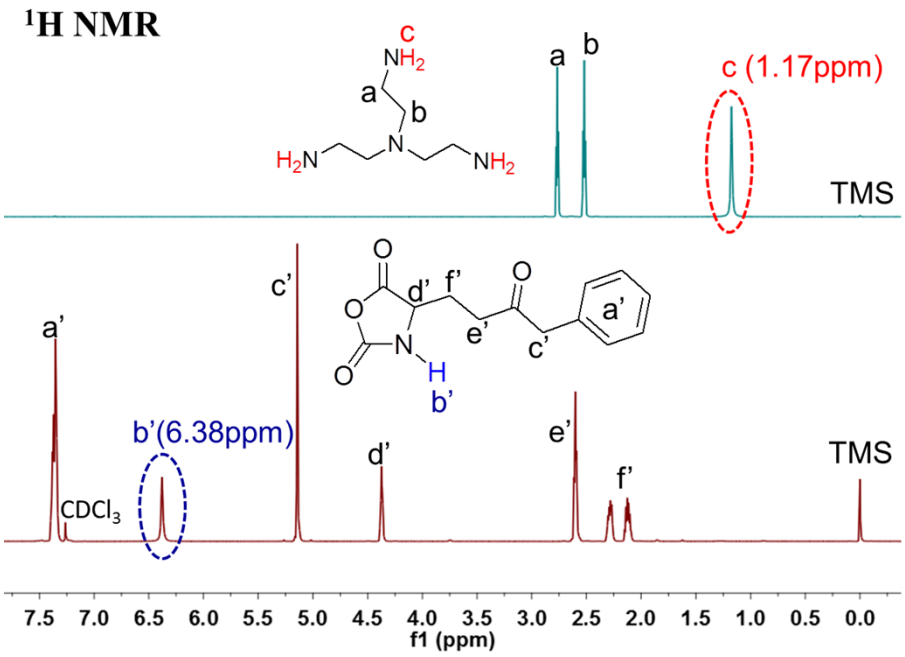

Figure S3. [1]H NMR spectra of TREN and Glu-NCA (600M, CDCl3, 298K).

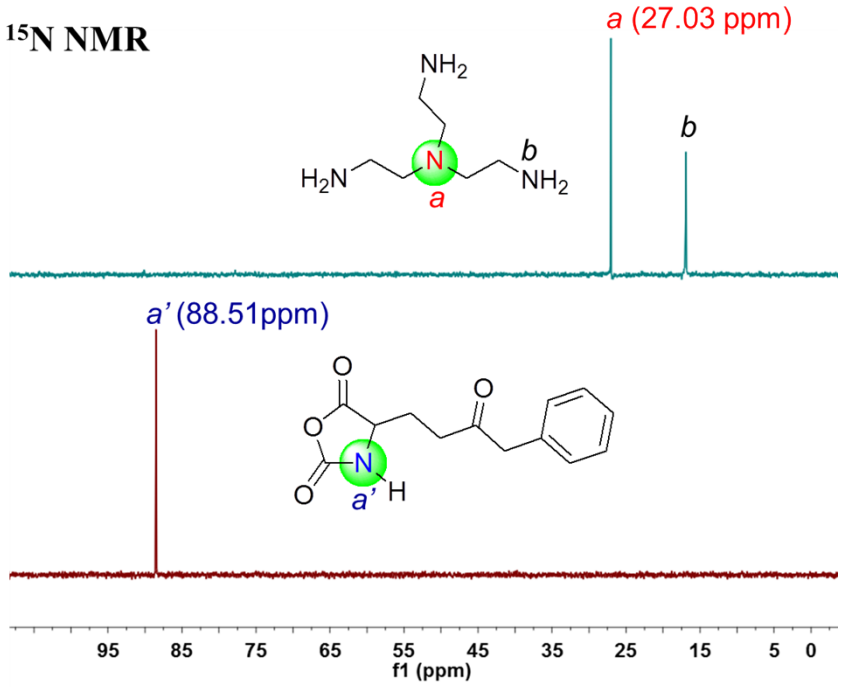

Figure S4. [15]N NMR spectra of TREN and Glu-NCA (CDCl3, 298K).

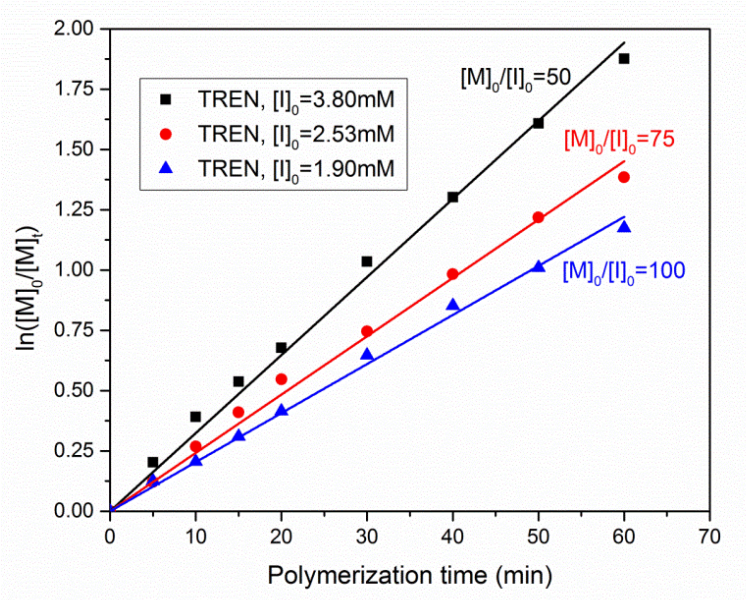

Figure S5. ln([NCA]$_0$/([NCA]$_t$) vs. time for the ROP of NCA initiated by TREN. Conditions: [NCA]$_0$ = 0.19 mM, DMF, 25 °C, [NCA]/[TREN] = 50 ([TETA]$_0$ = 3.80 mM, ■), 75 ([TREN]$_0$ = 2.53 mM, ●), 100 ([TREN]$_0$ = 1.90 mM, ▲).

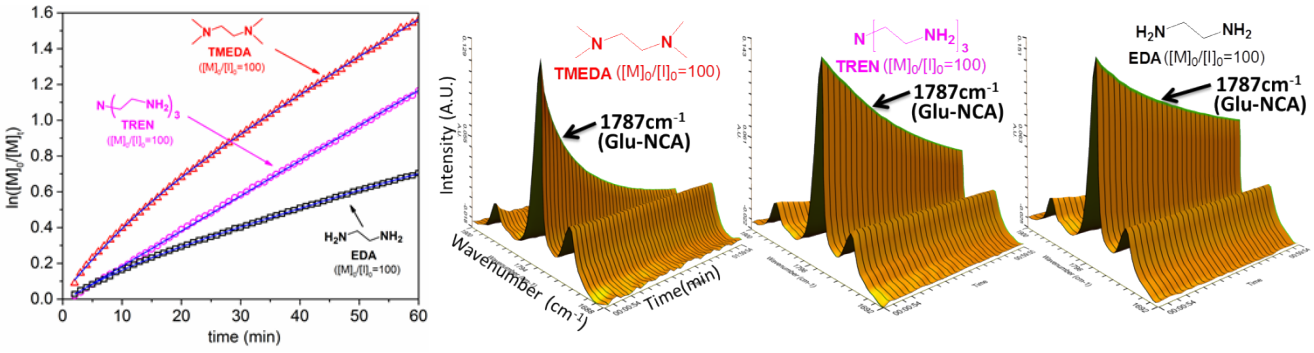

Figure S6. ln([NCA]$_0$/([NCA]$_t$) *vs* time for the ROP of Glu-NCA initiated by **TMEDA**, **TREN**, or **EDA** and corresponding 3D kinetic behavior profile obtained from *operando* IR for these polymerizations (the sampling interval of *operando* IR is 1 minute).

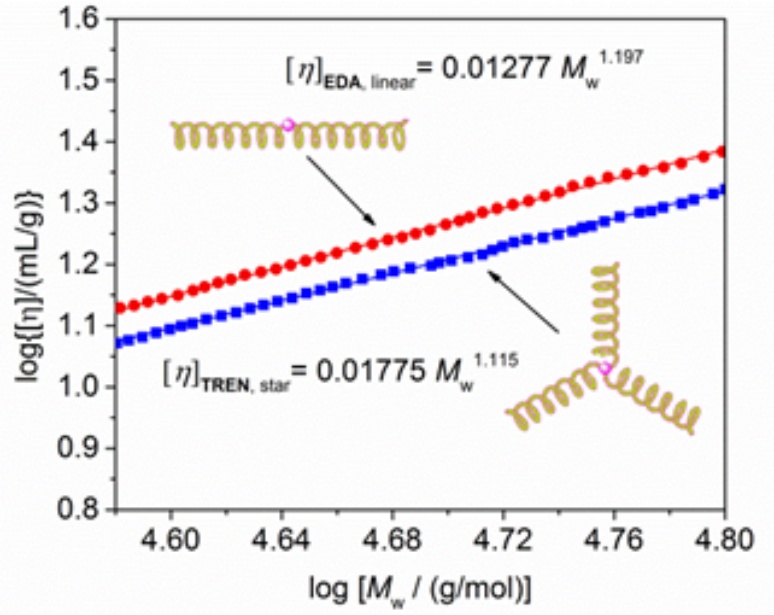

Figure S7. The Mark-Houwink-Sakurada plot of linear and star PBLGs with identical molecular weights and molecular weight distributions (Star and linear polymer were obtained by polymerization mediated by **TREN** and **EDA**, respectively).

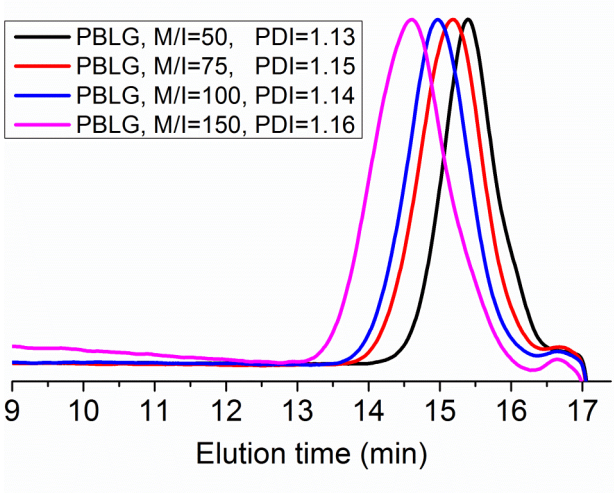

Figure S8. SEC profiles of PBLGs obtained from TREN-mediated polymerization of Glu-NCA with different [M]/[I] ratios (RI signals, monomer conversion >99%).

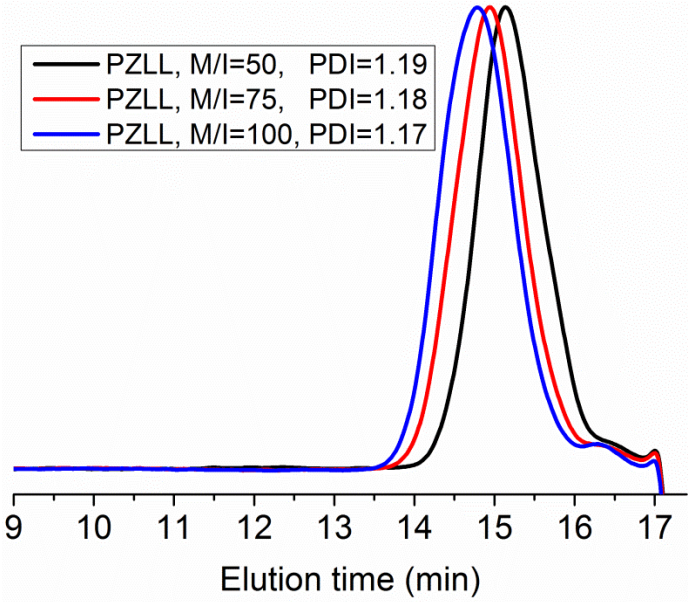

Figure S9. SEC profiles of PZLLs obtained from TREN-mediated polymerization of Lys-NCA with different [M]/[I] ratios (RI signals, monomer conversion >99%).

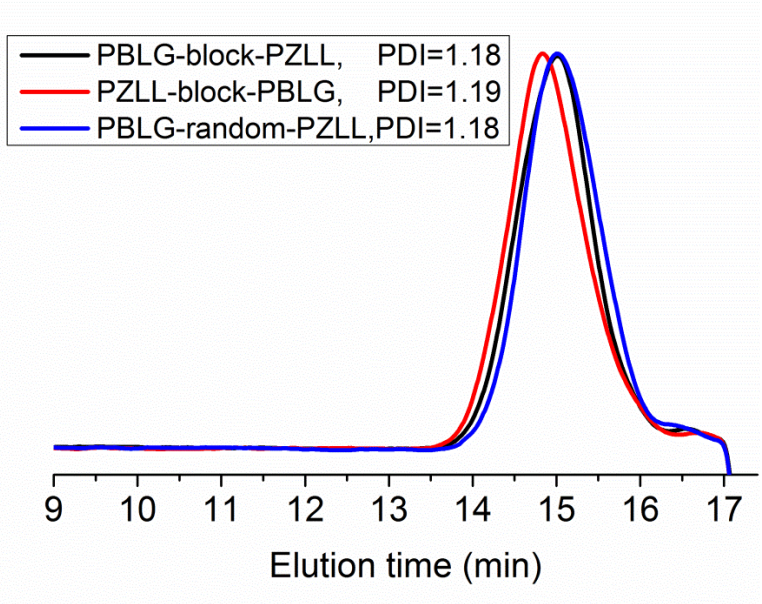

Figure S10. SEC profiles of PBLG-PZLL copolymer obtained from TREN-mediated copolymerization of Glu-NCA and Lys-NCA (RI signals, monomers conversion >99%).

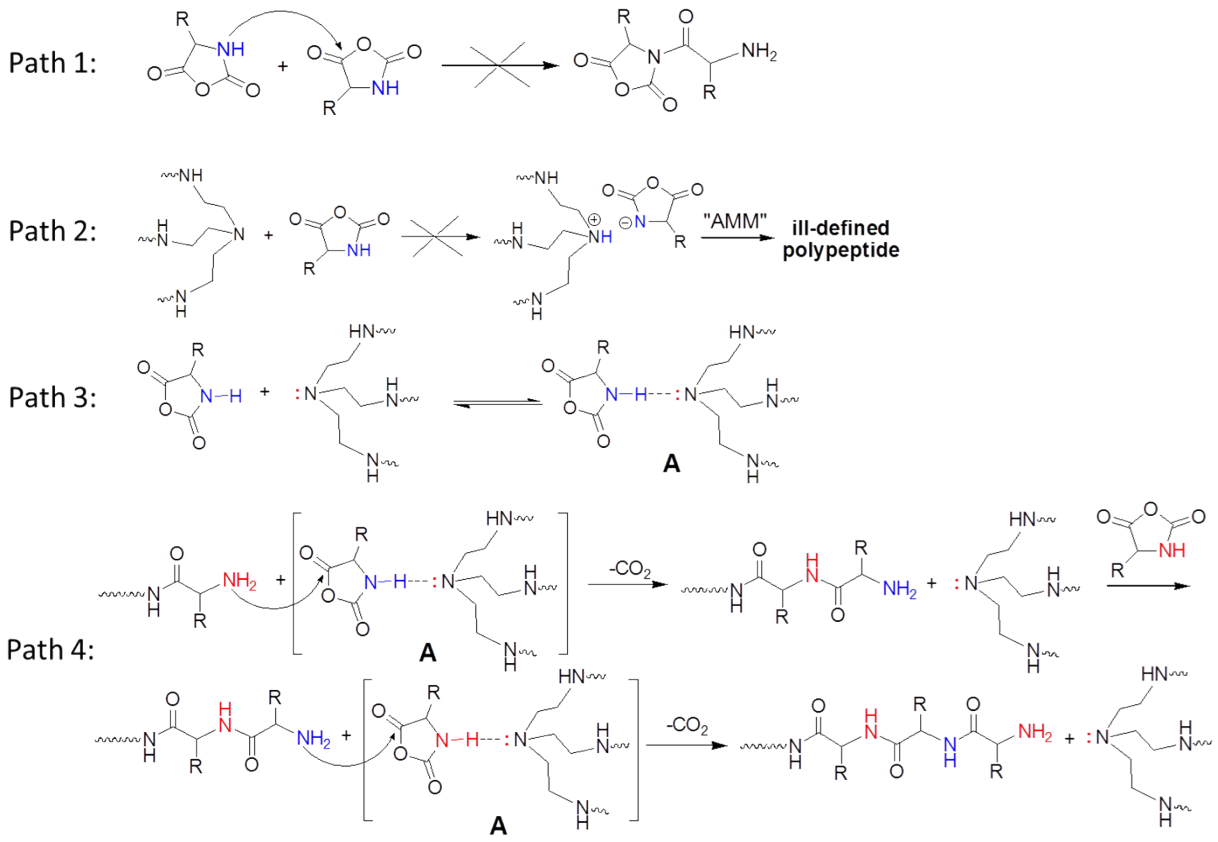

Figure S11. Possible reaction paths in the ROP of Glu-NCA initiated by **TREN**.

Proposed mechanism for the ROP of Glu-NCA initiated by TREN

No polypeptide is obtained in the absence of **TREN** (Figure 7, Path 1) indicating that the amido (-NH-) group of NCA has a very low basicity. In the presence of **TREN**, the polymerization proceeds fast after initiation by the primary amine groups of **TREN** that are more basic than the amido of NCA (Figure S5 and S6). The well-defined structures (low PDI, expected molecular weight) of the polypeptides produced suggest that AMM does not occur and that the tertiary amine of **TREN** could not abstract the proton of NCA (indicating that the "path 2" (Figure 7) is preventing from occurring during polymerization). Based on the ^{1}H NMR and ^{15}N NMR spectra of Glu-NCA and **TREN** we propose that the amido proton of the monomer gets activated by the tertiary amine of **TREN** through hydrogen bonding. The ROP of NCA is subsequently facilitated upon attack by the basic amine of **TREN** and by those of the growing ends, hence the a higher rate of polymerization under controlled living conditions.

References

(1) Lu, H.; Cheng, J. *J. Am. Chem. Soc.* **2007**, *129*, 14114-14115.