

Supporting Information

Hydrogels with Dual Thermo-Responsive Mechanical Performance

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Materials.

N-isopropylacrylamide (NIPAm, Aldrich), *N,N'*-methylenebisacrylamide (MBA, Aldrich), potassium peroxydisulfate (KPS, Aldrich), *N,N,N',N'*-tetramethylethylenediamine (TEMED, Aldrich), acryloyl chloride (97%, Aldrich) and glycylamide hydrochloride (98%, Aldrich) were used as received. All organic solvents were analytical grade and water was purified with a Millipore system combining inverse osmosis membrane (Milli RO) and ion exchange resins (Milli Q) for synthesis and purification.

Characterization.

Differential Scanning Calorimetry (DSC). The phase transition of hydrogels was investigated by differential scanning calorimetry using a DSC Q200 from TA instrument. Hydrogels at preparation state (ca. 80 mg), equilibrated with a reference filled with the same quantity of pure water, were submitted to temperature cycles between 10 and 70 °C under nitrogen atmosphere. The heating and cooling rates were fixed at 2 °C min⁻¹.

Nuclear magnetic resonance (NMR). For the characterization of monomer, the measurements were performed on a Bruker Avance III HD spectrometer operating at 400 MHz for ¹H, using a standard 5 mm broadband Smart probe at 25 °C.

The measurements at different temperatures were carried out on a Bruker Avance III HD spectrometer operating at 600 MHz for ¹H using a BBFO 5 mm probe, with 32 transients, 45 degree flip angle, 2.5 sec acquisition time, 2 sec relaxation delay. The temperature control was achieved by a Bruker BCU II unit and a build in temperature control unit. The sample was allowed to equilibrate for 10 minutes at each temperature prior to test. The ¹H chemical shifts were referred to residual HOD peak at each temperature.

Capillary electrophoresis (CE). The experiments were performed with a Beckmann P/ACE MDQ system equipped with a diode-array spectrophotometer (detection at wavelength 200 nm +/- 10 nm). The bare silica capillary (J & W Scientific) was 75 i.d. x 31 cm. The run

buffer was 25 mM, $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ at pH=7.1. Prior to measurements, the capillary was flushed with 0.1 M NaOH, rinsed 1 min with water, and equilibrated with the run buffer. Between runs, the capillary was rinsed for 1 min with the run buffer, then a short zone of sample diluted in the run buffer was hydrodynamically injected at the inlet (pressure of 0.2 psi, 4 s), possibly followed by similar injection of a 0.1 v% of DMSO (used as neutral marker) in the run buffer. Separation proceeded with 9kV voltage and 0.1 psi pressure applied at the inlet to mobilize analytes toward the detection window.

Inductively coupled plasma atomic emission spectroscopy. ICP-AES were performed on iCAP 6200 from Thermo Scientific.

Turbidimetry. Absorbance measurements were carried out at different temperatures with a UV-vis Hewlett-Packard 8453 spectrophotometer using a 0.2 cm path length quartz cell, in a wavelength range from 200 to 1100 nm and equipped with a temperature controller (± 0.1 °C). The hydrogel sample was directly synthesized in the UV-vis cell prior to the test. Turbidimetry curves were built by collecting the absorbance at 670 nm with a scanning rate of 0.5 °C min^{-1} . The UCST- and LCST-type cloud points were defined as the temperature where the transmitted intensity reaches 50 % of the incident intensity. In the case of PNAGA phase transition temperature, the heating curve has been chosen according to the work reported by Seuring.^[1]

Swelling measurements. Equilibrium swelling experiments were performed in pure water at designated temperature. The samples at their preparation state were initially cut and placed in a large excess of water which was exchanged twice a day. The swollen gels were weighed (m_t) and the swelling ratio at equilibrium (Q_e) were calculated as $Q_e = m_t/m_d$, m_d being the dry weight.

Rheology. The viscoelastic properties of hydrogels were studied in their preparation state, using a stress-controlled rheometer (AR 1000 from TA Instruments) equipped with a roughened plate/plate geometry (diameter 40 mm, angle 2, truncature 1500 μm). The experiments were performed in the linear viscoelastic regime, which was established for each sample by a stress sweep at 1 Hz. The temperature was controlled by a high power Peltier system that provides fast and precise adjustment of the temperature during heating and cooling stages. Typically, the experimental conditions were fixed at constant frequency (1 Hz) and shear stress (2 Pa). Particular care was taken to avoid the drying of the sample by using a homemade cover, which prevents water evaporation during the experiment. In these

conditions, dynamic moduli (G' and G'') were recorded between 2 and 70 °C by applying heating and cooling scans at 2 °C min⁻¹.

Large strain behavior in tension mode. Tensile tests were performed on a standard tensile Instron machine, model 5565, equipped with an environmental chamber allowing a precise control of the temperature. The device used a 10 N load cell (with a relative uncertainty of 0.16 % in the range from 0 to 0.1 N) and a video extensometer which follows the local displacement up to 120 mm (with a relative uncertainty of 0.11 % at full scale).

The gel samples used for mechanical tests were synthesized in home-made moulds consisting of two covered glass plates spaced by a stainless steel spacer of 2 mm thickness. The gels were then cut with a punch and their final dimensions are 30 mm x 4.9 mm x 2 mm. The length was taken constant ($L \sim 20$ mm) for all the tests and the gel strip was marked with two dots with a white marker, for their recognition by the video extensometer. For high temperatures or long-term experiments, tests were conducted in an immersion cell consisting of a paraffin oil bath surrounding the sample in order to isolate the samples from the environment (prevent evaporation or swelling). As a control experiment we initially checked the total immiscibility of the paraffin oil with the gel within the temperature range investigated in this study. All the tests were carried out at the fixed strain rate of 0.06 s⁻¹.

Synthesis of NAGA monomer.

A new procedure for NAGA monomer synthesis was adapted from Seuring et al.^[2] but greatly improved with the sake of enhancing total yield, facilitating purification process and avoiding the presence of ionic groups (**Figure S1**). The main change relates to the use of organic miscible cosolvent MeOH/Et₂O in the place of H₂O/Et₂O from the previous studies. In this case, the reaction is easier to process and the purification steps can be largely simplified (**Table S1**). For instance, time-consuming steps such as lyophilisation and column chromatography are no longer necessary. Moreover, in our case, even though acryloyl chloride can react with methanol, the corresponding product, methyl acrylate, could be simply removed by rotary evaporation or extraction. Therefore, we can work with an excess of acryloyl chloride to ensure that the glycinamide hydrochloride could be totally converted into NAGA monomer.

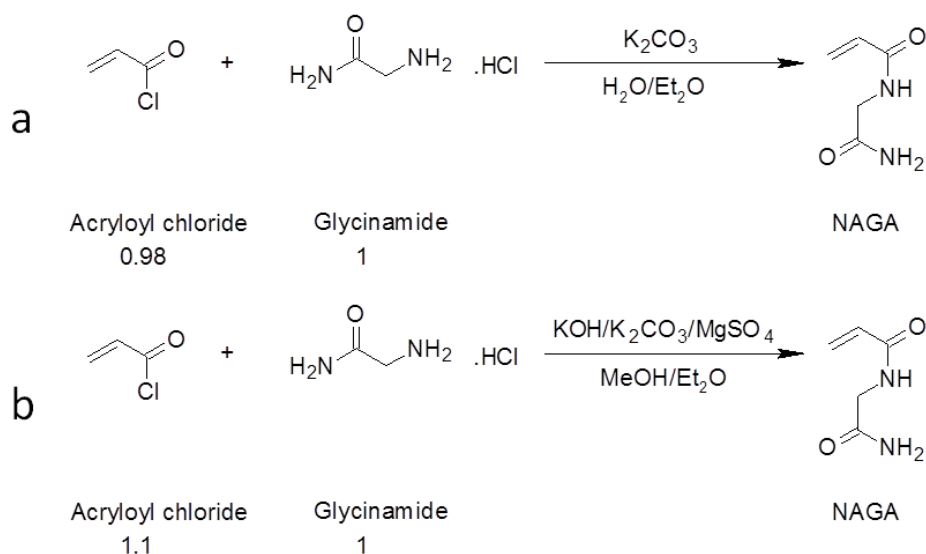


Figure S1. (a) Conventional synthesis procedure of NAGA monomer^[2] (b) Modified synthesis procedure of NAGA monomer.

Table S1 Comparison of work up steps between conventional method and revisited method

Previous method	Our method
	i. Removal of Et ₂ O by rotary evaporation
i. Removal of Et ₂ O by rotary evaporation	ii. Removal of methanol after filtration
ii. Removal of water by lyophilization ^a	iii. Extraction with acetone (twice) and removal of volatile solvent
iii. Extraction with acetone (6 times) and removal of volatile solvent ^a	iv. Recrystallization
iv. Column chromatography ^a	
v. Recrystallization	

^a steps are time-demanding and labor-consuming

Typically, in a 500 mL three-necked round-bottom flask, glycine hydrochloride (5.53 g, 50 mmol), potassium hydroxide (5.61 g, 100 mmol), potassium carbonate (2.07 g, 15 mmol) and magnesium sulphate (12.60 g, 105 mmol) were well dispersed in 50 mL of dry methanol. The solution was cooled in an ice bath and acryloyl chloride (4.98 g, 55 mmol) initially dissolved in 60 mL of diethyl ether was added dropwise over 2 h under vigorous stirring. The suspension was warmed to room temperature and further stirred for 3 h to fulfil the reaction. Then, diethyl ether was removed by rotary evaporation and the remaining suspension was gently warmed to 50 °C. After removing all the precipitate by filtration, the remaining volatile solvent was evaporated, and the residual solid was recovered with hot acetone to give rise to the crude product. NAGA monomer was finally recrystallized from isopropanol to yield pure product of 4.89 g, yield=76.4%. ^1H NMR (400 MHz, $\text{DMSO-}d_6$): δ =3.78 (d, J =6.0 Hz, 2H), 5.65 (dd, 1H, J_1 =2.1 Hz, J_2 =10.2 Hz), 6.13 (dd, 1H, J_1 =2.1 Hz, J_2 =17.1 Hz), 6.36 (dd, 1H, J_1 =10.2 Hz, J_2 =17.1 Hz), 7.07 (s, 1H), 7.40 (s, 1H), 8.31 (s, 1H) (**Figure S2**). CE: Acrylic acid impurities <0.01% wt (**Figure S3**), Potassium content: 0.7 ppm (ICP-AES).

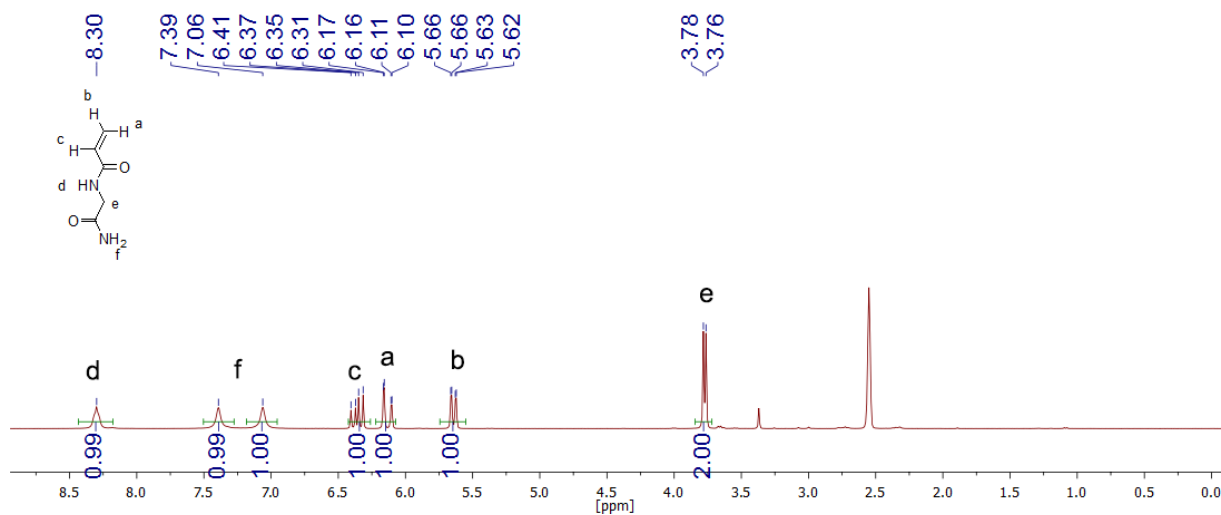


Figure S2. ^1H NMR spectra of NAGA monomer in $\text{DMSO-}d_6$. The NMR signals located at 2.55 and 3.4 ppm correspond to DMSO and water respectively.

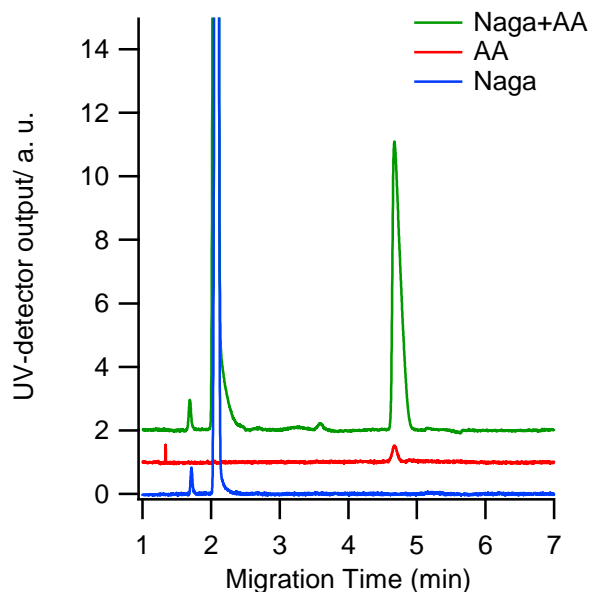


Figure S3. CE spectra of NAGA monomer (blue line), Acrylic acid (red line) and a mixture of both (green line), all in 25 mM $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ buffer at pH=7.1. The CE signals located at 2.06 and 4.67 min correspond to NAGA and Acrylic Acid respectively.

Hydrogel preparation

Synthesis of pure PNIPAm and PNAGA gels. Monomer and chemical cross-linker (stock solution of MBA) were initially dissolved in a given amount of water under nitrogen bubbling at 30 °C. Stock solutions of KPS and TEMED were separately prepared under nitrogen atmosphere and after 30 min, aliquots were added into the reaction medium. After fast mixing (2 min), the final formulation was rapidly transferred between glass plates of 2 mm width under nitrogen atmosphere and the reaction was left to proceed overnight at 30 °C. The resulting hydrogels were then cut with a die-cutter of rectangular or round shape and directly used for DSC and swelling experiments or stored into paraffin oil before mechanical testing in order to avoid any change in hydrogel composition induced by swelling or drying. For both gels, the weight ratio of “monomer/water” was constant, equal to 1/5 ($Q_0=6$), and the molar ratio of “monomer:MBA:KPS:TEMED” was set equal to 100:0.1:1:1.

Synthesis of PAN gels. First, the synthesis of PNIPAm macromonomer was achieved using a telomerization process with cysteamine hydrochloride followed by modification of the amino end-group with acrylic acid in order to get the vinyl function.^[3] The absolute characterization

of macromonomer by size exclusion chromatography gives average molar mass of $M_n=36$ kg mol⁻¹ ($D=1.4$).

Due to the transition temperatures of PNIPAm and PNAGA, the hydrogel synthesis was carried out under N₂ atmosphere at 30 °C, where both polymers are in coil conformations. Typically, NAGA monomer (1 g, 7.8 mmol), PNIPAm macromonomer (1 g, 0.028 mM) and chemical cross-linker MBA (1.20 mg, 0.1 mol% to monomer) were dissolved in a corresponding amount of water. After 30 min of deoxygenation with nitrogen bubbling at 30 °C, KPS (21.1 mg, 1 mol% to monomer) and TEMED (9.1 mg, 1 mol% to monomer) were dissolved separately in water, deoxygenated, and transferred subsequently into the aforementioned medium. The weight ratio “monomer/macromonomer/water” of gel was kept constant, equal to 1/1/10 ($Q_0=6$). After fast mixing (2 min), the final solution was rapidly transferred into different moulds under nitrogen atmosphere, either plates of 2 mm width or syringes of 5 mL. The reaction was left to proceed at 30 °C overnight.

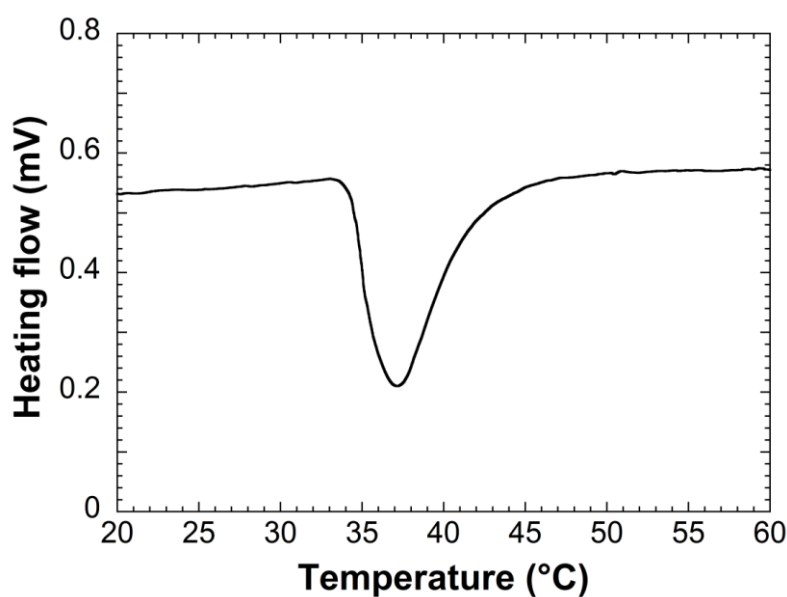


Figure S4 Thermogram of PAN hydrogels obtained by differential scanning calorimetry at 2 °C min⁻¹. The PNAGA phase separation cannot be determined with conventional DSC, due to the very low value of enthalpy.¹

Swelling behavior of pure PNIPAm and PNAGA gels.

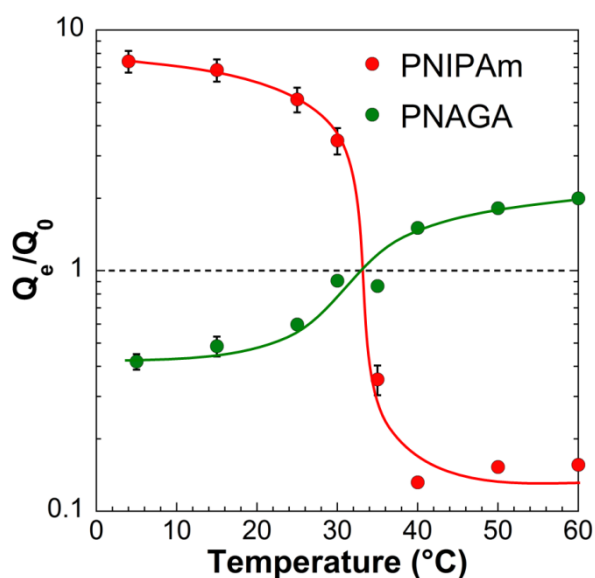


Figure S5. Swelling behavior of pure PNIPAm (red) and PNAGA (green) gels in water as a function of the temperature. Solid lines are used as guide lines. The dotted line corresponds to $Q_e/Q_0=1$. Both pure PNIPAm and PNAGA gels exhibit a volume change due to their phase transition.

Comparison of the rheological properties between single and dual thermo-responsive gels.

In order to investigate the impact of the PNAGA phase separation on the rheological properties, a comparison between dual thermo-responsive PAN gels (present work) and a single thermo-responsive gel, GPD-N,^[4, 5] was carried out as a function of temperature (**Figure S6**). GPD-N is a hydrophilic poly(*N,N*-dimethylacrylamide), PDMA, network (referred as GPD) grafted with thermoresponsive PNIPAm side-chains (denoted as-N) having both same average molar mass than PNIPAm macromonomers, same crosslinker amount and same weight ratio as used in this study. As shown in **Figure S6**, both PAN and GPD-N display very similar viscoelastic behavior at high temperature with a value of elastic modulus around 10 kPa, demonstrating the impact of the PNIPAm phase separation on the elastic properties. Note that the temperature at which the thermo-reinforcement starts as well as the observed hysteresis during the heating/cooling cycle ($2\text{ }^\circ\text{C min}^{-1}$) were very close, highlighting the similar topology. However, at low temperature, the elastic modulus of GPD-N remains very low (1 kPa) as both polymers PDMA and PNIPAm are in coil state. On the

other hand, a higher value of G' was observed for PAN (7 kPa) which clearly demonstrates the thermo-reinforcement of PAN gels at low temperature.

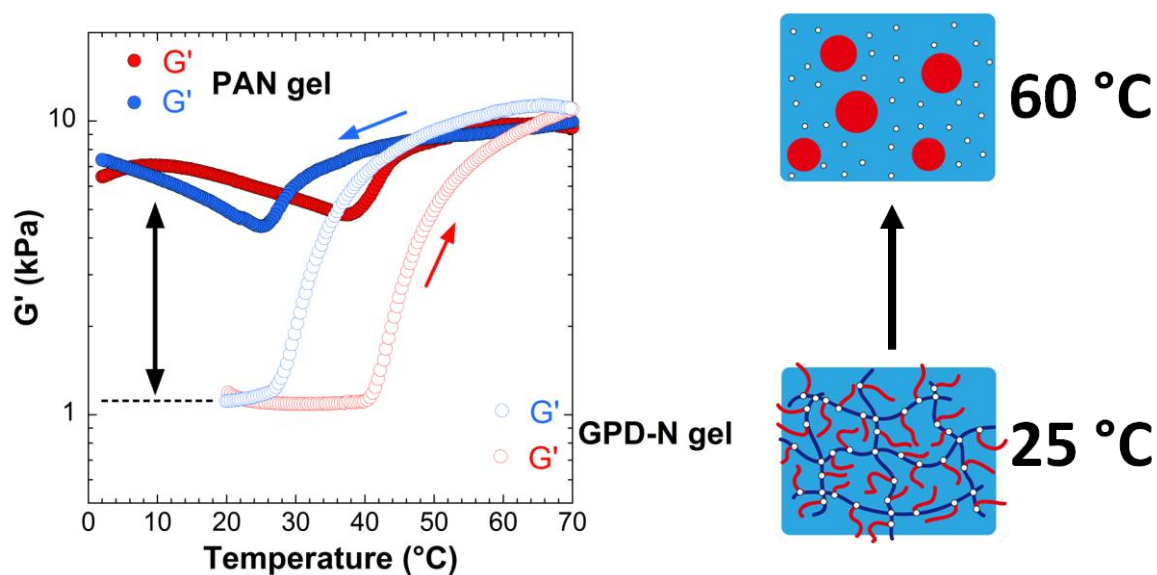


Figure S6. (left) Comparison of elastic moduli, G' , at 1 Hz at preparation state with a heating/cooling rate of $2\text{ }^{\circ}\text{C min}^{-1}$ (red, upon heating; blue, upon cooling) of dual thermo-responsive PAN gel (filled symbols) and singular thermo-responsive gel, GPD-N (empty symbols). (right) Structure of GPD-N at 25 and 60 $^{\circ}\text{C}$, where deep blue backbone represents PDMA, and red grafter stands for PNIPAm chains.

Tuning of the temperature-responsiveness of PAN hydrogels.

Turbidity experiments were carried out on PAN hydrogels swollen in Na_2SO_4 aqueous solution of different concentrations. The results were reported in **Figure S7**.

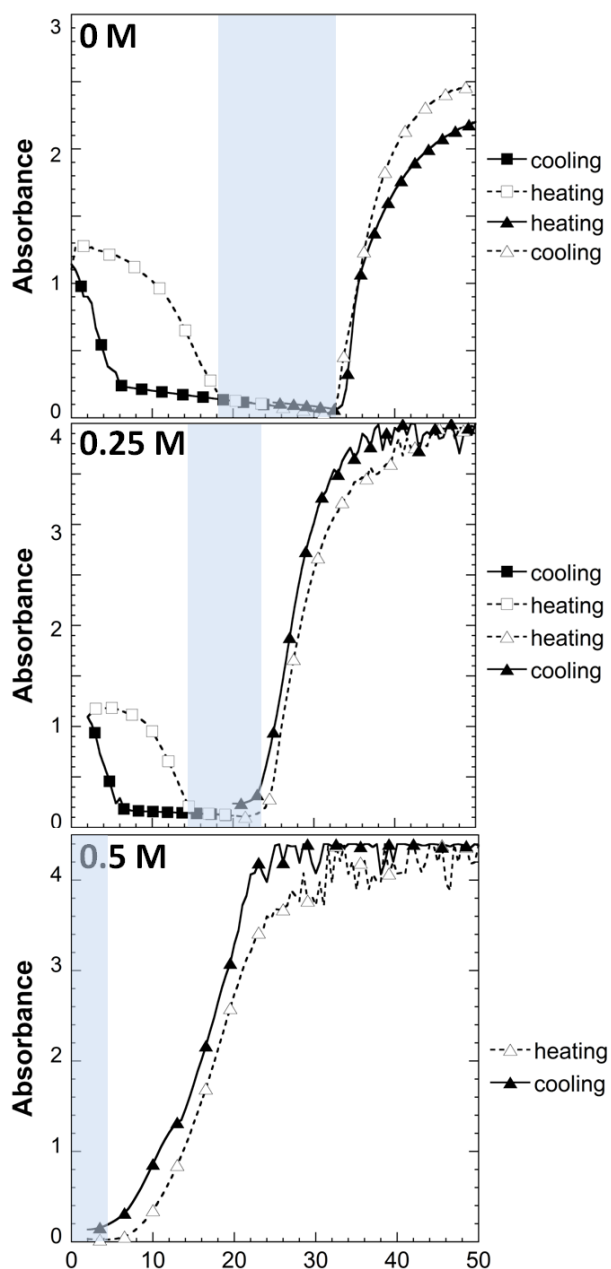


Figure S7. Turbidity curves of PAN gels swollen in Na_2SO_4 aqueous solution of different concentrations (0, 0.25 and 0.5 M) as a function of the temperature with a heating/cooling

rate of 0.5 °C min⁻¹.

References

- [1] J. Seuring, F. M. Bayer, K. Huber, S. Agarwal, *Macromolecules* **2012**, *45*, 374.
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