Structural insights into the mechanism of heat-set gel formation of polyisocyanopeptide polymers

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Keywords: Hofmeister effect, neutron scattering, hydrogel, heat-set gelation

One of the key factors influencing the mechanical properties of natural and synthetic extracellular matrices (ECM) is how large-scale 3D gel-like structures emerge from the molecular self-assembly of individual polymers. Here, structural measurements using small-angle neutron scattering (SANS) from ECM-mimicking oligo(ethylene glycol)-decorated polyisocyanopeptide (PIC) hydrogels are reported as a function of background ions across the Hofmeister series. More specifically, we examine the process of polymer assembly by probing the structural features of the heat-set gels and correlating them with their rheological and micro-mechanical properties. The molecular parameters obtained from SANS clearly show changes in polymer conformation which map onto the temperature-induced changes in rheological and micro-mechanical behavior. The scattering data further indicate the formation of larger structures in strong gels, while the structures of mechanically weak gel assemblies are similar to those of free flowing solutions. The formation of larger structures is linked to the formation
of cross-links (or bundles), whilst the onset of their detection in the SANS is putatively linked to their concentration in the gel. These insights provide support for the ‘hot-spot’ gelation mechanism of PIC heat-set gels. Finally, we find that formation of cross-links and heat-set gelling properties can be strongly influenced by ions in accordance with the Hofmeister series. In practice, these results have significance since ions are inherently present in high concentration during cell culture studies; this may therefore influence the structure of synthetic extracellular matrix networks and as such should be considered as one of the key parameters when designing PIC-based ECM-mimicking networks.

Artificial systems that exactly mimic the morphology of naturally occurring polymer networks are extremely rare.\textsuperscript{[1-3]} Natural networks consist predominantly of proteins and exhibit several levels of structural conformation and organization.\textsuperscript{[4-6]} It is these higher orders of structure that give these polymers the ability to dynamically control and adapt their physical and mechanical properties in response to their environment.\textsuperscript{[7,8]} In multicellular organisms, the protein polymer networks form the foundation of extracellular matrices (ECM), which provide cells with essential mechanical and (bio)chemical support. The ECM also play a crucial role in governing processes inside the cell, including proliferation, differentiation, and migration .\textsuperscript{[7, 9-11]} It is known that physical properties, such as stiffness and architecture of the ECM, play a role in triggering intra-cellular chemical signals through a process called mechano-transduction\textsuperscript{[12-14]}; however, the biophysical mechanism of mechano-transduction remains largely unclear - primarily with regards to how macroscale mechanical properties cascade down to the local mechanical environments of the cells.

In recent years, several different hydrogel systems have been developed to create artificial biogels for biomedical application.\textsuperscript{[15]} Despite noticeable progress in the field of synthetic biogels, most studies use commercially available naturally-derived ECMs such as Matrigel©, a mouse tumor lysate.\textsuperscript{[16-19]} The focus on nature-derived materials is chiefly stimulated by
differences between the behavior of biological and artificial matrices, both mechanical and chemical. Whilst incorporating key signaling components into biogels can successfully functionalize synthetic ECM-like matrices, the ability to mimic the architecture and mechanical properties of ECMs is severely limited. Typically, synthetic polymer-based hydrogels form a dense, homogeneous network; by contrast, semi-flexible biopolymers in natural ECM gels assemble to form a highly porous network made up of comparatively stiff polymer assemblies. In our previous research we described a synthetic ECM-mimicking system based on a helical ethylene-glycol-functionalized polyisocyanopeptide (PIC, Figure S3, Supplementary Info) and found that this gel closely resembles the mechanical properties of naturally-occurring polymer networks.[20-22] This synthetic PIC polymer forms soft thermo-responsive heat-set gels with rheological properties that closely mimic the strain-stiffening behavior of biogels,[23-25] such as actin,[26] collagen and intermediate filaments.[27] The benefit of PIC, as a well-defined synthetic material, is that complex rheological and functional properties of the PIC gels can be easily controlled and tuned to specific needs and applications. We previously demonstrated how their mechanical properties can be readily and predictably manipulated as a function of strain,[21] concentration, polymer length,[22] temperature[28, 29] as well as the addition of salts.[30] Recently, we also have demonstrated that small angle X-ray scattering techniques (SAXS) can be used as a major tool to determine the parameters of PIC gel network architecture as a function of PIC molecular weight, concentration and temperature.[31, 32] In this work, we address the current gap in knowledge about the initial stages of the heat-set gel formation in oligo(ethylene glycol)-decorated PIC polymers. We observe the associated processes across a broad range of scattering angles owing to the implementation of small-angle neutron scattering providing us with information on a wide range of lengthscales previously not available to SAXS. Furthermore we implement a fitting model specifically developed for the study of semiflexible polymers, previously not used for PIC polymers,[33] and by using this
model, we study how the heat-set gel formation and polymer bundle structure is affected by the presence of ions from the Hofmeister series.\[^{34, 35}\]

The Hofmeister series is a series of ions, first described in 1888, which are ordered based on their ability to precipitate proteins from aqueous solutions; an effect known as the Hofmeister effect.\[^{36}\] Since the initial report, the Hofmeister effect was not only found to influence protein precipitation or stabilization, but also many other processes, such as the lower critical solution temperature (LCST) of synthetic aqueous polymers.\[^{37}\] The precise origin of the Hofmeister series ion effect is still the object of ongoing discussion with the two major hypotheses being that these ions have an effect on (i) the “water structure” surrounding the proteins (polymers)\[^{38}\] or (ii) the way the salts bind to the proteins and its associated effect on the protein solvation shell.\[^{39}\]

Using a set of specifically synthesized PIC polymers with varying molecular weight, P1–P3,\[^{20}\] we have utilized a combination of oscillatory shear rheometry and SANS to probe the gel structure and their associated mechanical properties across a broad range of spatial dimensions, temperature and ionic environment. (Table 1).

Exploration of the dependence of the heat-set behavior on viscosity-derived average molecular weight ($M_{\eta}$) and polymer concentration revealed a qualitatively similar trend, with the gel strength increasing with increase in concentration and $M_{\eta}$. For PIC solutions in D$_2$O, Figure 1A shows the temperature dependence of the shear moduli ($G'$ and $G''$) evaluated at constant stress and frequency during a temperature ramp of 1 °C/min. Below ~15 °C, PIC solutions exhibit viscous behavior which then transitions, within a very short window of temperatures, to the elasticity dominated viscoelastic behavior. From there, the value of $G'$ undergoes a rapid increase within 5 °C. The inflection point of the $G'$ vs $T$ curve is used to define the effective gelling temperature. From ca. 22.5 – 24.0 °C, depending on $M_{\eta}$ and concentration, the positive slope of $G'$ versus temperature decreases, and above 25 °C this slope is approximately constant.
The heat-set behavior can also be further probed by monitoring the temperature-dependence of the viscoelasticity parameter \((\tan \delta = G''/G')\). The \(\tan \delta\) decreases rapidly from \(\sim 1\) to \(0.02 – 0.03\), passing through a minimum and then slowly trends upwards. At 37 °C, the values of \(\tan \delta\) converge at 0.03 – 0.04 for all \(M_{[n]}\) and concentrations studied. At 37 °C, the gels quickly reach their steady-state set strength \((G'_{\text{max}})\) within 300 s, with \(G'\) and \(G''\) remaining roughly constant. However, during the course of 30 min, the values of \(\tan \delta\) progressively decrease (Figure S1A, Supplementary Info), indicating that the evolution of viscoelastic behavior continues beyond the initial gel setting period. This suggests that molecular rearrangements of the gel structure persist long after the apparent set point of the gel. Small amplitude oscillatory shear (SAOS) spectra recorded at \(\gamma = 1\%\) post 30 min setting time show the characteristic behavior of physical gels. The values of \(G'\) remain constant within the frequency range 0.1 – 10 Hz, while the values of \(G''\) and, correspondingly, \(\tan \delta\) show a weak decrease with increase of frequency (Figure S1B, Supplementary Info). The stress ramp profiles \(f = 1\) Hz,\(^{[24]}\) follow typical behavior previously reported for the PIC gels (Figure S1C, Supplementary Info).\(^{[22]}\) For values of the oscillatory shear stress \((\sigma_{\text{osc}})\) below 40 – 50 Pa, the values of \(G'\) remain constant. A further increase in \(\sigma_{\text{osc}}\) above the critical stress \((\sigma_c)\) is consistent with the characteristic strain-stiffening effect reported previously by Rowan et al.,\(^{[20]}\) whereby the stiffness of the material increases with \(\sigma_{\text{osc}}\) until the point of the gel break (Figure S1C, Supplementary Info). The viscous and viscoelastic parameters provide additional insights into the gel behavior under applied stress; the dependency of \(G''\) and \(\tan \delta\) on \(\sigma_{\text{osc}}\) is found to be non-monotonous, with a clear minimum (for all \(M_{[n]}\)) at \(\sigma_{\text{osc}} \sim 10\) Pa. It is plausible that, at low values of stress, viscous creep is occurring which is associated with the diffusive re-arrangements of the network while, with greater stress, the creep is associated with the stress-induced rearrangements of the network, before, ultimately, the gel yields and breaks. Examining the effect of concentration using the polymer P2 (\(M_{[n]}=470\) g/mol) showed qualitatively similar behavior to that described for samples with
different $M_\eta$ (Figure S1D, Supplementary Info), suggesting that, within the concentration interval tested, the mechanism of gel formation remains consistent.

Analysis of rheological properties in heavy water provides key evidence of the physical nature of PIC gels, suggesting that formation of cross-linking junction zones is a dynamic process. As shown in Figure 2A, the scattering patterns of PIC solutions before and after gelling (30 min setting time) in the q-range $0.004 – 0.7 \text{ Å}^{-1}$ revealed a marked change in slope for $q < 0.1 \text{ Å}^{-1}$, where $q$ is the modulus of the scattering vector. We utilized the generalized Guinier-Porod model (GGP) to fit the data (Figure 2B, see Supplementary Info for model details).\textsuperscript{[40]} This approach is in contrast to our previous SAXS studies\textsuperscript{[29, 31]} which employed a Kholodenko model, discussed in more detail below. The choice of this model was twofold; the GGP model has both fewer fitting parameters than the previously employed models and showed consistent applicability across all solvent conditions, temperatures, concentrations and $M_\eta$; the use of fewer parameters is even more notable since the scattering data collected here extend to both lower and higher $q$ compared to previous studies thus resulting in far more rigorous testing of the applicability of scattering models over this extended $q$ range. The results of GGP fitting are summarized in Table 2. Briefly, for all $M_\eta$ and $c$ investigated we observe consistent and effectively identical values of radius of gyration ($R_G$) and dimensionality parameter ($3-s_G$). This suggests that changes in $M_\eta$ or $c$ do not elicit marked differences in the structure of the solutions, suggesting that the scattering signal is dominated by polymer chains. Temperature was found to have a strong but rather counter-intuitive effect. With increase in temperature, $R_G$ decreases by $\sim 10\%$ ($\sim 1\text{ Å}$ in absolute value), whilst $s_G$ increases from $1 \pm 0.05$ to $\sim 1.5 \pm 0.1$ (Figure 2B). The values of the dimensionality parameter suggest that at low temperature, PIC chains adopt a largely rod-like conformation in solution ($s_G = 1$). This result is consistent with the helical structure of PICs reported previously.\textsuperscript{[41]} With an increase of temperature, the conformation appears to be more coil like ($1 < s_G < 2$).\textsuperscript{[31]} One may speculate that such coil-
like values of $s_G \sim 1.5 \pm 0.1$ are associated with the chain curvature that emerges upon gelation. When the gel sets, pinning of the chains within junction zones can result in rigid chains adopting a curved, arch-like conformation. Since the distribution of junction zones is assumed to be random (certainly no scattering intensity peaks were observed to indicate the presence of periodic structures), this suggests that the rod-like chains must adopt some curved conformation in order to assemble into a percolated gel network (Figure 2D). For such an arched conformation, the angle between the chain segments separated by the distance $r$, $\langle \cos \theta \vec{u}(0), \vec{u}(r) \rangle$ will decay with increasing $r$, making such chains behave as if they have a finite persistence length (i.e., coil-like). Here we note that, for PIC polymers, this curved chain conformation, $y(r)$, is dominated by mechanical deformation (bending) rather than diffusive motion, which is characteristic of flexible polymers (i.e. entropic spring $\langle \cos \theta \vec{u}(0), \vec{u}(r) \rangle \sim e^{-r/l_p}$, where $l_p$ is the persistence length). In this case, the shape of the chain can be derived from the local balance of forces and bending moments: $M = -EI \frac{d^2 y(r)}{dr^2}$, $EI$ is flexural rigidity.\cite{33} In a general case, $y(r)$ takes the form of a polynomial function that can be approximated as $y \sim r^n$. In this case, we expect the angle between the chain segments to follow a power law rather than exponential decay:

$$\langle \cos \theta \vec{u}(0), \vec{u}(r) \rangle \sim \langle \cos \left( \tan^{-1} \left( \frac{dy}{dr} \right) \right) \rangle \sim \cos \left( \tan^{-1} (nr^{n-1}) \right) \sim \frac{1}{nr^{n-1}} \quad \text{(Eq. 1)}$$

This hypothesis is consistent with the finding that polymer models, such as Debye and extensions thereof covering semi-flexible polymers,\cite{42} as well as the Kholodenko model, failed to provide acceptable descriptions of the observed scattering.\cite{31, 43} Indeed, the Debye/Kholodenko models consider flexible molecules which adopt their equilibrium conformation by means of diffusive motion, and one may expect that these models would be less adequate for describing fibril-like PIC gels. We also note, that the question of applicability of the Debye/Kholodenko models should be considered in relation to the entire sets of data.
considered in this work, where the structures are probed not only over a broad $q$ and therefore size range but also across a broad range of conditions, such as solvent, $M_{\eta}$, concentration and temperature.$^{[31]}$ While individual scattering curves can be fitted using any of the above described models considered in this work, only the GPP model provided a systematic and non-contradictory result across the entire datasets.

Small changes in $R_G$ observed upon PIC gelation indicate that the scattering signal is dominated by the individual polymer chains. It is possible to infer from this result that the concentration of larger structures such as cross-links/junction zones is small and hence do not provide significant contribution to the scattering signal. With increasing polymer concentration, $R_G$ remains constant, suggesting that although the concentration of junction zones must increase so does the concentration of free polymer chains, and, therefore, the contribution to the scattering signal from cross-links in proportion to the signal from individual chains remains low. The small reduction in $R_G$ upon gelation may be attributed to de-swelling of PIC chains, as oligo-(ethylene glycol) side chains become progressively more hydrophobic.$^{[21]}$

To increase the contribution of the junction zones to the scattering, we employed a single concentration (6 mg/mL) of polymer P2 ($M_{\eta}=470$ g/mol) and selectively increased the density of the junction zones through the addition of sodium chloride.$^{[30]}$ Figure 1B shows the temperature dependence of the storage modulus ($G'$) and loss tangent ($\tan\delta$) as function of temperature and increase in NaCl concentration (with the same test parameters as in Figure 1A). It is apparent that, with increasing salt concentration, the gel strength progressively increases. We also note that after the initial stage of gel setting (marked by the rapid increase of $G'$), the dependence of $G'$ on temperature is characterized by the same value of slope for all salt concentrations tested, i.e. $\frac{\Delta G'}{\Delta T}$ is constant. Salt and temperature thus have an equivalent effect on $G'$ suggesting that, for a given temperature, the PIC gel in salt has a higher density of cross-links compared to the gel formed in water. This can be illustrated by constructing a
master curve, where the gelation curves for each salt concentration are plotted against temperature with an offset ($T + T_{\text{offset}}$), where the values of $T_{\text{offset}}$ are chosen empirically in order to overlap $G'$ moduli at higher temperatures (Figure S2, Supplementary Info).

The scattering data provide further insights; at 10 °C, we observe a small decrease in $R_G$ and a marked increase in $s_G$ with an increase of salt concentration (Figure 2C). This result is consistent with PIC chains becoming less hydrated (decrease of $R_G$) and more flexible (increase $s_G$) due to a decrease in water activity at high salt concentration. Upon gelation, at 37 °C, the values of $R_G$ progressively increase, whilst the values of $s_G$ gradually decrease (Figure 2C).

Based on previous studies of PIC gels,\textsuperscript{21,22} where bundle-like assemblies have been postulated to play a key role in gel formation, we attribute the increase of $R_G$ to the increasing contribution of junction-zone bundles to the scattering signal. Since no distinct decays were observed in the scattering curves, we conclude that the bundles exist in a distribution of sizes and that their thickness is not too dissimilar from that of a single chain, so that the overall scattering remains consistent with a one level GPP model. A simple empirical estimate based on a two level GPP model (i.e. using an approximation ($\phi$)-GPP$_1(R_G1)+(1-\phi)$-GPP$_2(R_G2) \sim$ GPP($R_{G,\text{effective}}$), where the volume fraction $0.05 < \phi < 0.5$ holds) indicates that the junction-zone bundles are likely to comprise several ($< 10$) individual chains. The decrease in $s_G$ can be rationalized assuming that the increase in the number concentration of junction zone results in the decrease of the average inter-junction distance. In such a scenario, single chains span across shorter distances and, hence, the chain conformation appears to be more rod-like, which corresponds to the lower values of $s_G$, i.e. $s_G = 1$ for rods.

The behavior of PIC gels in solutions of different sodium salts roughly follows the Hofmeister series, with gel strength ($G'_{\text{max}}$ @37 °C) following the sequence SCN$^- < I^- < D_2O < Cl^- < H_2PO_4^-(*)$. (* The PIC sample in sodium diphosphate gels at all temperatures above 0 °C, and it was not possible to measure its rheological properties without breaking the gel while loading.
into the rheometer). In addition to mineral salts, we have tested the effect of a common cell medium, DMEM (Dulbecco's Modified Eagle's Medium), on the gel properties of PICs. We find that the presence of DMEM results in a slight increase in gel stiffness compared to D$_2$O (Table 1), while being less stiff than the gels formed in 0.25 M NaCl solution, which is due to its comparatively low ionic strength of 0.13M originating from DMEM’s main salt component being NaCl.\textsuperscript{[44]}

Structurally, we see a clear trend with $R_G$ increasing with increasing gel strength, which is consistent with the hypothesis that larger bundles provide progressively stronger contribution to the scattering behavior. With decreasing gel strength, the pinning effect of the junction decreases enabling chains to retain their rod-like conformation; this is observed within the trend for the $s_G$ parameter. Overall, $s_G$ follows a non-monotonous trend (i.e., for a given temperature). For low gel strength, the chains, less hindered by junction zones, are able retain their rod-like conformation ($s_G \rightarrow 1$). With increasing gel strength, the chains become increasingly curved due to the pinning effect of the cross-linking junctions ($s_G \rightarrow 1.5$). A further increase in the number density of junctions results in reduced inter-junction distance and, hence, chains forming the inter-junction bridges appear to be more rod-like ($s_G \rightarrow 1$).

The gel strength ($G'_\text{max}$ @37 $^\circ$C) of PIC gels in chloride solutions of different cations followed the sequence Ca$^{2+} \approx$ Na $< K^+ \approx$ Mg$^{2+} < $ D$_2$O, which does not strictly follow the Hofmeister series. Although the cation effects are markedly weaker compared to the effect of anions, the deviation from the Hofmeister series may suggest that cations exhibit some more specific influence on PIC conformation. It is possible to surmise that the cation-specific effect may stem from the way the cations interact with the peptide or isocyanide functional groups. The structural behavior probed by SANS further confirms the observed trend that $R_G$ increases with increasing gel strength, whilst $s_G$ was found to be in the range 1.38 – 1.47 with no specific trend suggesting a small effect of cations on PIC gel strength.
These results provide important insights into the gelation of PIC polymers. We provide evidence that bundle formation during the initial stages of gelation is likely to be confined to the junction zones, with inter-junction bridges formed by individual PIC chains. In solution, PIC molecules adopt a rod-like conformation, which is likely due to their helical secondary structure. Upon gel assembly, PIC rods may adopt a curved conformation due to mechanical stresses induced by the pinning effect of the cross-links / junctions zones. This mechanical stress provides an important insight into why PIC gels display shear stiffening behaviour and have a unique set of ECM-mimicking properties. Unlike most biogels that are formed by the super-coiling\(^{[23]}\) of polymer chains or egg-box like junctions, PIC gels assemble through the formation of chain bundles that develop into junction zones within the gel network. This type of cross-link junction induces mechanical stresses on the PIC chains which, in turn, results in PIC gels existing in what essentially constitutes a pre-stressed state. Such polymer scaffolds would be characterized by the distribution of internal stresses that enable the long-range propagation of mechanical signals that are crucially important in the mechanosensing of cells.

**Supporting Information**
Supporting Information is available from the Wiley Online Library or from the author.

**Acknowledgements**
The authors acknowledge the support of the Australian Centre for Neutron Scattering, Australian Nuclear Science and Technology Organisation, in providing the neutron research facilities used in this work (Proposal ID 6540 & 7093); in particular, we acknowledge help of Dr Anna Sokolova, Dr Jitendra Mata and Dr Norman Booth for assistance with conducting preliminary measurements on BILBY (Proposal ID 7093). A.E.R acknowledges the financial support from Australian Research Council (ARC Laureate Fellowship FL160100139). G.E.Y acknowledges the financial support from the Australian Research Council Discovery Project Grant on Multiscale Viscoelastic Lubrication of Soft Matter Systems (DP180101919) and from the Biotechnology and Biological Sciences Research Council (BBSRC) grant (BB/T006404/1). The authors gratefully acknowledge Prof. Jason Stokes (University of Queensland) for many useful discussions and help with rheological measurements at the early stages of this work.

Received: ((will be filled in by the editorial staff))
Revised: ((will be filled in by the editorial staff))
Published online: ((will be filled in by the editorial staff))

11


### Table 1. Summary of the PIC rheological properties. The viscosity-derived average molecular weights $M_{\eta}\,[\text{kg/mol}]$ of P1, P2 and P3 are 360, 470 and 510 kg/mol, respectively.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Salt concentration [M]</th>
<th>Polymer concentration [mg/mL]</th>
<th>Storage modulus G$'$ [Pa]$^{a)}$</th>
<th>Gelation temperature [°C]</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>N/A</td>
<td>4</td>
<td>297.17</td>
<td>20.9</td>
</tr>
<tr>
<td>P2</td>
<td>N/A</td>
<td>4</td>
<td>321.91</td>
<td>19.2</td>
</tr>
<tr>
<td>P3</td>
<td>N/A</td>
<td>4</td>
<td>438.82</td>
<td>18.6</td>
</tr>
<tr>
<td>P1</td>
<td>N/A</td>
<td>8</td>
<td>1929.5</td>
<td>18.6</td>
</tr>
<tr>
<td>P2</td>
<td>N/A</td>
<td>8</td>
<td>1727.4</td>
<td>17.6</td>
</tr>
<tr>
<td>P3</td>
<td>N/A</td>
<td>8</td>
<td>1616.1</td>
<td>17.5</td>
</tr>
<tr>
<td>P2</td>
<td>N/A</td>
<td>6</td>
<td>305.46</td>
<td>19.0</td>
</tr>
<tr>
<td>P2</td>
<td>0.25 NaCl</td>
<td>6</td>
<td>1776.3</td>
<td>15.9</td>
</tr>
<tr>
<td>P2</td>
<td>0.5 NaCl</td>
<td>6</td>
<td>2298.9</td>
<td>13.7</td>
</tr>
<tr>
<td>P2</td>
<td>0.75 NaCl</td>
<td>6</td>
<td>3375.4</td>
<td>11.1</td>
</tr>
<tr>
<td>P2</td>
<td>1.0 NaCl</td>
<td>6</td>
<td>1552.7</td>
<td>$^b)$ ($\sim 10^6$)</td>
</tr>
<tr>
<td>P2</td>
<td>0.5 NaSCN</td>
<td>6</td>
<td>26.264</td>
<td>33.3</td>
</tr>
<tr>
<td>P2</td>
<td>0.5 NaI</td>
<td>6</td>
<td>209.28</td>
<td>25.7</td>
</tr>
<tr>
<td>P2</td>
<td>0.5 NaH$_2$PO$_4$</td>
<td>6</td>
<td>$^b)$</td>
<td>$^b)$ ($&lt; 0$)</td>
</tr>
<tr>
<td>P2</td>
<td>0.5 MgCl$_2$</td>
<td>6</td>
<td>1472.9</td>
<td>16.7</td>
</tr>
<tr>
<td>P2</td>
<td>0.5 KCl</td>
<td>6</td>
<td>1605.8</td>
<td>14.3</td>
</tr>
<tr>
<td>P2</td>
<td>0.5 CaCl$_2$</td>
<td>6</td>
<td>2109.9</td>
<td>13.3</td>
</tr>
<tr>
<td>P2</td>
<td>DMEM</td>
<td>6</td>
<td>1144.4</td>
<td>18.1</td>
</tr>
</tbody>
</table>

$^a)$ in D$_2$O at 37 °C; $^b)$ not possible to measure; $^c)$ estimate.
<table>
<thead>
<tr>
<th>Polymer</th>
<th>Salt concentration [M]</th>
<th>Polymer concentration [mg/mL]</th>
<th>R₀ at 10 °C [Å]</th>
<th>s₀ at 10 °C [-]</th>
<th>R₀ at 37 °C [Å]</th>
<th>s₀ at 37 °C [-]</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>N/A</td>
<td>4</td>
<td>10.0(0)</td>
<td>1.0(4)</td>
<td>10.2(2)</td>
<td>1.5(4)</td>
</tr>
<tr>
<td>P2</td>
<td>N/A</td>
<td>4</td>
<td>10.1(1)</td>
<td>1.0(1)</td>
<td>8.9(2)</td>
<td>1.5(0)</td>
</tr>
<tr>
<td>P3</td>
<td>N/A</td>
<td>4</td>
<td>10.0(1)</td>
<td>0.9(2)</td>
<td>10.5(1)</td>
<td>1.5(0)</td>
</tr>
<tr>
<td>P1</td>
<td>N/A</td>
<td>8</td>
<td>10.0(0)</td>
<td>0.9(7)</td>
<td>9.2(1)</td>
<td>1.4(6)</td>
</tr>
<tr>
<td>P2</td>
<td>N/A</td>
<td>8</td>
<td>10.1(1)</td>
<td>1.0(0)</td>
<td>9.4(4)</td>
<td>1.4(2)</td>
</tr>
<tr>
<td>P3</td>
<td>N/A</td>
<td>8</td>
<td>10.0(1)</td>
<td>0.9(8)</td>
<td>9.4(3)</td>
<td>1.4(2)</td>
</tr>
<tr>
<td>P2</td>
<td>N/A</td>
<td>6</td>
<td>10.0(0)</td>
<td>1.0(0)</td>
<td>9.1(4)</td>
<td>1.4(6)</td>
</tr>
<tr>
<td>P2</td>
<td>0.25 NaCl</td>
<td>6</td>
<td>9.7(1)</td>
<td>1.0(0)</td>
<td>12.1(1)</td>
<td>1.5(0)</td>
</tr>
<tr>
<td>P2</td>
<td>0.5 NaCl</td>
<td>6</td>
<td>9.2(2)</td>
<td>1.1(7)</td>
<td>16.1(2)</td>
<td>1.4(7)</td>
</tr>
<tr>
<td>P2</td>
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<tr>
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<td>1.3(2)</td>
<td>21.8(2)</td>
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<td>P2</td>
<td>0.5 NaSCN</td>
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<td>9.7(1)</td>
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<td>9.6(2)</td>
<td>0.9(2)</td>
<td>10.2(1)</td>
<td>1.3(3)</td>
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<tr>
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<td>27.1(1)</td>
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<tr>
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<td>1.4(4)</td>
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a) Porod exponent (d) was fixed at d = 4.
Figure 1. Small amplitude oscillatory shear test of PIC hydrogels as a function of temperature ($f_{osc} = 1 \text{ Hz}$, $\gamma = 1\%$). A) Temperature ramps of the storage (filled symbols) and loss moduli (open symbols) and tan\(\delta\) (half-filled symbols) for P1 – P3 polymers in D$_2$O. Temperature ramps of the storage modulus (filled symbols) and tan\(\delta\) (half-filled symbols) for P2 as a function of B) NaCl concentration; C) anion type; and D) cation type. In panels B – D, the loss moduli are not shown for clarity of presentation. Temperature ramps are not shown for P2 in 1M NaCl and 0.5M NH$_4$PO$_4$; these samples remain gelled down to the freezing point of water, making them highly sensitive to the sample history effects such as solution preparation method (i.e. mixing), storage conditions and sample loading artefacts during rheological tests. The salt concentrations in panels C and D were kept constant at 0.5 M, while DMEM was reconstituted in D$_2$O to follow the supplier-specified concentration of components.
Figure 2. A) SANS temperature series of P2 polymer (6 mg/mL in D2O). The inset shows the corresponding I(q)-q vs q plot, which assists in visualizing differences between scattering profiles recorded under different temperatures (30 min equilibration); B) Dependence of the fitting parameters of the GPP model as a function of temperature (P2, 6 mg/mL in D2O); $R_G$ (circles), $s_G$ (diamonds), Guinier amplitude (stars); C) Dependence of the $R_G$ (circles) and $s_G$ (diamonds) on NaCl concentration at 10 °C (open symbols) and 37 °C (filled symbols); D) Proposed model of the bundle formation during heat-set gel formation and the corresponding changes in the dimension parameter ($3 - s_G$), which are hypothesized to reflect the changes in the average curvature of PIC chains.