Supporting Information

Well-defined Synthetic Polymers with a Protein-like Gelation Behavior in Water

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A. Experimental procedures:

A.1. Chemicals. Acryloyl chloride (Alfa Aesar, 96%), glycine amide hydrochloride (Iris Biotech, 99.7%), potassium carbonate (Riedel-de Haën, 98-100%), diethyl ether (Merck, 99.7%), acetone (chem solute / Th. Geyer, >99%), VA-044 [2,2'-Azobis[2-(2-imidazolin-2-yl)propane]-dihydrochloride] (Wako Chemicals), benzyl mercaptan (Aldrich, 99%), sodium hydride (Acros, 60% in mineral oil), carbon disulfide (Acros, >99%) and 1,3-propane sultone (Fluka, >99%) were used as received. Phosphate buffered saline (PBS) solutions were prepared using pre-calibrated tablets (Fluka). Typically, one tablet was dissolved in 200 mL of deionized water to obtain a solution containing 137 mM of NaCl, 2.7 mM of KCl and 10 mM of the phosphate buffer (pH 7.4 at 25°C). The cell culture medium, tested in the present work, is the one typically used for the culture of mouse fibroblasts (cell line L929). It contains Dulbecco's Modified Eagle Medium (DMEM), 10% of Fetal Calf Serum (FCS) and antibiotics (penicillin and streptomycin).

A.2. Preparation of *N*-acryloyl glycinamide 1. The synthesis was adapted from the literature.[1] Glycine amide hydrochloride (5 Eq.) and potassium carbonate (10 Eq.) were dissolved in 50 mL of deionized water. The resulting solution was added to a three-neck round bottom flask. Acryloyl chloride (6 Eq.) was dissolved in 100 mL of diethyl ether. The flask with the aqueous solution was placed in an ice bath and cooled to 5°C. The diethyl ether

solution was added dropwise under vigorous stirring over 25 minutes in order to keep the temperature below 5°C. The ice bath was removed, and the solution was stirred at room temperature for two hours. Afterwards, the diethyl ether phase was removed, and 20 mL of water were added to the aqueous phase to dissolve the solids, which precipitated during the reaction. The resulting solution was transferred into a 1 L round bottom flask and concentrated under reduced pressure. 1 L of acetone was then added to the flask causing the precipitation of potassium carbonate. The solution was heated to 40°C and filtered under vacuum. The filtered solution was cooled to -25°C for several hours, thus leading to the formation of white crystals. The crystals were filtered, dissolved in deionized water and subsequently lyophilized (Yield = 72%). ^{1}H NMR (300 MHz, D_2O): δ 4.0 ppm (s, 2H, N-CH_2), 5.8 (m, 1H, CH_2=CH), 6.2-6.4 (m, 2H, CH_2=CH).

A.3. Preparation of sodium 3-(((benzylthio)carbonothioyl)thio)propane-1-sulfonate 2. Sodium hydride (1.139 g) was added to a 250 mL Schlenk flask and washed twice with 5 mL of hexane (previously dried over lithium aluminum hydride) under nitrogen atmosphere. Tetrahydrofuran (70 mL) and benzyl mercaptan (3.2 g) were added successively. The addition of the latter caused the precipitation of a white solid and formation of hydrogen gas. Subsequently, carbon disulfide (2.1 g) was added with a syringe causing the solution to turn yellow and to clear up. After 20 minutes the solution was cooled with a water bath and 3.1 g of 1,3-propane sultone solvated in approximately 6 mL of toluene were added dropwise. A yellow powder precipitated. The powder was separated using a P 40 filter frit and washed twice with 10 mL of toluene. The crude product was dried by flushing with dry nitrogen (Yield = 98%). ^{1}H NMR (600 MHz, D_2O): δ = 2.13 (quintet, 2H, CH_2), 2.97 (t, 2H, CH_2-SO_3^-), 3.51 (t, 2H, S-CH_2), 4.66 (s, 2H, phenyl-CH_2-S), 7.31-7.43 ppm (m, 5H, phenyl). ^{13}C NMR (150 MHz, D_2O): δ = 23.26 (CH_2), 35.07 (S-CH_2), 40.6 (phenyl-CH_2-S), 49.67 (CH_2-SO_3^-), 127.88 (CH phenyl), 128.89 (CH phenyl), 129.23 (CH phenyl), 135.75 (C phenyl).

A.4. Example of Reversible addition–fragmentation chain transfer polymerization of 1. *N*-acryloyl glycinamide **1** (100 Eq.), the RAFT chain-transfer agent **2** (1 Eq.) and the radical initiator VA-044 (0.3 Eq.) were added to a dry Schlenk tube. The tube was capped by a septum and thoroughly purged with nitrogen for at least 30 minutes. Then, degassed Milli-Q™ water was added through the septum with a degassed syringe (monomer concentration in water = 150 mg·mL^{-1}). The mixture was placed into an oil bath and stirred at 60°C. A ^{1}H NMR kinetic monitoring of the reaction indicated that very high monomer conversions were reached after one hour of polymerization. However, the reaction was stirred overnight in order to reach nearly quantitative monomer conversions. Afterwards, the reaction was stopped by opening the tube and exposing the reaction mixture to air. The crude solution was poured into

a dialysis tube (Roth, regenerated cellulose, MWCO 4000-6000) and dialyzed for some days against pure deionized water. Afterwards, the solution was lyophilized. The pure polymer appeared as a white powder (Yield > 95%).

B- Measurements and analysis:

B.1. Size exclusion chromatography (SEC). Molecular weights and molecular weight distributions have been determined by SEC performed at 70°C in dimethyl sulfoxide (DMSO) containing 5 g/L of LiBr as eluent, using a pre-column and one PSS-GRAL-LIN analytical column (particle size 10 µm) with a flow rate of 1 $mL \cdot min^{-1}$. The detection was performed with a RI- (Shodex RI-71) and a UV-Detector (Spectra-System UV 1000; 300 nm). For calibration, linear poly(methyl methacrylate) standards (PSS, Germany) were used. It should be noted that significant high molecular weight peaks (M_n ~ 200000-600000) can be observed in the DMSO chromatograms of poly(*N*-acryloyl glycinamide) if the SEC samples are not properly prepared. These shoulders are too high to be due to bimolecular termination and are attributed to the strong aggregation tendency of these polymers. This intermolecular aggregation was confirmed by dynamic light scattering measurements in the SEC eluent (Figure S2). Yet, the intensity of the aggregate peaks in SEC may be significantly minimized by preheating the solutions. For instance, molecular dissolution can be obtained if the samples are dissolved in the eluent at 80°C for some hours prior to analysis.

B.2. Nuclear Magnetic Resonance (NMR). ^{1}H NMR Spectra were recorded in D_2O or in DMSO-d_6 either on a Bruker Avance-600 operating at 600.24 MHz or on a Bruker Avance-300 operating at 300.13 MHz. The experimental average chain-length of the homopolymers was calculated from the D_2O spectra by comparing the integration of the benzyl protons of the α-chain end at 7.20-7.45 ppm to the integration of the polymer side-chain methylene protons (3.60-4.45 ppm) and to the integration of the polymer backbone protons, which resonate at 1.05-2.55 ppm. 2D [13C, 1H] Heteronuclear Single Quantum Coherence (HSQC) and Heteronuclear Multiple Bond Coherence (HMBC) experiments were performed in D_2O using a Bruker Avance-500 operating at 500.17 MHz (^{1}H)/125.78 MHz (^{13}C).

B.3. Dynamic Light Scattering (DLS). Dynamic Light Scattering was performed with a High Performance Particle Sizer (HPPS-ET 5002, Malvern Instruments, UK) using a light scattering apparatus equipped with a He-Ne (λ = 632.8 nm) laser and a thermo-electric Peltier temperature controller.

B.4. Rheology. The rheology measurements have been performed on an AR G2 rheometer (TA Instruments, Germany) equipped with a cone-and-plate geometry (cone angle 1° 00' 22'', cone diameter 40 mm), a Peltier plate (accuracy ± 0.1°C) for temperature control, and a solvent trap for avoiding water evaporation. The probed temperature range was 5°C to 40°C. The samples were first analyzed by an oscillatory stress sweep in the range of 0.01 Pa – 100 Pa at fixed temperatures (5°C, 25°C and 37°C) and oscillatory frequencies of 1 Hz and 2 Hz. The aim of this measurement was to find a shear stress region where the shear storage modulus (G') and the shear loss modulus (G'') are independent of the shear stress, i.e. the linear viscoelastic region. Afterwards, a frequency sweep at constant oscillatory stresses (0.2 Pa and 1 Pa as deducted from the oscillatory stress sweep) and temperatures (5°C, 25°C and 37°C) was conducted. Then the temperature sweep was done at a heating rate of 1 °C/min and two different frequencies (1 Hz and 2 Hz) and two stresses (0.2 Pa and 1 Pa). The four sets of parameters were used in order to ensure the reproducibility of the measurements and to validate the stress and frequency independence of the loss and storage modulus. Finally the gel point and thus the gelation temperature was deducted from the temperature sweep (G'; G'' vs. T) curve. The gelation temperature was found at the intersection of the G' and G'' curves.

B.5. Tube inversion method. The CGC of the polymers was measured at room temperature using the tube inversion method. Polymer solutions (various concentrations in deionized water or PBS) were prepared in standardized vials. Afterwards, the vials were turned upside down. In such experiments, the gel state is defined as a non-flowing semisolid.

C- Additional data and Figures:

Table S1. Properties of the homopolymers prepared by RAFT polymerization.

$DP_{n\ th}$[a]	M_n[b] [g·mol^{-1}]	M_w/M_n[c]	DP_n	CGC[f] [wt%]
100	16900	1.36	87[d]	> 20
200	38000	1.24	209[d]	> 10
300	58900	1.24	325[e]	~ 7.0
400	83000	1.19	458[d]	~ 5.5
500	92500	1.21	567[e]	~ 4.5

[a] Theoretical average chain-length $DP_{n\ th}$ = [**1**]/[**2**]. [b] Number average molecular weight measured by SEC in DMSO for the main peak of the chromatograms. [c] Molecular weight distribution measured by SEC in DMSO for the main peak of the chromatograms. [d] Estimated from 300 MHz ^{1}H NMR spectra in D_2O. [e] Estimated from 600 MHz ^{1}H NMR spectra in D_2O. [f] Critical gelation concentration measured at room temperature using the tube inversion method in phosphate buffered saline solution.

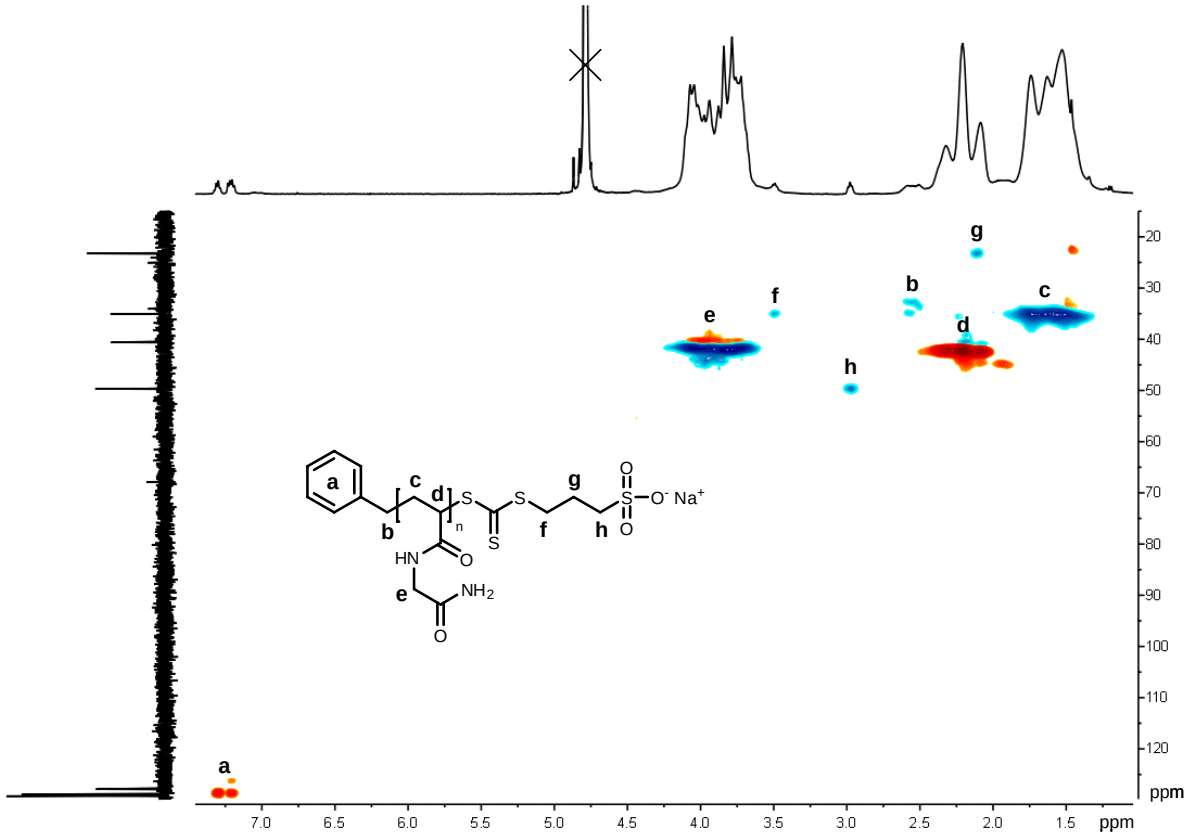

Figure S1. 2D [13C, 1H] HSQC experiment recorded in D_2O for a homopolymer with an average chain length of 100. The x-axis shows the ^{1}H NMR spectrum of the homopolymer. For comparison, the y-axis shows the ^{13}C NMR spectrum of the RAFT chain transfer agent **2**.

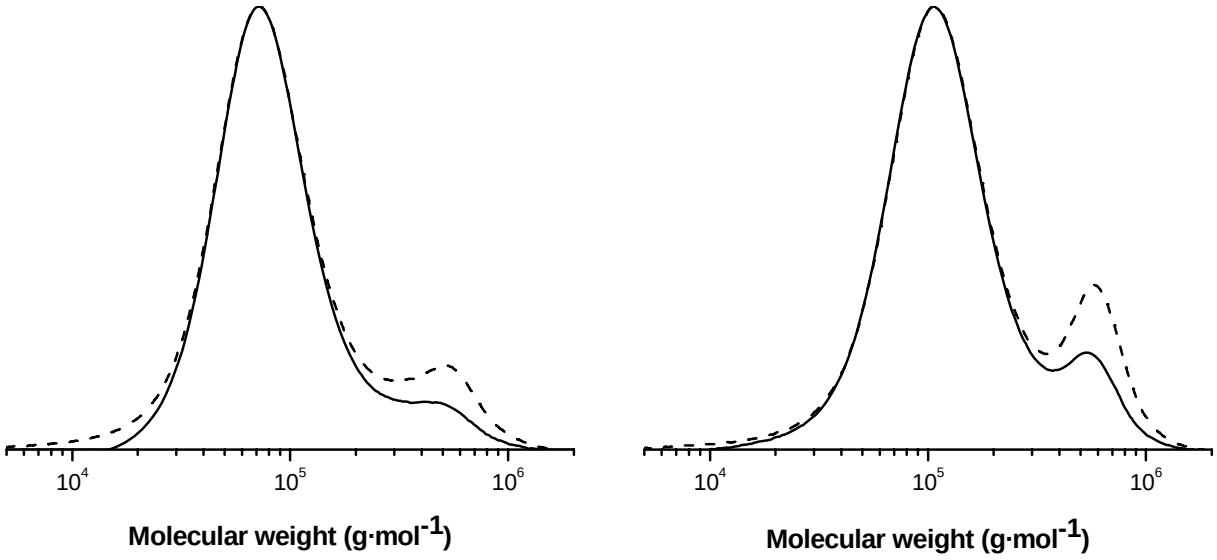

Figure S2. SEC chromatograms recorded in DMSO at 70°C for homopolymers of **1** with an average chain length of 300 (left) or 500 (right). Prior to measurement, the samples were dissolved in hot DMSO for 30 minutes (dashed lines) or several hours (full lines).

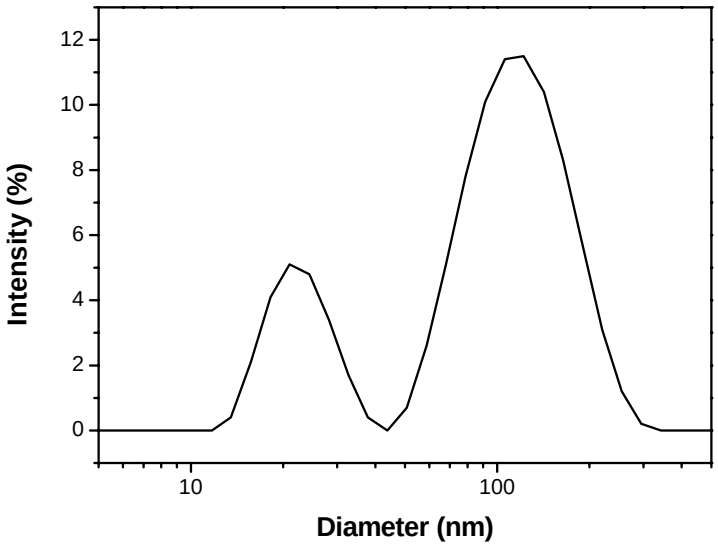

Figure S3. Intensity size distribution measured by dynamic light scattering at 70°C for a DMSO solution of a homopolymer of **1** with an average chain length of 500.

References and notes.

1. Haas, H. C.; Schuler, N. W., *J. Polym Sci. Part B: Polym. Lett.* **1964,** 2, (12), 1095-1096.