Supporting information

Unusual Temperature-Induced Swelling of Ionizable Poly(N-isopropylacrylamide)-Based Microgels: Experimental and Theoretical Insights into its Molecular Origin

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Materials and Methods

Materials.

All chemicals were purchased from Sigma-Aldrich and were used as received without further purification, unless otherwise specified. N-isopropylacrylamide (NIPAm), N,N'-methylene-bis-acrylamide (MBA), ammonium persulfate (APS). Methacrylic acid (MAA) was purified by distillation.

Microgel synthesis and characterization.

Synthesis. Microgel synthesis was performed according to Zavgorodnya, et al.1 100 mL solution containing 12 mmol of NIPAm and 0.28 mmol of BIS in Milli Q water was heated up to 70 °C with magnetic stirring and purged with N2 in a three-neck 250 mL round-bottom flask for 1h. Thereafter, 1.72 mmol of MAA was added and the solution was stirred during 5 min. Finally, the reaction was initiated by adding 0.046 g of APS dissolved in 1 mL of Milli Q water at room temperature. After 4 hours, microgels were purified by centrifugation/re-suspension in water three times. 1H NMR spectrum and assignments of resonance signals are shown in Figure S1.
The microgel composition of the microgel was estimated from $^1$H NMR spectrum: 65% NIPAm and 35% MMA (0.65:0.35).

**Figure S1.** $^1$H NMR spectrum with assignments for a P(NIPAm-co-MAA) microgel.

_Dinamic light scattering (DLS) measurement._

The microgel particle size and size distribution of PNIPAm-based microgels were determined by dynamic light scattering (DLS) as a function of temperature using a Zetasizer Nano-ZS90 instrument (Malvern Instruments Ltd.). Figure S2 shows the variation of the hydrodynamic diameter, $D_H$, as a function of temperature when microgels are in pure water at 1 and 5 mg/mL concentration.
Figure S2. Swelling curves of P(NIPAm-co-MMA) microgels at different microgel concentrations in pure water resulting from dynamic light scattering experiments.

In order to complete the DLS results, polydispersity (PDI) values for microgel suspensions at 14, 18 and 40°C are present in table S1.

Table S1. PDI values for microgel suspensions at 14, 18 and 40°C.

<table>
<thead>
<tr>
<th>Microgel concentration</th>
<th>14°C</th>
<th>28°C</th>
<th>40°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mg/ml</td>
<td>0.240</td>
<td>0.136</td>
<td>0.028</td>
</tr>
<tr>
<td>2.5 mg/ml</td>
<td>0.292</td>
<td>0.242</td>
<td>0.053</td>
</tr>
<tr>
<td>5 mg/ml</td>
<td>0.180</td>
<td>0.248</td>
<td>0.045</td>
</tr>
</tbody>
</table>

Figure S3 shows photographs of the microgel suspensions at 14, 28 and 40 °C. The clearly visible differences in turbidity of the microgel suspensions indicate the collapsed state at 40°C, while at 14 and 28°C the solution appearance is similar.
NMR relaxation experiments

Relaxation measurements were performed in a Brukerminispec spectrometer operating at 20 MHz for $^1$H equipped with a BVT3000 sample temperature controller with 0.01 °C stability. Samples consisting of 0.7 mL of the microgels solutions were placed in a 10 mm NMR tube. Temperature was increased from 17 °C to 31 °C every 2°C, allowing the system to thermally stabilize for at least one hour between each measurement.

Transverse proton relaxation times ($T_2$) were measured using a Carr–Purcell–Meiboom–Gill\textsuperscript{2,3} (CPMG) sequence which was applied after a magnetization inversion water suppression pulse. The water suppression waiting time was adjusted for each temperature. The CPMG parameters were: echo time 0.5ms, number of echoes 10,000 echoes and the length of the 90° radiofrequency pulse was 2.5 Ds. With this echo time it can be assured that the detected signal arises only from water molecules and not from the polymer network, whose relaxation times were determined to be on the order of microseconds. The resulting CPMG decay presents a multiple exponential decay, and the $T_2$ distribution functions were obtained by using an inverse Laplace transform (ILT) algorithm based on the Tikhonov regularization method\textsuperscript{4} provided by Dr. Petrik Galvosas from the Victoria University of Wellington, New Zealand.
**Molecular theory**

To gain understanding on what nanometer-scale interactions determine the unusual behavior of pNIPAm-co-MAA microgels, we use a theory that accounts for specific molecular details of the polymer network as well as its conformational degrees of freedom. This molecular theory explicitly considers size, shape and charge of all species, their physical interactions as well as the acid-base equilibrium of each titratable MAA unit of the polymer network. The formation of hydrogen bonds between NIPAm and MAA is described using an effective ligand-receptor binding interaction. The method does not assume the state of protonation and binding of each group, but it predicts them depending on the local molecular organization and the optimal conditions that minimize the total free energy of the system. This theoretical approach was first introduced to investigate the response of hydrophilic polyacid gels to changes in the pH and salt concentration of a buffer solution\(^1\).

We are most interested in showing that the interplay, inside each individual microgel, between the physical interactions and chemical equilibriums (acid-base and ligand-receptor) can qualitatively explain the behavior observed in our DLS and NMR experiments. Thus, we consider a single microgel, modeled as a large polymer network. When discussing results, the interaction with other microgels is thought as external force acting on the polymer network, such that its balance with the intra-microgel interactions that we account for determines the equilibrium size (or equivalently, the polymer volume fraction) of the microgel.

Let us then consider a polymer network composed by both MAA and NIPAm segments. The polymer is in contact with a buffer solution that contains water (\(w\)), hydroxide ions (\(OH^-\)), protons (\(H^+\)), salt cations (+) and anions (−) due to the complete dissociation of the added salt (KCl, for example). This solution provides a bath for all of the free (or mobile) species, fixing their chemical potentials that are the same in all regions of space, in the bath solution and inside the microgel. The initial step in this theoretical procedure consists in writing down the total Helmholtz free energy of the system:

\[
F = -TS_N - TS_T + F_B + F_{AB} + U_E
\]

where \(T\) is the temperature. The first term describes the conformational entropy of the network, which can be expressed as:
\[ S_N = -k_B \sum_{\alpha_N \in \{\alpha_N\}} P(\alpha_N) \ln P(\alpha_N) \]

where \( k_B \) is the Boltzmann constant, and \( P(\alpha_N) \) is the probability of finding the polymer network in its molecular conformation \( \alpha_N \). A conformation is defined by the position of all segments of the network. The symbol \( \{\alpha_N\} \) denotes the set of all conformations of the polymer. The next term in the free energy includes the translational entropy of mobile molecules plus the self-energy of each species. This entropic contribution can be written as:

\[ S_T = -k_B \sum_{i \in \{w, OHH^+, H^-, +\}} \int_V d\mathbf{r} \rho_i(\mathbf{r}) \left[ \ln(\rho_i(\mathbf{r}) v_w) - 1 + \beta \mu_i^0 \right] \]

where \( \rho_i(\mathbf{r}) \) and \( \mu_i^0 \) are respectively the local number density and the standard chemical potential of free species \( i \) (with \( i \in \{w, OHH^+, H^-, +\} \)). The volume of the system is \( V \), \( v_w \) is the volume of a water molecule, and \( \beta = \frac{1}{k_B T} \).

The next contribution to \( F \) results from the equilibrium binding between NIPAm and protonated MAA units of the network. This reaction can be expressed as:

\[ MAA_{AH} + NIPAm \rightleftharpoons MAA :: NIPAm \]

where \( MAA_{AH} \) denotes a protonated MAA segment and \( MAA :: NIPAm \) represents the complex between the two units. The chemical free energy that accounts for this binding at equilibrium can be written as:

\[ \beta F_B = \int_V d\mathbf{r} \langle \rho_{MAA}^p(\mathbf{r}) \rangle \left[ f_B(\mathbf{r}) (\ln f_B(\mathbf{r}) + \beta \mu_B^0 - \beta \mu_F^0) + (1 - f_B(\mathbf{r})) \ln(1 - f_B(\mathbf{r})) \right] \]

\[ + \int_V d\mathbf{r} \langle \rho_{NIPAm}(\mathbf{r}) \rangle \beta \mu_F^0 \]

where \( \mu_F^0 \) is the standard chemical potential of the unbound NIPAm unit and \( \mu_F^0 \) is that of the complex. Angle brackets in the last equation represent an ensemble average over the set of network conformations. Then,
\[
\langle \rho_{\text{NIPAm}}(r) \rangle = \sum_{\alpha_N \in \{\alpha_N\}} P(\alpha_N) \rho_{\text{NIPAm}}(\alpha_N, r)
\]

is the average local density of NIPAm segments, while that of MAA units is

\[
\langle \rho_{\text{MAA}}(r) \rangle = \sum_{\alpha_N \in \{\alpha_N\}} P(\alpha_N) \rho_{\text{MAA}}(\alpha_N, r)
\]

where \( \rho_{\text{NIPAm}}(\alpha_N, r) \) and \( \rho_{\text{MAA}}(\alpha_N, r) \) are the local density of NIPAm and MAA units, respectively, when the network is in its conformation \( \alpha_N \). The local quantity \( \langle \rho_{\text{MAA}}(r) \rangle \) gives the density of MAA units independently of their chemical states (bound, protonated or deprotonated). Similarly, \( \langle \rho_{\text{NIPAm}}(r) \rangle \) does not consider the local binding state (free or bound) of NIPAm units.

Moreover, we introduce the local density of paired MAA units, \( \rho_{\text{MAA}}^p(\alpha_N, r) \), which gives the local density of MAA segments that have at least one NIPAm unit within a distance \( l_{\text{pair}} \), for network conformation \( \alpha_N \). In our theory, only paired MAA segments can bind NIPAm. Thus, the ensemble average density of paired MAA segments is given by

\[
\langle \rho_{\text{MAA}}^p(r) \rangle = \sum_{\alpha_N \in \{\alpha_N\}} P(\alpha_N) \rho_{\text{MAA}}^p(\alpha_N, r)
\]

Note that the three conformation-dependent local densities \( \rho_{\text{NIPAm}}(\alpha_N, r) \), \( \rho_{\text{MAA}}(\alpha_N, r) \) and \( \rho_{\text{MAA}}^p(\alpha_N, r) \) are inputs of this theory. A molecular model of the polymer network of interest must supply these quantities, for each \( \alpha_N \in \{\alpha_N\} \) and at each \( r \in V \). Then, these three functions introduce the molecular information of the network into the present theoretical approach.

In the binding free energy, \( f_B(r) \) is the local degree of binding that gives the fraction of paired units that are bound. Namely, the local density of bound MAA segments is

\[
\langle \rho_{\text{MAA}}^b(r) \rangle = f_B(r) \langle \rho_{\text{MAA}}^p(r) \rangle
\]

The following term in the Helmholtz free energy is the chemical free energy that describes the acid-base equilibrium of (unbound) MAA units. This contribution can be expressed as:
\[ \beta F_{AB} = \int_V dr \left( \langle \rho_{\text{MAA}}(r) \rangle - f_B \langle \rho^p_{\text{MAA}}(r) \rangle \right) \left( f_d(r) \ln f_d(r) + \beta \mu_{A^-}^0 \right) \]
\[ + \left( 1 - f_d(r) \right) \left[ \ln \left( 1 - f_d(r) \right) + \beta \mu_{A^H}^0 \right] \]

where \( \mu_{A^H}^0 \) and \( \mu_{A^-}^0 \) are the standard chemical potentials of the protonated and deprotonated MAA unit, respectively. The local degree of dissociation, \( f_d(r) \), gives the fraction of unbound units that are charged; then,

\[ \langle \rho_{\text{MAA}}^{A^-}(r) \rangle = f_d(r) \left( \langle \rho_{\text{MAA}}(r) \rangle - f_B \langle \rho^p_{\text{MAA}}(r) \rangle \right) \]

gives the local density of charged MAA segments, while that of protonated units is given by

\[ \langle \rho_{\text{MAA}}^{A^H}(r) \rangle = \left( 1 - f_d(r) \right) \left( \langle \rho_{\text{MAA}}(r) \rangle - f_B \langle \rho^p_{\text{MAA}}(r) \rangle \right) \]

The last contribution to the free energy is the electrostatic energy,

\[ \beta U_E = \int_V dr \left[ \langle \rho_q(r) \rangle \beta \psi(r) - \frac{1}{2} \beta \epsilon \left( \nabla \psi(r) \right)^2 \right] \]

where \( \psi(r) \) is the electrostatic potential, and \( \epsilon \) denotes the medium dielectric permittivity. The local charge density is

\[ \langle \rho_q(r) \rangle = \sum_{i \in \{\text{OH}^-,\text{H}^+,\text{H}^-,\text{H}^+\}} q_i \rho_i(r) + \left( \langle \rho_{\text{MAA}}(r) \rangle - f_B \langle \rho^p_{\text{MAA}}(r) \rangle \right) f_d(r) q_{A^-} \]

where \( q_i \) is the electric charge of free species \( i \), and \( q_{A^-} \) is that of the deprotonated MAA unit.

Therefore, with all the previous expressions, the Helmholtz free energy can be explicitly written as:
\[
\beta F = \sum_{\alpha_N \in \{\alpha_N\}} P(\alpha_N) \ln P(\alpha_N) + \sum_{i \in \{w,OH^-,H^+,-,+\}} \int_V dr \, \rho_i(\mathbf{r}) [\ln(\rho_i(\mathbf{r}) v_w) - 1 + \beta \mu_i^0] \\
+ \int_V dr \left( (\rho_{MAA}(\mathbf{r}) - f_B(\mathbf{r}) \langle \rho_{MAA}^p(\mathbf{r}) \rangle) [f_d(\mathbf{r}) (\ln f_d(\mathbf{r}) + \beta \mu_A^0 - \mu^0)] \\
+ (1 - f_d(\mathbf{r})) [\ln(1 - f_d(\mathbf{r}) + \beta \mu_A^0 - \mu^0)] \\
+ \int_V dr \rho_{MAA}^p(\mathbf{r}) \{f_B(\mathbf{r}) (\ln f_B(\mathbf{r}) + \beta \mu_B^0 - \mu_B^0) \\
+ (1 - f_d(\mathbf{r})) [\ln(1 - f_d(\mathbf{r}) + \beta \mu_A^0 - \mu^0)] \} + \int_V dr \rho_{NIPAm}(\mathbf{r}) \beta \mu_B^0 \\
+ \int_V dr [\langle \rho_q(\mathbf{r}) \rangle \beta \psi(\mathbf{r}) - \frac{1}{2} \beta \epsilon \langle \nabla \psi(\mathbf{r}) \rangle^2] \\
\]

Two physical constraints must be satisfied by this free energy. First, at each point of space, the volume must be completely filled by some of the molecular species. This is the local incompressibility constraint that accounts for inter-molecular repulsions (excluded volume interactions), which can be expressed as:

\[
\sum_{i \in \{w,OH^-,H^+,-,+\}} v_i \rho_i(\mathbf{r}) + \langle \rho_{MAA}(\mathbf{r}) \rangle v_{MAA} + \langle \rho_{NIPAm}(\mathbf{r}) \rangle v_{NIPAm} = 1
\]

where \( v_{OH^-}, v_{H^+}, v_- \) and \( v_+ \) are the molecular volumes of corresponding free species, and \( v_{MAA} \) and \( v_{NIPAm} \) are the volumes of the MAA and NIPAm segments, respectively. In addition, system must be electroneutral, which implies

\[
\int_V dr \langle \rho_q(\mathbf{r}) \rangle = 0
\]

The microgel is in equilibrium with a homogeneous buffer solution that provides a bath for all of the free species. The proper thermodynamic potential to describe the system is the Lagrange transform of the free energy having as independent variables the chemical potentials of the mobile species (excluding water molecules due to the incompressibility constraint). Then, we use the semi-grand canonical potential
\[ \Omega = F - \sum_{i \in \{\text{OH}^{-}, \text{H}^{+}, +, -\}} \mu_i N_i \]

where \( N_i \) is the total number of molecules of the given species in the system, and \( \mu_i \) represents its chemical potential, which must be identical inside the microgel and in the bath solution. In the present formalism, \( \Omega \) can be more explicitly written as

\[ \Omega = F - \sum_{i \in \{\text{OH}^{-}, +, -\}} \mu_i \int_V d\mathbf{r} \rho_i(\mathbf{r}) \]

\[ - \mu_H^+ \int_V d\mathbf{r} \left[ \rho_H^+(\mathbf{r}) + f_d(\mathbf{r})(\langle \rho_{\text{MAA}}(\mathbf{r}) \rangle - f_B(\mathbf{r})\langle \rho_{\text{MAA}}^p(\mathbf{r}) \rangle) + f_B(\mathbf{r})\langle \rho_{\text{MAA}}^p(\mathbf{r}) \rangle \right] \]

where the last integral properly accounts for the total number of protons in the system, including those that protonate the unbound, uncharged MAA units (\( \text{MAA}_{\text{AH}} \)).

The unknowns in \( \Omega \) consist of the probability distribution of network conformations, \( P(\alpha_N) \), the local densities, \( \rho_w(\mathbf{r}) \), \( \rho_{\text{OH}^{-}}(\mathbf{r}) \), \( \rho_{\text{H}^{+}}(\mathbf{r}) \), \( \rho_{-}(\mathbf{r}) \) and \( \rho_{+}(\mathbf{r}) \), the local degree of binding, \( f_B(\mathbf{r}) \), the local degree of charge, \( f_d(\mathbf{r}) \), and the position-dependent electrostatic potential, \( \psi(\mathbf{r}) \). Optimizing \( \Omega \) with respect to each of these functions, while considering the two aforementioned constrains, leads to expressions for each function. Then, the function to optimize is

\[ \beta \Phi = \beta \Omega - \int_V d\mathbf{r} \beta \pi(\mathbf{r}) \left( - \sum_{i \in \{w, \text{OH}^{-}, \text{H}^{+}, -, +\}} v_i \rho_i(\mathbf{r}) + \langle \rho_{\text{MAA}}(\mathbf{r}) \rangle v_{\text{MAA}} + \langle \rho_{\text{NIPAm}}(\mathbf{r}) \rangle v_{\text{NIPAm}} - 1 \right) \]

where \( \pi(\mathbf{r}) \) are the local Lagrange multipliers that are introduced to satisfy the incompressibility constraint. Global charge neutrality, meanwhile, can be satisfied with the proper choice of boundary conditions for the electrostatic potential and its derivatives. Thus, the explicit function to optimize is
\[
\beta \Phi = \sum_{\alpha_N \in \alpha_N} P(\alpha_N) \ln P(\alpha_N) + \sum_{i \in \{w,\alpha_N^{-},\alpha_N^{+}\}} \int_V \! d\mathbf{r} \, \rho_i(\mathbf{r}) \left[ \ln(\rho_i(\mathbf{r}) v_w) - 1 + \beta \mu_i^0 \right] \\
+ \int_V \! d\mathbf{r} \left( \rho_{\text{MAA}}^p(\mathbf{r}) - f_B(\mathbf{r}) \rho_{\text{MAA}}^p(\mathbf{r}) \right) \left[ f_d(\mathbf{r}) \ln f_d(\mathbf{r}) + \beta \mu_d^0 \right] \\
+ (1 - f_d(\mathbf{r})) \left[ \ln(1 - f_d(\mathbf{r})) + \beta \mu_d^0 \right] \\
+ \int_V \! d\mathbf{r} \left( \rho_{\text{MAA}}^p(\mathbf{r}) \right) \left[ f_B(\mathbf{r}) \ln f_B(\mathbf{r}) + \beta \mu_f^0 - \beta \mu_d^0 + (1 - f_B(\mathbf{r})) \ln(1 - f_B(\mathbf{r})) \right] \\
+ \int_V \! d\mathbf{r} \left( \rho_{\text{NIPAm}}(\mathbf{r}) \right) \beta \mu_f^0 + \int_V \! d\mathbf{r} \left[ \langle \rho_q(\mathbf{r}) \rangle \beta \psi(\mathbf{r}) - \frac{1}{2} \beta \epsilon \nabla \psi(\mathbf{r})^2 \right] \\
- \sum_{i \in \{\alpha_N^{-},\alpha_N^{+}\}} \beta \mu_i \int_V \! d\mathbf{r} \rho_i(\mathbf{r}) \\
- \beta \mu_h^+ \int_V \! d\mathbf{r} \left[ \rho_{H^+}(\mathbf{r}) + f_d(\mathbf{r}) \left( \langle \rho_{\text{MAA}}(\mathbf{r}) \rangle - f_B(\mathbf{r}) \langle \rho_{\text{MAA}}^p(\mathbf{r}) \rangle \right) + f_B(\mathbf{r}) \langle \rho_{\text{MAA}}^p(\mathbf{r}) \rangle \right] \\
- \int_V \! d\mathbf{r} \beta \pi(\mathbf{r}) \left( \sum_{i \in \{w,\alpha_N^{-},\alpha_N^{+}\}} v_i \rho_i(\mathbf{r}) + \langle \rho_{\text{MAA}}(\mathbf{r}) \rangle v_{\text{MAA}} + \langle \rho_{\text{NIPAm}}(\mathbf{r}) \rangle v_{\text{NIPAm}} - 1 \right)
\]

Optimization of $\beta \Phi$ with respect to the density of the free species yields

\[
\rho_i(\mathbf{r}) = \frac{e^{\beta \mu_i - \beta \mu_i^0}}{v_w} \exp( - \beta \pi(\mathbf{r}) v_i - \beta \psi(\mathbf{r}) q_i ) = \frac{\rho_{i \text{bat} h}}{v_w (v_w \rho_{i \text{bat} h}^\text{bat}h)^{v_i/v_w}} \exp( - \beta \pi(\mathbf{r}) v_i - \beta \psi(\mathbf{r}) q_i )
\]

where $\rho_{i \text{bat} h}$ is the density of species $i$ in the bath solution. These densities of the mobile species are completely determined by the pH and salt concentration of bath solution. The last expression holds for all free species, including water with $q_w = 0$.

Moreover, assuming that the medium permittivity is constant, we can express the probability of network conformations as:

\[
P(\alpha_N) = \frac{1}{Q} \exp \left[ - \int_V \! d\mathbf{r} \, \rho_{\text{MAA}}^p(\alpha_N,\mathbf{r}) \ln(1 - f_B(\mathbf{r})) - \int_V \! d\mathbf{r} \, \rho_{\text{MAA}}(\alpha_N,\mathbf{r}) \left( \ln f_d(\mathbf{r}) + \beta \psi(\mathbf{r}) q_A - \right) \right. \\
\left. - \int_V \! d\mathbf{r} \, \rho_{\text{MAA}}(\alpha_N,\mathbf{r}) v_{\text{MAA}} + \rho_{\text{NIPAm}}(\alpha_N,\mathbf{r}) v_{\text{NIPAm}} \right] \beta \pi(\mathbf{r})
\]
where the factor $\frac{1}{Q}$ ensures that

$$\sum_{\alpha N \in \{\alpha N\}} P(\alpha N) = 1$$

For the local degree of dissociation of unbound MAA units, we obtain

$$\frac{f_d(\mathbf{r})}{1 - f_d(\mathbf{r})} = \frac{v_w p_w^{bat h} v_{H+}/v_w}{v_w p_w^{bat h}} K_a^0 \exp(-\beta \psi(\mathbf{r}) q_A^-)$$

where $K_a^0$ is the dimensionless thermodynamic equilibrium constant of the acid-base reaction of MAA units. This quantity is related to the standard chemical potentials of protons, and protonated and deprotonated MAA units, because

$$K_a^0 = \exp(\beta \mu_{AH}^0 - \beta \mu_{H^+} - \beta \mu_{A^-}^0)$$

The local degree of binding of paired MAA segments is

$$\frac{f_B(\mathbf{r})}{1 - f_B(\mathbf{r})} = e^{-\beta \Delta G^0} (1 - f_d(\mathbf{r}))$$

where $\Delta G^0$ is the standard Gibbs free energy of the binding reaction. This equation clearly shows the strong coupling that exists between binding and acid-base equilibriums. The standard free energy of binding relates to the standard chemical potentials of free and bound species via

$$\Delta G^0 = \mu_B^0 - \mu_{AH}^0 - \mu_{H^+}^0 - \mu_{A^-}^0$$

Finally, variation of $\Phi$ with respect to the electrostatic potential yields the Poisson equation:

$$\epsilon \nabla^2 \psi(\mathbf{r}) = -\langle \rho_q(\mathbf{r}) \rangle$$

This last equation together with the explicit expressions for charge density and degree of binding shows that the electrostatic interactions are strongly coupled to the chemical state of the different segments. At this point, all of the functions that compose the thermodynamic potential can be expressed in terms of input quantities and the local interaction potentials, $\pi(\mathbf{r})$ and $\psi(\mathbf{r})$. These interaction potentials can be
obtained through replacing the explicit expressions for the different functions, and numerically solving
the incompressibility constraint and the Poisson equation at each point of space.

Once \( \pi(\mathbf{r}) \) and \( \psi(\mathbf{r}) \) are known any thermodynamic quantity of interest can be derived from the
thermodynamic potential, which is now determined. In addition, structural properties can be calculated
using the conformations of the network and their distribution of probability. For example, the fraction
of MAA segments that are bound is given by

\[
\langle x_{bnd} \rangle = \frac{\int \mathbf{r} \, d\mathbf{r} \rho_{MAA}^B(\mathbf{r})}{\int \mathbf{r} \, d\mathbf{r} \rho_{MAA}(\mathbf{r})}
\]

while the fraction of dissociated MAA units can be determined from

\[
\langle x_{dis} \rangle = \frac{\int \mathbf{r} \, d\mathbf{r} \rho_{MAA}^A(\mathbf{r})}{\int \mathbf{r} \, d\mathbf{r} \rho_{MAA}(\mathbf{r})}
\]

The theory has been presented in a general fashion. To obtain results using this method, we
need to provide the molecular information of all species in the system. In particular, the molecular
details of the P(NIPAm-co-MAA) network are incorporated via the conformation-dependent local
densities \( \rho_{NIPAm}(\alpha_N, \mathbf{r}) \), \( \rho_{MAA}(\alpha_N, \mathbf{r}) \) and \( \rho_{MAA}^B(\alpha_N, \mathbf{r}) \), which must be given \( \forall (\alpha_N, \mathbf{r}) \). To obtain the
conformations of the network, we perform Molecular Dynamics (MD) simulations.

In our calculations, we consider a network in a cubic box with full periodic boundary
conditions. The periodicity of the electrostatic potential at the walls of the calculation box ensures
electroneutrality of the system. The independent variables of a single calculation are the pH and salt
concentration of the bath solution. In addition, the strength of the MAA-NIPAm binding interaction
must be provided. In the qualitative model we are using, the main effect of an increasing temperature is
reducing the effective strength of this interaction. Thus, we incorporate the effect of temperature into
the value of \( \beta \Delta G^0 \).

*Molecular model.* Calculation of results using the molecular theory requires defining a molecular
model of the P(NIPAm-co-MAA) microgel network. The particular architecture that we have
considered in this work is illustrated in Figure S4. This regular polymer network is composed of 25-
segment long polymer chains connected at six-coordinated nodal units (crosslinks). We have performed all calculations in a cubic box of volume $L^3$ having $N_x = 8$ crosslinks and a total of $N_{seg} = 608$ segments; full periodic boundary conditions are imposed in each calculation. Segments are randomly labeled as either MAA or NIPAm (except crosslinks), the only constraint is the MAA:NIPAm ratio to be 0.35:0.65, as in the experimental system. Thus, the calculation box contains $N_{NIPAm} = 378$ and $N_{MAA} = 222$ segments. The segment length is $l_{seg} = 0.5 \, nm$ for all types of units. Then, the molecular volume of a segment is $v_{NIPAm} = v_{MAA} = v_{seg} = \frac{\pi}{6} l_{seg}^3 = 0.0655 \, nm^3$, and the polymer volume fraction is $\phi_p = \frac{(N_x + N_{NIPAm} + N_{MAA})v_{seg}}{L^3} = \frac{N_{seg}v_{seg}}{L^3}$.

Figure S4. The scheme illustrates the molecular model of polymer network used to describe P(NIPAm-co-MAA) microgels. Random NIPAm-co-MAA copolymer chains are interconnected at crosslinking segments. The two panels show different polymer volume fractions, $\phi_p$. Orange tubes enclose the calculation box; full-3D periodic boundary conditions are applied in the calculations. This same graph has been included in the article, and it is reproduced here in the interest of making this section self-contained.

A molecular conformation of the network is defined by the spatial position of all its segments. We obtain the different molecular conformations of the network performing Molecular Dynamics
simulations, which are described in detail in Longo et al.\textsuperscript{1,2} Two different box sizes have been considered, such that the polymer volume fractions are $\phi_p = 0.002$ and $\phi_p = 0.01$ (see Fig. S3). We have arbitrarily chosen these volume fractions to represent the relatively compressed and swollen states of microgels observed in our DLS experiments at 24 and 28 °C, respectively, for 5mM salt solutions. The ratio between these volume fractions is equivalent to that observed in the experiments. Each $\phi_p$ considered requires an independent set of MD simulations.

The molecular conformations of the network enter the theoretical calculations \textit{via} the three conformation-dependent local densities: $\rho_{\text{NIPAm}}(\alpha_N, r)$, $\rho_{\text{MAA}}(\alpha_N, r)$ and $\rho_{\text{MAA}}^p(\alpha_N, r)\psi(\alpha_N, r)$, for NIPAm, MAA, and paired MAA units, respectively. To obtain these quantities, we discretize the volume of the box and count the number of different units in each discrete cell for every conformation. In the density of paired MAA, we only consider those segments that have a NIPAm unit within a distance equal to $l_{\text{pair}} = 1.5\ l_{\text{seg}}$. Details of the methodology used to numerically solve the equations of the theory can be found in Longo et al.\textsuperscript{5,6}.

The electric charge of a dissociated MAA segment is $q_{A^-} = -e$, where $e$ is the absolute value of the electron charge. The acid-base equilibrium of MAA units is described using $pK_a = 4.65$; to describe water self-dissociation we use $pK_w = 14$. The permittivity of the aqueous medium is constant and equal to $\epsilon = \epsilon_w\epsilon_0$, where $\epsilon_w = 78.5$ is the relative permittivity of water at room temperature and $\epsilon_0$ denotes the vacuum permittivity. Monovalent salt ions are modeled using $v_+ = v_- = 0.0335\ n m^3$ and $q_+ = -q_- = e$. For the rest of the free species in the solution, we use $v_{H^+} = v_{\text{OH}^-} = v_w = 0.03\ n m^3$ and $q_{H^+} = -q_{\text{OH}^-} = e$.

References


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(6) Longo, G. S.; Olvera de la Cruz, M.; Szleifer, I. ACS Nano 2013, 7 (3), 2693–2704.