

Supplementary Information for

Green solvent-to-polymer upgrading approach to water-soluble LCST poly(*N*-substituted lactamide acrylate)s

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1. Materials and methods

Materials. The following chemicals were purchased from Merck and used as received: 2-(aminomethyl) tetrahydrofuran (THFA, 97%), ammonium persulfate (APS, 98%), anhydrous acrylic acid (AA, 99%), α -bromoisobutyryl bromide (BiBB, 98%), tris[2-(dimethylamino)ethyl]amine ligand (Me₆-TREN), propylphosphonic anhydride solution (T3P[®], 50 wt. % in ethyl acetate), *N,N'*-methylenebis(acrylamide) (MBA, 99%), natural ethyl lactate (EL, 98%), *N,N,N',N'*-tetramethylethylenediamine (TEMED, 99%), triethylamine (TEA, $\geq 99\%$), and dialysis tube benzoylated (MWCO 2,000). The following chemicals were purchased from Scharlab and used as received: acid acetic glacial (synthesis grade), ethanol absolute (EssentQ), ethyl acetate (AcOEt, EssentQ), hexane (Hx, EssentQ), basic aluminium oxide (Al₂O₃), acetone (EssentQ), and deuterium oxide (D₂O, 99.8%). Deuterated chloroform (CDCl₃, 99.8%) and acetone (acetone-*d*₆, 99.8%) were purchased from Eurisotop. Triethylamine (TEA, $\geq 99\%$, Merck) was distilled prior to use from CaH₂. 2-Methyltetrahydrofuran (Me-THF, 99.5%, Merck) and diethylether (Et₂O, synthesis grade, Scharlab) were distilled prior to use from sodium/benzophenone. Sea water (SW) was collected from Arrabassada beach (Tarragona, Spain). Copper (I) bromide (Cu(I)Br, Merck) was stirred with glacial acetic acid for 30 min, filtered and subsequently washed three times with ethanol absolute and anhydrous diethylether, dried under vacuum, and stored under Ar. Agnique[®] AMD 3L (2-hydroxy-*N,N*-dimethyl propanamide) was kindly donated by BASF SE (Ludwigshafen, Germany) and used as received.

Methods. Proton (¹H NMR) and carbon (¹³C NMR) nuclear magnetic resonance spectra were recorded on a 400 MHz (for ¹H) and 100.6 MHz (for ¹³C) Varian VNMR-S400 NMR instrument at 25 °C in indicated deuterated solvent. All chemical shifts are quoted on the δ scale in ppm using the residual solvent as internal standard (¹H NMR: CDCl₃ = 7.26, D₂O = 4.79, (CD₃)₂CO = 2.05, and ¹³C NMR: CDCl₃ = 77.16, (CD₃)₂CO = 29.84, (CD₃)₂CO = 206.26). Infrared spectra were recorded on a FTIR-680 PLUS

spectrophotometer with a resolution of 4 cm^{-1} in the transmittance mode. An attenuated total reflection (ATR) device with thermal control and a diamond crystal (Golden Gate heated single-reflection diamond ATR, Specac Teknokroma) was used. Absorption maxima (ν_{max}) are reported in wavenumbers (cm^{-1}). Gas chromatography (GC) analysis were carried out on an Agilent 7820A GC system equipped with a FID detector, using dichloromethane as eluent. Molecular weight analysis was performed via size exclusion chromatography (SEC) using an Agilent 1200 series system equipped with a precolumn (PLgel 5 μm Guard column) and a two-serial column system (2x PLgel 5 μm MIXED-D) and with an Agilent 1100 series refractive index detector. Chromatograms were carried out in *N,N*-dimethyl formamide (DMF), HPLC grade, containing 0.05% (w/w) of LiBr with a flow rate of $1\text{ mL}\cdot\text{min}^{-1}$ at $50\text{ }^{\circ}\text{C}$. Samples were filtered through $0.22\text{ }\mu\text{m}$ Teflon syringe filter and $20\text{ }\mu\text{L}$ of the polymer solution was injected using a manual sample injector Rheodyne Model 7125. The calibration curves for GPC analysis were obtained with poly(methyl methacrylate) (PMMA) standards purchased from PSS Polymer Standards Service GmbH. The molecular weights were calculated using the universal calibration principle and Mark-Houwink parameters. Toluene was used as flow rate marker. Differential scanning calorimetry (DSC) measurements were carried out on a Mettler DSC3+ instrument using N_2 as a purge gas ($50\text{ mL}\cdot\text{min}^{-1}$) at a scanning rate of $20\text{ }^{\circ}\text{C}\text{ min}^{-1}$ in the -80 to $150\text{ }^{\circ}\text{C}$ temperature range for three cycles, and the second heating curve was used to determine the glass transition temperature (T_g) in the middle of the step transition. Polymer (5 to 10 mg) were encapsulated in aluminium pans before measurements. Calibration was made using an indium standard (heat flow calibration) and an indium-lead-zinc standard (temperature calibration). Dynamic light scattering (DLS) measurements were carried out at targeted temperatures using Zetasizer Ultra from Malvern Instruments equipped with a He-Ne laser. Optical rotation measurements were conducted on a PerkinElmer 241 MC polarimeter with a path length of 10 cm and are reported with implied units of $10^{-1}\text{ deg}\cdot\text{cm}^2\cdot\text{g}^{-1}$. The phase separation temperature or cloud point

temperature (T_{cp}) of the synthesized polymers was analysed on a UV-2401PC UV-VIS Recording Spectrophotometer using a wavelength of 500 nm in transmittance mode, in which the T_{cp} was determined at 50% change of the transmittance. MS were run on an Exactive Orbitrap™ mass spectrometer from Thermo Scientific (Bremen, Germany), with a heated electrospray ionization (HESI) source and a higher-energy collisional dissociation (HCD) cell to fragment the analytes. Samples were ionized in positive mode using the following parameters: spray voltage, 2 kV; skimmer voltage, 20 V; capillary voltage, 25 V; and tube lens voltage, 20 V. Gas flow rates were: sheath gas, 20 AU (adimensional units) and auxiliary gas, 10 AU; and, the heater and capillary temperatures were 350°C. The probe position settings were side to side 0, vertical C, and micrometer 0.5.

2. Additional experimental procedures

Synthesis of DMLA monomer from DML solvent. Acrylic acid (7.4 mL, 108 mmol), TEA (39.0 mL, 280 mmol) and T3P® (68 mL, 114 mmol) were added to a solution of DML (11 mL, 90 mmol) in anhydrous Me-THF (100 mL). The mixture was stirred for 24 hours at room temperature under inert atmosphere. The reaction was monitored by ^1H NMR. When full conversion was achieved, the solvent was eliminated under *vacuo*, and the residue was purified by vacuum distillation in the presence of 5 w/w % of hydroquinone. The obtained colourless oil was filtered throughout a small column of basic alumina to remove hydroquinone traces to afford DMLA (12.91 g, 84 %). $[\alpha]_{\text{D}}^{20} = -6.320 \text{ deg}\cdot\text{dm}^{-1}\cdot\text{cm}^3\cdot\text{g}^{-1}$ (52.8 $\text{mg}\cdot\text{mL}^{-1}$, MeCN). ^1H NMR (401 MHz, Chloroform-*d*) δ 6.46 (dd, $J = 17.4, 1.4$ Hz, 1H), 6.18 (dd, $J = 17.3, 10.4$ Hz, 1H), 5.87 (dd, $J = 10.4, 1.4$ Hz, 1H), 5.47 (q, $J = 6.7$ Hz, 1H), 3.07 (s, 3H), 2.97 (s, 3H), 1.47 (d, $J = 6.8$ Hz, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 170.06, 165.65, 131.69, 127.81, 67.00, 36.77, 35.92, 16.63. HRMS (HESI-Orbitrap) calculated for $[\text{M} + \text{Na}]^+ \text{C}_8\text{H}_{13}\text{NNaO}_3^+$ (m/z): 194.0787; found: 194.0789.

Homopolymerizations of THFLA and DMLA via aqueous SET-LRP. The homopolymerization of THFLA under the following conditions $[\text{THFLA}]_0/[\text{DMLBr}]_0/[\text{Cu(I)Br}]_0/[\text{Me}_6\text{TREN}]_0 = 40/1/0.8/0.6$ is described. This procedure is generic for all the homopolymerizations conducted herein. A Schlenk tube was charged with Cu(I)Br (5.8 mg, 40 μmol), sealed, and deoxygenated through three Ar/vacuum cycles. At the same time, H₂O (1 mL) and Me₆TREN (7.76 μL , 29 μmol) were charged to a vial and the mixture was bubbled with argon for 10 min. Then, the degassed ligand/water solution was cannula transferred to the Cu(I)Br containing Schlenk tube. The resultant blue suspension containing Cu(0)/Cu(II)Br mixture was stirred for 5 min at room temperature and then cooled down with an ice bath. Simultaneously, to a vial also immersed in ice bath, a solution of DMLBr (12.95 mg, 48 μmol) and THFLA (448.9 mg, 1.96 mmol) in water (1.2 mL) was deoxygenated with argon for 15 min. Next, the monomer/initiator solution was cannula transferred to the Schlenk tube with Cu(0)/Cu(I)Br₂/Me₆TREN catalyst. The Schlenk tube was sealed, and the mixed solution was allowed to polymerize at 0 °C for 1 h. Sample of the reaction mixture was then withdrawn for ¹H NMR and GPC analysis. Catalyst residues were removed by filtration through a column of basic alumina prior to SEC analysis. The sample for ¹H NMR spectroscopy was directly diluted with acetone-*d*₆, which confirmed > 99% conversion according to comparison of the integrals of vinyl signals (5.5-6.5 ppm) to the lactamide methine (-CH-) signal at 5-5.5 ppm. The reaction was then quenched opening the Schlenk tube to the air. Next, the reaction mixture was dialyzed (MWCO 2,000) against acetone, refreshing the solvent 3 to 4 times for 2 days. Finally, the solvent was removed to recover the synthesized polymer as a white solid.

Preparation of poly(THFLA-*r*-DMLA) LCST-type hydrogels. A solution of THFLA (473.1 mg, 2.1 mmol), DMLA (356.9 mg, 2.1 mmol) and TEMED (12 μL , 81 μmol) in water (5 mL) was charged in a vial. Then, a freshly prepared solution (1 mL) of MBA (32.0 mg in 8 mL H₂O) was added. Next, the vial was immersed in an ice/water bath, sealed with a rubber septum, and the solution was degassed by argon

bubbling for 10 minutes. After that, a freshly prepared aqueous solution (2 mL) of APS (80.7 mg in 8 mL H₂O) was added. The mixture was stirred vigorously for 30 seconds and then was transferred to various coin-like cylindrical moulds under argon flow protection. The moulds were sealed and kept at 5 °C for 24 h to allow polymerization. After that time, the hydrogels were separated carefully from the moulds and rinsed with water several times. To remove unreacted monomer, the hydrogels were soaked in water that was changed for fresh water every hour for 2 days. Next, hydrogels were placed in a previously weighted petri dishes, soaked in water and stored in the fridge (5 °C) for 24 h to reach swelling equilibrium. Petri dishes containing water and hydrogels were incubated to targeted temperature for 1 h. After that, water was removed from the petri and the hydrogels were carefully wiped and weighted (W_s). Finally, hydrogels were freeze-dried and weighted (W_d). Swelling ratio (SR) was calculated using the Equation 1.

$$SR (\%) = \frac{W_s - W_d}{W_d} \times 100 \quad (1)$$

Cultivation of cells

Normal human dermal fibroblasts (NHDF, ATCC) and J774A1 cells (ATCC) were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco™) supplemented with 10% fetal bovine serum (FBS, PAN-Biotech) and 1% pen/strep (AntibioticAntimycotic (100X) Gibco®). Cultivation was carried out at 37 °C and in humidified 5% CO₂ atmosphere.

Cytotoxicity studies

Cytotoxicity was assessed by determining cell viability using the MTS assay (The CellTiter 96® Aqueous One Solution Cell Proliferation Assay, Promega) after 24 and 72 hours of incubation in direct contact. For the experiments, NHDF were harvested by trypsin treatment (Gibco™) and seeded in 48-well plates (Greiner bio-one) at a density of 40,000 cells/well. J774A1 cells were harvested by scraping and seeded in 48-well plates at a density of 60,000 cells/well. Cells were then cultured until 80-90% confluence.

Hydrogel samples (d = 1 cm, h = 0.5 cm) were washed in PBS (3 x 5 min) and sterilized by UV disinfection (254 nm) for 1 hour. Subsequently, hydrogels were washed again with PBS (3 x 5 min) and then incubated in sterilized DMEM for 2 hours. The hydrogels (N = 6) were then carefully placed on top of the cells and incubated for 24 or 72 hours at 37 °C in a humidified atmosphere with 5% CO₂. Cells treated with culture medium only serve as negative controls (non-cytotoxic), while cells treated with latex (1 cm²) and LPS (10³ ng) serve as positive controls (cytotoxic) for NHDF and J774A1 experiments, respectively. After incubation, the hydrogels were carefully removed, the cell culture medium was replaced with 200 µl of fresh medium to equalize the volumes in each well and 40 µl of MTS reagent (100/20) was added. The well plates were then allowed to incubate for 3 hours. Combined medium was then transferred into 96-well plates and the absorbance was recorded at 490 nm using the SpectraMax[®] M3 multimode microplate reader. The collected absorbance data were normalized, and the negative control was set as 100% reference.

LIVE/DEAD staining

LIVE/DEAD staining was performed after 72 hours of incubation to visualize cell morphology and viability of NHDF. The LIVE/DEAD[™] Cell Imaging Kit (Invitrogen TM) was prepared as recommended by the supplier. Briefly, 1 mL of the cell dye Calcein AM was pipetted into 1 µg of BOBO-3 iodide and then mixed with 1 mL of PBS. 200 µL of LIVE/DEAD staining solution was added to each well, and incubation proceeded for 10 min at room temperature. Images were acquired by confocal laser scanning microscopy using a TCS SP8 (Leica Microsystems CMS GmbH, Mannheim, Germany) equipped with an argon laser (10% intensity). The excitation wavelengths were set to 488 nm and 570 nm. The emitted fluorescence was detected with PMT detectors at 515 nm and 602 nm.

3. Aminolysis of EL solvent with THFA

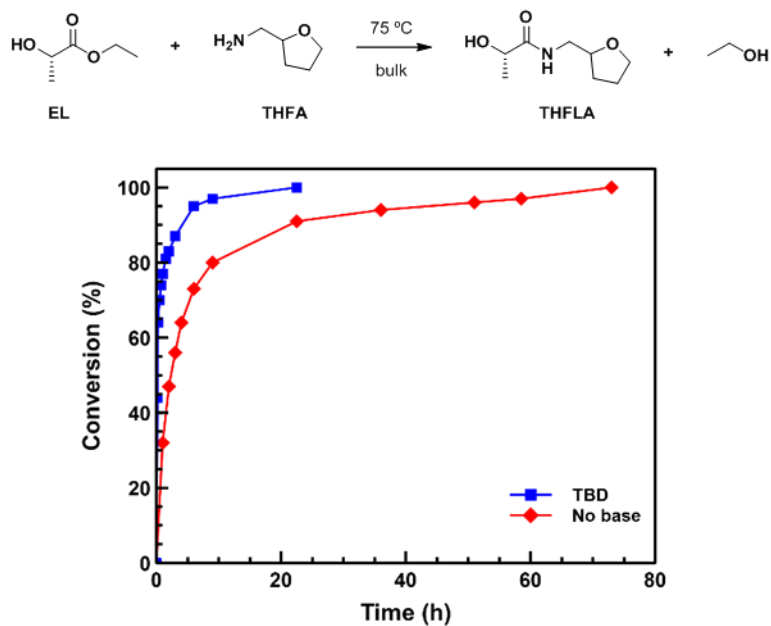


Figure S1. General scheme and conversion as a function of reaction time for the bulk aminolysis of EL solvent with tetrahydrofurfuryl amine in the presence or not of TBD.

4. Structural characterization of THFLA and DMLBr

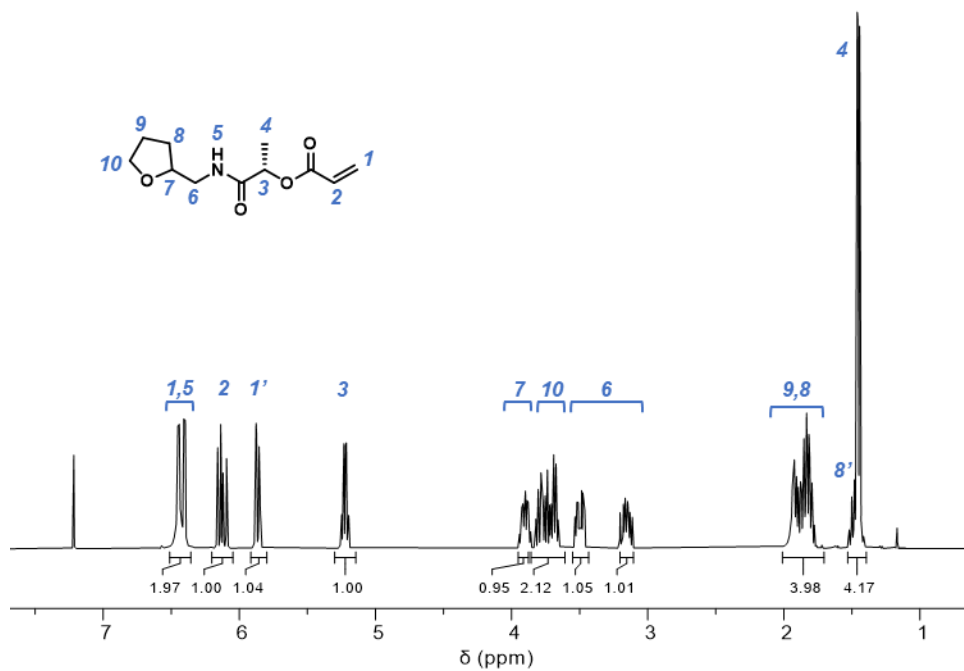


Figure S2. ¹H NMR spectrum of THFLA in CDCl₃.

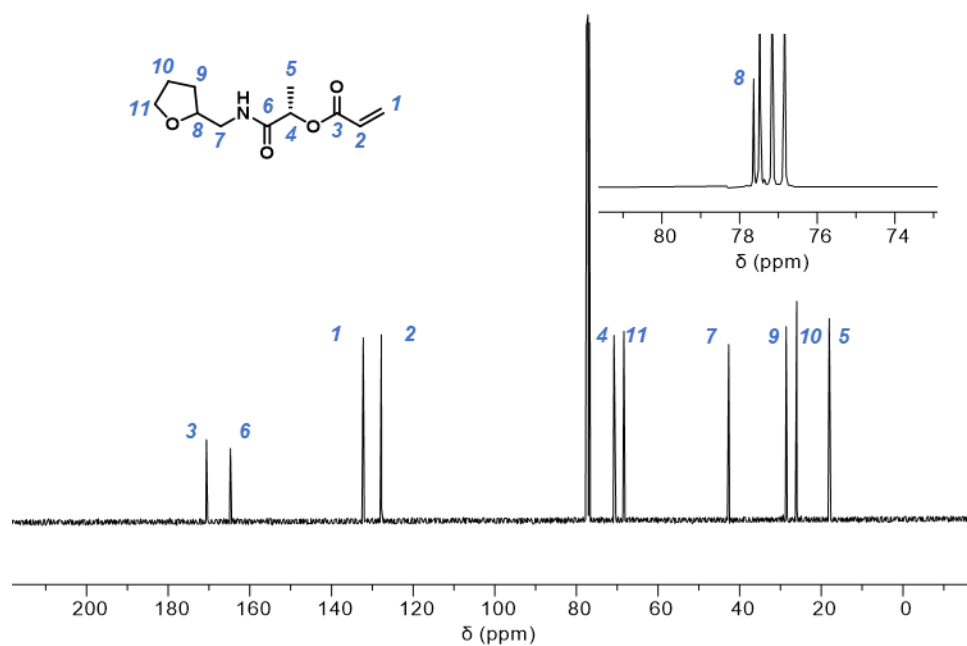


Figure S3. ¹³C NMR spectrum of THFLA in CDCl₃.

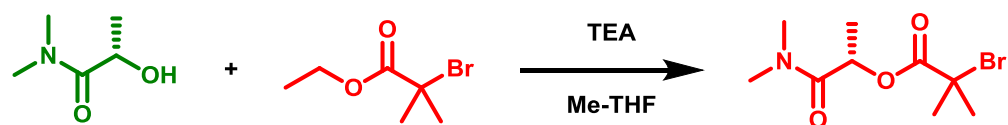


Figure S4. Synthesis of the water-soluble initiator DMLBr from DML solvent.

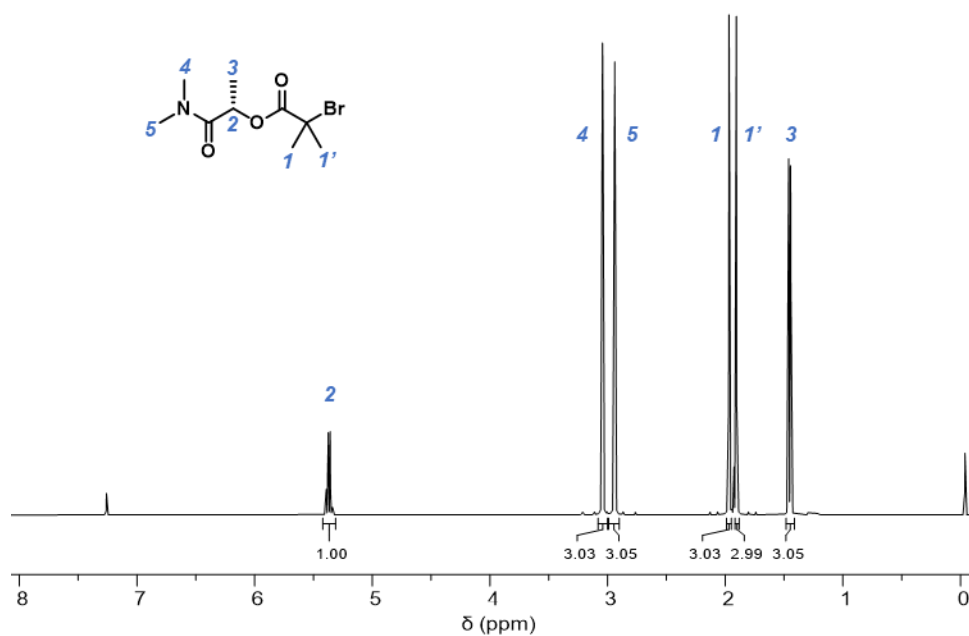


Figure S5. ^1H NMR spectrum of DMLBr in CDCl_3 .

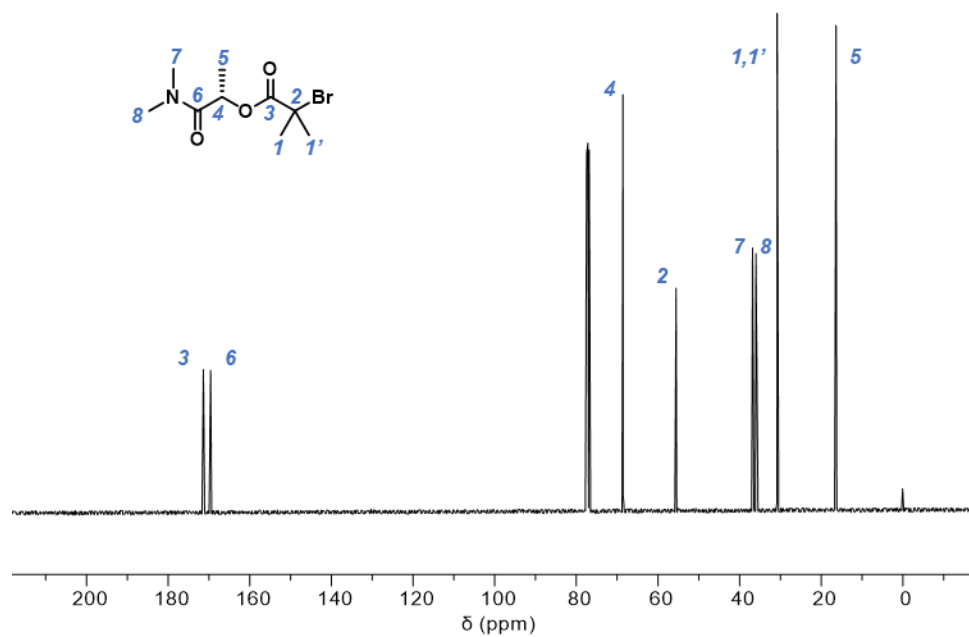


Figure S6. ^{13}C NMR spectrum of DMLBr in CDCl_3 .

5. Homopolymerization of THFLA and DMLA by aqueous SET-LRP

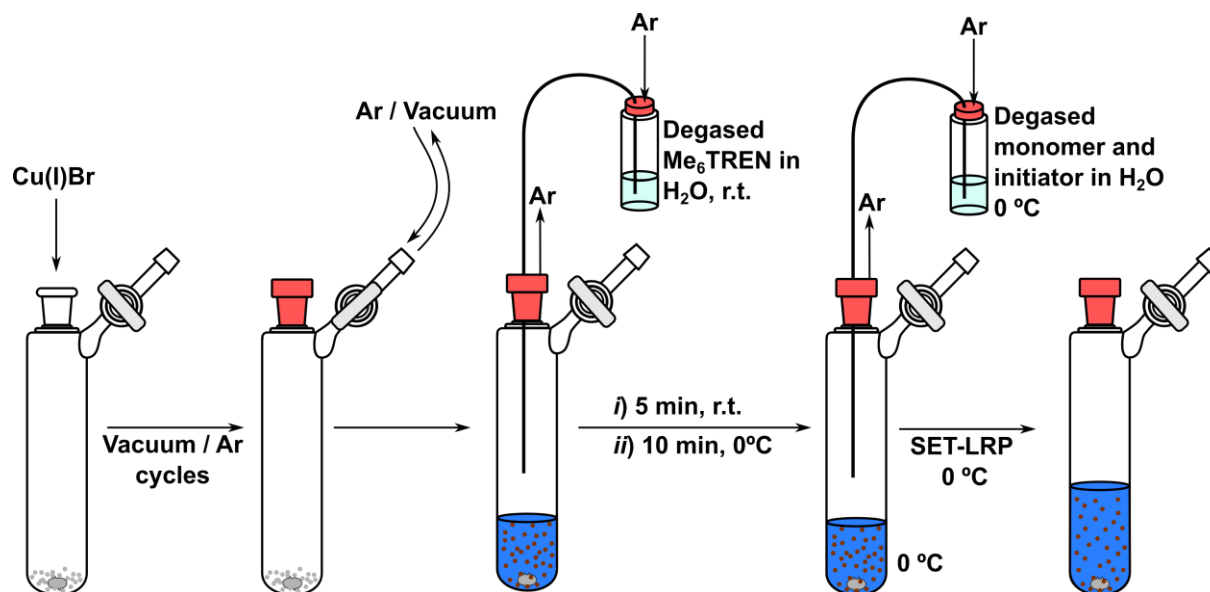


Figure S7. Representative schematic procedure for the aqueous SET-LRP catalysed by Cu(0) generated in situ by water disproportionation of Cu(I)Br .

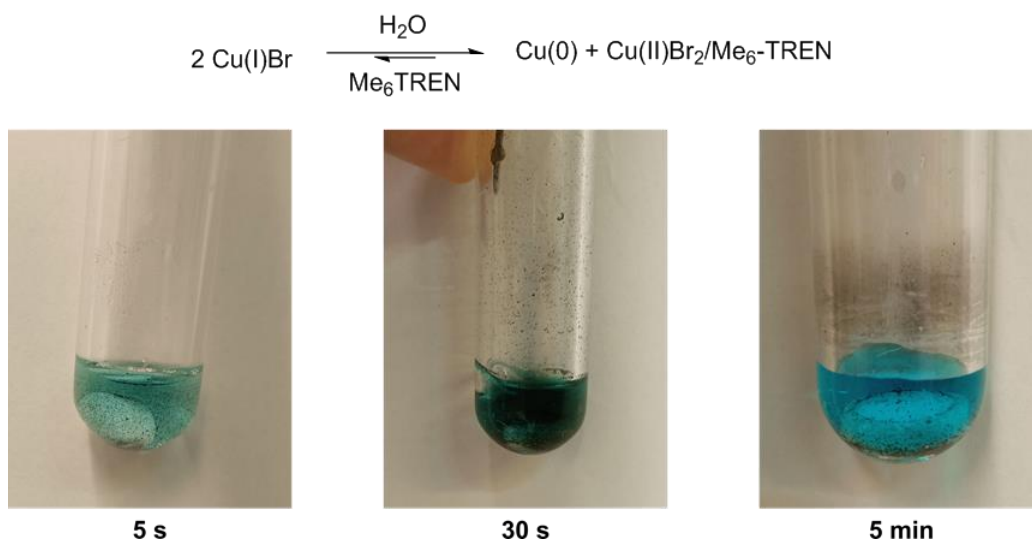


Figure S8. Visual observation of the formation of nascent Cu(0) and $\text{Cu(II)Br}_2/\text{Me}_6\text{-TREN}$ complexes by the disproportionation of Cu(I)Br in the presence of $\text{Me}_6\text{-TREN}$.

Table S1. Aqueous SET-LRP homopolymerization of THFLA.

Entry	[M]:[I]:[Cu(I)Br]:[L]	Conv. (%) ^a	$M_n^{\text{th } b}$	$M_n^{\text{NMR } a}$	$M_n^{\text{SEC } d}$	M_w/M_n^c	$T_{\text{cp}} (\text{°C})^d$
1	20:1:0.6:0.4	94	4,600	5,400	5,900	1.08	23.0
2	40:1:0.8:0.6	100	9,600	9,900	11,200	1.14	18.1
3	80:1:1.2:0.8	> 99	18,600	18,000	19,400	1.13	15.8
4	160:1:1.6: 1	100	36,600	35,700	37,300	1.11	14.5
5	320:1:2:1.2	94	66,800	67,300	64,000	1.16	12.7

^a Determined by ¹H NMR after 30 min (entries 1 and 2) or 1 h (entries 3-5). ^b $M(\text{th}) = 227.26 \times [\text{THFLA}]_0/[\text{DMLBr}]_0 \times \text{conv.} + 266.14$. ^c Determined by SEC using PMMA standards. ^d Determined by UV/Vis spectroscopy (5 mg·mL⁻¹).

Table S2. Aqueous SET-LRP homopolymerization of DMLA.

Entry	[M]:[I]:[Cu(I)Br]:[L]	Conv. (%) ^a	$M_n^{\text{th } b}$	$M_n^{\text{SEC } c}$	M_w/M_n^c
1	20:1:0.4:0.4	> 99	4,800	6,000	1.17
2	40:1:0.8:0.6	100	7,100	7,900	1.08
3	80:1:1.6: 1	> 99	14,000	14,900	1.05
4	160:1:1.6: 1	95	27,000	28,200	1.07
5	320:1:2:1.2	99	55,100	46,300	1.08
6	640:1:4.8:2.8	98	109,800	82,800	1.08

^a Determined by ¹H NMR after 30 min (entries 1 and 2) or 1 h (entries 3-6). ^b $M(\text{th}) = 171.2 \times [\text{DMLA}]_0/[\text{DMLBr}]_0 \times \text{conv.} + 266.14$. ^c Determined by SEC using PMMA standards.

Table S3. T_{cp} for aqueous solution of poly(THFLA-*r*-DMLA) (51 % mol DMLA content) at different DP, polymer, and salt concentration.

Entry	DP	$M_n^{\text{SEC } a}$	Copolymer conc. (mg·mL ⁻¹)	Salt conc. (mol·L ⁻¹)	$T_{\text{cp}} (\text{°C})^c$
1	40	11,000	5	1 ^b	24.5
2	40	11,000	5	SW ^d	31.5
3	160	33,400	5	-	35.6
4	40	11,000	5	0.2 ^b	38.3
5	40	11,000	5	-	43.5
6	40	11,000	2	-	45.9
7	40	11,000	1	-	50.0
8	20	4,300	5	-	56.0
9	40	11,000	5 ^e	-	42.5
10	40	11,000	5 ^f	-	-

^a Determined by ¹H NMR after 1 h. ^b NaCl concentration. ^c Determined by UV/Vis spectroscopy. ^d Copolymer dissolved in freshly collected sea water (SW). ^e Copolymer dissolved in an aqueous HCl solution at pH = 4.0. ^f Copolymer dissolved in an aqueous NaOH solution at pH = 10.0.

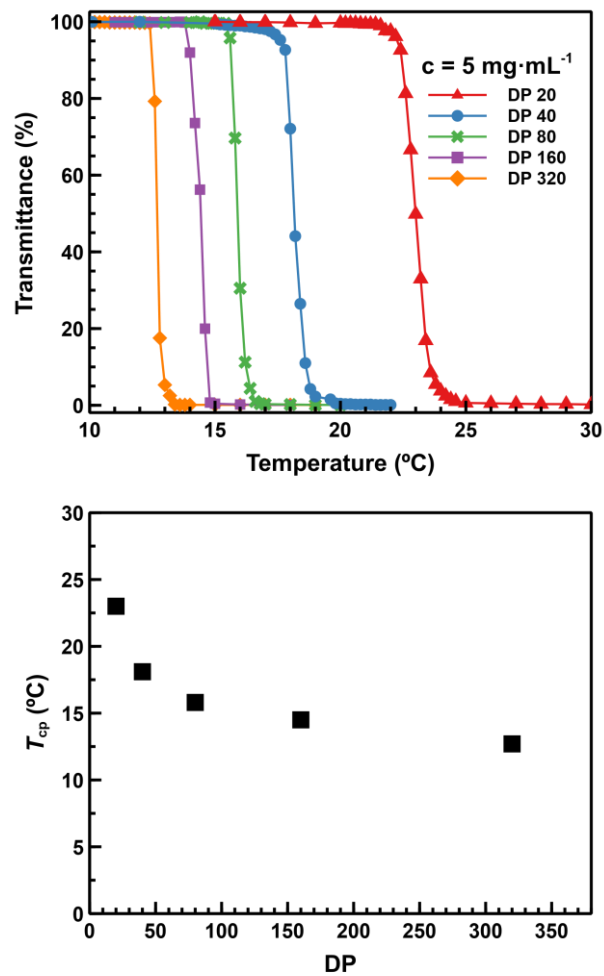


Figure S9. Relationship between LCST behavior (T_{cp}) determined by UV/Vis spectroscopy and DP for aqueous solution of poly(THFLA) ($c = 5 \text{ mg} \cdot \text{mL}^{-1}$).

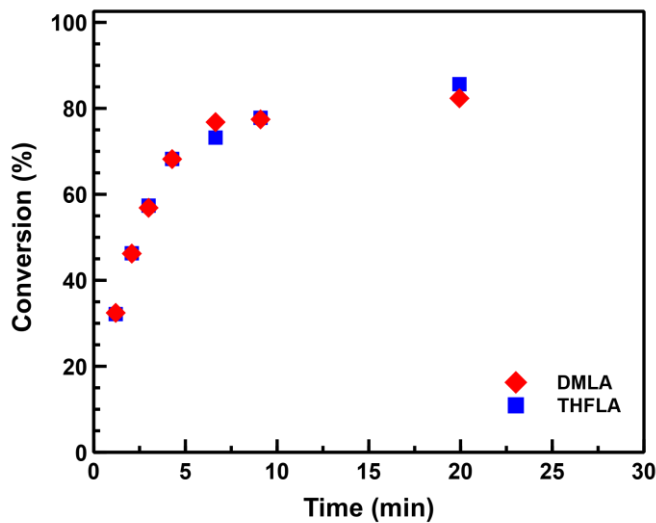


Figure S10. Kinetic monitoring by GC of the equimolar copolymerization of THFLA and DMLA by aqueous SET-LRP under the following reaction conditions ($[DMLA]_0 + [THFLA]_0 / [DMLBr]_0 / [Cu(I)Br]_0 / [Me_6TREN]_0 = 40/1/0.8/0.6$).

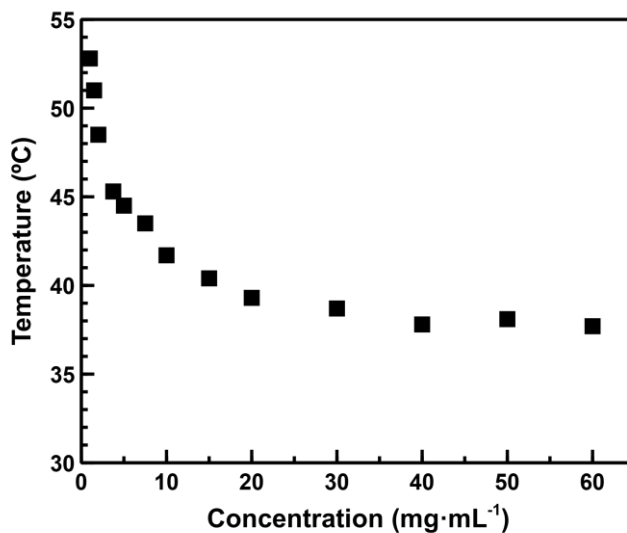


Figure S11. Temperature-concentration phase diagram showing the LCST-type transitions of for poly(THFLA-r-DMLA) (51 mol% DMLA) in water.

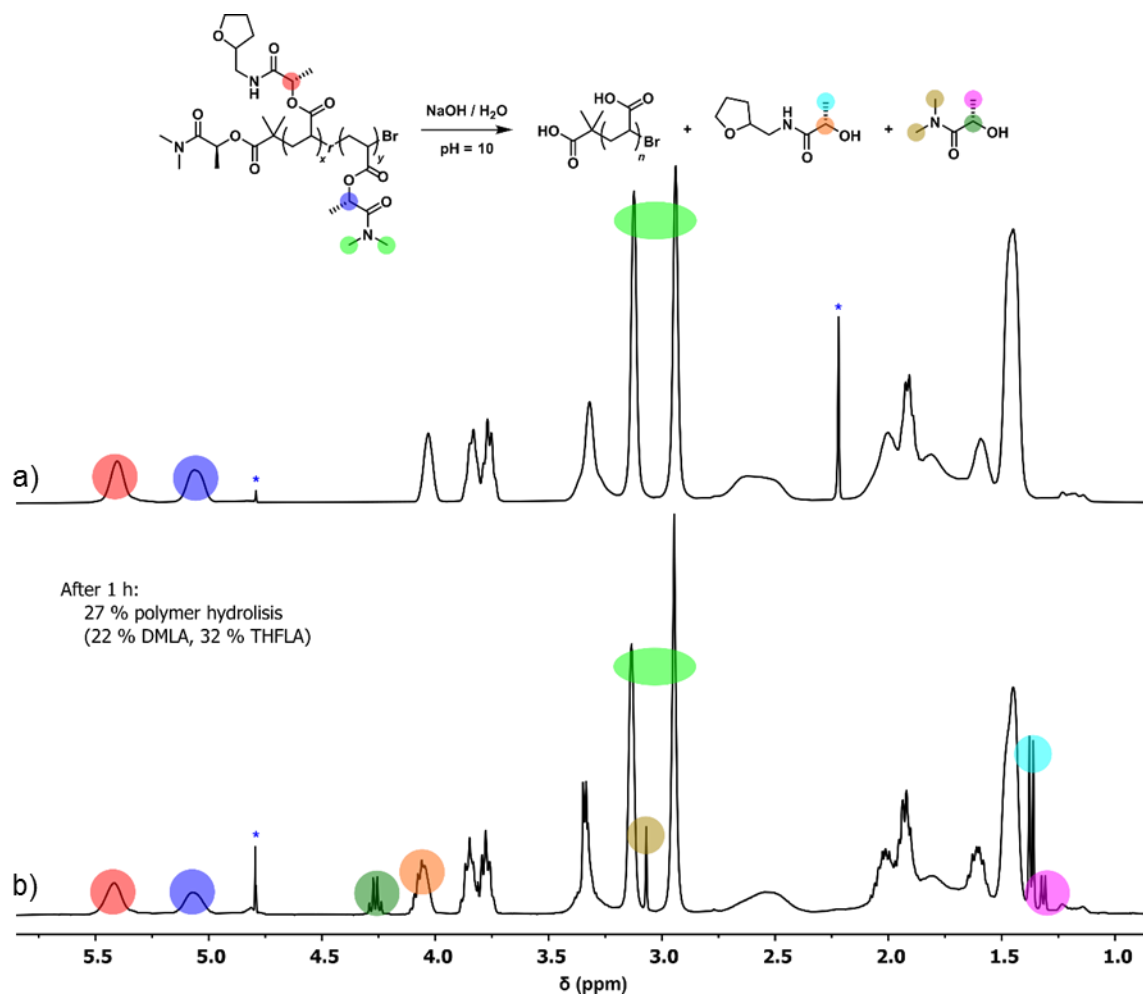


Figure S12. ^1H NMR spectrum in D_2O of poly(THFLA-*r*-DMLA) containing 40% DMLA ($M_n = 11,300 \text{ g mol}^{-1}$, $\bar{D} = 1.11$) (a) before and (b) after 1 h under stirring in an aqueous basic (NaOH , $\text{pH} = 10.0$) solution ($5 \text{ mg}\cdot\text{ml}^{-1}$).

6. Cytotoxicity studies

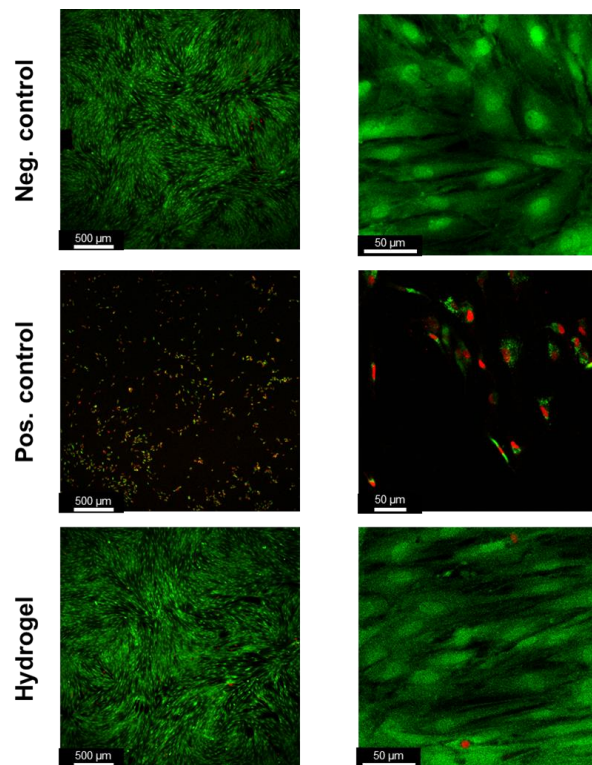


Figure S13. Photomicrographs of human fibroblast cell cultures after exposure to hydrogel discs, positive, and negative controls. Morphology was assessed by LIVE/DEAD staining after 72 h of direct contact of samples with the cells.