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Salt-Induced Shape-Memory Effect in Gelatin-based Hydrogels

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ABSTRACT

Hydrophilic biopolymers display a strong tendency for self-organization into stable secondary, tertiary, and quaternary structures in aqueous environments. These structures are sensitive to changes in external conditions, such as temperature, pH or ions/salts, which may lead to molecular and/or macroscopic transitions. Here, we report on biopolymer-based stimuli-sensitive switchable

matrices showing a shape-memory function as an output being alternatively switched by two different input signals, such as environmental changes in salt concentration or temperature.

This was realized by implementing a shape-memory function in hydrogels based on the coil-to-helix transition of protein chains in gelatin-based networks. The hydrogels exhibited mechanical properties similar to that of soft tissue (storage modulus $G' = 1-100$ kPa) and high swelling capabilities ($Q = 1000-3000$ vol%). In these gelatin-based networks, the covalent netpoints defined the permanent shape while after deformation helicalization of the gelatin acted as reversible stimuli-sensitive switches providing additional crosslinks capable of fixing the deformed temporary shape. By using either chaotropic salts to suppress gelatin helicalization or kosmotropic salts to support conformational changes of gelatin toward a helical orientation, these additional crosslinks could be cleaved or formed. In bending experiments, the strain fixity (R_f) and strain recovery ratios (R_r) were determined. While R_f ranged from 65 to 95% and was depending on the network composition, R_r were independent of the hydrogel composition with values about 100%. In addition, R_f and R_r were independent of the type of chaotropic salt that was used in this study, showing equal R_f and R_r values for $MgCl_2$, $NaSCN$, and $Mg(SCN)_2$.

INTRODUCTION

The design of polymeric matrices that can responsively change shapes rely on information encoded into their structure, creating functional physical behavior, enabling directed and on demand changes of the macroscopic appearance, for example, a shape-memory and an actuation capability.¹ This can be described using the input-function-output relationship. The input (denominated as stimulus) is an inducing environmental signal causing that the internal structural barrier is removed, resulting in the output, the recovery of the original shape. In order to implement

the functionality of a directed movement into a switchable matrix, several requirements have to be fulfilled. The polymer matrix has to be crosslinked to enable a permanent shape of the switchable matrix. Furthermore, a stimuli-sensitive molecular switch has to be implemented into the matrix in order to translate the signal to the macroscopic device. The coupling of the function of the stimuli-sensitive switch to the macroscopic dimension of the switchable matrix can be realized by direct connections to the polymer matrix. The external stimulus is directly connected with the molecular basis for shape fixation and/or movement.² Reprogrammable macroscopic-directed shape-shifts in hydrogels were realized by transferring strategies for shape fixation and recovery to hydrophilic networks containing water³, by employing dipole-dipole interactions⁴, or, in somewhat misnamed approaches, relying on swelling effects⁵. Hydrophilic biopolymers can often be used to form hydrogels^{6, 7} and display a strong tendency to self-organize into specific three-dimensional conformations⁸. This ability of biopolymers is used to implement a shape-memory function in biopolymer-based hydrogels.^{9, 10} Such changes in secondary, tertiary, and quaternary structures are especially well investigated in proteins and their fragments, being sensitive to changes in their external environment. One example of protein self-organization is the temperature-dependent equilibrium of collagen-related sequences between a random coil structure and single as well as triple helical conformations.¹¹⁻¹³ In bioengineered proteins with terminal collagen-like peptide sequences, temperature-dependent triple helicalization could be employed to form temporary netpoints for the fixation of a temporary shape.⁹ Moreover, other than temperature, pH or ions influence protein folding.¹⁴⁻¹⁶ Hofmeister was the first to report on the influence of ions on the solubility of proteins which was later found out to be directly connected to the protein's three-dimensional structure.¹⁷ It was shown that ions such as CNS⁻, Ba²⁺, I⁻, Ca²⁺, and Mg²⁺ support a random coil structure, whereas ions such as SO₄²⁻, NH₄⁺, N(CH₃)₄⁺, CH₃COO⁻, Rb⁺, and K⁺

encourage a helical conformation.¹⁸ This phenomenon was based on the so-called chaotropic (former series of ions) and kosmotropic (latter examples of ions) effect of ions on biomacromolecules such as proteins or DNA.^{19,20} A kosmotropic effect causes secondary structure reinforcement by supporting H-bond formation, while a chaotropic ion accumulation to the macromolecule is perturbing the H-bonds leading to disaggregation of the secondary structure.^{18,}
²¹ The different efficacy of ions to act chaotropic or kosmotropic has been described in the so-called Hofmeister series. In gelatin-based hydrogels, the influence of salts on gelatin chain aggregation was studied and employed in order to implement the shape-memory function.^{22, 23} However, this effect could only be realized using kosmotropic ions in physically crosslinked polymer networks. A shape-memory capability sensitive toward ions could be realized in hydrogels by swollen polymer networks, which provide ion coordinative groups.²⁴ In these systems, the ions become a part of the temporary crosslinks fixing the temporary shape and could potentially be leached out. In contrast, we aimed at a shape-memory hydrogel system, in which the temporary crosslinks are an integral part of the polymer network and could not be leached out.

We explored whether, in addition to kosmotropes, the chaotropic properties of salt solutions could be used for shape fixation and/or trigger a directed shape change of protein-based shape-memory hydrogels by stabilizing or destabilizing temporary netpoints based on hydrogen-bonded atom groups. For this purpose, a polymer matrix based on gelatin, a derivative of collagen, was employed, in which the formation and dissolution of triple helices acted as temporary netpoints to enable the reprogrammable shape. Gelatin-based networks with a shape-memory capability could be realized by covalent crosslinking upon irradiation with an electron beam, by coordinative crosslinking using Ureidopyromidone (Upy) coordination motifs, in blends from gelatin and alginate, in which the alginate compounds provide the switching function as well as in

interpenetrating networks from crosslinked polyacrylamide (PAM) and the physically crosslinked gelatin network enhanced with oxidized cellulose nanofibers.²⁵⁻²⁸ A permanent network is a necessary prerequisite for the later entropy-elasticity. We speculated that such a covalent polymer network could be realized employing the thiol Michael-type addition, which was demonstrated to be successful in other nonswollen SMP systems.^{29, 30}

In the following, the hydrogel synthesis and the implementation of the SME as well as characterizations on the helix formation and studies on the influence of salts on the hydrogel properties are shown. In addition to the active movement as the output in biopolymer-based smart materials using salts as one input signal, the temperature-dependency of the system is demonstrated, extending the number of input signals.

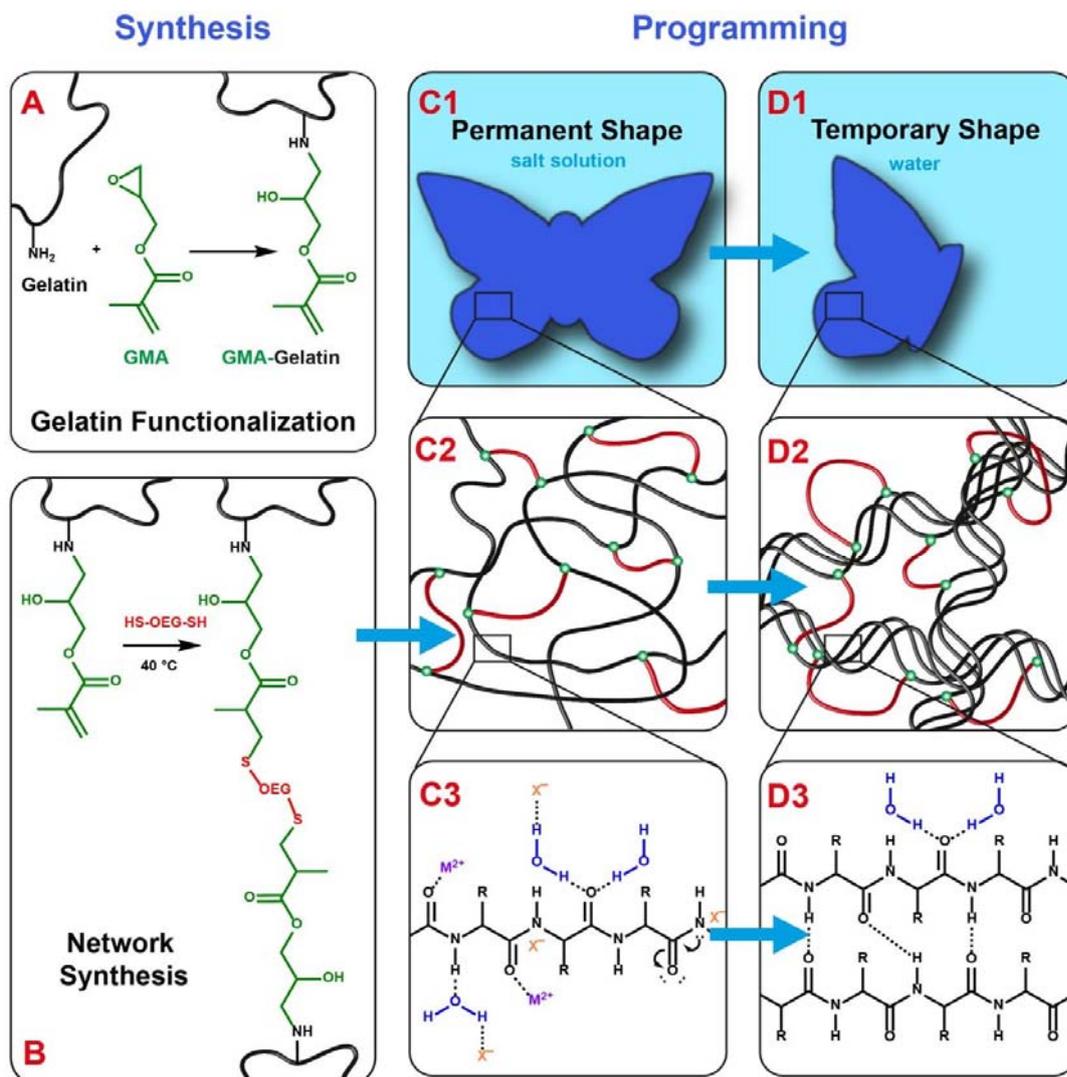


Fig. 1: Schematic overview about the synthesis and programming steps for implementing an SME into gelatin-based hydrogels: (A) Gelatin functionalization with glycidyl methacrylate (GMA). (B) Synthesis of gelatin-based networks by thiol-ene Michael-addition; gelatin (black), glycidyl methacrylate (green), oligo(ethylene glycol) (OEG) dithiol (red). (C) Network in the permanent shape: (C1) macroscopic view, (C2) schematic view with a random coil orientation of the gelatin main-chain (black), crosslinked with OEG (red) where the covalent netpoints (green) were previously formed by thiol-ene reaction, (C3) expected mechanism of ion coordination along the

gelatin backbone inhibiting the gelatin-gelatin interaction. (D) Network in the temporary shape after the programming process (deformation): (D1) macroscopic view, (D2) schematic view with the gelatin main-chain (black) in a helical conformation acting as temporary netpoints, crosslinked with OEG (red) where the covalent netpoints (green) were previously formed by the thiol-ene reaction, (D3) expected mechanism of gelatin-gelatin intramolecular interactions allowing the helicalization to fix the temporary shape.

EXPERIMENTAL SECTION

Materials

Gelatin (type A, 200 bloom), and comassie blue were purchased from Fluka (Steinheim, Germany). Glycidyl methacrylate (GMA), OEG1000, OEG3400, 2,4,6-trinitrobenzenesulfonic acid (TNBS), sodium chloride, sodium thiocyanate, sodium bicarbonate, ethanol, hydrochloric acid, diethylether, DMSO-D₆, Mg(SCN)₂, and D₂O were obtained from Sigma-Aldrich (Munich, Germany). Sodium carbonate and magnesium chloride were purchased from Merck (Darmstadt, Germany). All solvents and reagents were used without further purification. If not indicated otherwise, water refers to distilled water.

Synthesis

Gelatin Functionalization with GMA: Gelatin was solubilized in bicarbonate buffer (0.05 M, pH 9.6: 1.59 g sodium carbonate and 2.93 g sodium bicarbonate in 1 L Millipore® water) as a 10 wt% solution at 50 °C. GMA was added to this solution using a dropping funnel, and the reaction was allowed to stir at 50 °C for 3 h. The reaction product was obtained by precipitation from a fivefold volume excess of ethanol at room temperature (*RT*), in which it was stirred overnight for further

extraction of unreacted GMA. The GMA-functionalized gelatin (Gel-GMA) was cut into smaller pieces (1 cm x 1 cm), which were dried at 40 °C under reduced pressure. The degree of functionalization (DF) was determined by TNBS assay.

Polymer Network Synthesis: The polymer networks were synthesized by solubilizing the GMA functionalized gelatin in different concentrations (10, 20 wt%) in distilled water and addition of OEG dithiols with varying molar masses ($M_w = 1000, 3400 \text{ g}\cdot\text{mol}^{-1}$) solubilized in water (10, 20 wt%). The reaction mixture was stirred for one minute and casted into a Petri dish, followed by evaporation of the water at 40 °C. The dry polymer networks were washed with water for 24 hours, frequently exchanging the water to remove all unreacted reagents, before samples were further characterized.

Methods

TNBS Assay: For the determination of the DF of gelatin with GMA, the TNBS assay for the detection of free amino groups in gelatin was used. Gelatin (11 mg) was solubilized in 1 mL of 4% NaHCO_3 (pH 8.5) and 1 mL of 0.5 % TNBS. The reaction mixture was shaken at 40 °C for 4 h. Then, hydrochloric acid (6 M, 3 mL) was added, and the mixture was heated at 120 °C for 1 h to hydrolyze and dissolve any insoluble material. The samples were cooled to RT and diluted with 5 mL of water. The hydrolyzed solution was extracted with 20 mL (3x) ethyl ether to remove excess of unreacted TNBS. An aliquot (5 mL) of the aqueous phase was removed and heated for 20 min in a hot water bath to evaporate the residual ether. The aliquot was diluted with 15 mL of water, and the absorbance measured at 346 nm. All experiments were performed in triplicates and read against a blank, prepared by the same procedure as the samples, with the exception that the hydrochloric acid was added before the addition of TNBS, avoiding any reaction of TNBS with the gelatin. In this spectrophotometric method, TNBS reacts with primary amino groups of the

gelatin at an alkaline pH, forming a trinitrophenyl derivative, which can be detected by UV spectrometry, and the DF can be calculated using equations I and II, where n_{NH_2} is the relative content of amino group in the sample in $\text{mol}\cdot\text{g}^{-1}$, A is the measured absorbance at 346 nm, V is the volume of the aliquot (0.02 L), (1.46×10^4) is the absorption coefficient of the 2,4,6-trinitrophenyl derivative of lysine functionalized on the ϵ -amino group, l is the cell path length (1 cm), m is the dry sample weight, half of which is diluted to 20 mL, hence the factor 2 in the numerator, and $n_{NH_2^0}$ is the amount of free amino groups in gelatin before functionalization ($3.48 \times 10^{-4} \text{ mol}\cdot\text{g}^{-1}$).

$$n_{NH_2} = \frac{2 \cdot A \cdot V}{1.46 \times 10^4 \cdot l \cdot m} \quad (\text{Equation I})$$

$$DF = 100 - \frac{n_{NH_2}}{n_{NH_2^0}} \times 100\% \quad (\text{Equation II})$$

Nuclear Magnetic Resonance: Nuclear magnetic resonance spectra were recorded at 25 °C on a NMR Bruker system (DRX 500 Avance, Bruker Biospin GmbH, Rheinstetten, Germany) and analyzed with ACD Labs 12.0. The samples were dissolved or swollen in D₂O and relaxation time was set to 10 s.

Gel Content: The gel content of the polymer networks was determined by washing the networks in water for 24 h at RT and is calculated by dividing the dry weight of the sample after extraction (m_{extr}) through the dry weight before extraction (m_{iso}). The measurement was performed with five replicates.

$$G = \frac{m_{\text{extr}}}{m_{\text{iso}}} \cdot 100\% \quad (\text{Equation III})$$

Volumetric Degree of Swelling: The swelling behavior of gelatin hydrogels was investigated in water or salt solution at 4, 37, and 55 °C. Dry hydrogel discs were immersed in swelling medium for 24 h at RT to allow equilibrium swelling, which was previously studied in kinetic swelling

experiments. The swollen hydrogels were incubated in the swelling medium at different temperatures (4, 37, and 55 °C) for 12 h to allow the self-organization of the polymer chains. Afterwards, the hydrogels were taken out of the swelling medium, and the gel surface was gently blotted with filter paper to remove the unbound water on the surface. The weight of the swollen hydrogel disc (m_{sw}) was measured. Salt-swollen hydrogels were washed with water. Afterwards, the samples were dried until a constant weight was achieved, and the dry weight (m_d) was measured. The volumetric degree of swelling (Q) was calculated using equation IV. All measurements were performed in six repetitions. The density of the dry polymer films (ρ_{polymer}) was determined by pycnometry. ρ_s is the density of the swelling medium.

$$Q = 1 + \rho_p \cdot \left(\frac{m_{sw}}{m_d \cdot \rho_s} - \frac{1}{\rho_s} \right) \cdot 100\% \quad (\text{Equation IV})$$

Morphology of the Networks: Wide-angle X-ray scattering (WAXS) measurements were investigated on a Bruker D8 Discover with a 2D-detector from Bruker AXS (Karlsruhe, Germany). WAXS images were collected from gelatin films in transmission geometry with a collimator-opening of 0.8 mm at a sample-to-detector distance of 15 cm. The X-ray generator was conducted at a voltage of 40 kV and a current of 40 mA, which produces Cu- K_α radiation with a wavelength of $\lambda = 0.154$ nm. The 2D-detector (Hi-Star, 1024 x 1024 pixel mode) was calibrated with corundum standard (Al_2O_3) and placed at a distance of 15 cm.

Thermal Properties: Micro-Scanning calorimetry (μ -SC) measurements were performed on a μ -SC micro calorimeter (Setaram, France). Swollen gelatin networks were heated from 4 to 60 °C with an underlying heating rate of 1 K·min⁻¹, followed by a cooling cycle from 60 to 4 °C with a cooling rate of 1 K·min⁻¹. Then, the sample was again heated to 60 °C with a heating rate of 1 K·min⁻¹, followed by an isothermal run at 4 °C for 12 h to study the helicalization process.

Mechanical Properties: Rheological measurements were performed on a Haake Rheowin Mars II and Haake Rheowin Mars III (Thermo Scientific, Karlsruhe, Germany) using a 20 mm plate-plate geometry. A solvent trap was disposed above in order to avoid water evaporation. During the stress sweep measurements (from 0.1 to 100 Pa) and frequency sweep measurements (from 0.01 to 100 Hz) at 4, 37, and 55 °C, the parameters for the linear viscoelastic region (4 Pa, 1Hz) were determined. Time sweep measurements were performed at 4 or 55 °C for 5 min to 12 h, depending on the results that were expected. Temperature ramp measurements were performed using a controlled stress of 4 Pa and a frequency of 1 Hz because at these values, the systems were found to be in the linear viscoelastic range. Each run was carried out for 1 h at a heating rate of 1 K·min⁻¹. For all measurements, a constant force of 1 N was applied. For kinetic studies on the formation of helices, the hydrogels were incubated in water until equilibrium swelling, followed by incubation in salt solution. Afterwards, the hydrogels were transferred to the rheometer, and G' was measured as a function of t_{salt} . For kinetic studies on the helix dissociation, the hydrogels were incubated in salt solution until equilibrium swelling, followed by washing with water which was regularly exchanged. Afterwards, the hydrogels were transferred to the rheometer, and G' was measured as a function of t_{water} .

Determination of Netpoint Density: The netpoint density ν_c of the hydrogels was calculated from rheological measurements using equation V with G_R , R , and T representing the shear modulus, the universal gas constant, and the temperature, respectively. G_R was calculated from the storage modulus (G') and the loss modulus (G'') according equation VI:

$$G_R = \sqrt{G'^2 - G''^2} \quad (\text{Equation V})$$

$$v_c = \frac{G_R}{R \cdot T} \quad (\text{Equation VI})$$

Quantification of the SME: The shape-memory properties were determined by calculation of strain fixity R_f and strain recovery ratio R_r from the samples cut into stripes and heated to 55 °C. The initial angle (θ_i) of the sample was determined. Then, the samples were programmed by deformation by bending in the middle of the stripe and fixed between aluminum foil, after determining the angle of the programmed sample (θ_p). The samples were furthermore cooled at 4 °C for a minimum of 8 h, in order to fix the temporary shape, followed by determining the angle of the fixed sample (θ_f). In order to allow the recovery of the permanent shape, the aluminum foil was removed, and the samples were incubated in 5M MgCl₂, NaSCN or Mg(SCN)₂ solutions at 4 °C to recover the permanent shape. The angle of the recovered shape was determined (θ_r). R_f and R_r were calculated according to equations VII and VIII.

$$R_f = \frac{\theta_f - \theta_p}{\theta_i - \theta_p} \cdot 100\% \quad (\text{Equation VII})$$

$$R_r = \frac{\theta_f - \theta_p}{\theta_f - \theta_r} \cdot 100\% \quad (\text{Equation VIII})$$

RESULTS AND DISCUSSION

Gelatin-based hydrogels were synthesized by thiol-ene Michael-addition of methacrylated gelatin and oligo(ethylene glycol) (OEG) dithiols. In order to allow a chemoselective crosslinking reaction, gelatin was first functionalized at the lysine residues with glycidyl methacrylate (GMA) (Figure 1A).³¹ The degree of GMA-functionalization was investigated by NMR studies and a

photometric assay based on the reaction of trinitrobenzene sulfonic acid with the remaining free amino groups of lysine in gelatin (TNBS assay), showing high degrees of methacrylation around 94 mol-%. In a second step, GMA-gelatin was reacted with OEG dithiols of different chain lengths ($M_w = 1000$ and $3400 \text{ g}\cdot\text{mol}^{-1}$) resulting in network formation under mild reaction conditions (Figure 1B). This synthesis was performed in water and did not require addition of a catalyst, avoiding a difficult catalyst removal after network formation. In order to determine the influence of molecular composition on the degree of swelling and the mechanical properties and to investigate the shape-memory capability of these systems, the hydrogel networks were prepared with different molar amounts and chain lengths of OEG as well as different polymer concentrations during crosslinking. The hydrogels are referred to in the following as $G_x_OEGy(z)$, where x depicts the polymer concentration in the hydrogel synthesis, y describes the chain length of the OEG crosslinker, and z is attributed to the molar ratio of the crosslinker (thiol/methacrylate) used in the crosslinking reaction. The successful crosslinking reaction was demonstrated by NMR studies, showing the disappearance of the peaks corresponding to the protons of the methacrylic double bond at 5.5-6.5 ppm after network formation (Figure S1, Supporting Information). Unfortunately, a successful crosslinking reaction could not be confirmed by Fourier transform infrared (FT-IR) investigations (Figure S2, Supporting Information) as the spectrum is dominated by the bands associated to gelatin. Furthermore, the crosslinking reaction yielded in high gel contents above 70%, independent of the polymer concentration (Figure S3, Supporting Information).

The conformational changes of the gelatin chains between random-coil and helical organization were studied by WAXS measurements (Figure S4, Supporting Information), rheology (Figure S5, Supporting Information), and differential scanning microcalorimetry (μ -DSC) (Figure S6,

Supporting Information). The studies on the morphology of the dry polymer networks by WAXS showed a peak at a scattering angle $2\theta = 8^\circ$, which indicated that the gelatin chains could adopt a triple helical structure in the networks, which was a requirement for the formation of temporary netpoints to implement a SME in gelatin-based hydrogels. In rheological measurements for evaluations on the mechanical properties of the hydrogels, a decrease of storage modulus (G') was observed at 20-40 °C (depending on the composition), further referred as T_{trans} , which was connected to the dissolution of gelatin triple helices.³² During heating, the gelatin chains undergo an order-disorder transition by cleaving the thermo-reversible physical netpoints by dissociating the helices that were stabilized by hydrogen bonds. The cooling below the helix-to-coil transition temperature led to an increase in G' , which corresponds to the reformation of additional physical netpoints by helicalization of the gelatin. G' was evaluated as a function of cooling time at 4 °C, where after a sharp increase of G' within 30 min a further increase of G' was observed, reaching a stable plateau after approximately 8 h. This time period is slightly longer compared to noncrosslinked gelatin capable to solidify freely, similar to a jelly pudding. This observation indicates that the initiation of helicalization is fast but that the propagation of the helices requires a longer cooling period. This is in accordance to previous studies showing that the formation of the triple-helix nucleus occurs rapidly, while the overall helix content is a result of triple helix lengthening, where the cis-trans isomerization remains the rate-limiting step of helix propagation.³³⁻³⁵ In addition to the data obtained by rheology, μ -DSC experiments showed that the hydrogels exhibited a thermal transition at 10-37 °C, which was attributed to the shift between a helical conformation and a random-coil organization.

Based on the shear moduli obtained from rheological measurements, the crosslink density (ν_c) of the hydrogels was calculated on the basis of theories by Flory and Rehner for the rubber-

elasticity of polymer networks.³⁶ ν_c was influenced by the hydrogel composition as well as by the temperature (Table S1, S2, Supporting Information). ν_c was decreased from $9.1 \pm 0.1 \text{ mol} \cdot \text{m}^{-3}$ to $3.8 \pm 0.1 \text{ mol} \cdot \text{m}^{-3}$ by increasing the molar thiol-to-methacrylate ratio (z) above 1. At $z = 1$ an equal number of reactive groups were present during the synthesis, resulting in the highest crosslinking efficiency. This was in accordance with the gel content, which was the highest at $z = 1$ (Figure S3, Supporting Information). Accordingly, an increasing z led to a decrease in ν_c , attributed to the increasing number of dangling thiol groups. Furthermore, an increase in chain length of the crosslinker from $1000 \text{ g} \cdot \text{mol}^{-1}$ to $3400 \text{ g} \cdot \text{mol}^{-1}$ led to a decrease of ν_c to $2.8 \pm 0.1 \text{ mol} \cdot \text{m}^{-3}$ according to the increased mesh size of the polymer network, which consequently also increased the swellability and decreased the G' . Additionally, ν_c was affected by variation of the temperature. With increasing temperature, ν_c was decreased, attributed to the decreasing number of physical netpoints by disaggregation of the gelatin helices. Above T_{trans} , ν_c was defined by the covalent netpoints which were formed during the crosslinking. Below T_{trans} , not only the covalent netpoints, but also the physical netpoints contributed to the entire number of netpoints.

The hydrogels exhibited equal swelling capabilities below and above T_{trans} (T_{low} and T_{high} , respectively). As at T_{low} the gelatin chains adopt a helical orientation, while at T_{high} a random-coil orientation is favored, the swelling data indicated that the presence of helices has no influence on the swelling behavior of the hydrogels (Figure S6, Supporting Information). However, the swelling capability of the hydrogels in water was dependent on their composition, showing an increase with increasing chain length of the OEG crosslinker and when increasing the molar ratio between thiol and methacrylate above 1:1 (Figure S7, Supporting Information). The conformation-independent swelling behavior of the hydrogels was required to be proven in order to assure that the swelling has no influence on the SME (Figure S8, Supporting Information).

In order to prove the hypothesis that the required temporary netpoints for the SME can be induced by salts, the following strategy was chosen: equilibrium swollen gelatin hydrogels were deformed in a chaotropic salt solution at temperatures below T_{trans} ($T_{\text{low}} = 4\text{ }^{\circ}\text{C}$) and incubated in water to fix the temporary shape. Finally, the permanent shape was recovered by addition of salts (Figure 1C, 1D). In this way, the permanent shape of the hydrogels in salt solution should only comprise the permanent netpoints, formed by the thiol-ene reaction, as helicalization was expected to be suppressed by the chaotropic ions. The removal of these ions should lead to a helicalization of the gelatin chains resulting in the creation of temporary netpoints that stabilize the macroscopically deformed shape. The recovery of the permanent shape should then be observed after addition of the chaotropic salts, attributed to the entropy elasticity of the polymer chains after cleavage of the temporary netpoints by perturbing the hydrogen bonding, which led to the disintegration of the helices. All experiments were performed at T_{low} , in order to assure the helix formation in water. The salts employed (MgCl_2 , NaSCN , and $\text{Mg}(\text{SCN})_2$) were selected according to their position in the ‘Hofmeister’ series as chaotropic, with MgCl_2 as a representative salt with a chaotropic cation, NaSCN with a chaotropic anion and $\text{Mg}(\text{SCN})_2$ having both the chaotropic cation and anion, respectively.^{17, 21} The SME could be successfully shown according to the described strategy (Figure 2A) and could be repeated in several cycles, showing the reprogrammability of the hydrogels. NaCl was used as reference salt, and the presence of this salt did not result in an SME, attributed to Na^+ and Cl^- having no chaotropic effect on gelatin.

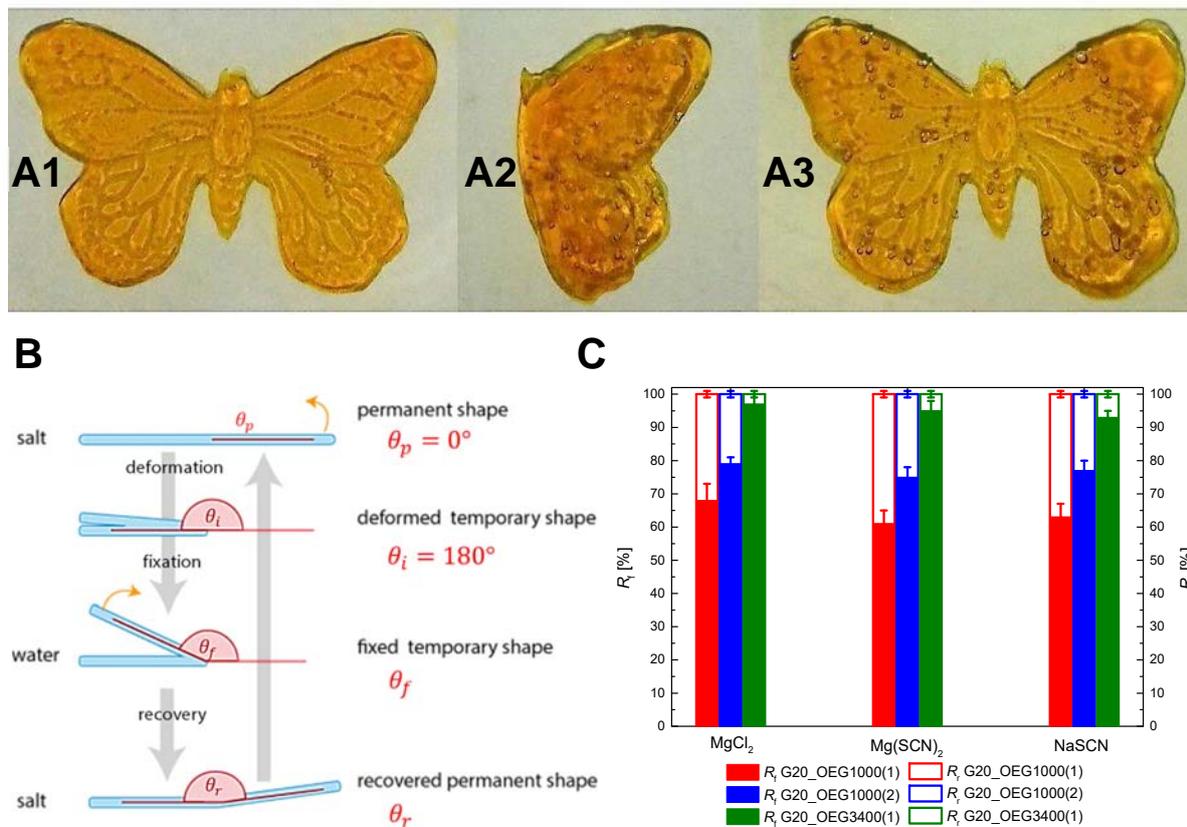


Fig. 2: A) SME demonstrating hydrogel (G20_OEG3400(1)): (A1) permanent shape of the hydrogel demonstrating a butterfly is incubated in MgCl₂ solution, (A2) deformed temporary shape is fixed in water, (A3) permanent shape is recovered in MgCl₂ solution. For better visualization, the hydrogel was stained with fluorescein, which had no influence on the SME. Especially in (A3), gas bubbles of the medium adhering on the sample can be noticed. (B) Schematic illustration of bending experiments for the determination of strain fixity and strain recovery ratios. (C) R_r - and R_r of gelatin hydrogels of different compositions using salt solutions.

For investigations on the shape-memory properties, the strain fixity (R_f) and strain recovery ratios (R_r) were determined in the bending mode, as shown in Figure 2B. R_f was depending on the network composition, ranging from 65 to 95% (Figure 2C). R_f was increasing with an increasing thiol-to-methacrylate ratio, attributed to a lower crosslinking density, allowing the formation of helices in the network in a higher amount than in more densely crosslinked systems. This is in accordance with the observation that R_f was increasing with increasing OEG chain length, which likewise led to an increasing mesh size of the polymer network. This behavior corresponds to the postulated molecular basis for form fixation based on helices acting as temporary netpoints. The hydrogels exhibited R_r values about 100%, independent of the hydrogel composition. In addition, R_f and R_r were independent of the type of chaotropic salt that was used in this study, showing equal R_f and R_r values for $MgCl_2$, $NaSCN$, and $Mg(SCN)_2$, as the waiting time periods in the shape-memory experiments were selected sufficiently long to exclude kinetic effects.

The influence of salts on the helicalization in the hydrogels was investigated by rheology (Figure 3A). Therefore, the networks were equilibrated in salt solution ($c= 5$ M) and water at T_{low} , and the storage moduli were evaluated as a function of temperature. The network swollen in water showed a clear decrease in the storage modulus between 20 and 40 °C from 7 to 2.5 kPa, according to the dissolution of helices at T_{trans} . In contrast, the hydrogels swollen in salt solutions exhibited a temperature independent plateau of $G' \sim 2$ kPa, implying that the addition of chaotropic salts suppressed the formation of helices in the hydrogels. This was observed for all chaotropic salts that were studied. According to the data obtained from rheology, the crosslinking density of the hydrogels in the different swelling media was calculated (Table S3, Supporting Information). In water swollen hydrogels, ν_c decreased by increasing temperature according to the decreasing number of netpoints, as helices were dissolved above T_{trans} . The hydrogels swollen in the

chaotropic salt solutions showed v_c values in the same range of the water swollen hydrogels at T_{high} , which clearly showed that the number of netpoints was decreased by addition of salts, related to the helix dissociation.

In addition, the influence of salt concentration on the helix suppression was investigated. Therefore, the hydrogels were equilibrated in MgCl_2 , NaSCN , and $\text{Mg}(\text{SCN})_2$ solutions with different salt concentrations (0.1-7.5 M) at T_{low} (Figure 3B). G' of the hydrogels incubated in salt solutions was compared with G' of hydrogels in water at T_{low} and T_{high} as reference values for maximal helicalization and helix suppression. With increasing salt concentration, G' was decreasing, attributed to the decreasing netpoint density by dissolution of the helices (Table S4, Supporting Information). In the above-mentioned salt concentrations of 5 M for MgCl_2 and 3.5 M for NaSCN and $\text{Mg}(\text{SCN})_2$ the hydrogels exhibited constant G' values which were comparable to the G' of hydrogels in water at T_{high} ($G' = 2.5$ kPa), indicating that the formation of all helices was inhibited at these concentrations. The nonchaotropic sodium chloride was studied as reference salt, which showed, as hypothesized, no influence on the mechanical properties (Figure 3A).

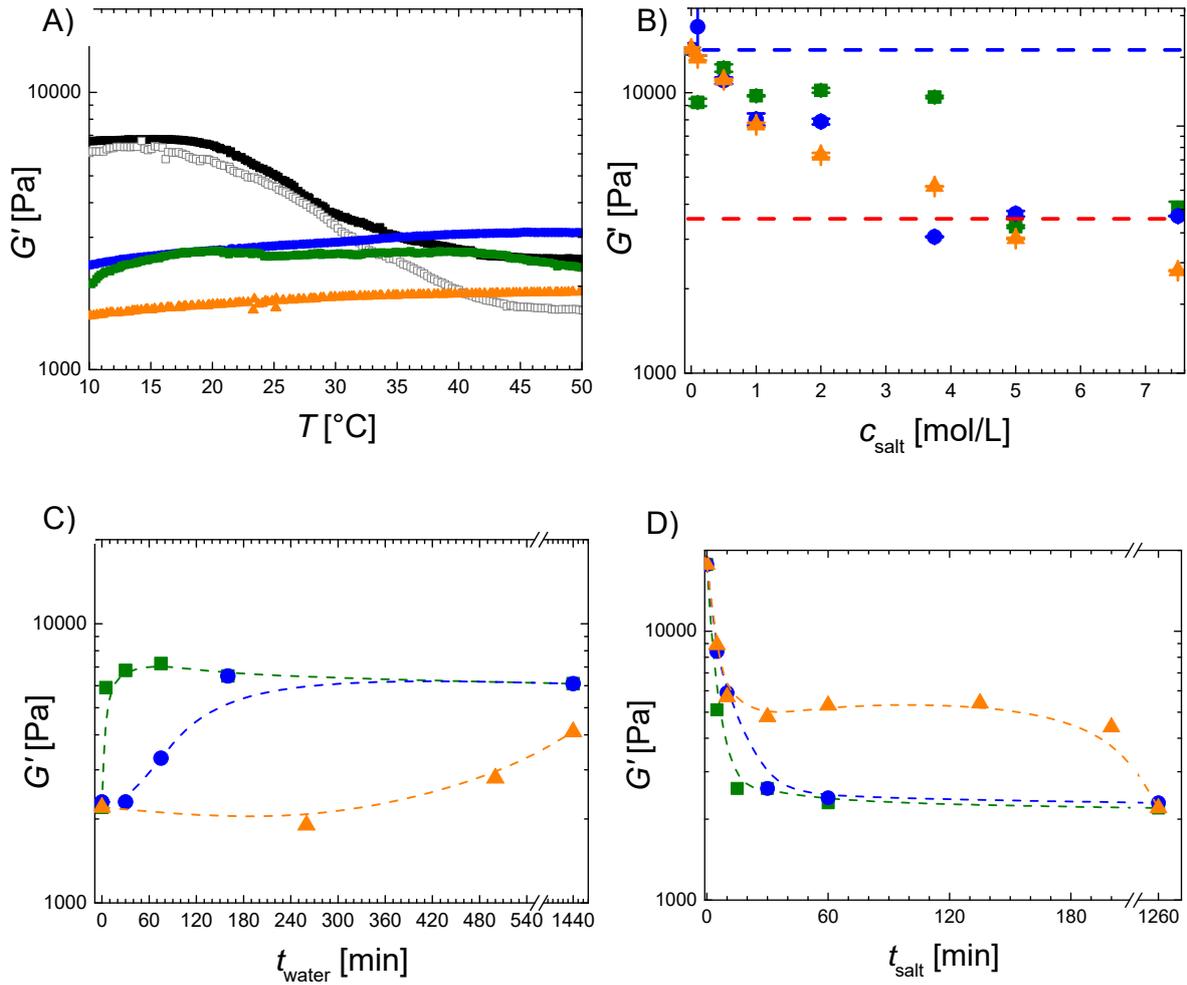


Fig. 3: (A) G' of G20_OEG3400(1) hydrogels incubated in chaotropic salt solutions (MgCl₂ (green ■), Mg(SCN)₂ (orange ▲), and NaSCN (blue ●), NaCl (□), and water (■)) as a function of temperature. (B) Influence on the salt concentration of MgCl₂ (green ■), Mg(SCN)₂ (orange ▲), and NaSCN (blue ●) on G' of G20_OEG3400(1) hydrogels at 4 °C. The blue dashed line is referred to G' of hydrogels in water at 4 °C and the dashed red line is referred to G' of hydrogels at 55 °C in water. (C) Kinetic study on G' of G20_OEG3400(1) hydrogels in MgCl₂ (green ■), Mg(SCN)₂ (blue ●), and NaSCN (orange ▲) solutions after washing with water at 4 °C; the dashed lines are guidelines to the eye. (D) Kinetic study at 4 °C on G' of G20_OEG3400(1) hydrogels in water

after addition of MgCl_2 (green ■), $\text{Mg}(\text{SCN})_2$ (blue ●), and NaSCN (orange ▲); the dashed lines are guidelines to the eye.

The kinetics of helix formation, related to the shape fixation of the temporary shape, were studied by rheology (Figure 3C), recording the G' of salt solution equilibrated hydrogels as a function of incubation time in water (t_{water}). After 24 hours, G' of the hydrogels reached a plateau at 7 kPa, revealing triple-helicalization of the gelatin chains. Hydrogels priorly incubated in salt solutions with Mg^{2+} ions showed a faster increase of G' ($t_{\text{water}} = 2\text{-}3$ h) compared to hydrogels in NaSCN solution ($t_{\text{water}} = 24$ h). Nevertheless, a complete recovery of G' was observed for all chaotropic salts. The kinetics of helix dissolution, related to the shape recovery, were studied (Figure 3D) by evaluation of G' of water-swollen hydrogels as a function of incubation time in salt solution (t_{salt}). G' was decreasing from 20-2 kPa with increasing t_{salt} , related to the increasing salt concentration in the hydrogels. As a consequence, the amount of hydrogen bond disturbing ions was increased, resulting in an increased suppression of helices and consequently a decrease in netpoint density. Hydrogels in Mg^{2+} -based salt solutions exhibited a stable plateau of G' after $t_{\text{salt}} = 30$ min, attributed to the entire suppression of the helices. NaSCN -incubated hydrogels reached the plateau of G' after $t_{\text{salt}} = 24$ h, also indicating the complete helix disaggregation. The decrease of G' until a plateau at 2 kPa was equal to the G' plateau of hydrogels incubated in water at T_{high} , where the helices acting as physical netpoints were disintegrated. This rheological study was in agreement with the shape-memory experiments, where the time for the fixation of the deformed shape was higher compared to the recovery time, as the formation of helices required more time than the dissolution. By comparison of the salts investigated in this study, all employed chaotropic salts

realized the dissociation of the helices. However, salts with Mg^{2+} as chaotropic ions showed faster kinetics than NaSCN, which might be attributed to the increased binding affinity of the double charged magnesium cations to the gelatin backbone in comparison to single charged sodium cations.

In addition to the presented strategy of using chaotropic salts to suppress gelatin helicalization, a second strategy was investigated. Here, kosmotropic salts were employed that were expected to support conformational changes of gelatin toward a helical orientation. The shape-memory experiments were performed at $T_{\text{high}} = 55\text{ }^{\circ}\text{C}$ by deformation of water-swollen hydrogels, that were then fixed in kosmotropic salt solution and recovered by reconditioning in water. For this strategy, Na_2SO_4 and NH_4Cl were used, and the SME was successfully realized. However, the hydrogels were shrinking after incubation in salt solution which might be attributed to a complex formation of the ions with the gelatin backbone leading to additional netpoints that limited the swellability of the hydrogels. Hence, the extreme change in Q does not clearly indicate that the SME was based on helices forming the additional shape-fixing netpoints but also other effects might have occurred that enabled the fixation of the temporary shape.

CONCLUSION

By transferring the knowledge of the influence of ions on the conformations of macromolecules to protein-based networks, a directed actuation induced by two different environmental signals could be successfully implemented in gelatin-based hydrogels, based on information encoded in their molecular structure. Here, an alternative mechanism for the SME as an output function in chemically crosslinked polymer networks was presented by inducing conformational changes in the network chain segments leading to the formation and dissolution of temporary netpoints. This

principle may be transferred to other macromolecules that undergo conformational changes by variation of ions in their external environment, resulting in stimuli-sensitive physical netpoints that are necessary for the fixation of temporary shapes. These studies lead to inspirations for the design of shape-memory hydrogels by extending the existing mechanisms with approaches based on conformational changes of macromolecules.

ASSOCIATED CONTENT

Supporting Information.

Figures: ¹H-NMR, FT-IR spectra, Gel content, WAXS spectra, Storage modulus, μ -SC, Volumetric degree of swelling, Tables: gel content and degree of swelling, calculated netpoint densities.

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Notes

The authors declare the following competing financial interest: A.L. and M.B. are co-inventors on patents in the field of polymer-based shape-memory materials.

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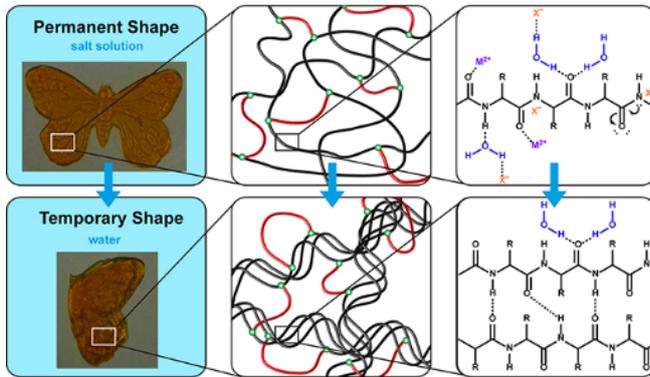
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For Table of Contents Use Only



Supporting information for

Salt-Induced Shape-Memory Effect in Gelatin-based Hydrogels

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Lendlein

Supporting data

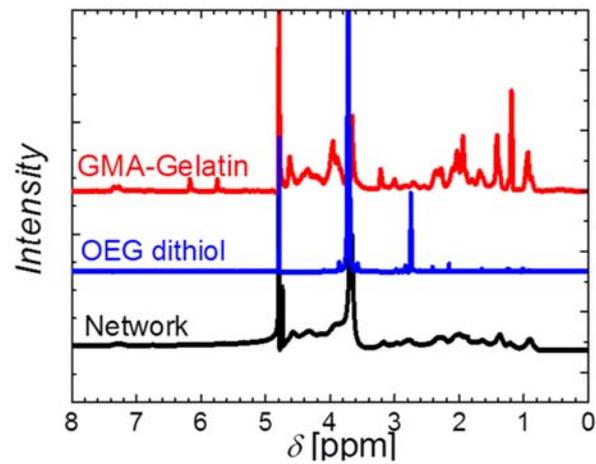


Fig. S1: ¹H-NMR of GMA-gelatin (red), OEG dithiols $M_w = 1000 \text{ g}\cdot\text{mol}^{-1}$ (blue) and the polymer network synthesized from both (black).

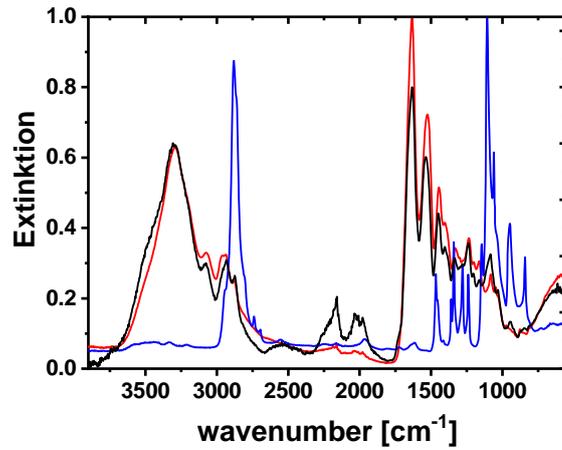


Fig. S2: FT-IR spectra of GMA-gelatin (red), OEG dithiols $M_w = 1000 \text{ g}\cdot\text{mol}^{-1}$ (blue) and the polymer network synthesized from both (black).

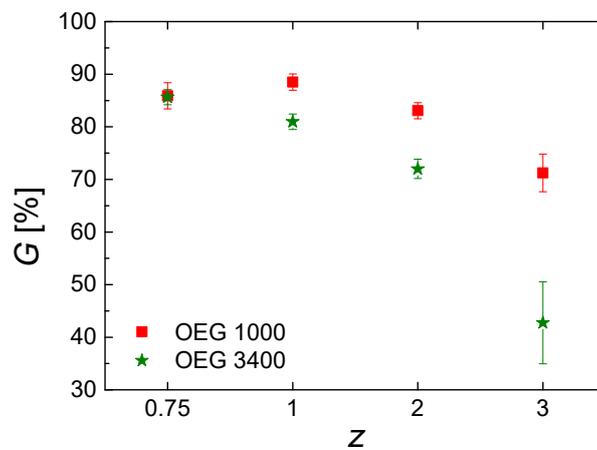


Fig. S3: Gel content (G) of gelatin networks, crosslinked with OEG dithiols of different chain length: OEG $M_w = 1000 \text{ g}\cdot\text{mol}^{-1}$ (■), and OEG $M_w = 3400 \text{ g}\cdot\text{mol}^{-1}$ (★), as function of the thiol-to-methacrylate ratio (z).

Table S1: Gel content (G) and volumetric degree of swelling (Q) at defined temperatures for different hydrogel compositions.

<i>Sample ID</i>	G [wt%]	$Q^{4\text{ }^\circ\text{C}}$ [vol%]	$Q^{37\text{ }^\circ\text{C}}$ [vol%]	$Q^{55\text{ }^\circ\text{C}}$ [vol%]
G20_OEG1000(0.75)	86 ± 2	1180 ± 80	1110 ± 80	1030 ± 60
G20_OEG1000(1)	89 ± 2	1080 ± 30	1010 ± 50	940 ± 50
G20_OEG1000(2)	83 ± 2	1630 ± 90	1610 ± 120	1620 ± 30
G20_OEG1000(3)	71 ± 4	3050 ± 210	3050 ± 360	2680 ± 30
G20_OEG3400(0.75)	86 ± 1	1340 ± 40	1280 ± 40	1290 ± 130
G20_OEG3400(1)	81 ± 1	1620 ± 70	1630 ± 120	1640 ± 170
G20_OEG3400(2)	72 ± 2	2410 ± 150	2390 ± 200	2400 ± 130
G20_OEG3400(3)	42 ± 8	2890 ± 100	2970 ± 160	2900 ± 160

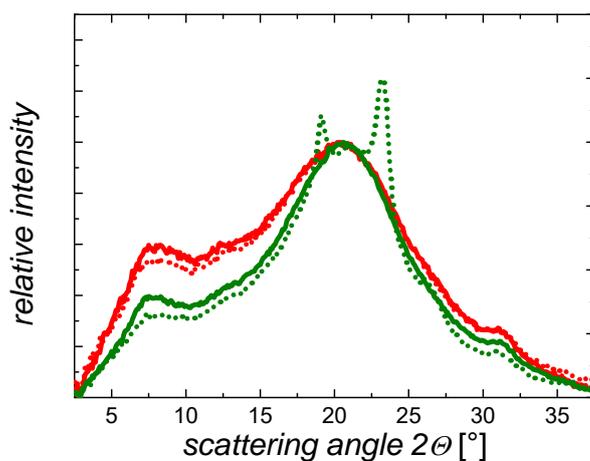


Fig. S4: WAXS spectra of gelatin networks: G20_OEG1000(1) (—), G20_OEG1000(2) (⋯), G20_OEG3400(1) (—), G20_OEG3400(2) (⋯).

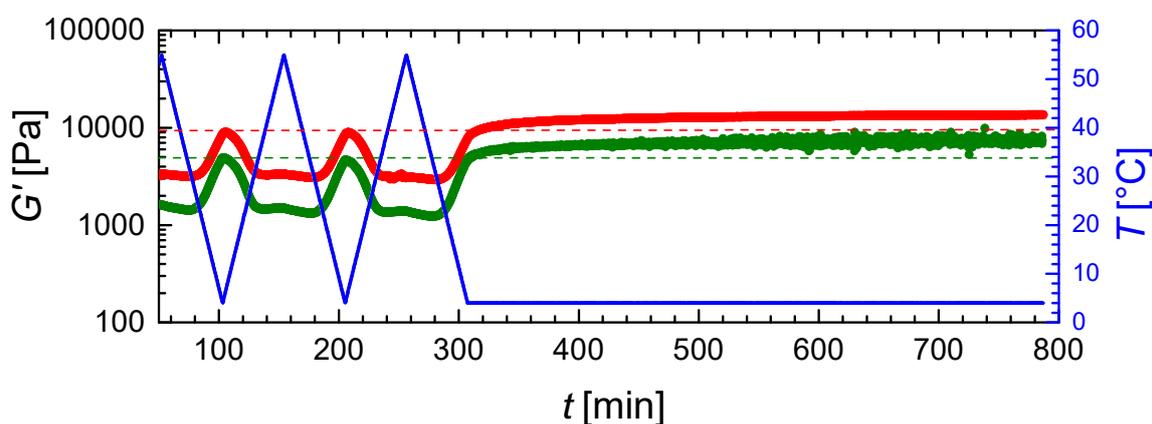


Fig. S5: Storage modulus (G') of gelatin networks, crosslinked with OEG dithiols of different chain length: OEG $M_W = 1000 \text{ g}\cdot\text{mol}^{-1}$ (●), OEG $M_W = 3400 \text{ g}\cdot\text{mol}^{-1}$ (●) with $z = 1$, as function of time after applying heating and cooling cycles (—).

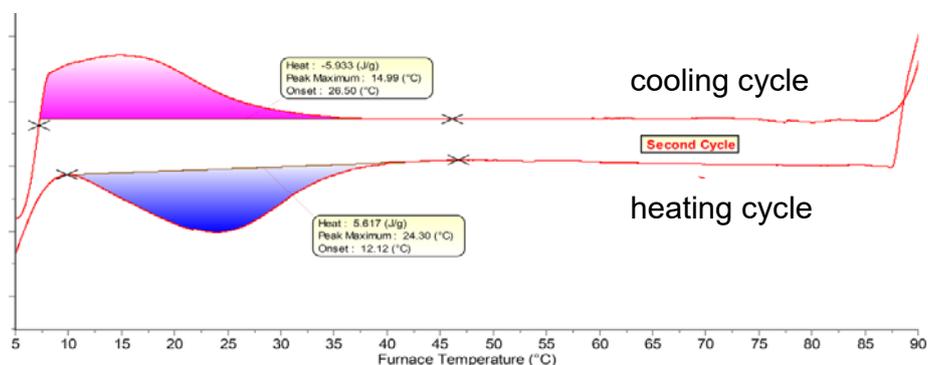


Fig. S6: Study on helix disaggregation (heating cycle) and helix formation (cooling cycle) of G20_OEG3400(1) measured by μ -DSC.

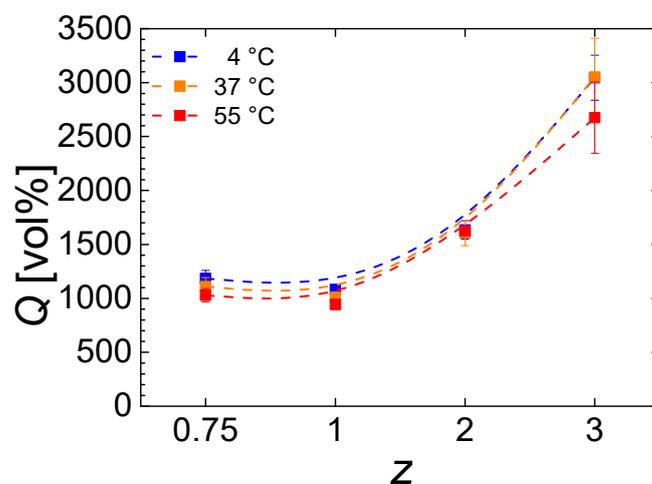


Fig. S7: Volumetric swelling (Q) of gelatin networks, crosslinked with OEG dithiols with a polymer chain length of $M_W = 1000 \text{ g}\cdot\text{mol}^{-1}$ as function of the thiol-to-methacrylate ratio (z), determined in the equilibrium swollen state at $4 \text{ }^\circ\text{C}$ (■), $37 \text{ }^\circ\text{C}$ (■), and $55 \text{ }^\circ\text{C}$ (■).

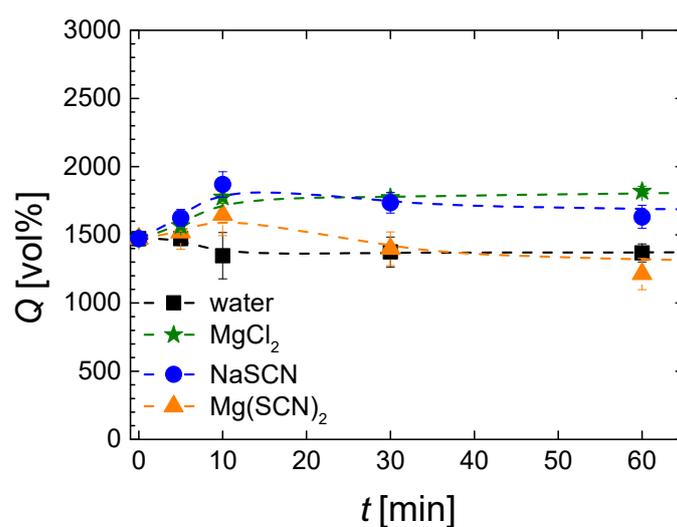


Fig. S8: Volumetric degree of swelling (Q) of G20_OEG3400(1) in 5 molar salt solutions as a function of time: water (■) MgCl_2 (★), $\text{Mg}(\text{SCN})_2$ (▲), and NaSCN (●). The influence of salts on the volumetric degree of swelling was investigated, in order to assure that the swelling has no influence on the shape-memory effect. The kinetic swelling studies were performed in 5 M salt solutions. As only a slight increase in the volumetric swelling behavior was observed when salts were added to the hydrogels, these studies indicate that the salts have no influence on the recovery of the permanent shape, which takes place within the first 30 minutes. This observation was independent of the type of salt that was used.

Table S2: Calculated netpoint density of hydrogels based on rheological measurements under variation of hydrogel composition and temperature.

Network composition	T [°C]	ν_c [mol·m ⁻³]
G20_1000(1)	4	9.1 ± 0.1
G20_1000(2)	4	3.8 ± 0.1
G20_3400(1)	4	2.8 ± 0.1
G20_3400(2)	4	1.8 ± 0.1
G20_1000(1)	55	5.4 ± 0.1
G20_1000(2)	55	3.3 ± 0.1
G20_3400(1)	55	0.9 ± 0.1
G20_3400(2)	55	0.8 ± 0.1

Table S3: Calculated netpoint density of G20_OEG3400(1) hydrogels based on rheological measurements under variation of swelling medium and temperature.

Swelling medium	T [°C]	ν_c [mol·m ⁻³]
H ₂ O	4	2.8 ± 0.1
H ₂ O	55	0.9 ± 0.1
5 M MgCl ₂ solution	4	1.0 ± 0.1
5 M NaSCN solution	4	0.9 ± 0.1
5 M Mg(SCN) ₂ solution	4	0.6 ± 0.1

Table S4: Calculated netpoint density of G20_OEG3400(1) hydrogels based on rheological measurements under variation of the salt concentration.

c_{salt} [mol·L ⁻¹]	ν_c [mol·m ⁻³]	ν_c [mol·m ⁻³]	ν_c [mol·m ⁻³]
	in MgCl ₂	in NaSCN	in Mg(SCN) ₂
0.1	4.0	7.5	5.8
0.5	4.0	4.8	4.8
1.0	4.2	3.5	3.3
2.0	4.4	3.4	2.6
2.5	4.2	1.3	2.0
5.0	1.4	1.6	1.3
7.5	1.7	1.6	1.0