Supplementary Material

Polyisocyanopeptides Hydrogels as Responsive Biomimetic Cytoskeletal Networks

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Synthesis and characterisation

Dichloromethane and chloroform were distilled over CaCl2. Tetrahydrofuran, diethyl ether and toluene were distilled over sodium, in presence of benzophenone. Monomers 1-3 were prepared following described procedures.1 H NMR (yielding only broad signals for the polymers) were recorded on Bruker AC-300 MHz instruments operating 300 MHz. FT-infrared spectra of the pure compounds were recorded on a ThermoMattson IR300 spectrometer equipped with a Harrick ATR unit. Solution IR spectroscopy was carried out in sealed KBr cuvette (1 mm) on a Bruker Tensor 27 spectrometer operated with Opus software. Solutions of monomers and polymers were prepared in chloroform, tetrahydrofuran, or toluene at a concentration of approximately 30 mM.
Synthesis
The synthesis of polymers P1-P3 is shown in scheme S1. The experimental procedures are given below.

Synthesis of polymer P1. To a solution of 1 (40 mg, 15 mmol) in freshly distilled toluene (3 mL) was added a solution of Ni(ClO4)2.H2O (0.147 mol/L, 10 μL) in toluene/methanol (7:3). The reaction mixture was vigorously stirred under air in a sealed flask, for two hours. The solvent was evaporated and the crude polymer was redissolved in CHCl3 (3 mL), precipitated in diethyl ether (10 mL) and collected by centrifugation (3500 rpm, 7 min). This procedure was repeated twice and the polymer was dried under vacuum to yield P1 (29.2 mg, 10.9 mmol, 73%) as a yellow-brown glassy solid. 1H NMR (CDCl3, 300 MHz): 3.74−3.36 (br m, 13H); 1.60 (br s, 6H); FT-IR (cm–1, ATR): 3263 (N−H); 2927, 2880 (C−H); 1740 (C=O); 1656 (C=O amide I); 1531 (N−H amide II); 1214 (C−O ester); 1108 (C−O ethers) [α]D20: +75 (c 0.03; CH2Cl2); Mn = 478 kDa, Mw = 716 kDa.

Synthesis of polymer P2. The procedure as described for P1 was used with the exception that tetrahydrofuran was used to redissolve the polymer during the precipitation process. The reaction was carried out with two different monomer:Ni2+ ratios:
P2a: Using 2 (40 mg, 12.6 mmol), Ni(ClO4)2.H2O solution (0.126 mol/L, 10 μL), in toluene (2 mL). Purification of the crude mixture yielded P2a (33.7 mg, 10.7 mmol, 85%) as a deep yellow glassy solid. 1H NMR (CDCl3, 300 MHz): 3.68−3.34 (br m, 17H); 1.58 (br s, 6H); FT-IR (cm–1, ATR): 3260 (N−H); 2917, 2875 (C−H); 1740 (C=O); 1657 (C=O amide I); 1530 (N−H amide II); 1210 (C−O ester); 1108 (C−O ethers); [α]D20: +105 (c 0.03; CH2Cl2); Mn = 830 kDa, Mw = 1327 kDa.
P2b: Using 3 (41 mg, 12.9 mmol), Ni(ClO4)2.H2O solution (1.29 mmol/L, 10 μL), in toluene (2 mL). Purification of the crude mixture yielded P2b (32.0 mg, 10.1 mmol, 78%) as a pale yellow glassy solid. 1H NMR (CDCl3, 300 MHz): 3.71−3.35 (br m, 17H); 1.63 (br s, 6H); FT-IR (cm–1, ATR): 3261 (N−H); 2917, 2879 (C−H); 1740 (C=O); 1657 (C=O amide I); 1530 (N−H amide II); 1210 (C−O ester); 1105 (C−O ethers); Mn = 2306 kDa, Mw = 3458 kDa.

Synthesis of polymer P3. The procedure as described for P2 was used. The reaction was carried out with two different monomer:Ni2+ ratios:
P3a. Using 3 (50.1 mg, 13.8 mmol), Ni(ClO4)2.H2O solution (0.138 mol/L, 10 μL), in toluene (2.5 mL). Purification of the crude mixture yielded P3a (45.2 mg, 12.5 mmol, 90%) as a deep yellow glassy solid. 1H NMR (CDCl3, 300 MHz): 3.71−3.35 (br m, 21H); 1.63 (br s, 6H); FT-IR (cm–1, ATR): 3261 (N−H); 2917, 2879 (C−H); 1740 (C=O); 1656 (C=O amide I); 1529 (N−H amide II); 1213 (C−O ester); 1109 (C−O ethers) [α]D20: +175 (c 0.01; CH2Cl2); [α]D20: +169 (c 0.03; H2O); Mn = 249 kDa, Mw = 323 kDa.
P3b. Using 3 (56.3 mg, 15.6 mmol), Ni(ClO4)2.H2O solution (1.56 mmol/L, 10 μL), in toluene (2.8 mL). Purification of the crude mixture yielded P3b (43.9 mg, 12.2 mmol, 78%) as a pale yellow glassy solid. 1H NMR (CDCl3, 300 MHz): 3.71−3.35 (brm, 14H); 1.63 (brs, 6H); FT-IR (cm–1, ATR): 3260 (N−H);
2917, 2875 (C–H); 1740 (C=O); 1657 (C=O amide I); 1530 (N–H amide II); 1210 (C–O ester); 1105 (C–O ethers); $M_n = 1589$ kDa, $M_w = 2702$ kDa.

**Circular dichroism measurements**

Polymers P1–P3 were subjected to CD analysis in dichloromethane and in water. The spectra in both solvents are very similar. Figure S1 shows the CD traces of P1-P3 in DCM.

![Figure S1](image)

**Figure S1** | CD spectra of P1-P3 recorded in DCM (20 °C, 1 mg mL$^{-1}$).

Temperature dependent CD was recorded in water (Figure S3). In a stability experiment, a spectrum was recorded at every temperature after cooling the sample 5 °C and subsequently heating it to the desired temperature. The CD signal recovers completely at intermediate temperatures and also at the gel transition, no changes in the helical structure of the polymer are visible. At $T = 72$ °C the CD signal decreases abruptly and a cooling cycle of the sample the sample did not result in recovery of the
signal, indicating permanent damage to the helical structure. After reaching 74 °C, the sample was cooled to 5 °C and kept for three days before measuring again, but the CD signal did not recover.

AFM Analysis

AFM experiments were performed using a dimension 3100 or Multimode microscope operated with nanoscope III or nanoscope IV control units (Digital Instruments). Solutions of P1-P3 (~10⁻⁶ M in CHCl₃) were spin-coated (1600 RPM) onto freshly cleaved Muscovite Mica to determine the contour length (lᵣ) of isolated polymers chains. P2b hydrogels were deposited by direct contact with freshly cleaved HOPG or Mucsvite Mica. All images were recorded with the AFM operating in Tapping Mode™ in air at room temperature, with a resolution of 1024 × 1024 pixels, using moderate scan rates (1–1.5 lines/sec). Commercial tapping-mode golden-coated silicon tips (NT-MDT) were used with a typical resonance frequency around 300 kHz. Polymer chain lengths were evaluated using NeuronJ plugin (v1.4.1 by E. Meijering) run on ImageJ (v1.43i) software. The polymer chains heights and widths were measured using the Nanoscope software (v6.14r1) from Digital Instruments. Raw pictures were compensated for drift using the Nanoscope software (v6.14r1); no other processing was applied on the data used for the analysis. 3D rendering of the pictures was done with SurfaceJ plugin (v1.2 M. Abramoff ImageJ (v1.43i) software.

Figure S4 | Representative AFM micrograph of individual polymer chains spin-coated from dilute CHCl₃ solutions onto mica: (A) P1; (B) P2a; (C) P2b; (D) P3a and (E) P3b. For all images, the scale bar is 1 μm and the vertical scale is 2 nm.
Figure S5 | AFM images of P2b obtained from sample of different polymer concentration: (A) 0.2 mg/mL and (B) 2 mg/mL. Both images 10 x 10 μm. Note that feature height of images (A) and (B) are closely corresponding (~1.2 nm); (C) CryoSEM image of P2b (scale bar 1 μm).

Bundle dimensions from AFM measurements. To measure bundle dimensions as a function of concentration, gels prepared at concentrations ranging from 0.13 mg mL^{-1} were transferred to mica substrates and subjected to AFM microscopy. Statistical analysis of the images yielded distributions of bundle heights and bundle widths. The height profiles in samples prepared from a simple gel transfer showed strong variations with the position in the sample and was found to be unreliable. Better data was obtained from the width profiles. Despite the broad distributions per sample, the gels at all concentrations typically showed ~30 nm wide bundles.

Figure S6 | Bundle width (left) and height (right) determined from statistical analysis of AFM results at c = 0.13 – 3.0 mg mL^{-1}. The sample size of the analysis is shown in the top panel.

Particle tracking analysis

Particle tracking studies were carried out on a Nanosight LM10-HS instrument equipped with an Electron Multiplication Charge Coupled Device (EMCCD) camera mounted on an optical microscopic system to track light scattered by particles that are present in a focused (80 μm) beam generated by a single mode laser diode with a 60 mW blue laser illumination (405 nm). The cold polymer solutions containing platinum nanoparticles (size concentration) in a concentration were injected in a sample chamber of 0.5 ml size from which a volume of 120x80x20 microns was visualized under the microscope. The Brownian motion of the nanoparticles was tracked at 30 frames/s. NTA 2.2 software
was used to evaluate the mean squared displacements of each visible particle (calibration 166 nm/pixel). Averaging over multiple particles ($N > 50$) yielded an average diffusion coefficient

![Graph showing diffusion coefficient as a function of concentration](image)

**Figure S7** | Tracking analysis. Average diffusion coefficient as a function of concentration of P2b at $T = 22^\circ$C.

**Vial inversion test**

![Image of vials with different concentrations](image)

**Figure S8** | Room temperature gel inversion test of P2b at $c = 2.0 - 0.031$ mg mL$^{-1}$ (or 0.2 – 0.003 wt-%). Gels at concentrations as low as $c = 0.063$ mg mL$^{-1}$ pass the vial inversion test.

**Rheology**

**Instrumental.** Rheological measurements were performed using a TA Instruments Ares G2 rheometer in a ~20 mL couette configuration with temperature control using a peltier element. Samples were
prepared by mixing the appropriate amount of polymer in demi water (20 mL) and regular vortexing the mixture over time (at least 24 hours) until a homogeneous solution was obtained. Solutions of P2b were prepared in refrigerated (4 °C) conditions to avoid early gel formation. The measurements in the linear response regime were conducted at 4 % strain at different frequencies between 0.5 and 5 Hz. The data depicted in the manuscript is recorded at 1 Hz. Temperature scans were recorded at a heating or cooling rate of 2 °C min⁻¹. The measurements in the nonlinear regime were taken at constant temperature (15 minutes equilibrated) and at different frequencies (0.5-5 Hz). Again, the data in the manuscript was recorded at 1 Hz.

**Strain and Frequency sweeps.** Figures S7 and S8 show a strain and frequency sweep sweep of P2b (1 mg mL⁻¹) at T = 5 °C (solution) and T = 50 °C (gel). The moduli are constant for strains up to 10 %. We typically measured our gels at 4% strain, to make sure that also in the solution regime of the experiment, reliable data is obtained. Displayed data in Figure S7 has been recorded at an angular frequency \( \omega = 6.3 \) rad s⁻¹ (or \( f = 1 \) Hz). The frequency sweep in the linear regime (Figure S8) was recorded at 4 % strain. The moduli \( G' \) and \( G'' \) scale with the frequency in the cold solution, showing a power law behaviour \( (\propto \omega^{0.6}) \) and are nearly constant over five decades in the gel.

![figure S9](image)

**Figure S9 |** Strain sweeps of P2b (1 mg mL⁻¹) at \( T = 5 \) °C (solution) and \( T = 50 \) °C (gel) at \( f = 1 \) Hz.

![figure S10](image)

**Figure S10 |** Frequency sweeps of P2b (1 mg mL⁻¹) at \( T = 5 \) °C (solution) and \( T = 50 \) °C (gel) at \( \gamma = 4\% \).

**Gel temperature.** The gel temperature has been defined as the onset of the step in \( G' \) at the LCST. Alternatively, one can decide to use the crossover temperature where \( G' = G'' \). We find that the latter shows a much stronger concentration dependence, since the absolute values of \( G' \) and \( G'' \) are respond differently to different concentrations. In addition, the crossover definition also depends on the frequency that is applied, whereas the onset temperature (or \( G' \) in general) is much less sensitive to different frequencies. The transition temperatures using both definitions are shown in Figure S6 (\( f = 1 \) Hz).
Figure S11 | Moduli $G'$ and $G''$ of P2b ($c = 1 \text{ mg mL}^{-1}$) as a function of temperature, measured to 65 °C.

Figure S12 | Gelation temperatures of P2b as a function of concentration $c$ determined as the onset of the increase in $G'$ and the crossover of $G'$ and $G''$. For the former, a relative constant gelation temperature is observed. The large error in the experimental data at $c = 0.05 \text{ mg mL}^{-1}$ prevented a reliable value at this low concentration. The steeper increase in transition temperature when determined as the crossover temperature is the result of the stronger dependence of the absolute values of $G''$ on $c$ than $G'$ on $c$.

Figure S13 | Storage modulus of P2b ($c = 1 \text{ mg mL}^{-1}$) as a function of temperature for 6 sequential heating and cooling runs. The transition temperature remains unaffected.

**Single Molecule Force Microscopy Analysis**

Single Molecule Force Microscopy measurements were conducted on a JPK Nanowizard 1 AFM running software version 3.4.18 using Olympus Biolever BL-AC40TS-C2 cantilevers with a resonance frequency of 100-130 kHz in air and ~30 kHz in water. The cantilevers were cleaned with concentrated sulfuric acid, neutralized with MilliQ, washed with ethanol, dried and finally put in an ozone-cleaner for at least half an hour. The temperature of the sample was controlled with a peltier element mounted underneath the glass sample holder. To minimize the chance of probing multiple polymers simultaneously, samples were prepared by spin-coating (3000 rpm) dilute polymer solutions (~3mM) in dichloromethane on a freshly cleaved muscovite mica surface. Subsequently, these samples were rinsed with MilliQ to remove any non-adsorbed polymer. The resulting polymer density and morphology were then analyzed using tapping mode AFM in air. The force spectroscopy measurements were performed in MilliQ. The polymers were attached to the AFM tip by non-specific adsorption. The tip was lowered with a speed of 2 μm/s until it made contact with the sample, pressed onto the sample with a force of a few nN for one second and retracted from the surface with a speed of 2 μm/s. Due to the low polymer
density on the surface, a polymer was adsorbed to the AFM tip in less than 1% of the approach-retract cycles. This low interaction frequency also indicates that the chance of adsorbing multiple polymers to the tip simultaneously is extremely low. Curves that showed artifacts such as large nonspecific adhesion or multiple rupture events resulting from the simultaneous pulling of several molecules or multipoint attachment of one polymer were discarded using a custom Matlab program and JPK data processing version 3.4.18. These programs were also used to fit the statistical polymer models to the selected force-distance curves.

Analysis of the selected force-distance curves revealed a large variation in the contour length. This can be easily explained by the relatively high polydispersity of the sample. Furthermore, the polymers adsorb to both the tip and the surface at random positions along the polymer chain. To obtain the persistence length, the force-distance curves were fitted to the Extensible Worm Like Chain (EWLC) model using least squares fitting. All parameters were fitted for each individual force-distance curve. The distribution of the persistence length $l_p$ for P2b at different temperatures is shown in Figure S7, indicating a broad distribution. Temperature sweeps from 10 to 60 °C with steps of 10 °C showed that this effect persisted at all temperatures. The average $l_p$ value is plotted versus temperature and fitted to $e^{\beta T}$ with an exponent $\beta$ of 0.041 (see Figure 3D in the manuscript).

The absolute values of the $l_{p,0}$ are low in comparison to other materials based on polyisocyanopeptides. This is attributed to solvent effects. Water, the solvent used to carry out the SMFS measurements of P2b is believed to weaken the hydrogen bonds that cause the high stiffness of the polymers. As the temperature increases and water is slowly forced out of the polymer by hydrophobic effects of the ethyleneglycol tails, the core stiffens further. Measurements were also carried out a nonprotic solvent, like 1,1,2-trichloroethane. A temperature sweep shows a persistence length $l_{p,0}$ of 34 nm with a negligible temperature dependence. Measurements in dioxane, a hydrogen bond acceptor, but not a donor, shows $l_{p,0}$ values around 12 nm that are also constant with temperature.

Figure S14 | Persistence length distribution of P2b at temperatures ranging from 10 to 60°C.
Semi-flexible network theory

Previously published theories on rheology of semi-flexible networks, based on the Extensible Worm-like Chain model were used for this work. The plateau modulus $G_0$ and the critical stress $\sigma_c$ of a gel composed of bundles of $N$ polymer chains are dependent on the persistence length of the bundle $l_{p,B}$ and the contour length, or the length between crosslinks $l_c$ when this is smaller than the contour length:

\[
G_0 = 6\chi c N k_B T \frac{l_{p,B}^2}{l_c^3} \tag{1}
\]

and

\[
\sigma_c = \chi c N k_B T \frac{l_{p,B}}{l_c^2} \tag{2}
\]

Here, $c$ is the concentration, $k_B$ is the Boltzmann’s constant, $T$ is the absolute temperature and $\chi$ is composed of (molecular) constant. The related bundle density $\rho$ (in length per volume) can also be used:

\[
\rho = \chi c N = \frac{l_M N_A c}{M} \frac{c}{N} \tag{S1}
\]

where $l_M$ is the length per monomer unit projected along the polymer backbone ($l_M = 0.25$ nm), $M$ is the molecular weight of a monomer (for P2: $M = 0.316$ kg mol$^{-1}$) and $N_A$ is Avogadro’s number. When bundling is independent on the concentration as holds for our materials (see manuscript), the pore size can be estimated by calculating the mesh size $\xi$:

\[
\xi = \frac{1}{\sqrt{\rho}} \tag{S2}
\]

In a semi-flexible network with relatively flexible bundles, the $\xi$ ($\xi \propto c^{-0.5}$) should roughly correspond to $l_c$. This, we also observed experimentally ($l_c \propto c^{-0.4}$) and can be rationalised by considering that other contributions that affect (ie decrease) $\xi$, but not $l_c$ will be entanglements of bundles. If entanglement have a significant impact, $l_c$ is expected to be much larger than $\xi$, which is experimentally not found (P2b, $c = 1$ mg mL$^{-1}$, $T = 30$ °C: $\xi = 140$ and $l_c = 110$ nm), vide supra.

Equations S3 and S4 combine eqs. 1 and 2 with S1:

\[
G_0 = \frac{6 l_M c}{M N} RT \frac{l_{p,B}^2}{l_c^3} \tag{S3}
\]

and

\[
\sigma_c = \frac{l_M c}{M N} RT \frac{l_{p,B}}{l_c^2} \tag{S4}
\]

We observed an exponential increase with temperature in the (narrow) experimental temperature window and we insert a phenomenological correction for that in eqs. S5 and S6 where $\beta$ (K$^{-1}$) is a constant. This temperature contribution to $G_0$ and $\sigma_c$ directly results from the stiffening of the individual polymer chains which is included in $l_{p,B}$ (vide supra). Since $G_0$ scales with $l_{p,B}^2$ the exponent also includes a prefactor 2 yielding: $e^{2\beta T}$. In contrast, $\sigma_c$ scales linear with $l_{p,B}$, resulting in the exponent $e^{\beta T}$.

\[
G_0 = \frac{6 l_M c}{M N} \frac{l_{p,B}^2}{l_c^3} RT e^{2\beta T} \tag{S5}
\]

and
\[
\sigma_c = \frac{l_m c \ell_{\text{p,B}}}{M N \ell_z^2} R T e^{\beta T}
\]  

(S6)

Taking together equations S5 and S6, one can calculate \( l_{\text{p,B}} \) and \( l_c \) from the experimentally obtained \( G_0 \) and \( \sigma_c \):

\[
l_{\text{p,B}} = \frac{1}{36 \frac{l_m c \ell_z^2}{M N \sigma_c^2}} R T e^{-\beta T} \tag{S7}
\]

and

\[
l_c = 6 \frac{l_{\text{p,B}} \sigma_c}{\rho_0} e^{\beta T} \tag{S8}
\]

The only unknown quantity in eqs. S7 and S8 is the bundle number \( N \). This was estimated by AFM experiments \( (N = 6.9) \), but this can also be calculated using the single molecule force spectroscopy (SMFS) measurements. We determined in the manuscript that for the low bundle numbers, the material is in the tight bundle regime, which gives a square \( N \) dependency:

\[
l_{\text{p,B}} = l_{\text{p,0}} N^2 \tag{S9}
\]

The temperature dependence of \( l_{\text{p,0}} \) is identical to that of \( l_{\text{p,B}} \) (exponential with the same constant \( \beta \)), which tells us that the stiffening of the gel is directly related to the stiffening of the individual polymers (and also that \( N \) does not vary with temperature as would be expected for the gel). Obviously, the temperature dependence of the gel is quadratic, since \( G_0 \) scales with \( l_c^2 \). Taking together eqs. S7 and S8 with S9, we obtain two equations with only two unknown quantities: \( N \) and \( l_c \):

\[
G_0 = \frac{6 l_m N^3 c}{l_z^2} R T l_{\text{p,0}}^2 e^{2\beta T} \tag{S10}
\]

and

\[
\sigma_c = \frac{l_m N \xi c}{l_z} R T \ell_{\text{p,0}} e^{\beta T} \tag{S11}
\]

Both have been calculated for different temperatures and concentrations and give very consistent results:

\( N = 9.1 \) (close to 6.9 estimated by AFM) and \( l_c = 110 \text{ nm} \) (at \( c = 1 \text{ mg mL}^{-1} \)), close to the mesh size (\( \xi = 140 \text{ nm} \) at this concentration). Reduced versions of eq. S10 and S11, highlighting the different design parameters for efficient gel formation is shown in the manuscript are:

\[
G_0(T) \propto N^3 \frac{c}{\ell_z} R T l_{\text{p,0}}^2 \tag{3}
\]

\[
\sigma_c(T) \propto N \frac{c}{\ell_z} R T l_{\text{p,0}} \tag{4}
\]

The predictions of Figure 4 are made with the assumption that the mesh size and the length between crosslinks are very close: \( \xi \approx l_c \), which results, together with eq. S2 in:

\[
G_0 \approx \frac{6 l_m N^3 \xi c}{\ell_z^2} R T l_{\text{p,0}}^2 e^{2\beta T} \approx \frac{6 l_m N^3 \sqrt{p^3 c}}{\ell_z^2} R T l_{\text{p,0}}^2 e^{2\beta T} \tag{S12}
\]

and
\[ \sigma_c \approx \frac{i M}{m} N \xi^2 R T l_{p,0} e^{\beta T} \approx \frac{i M}{m} N p c R T l_{p,0} e^{\beta T} \]  
(S13)

together with eq. S1, this leads to:

\[ G_0 \approx 6 \left( \frac{N A l_{SM}}{M} \right)^2 \sqrt{N^3 c^5} k_B T l_{p,0} e^{2 \beta T} \]  
(S14)

and

\[ \sigma_c \approx \left( \frac{N A l_{SM}}{M} \right)^2 c^2 k_B T l_{p,0} e^{\beta T} \]  
(S15)

which have been used to plot Figure 4. Equations S14 and S15 show that \( G_0 \) is dependent on \( N, c \) and \( l_{p,0} \), while \( \sigma_c \) only scales with \( c \) and \( l_{p,0} \). In the reduced form, analogous to eqs. 3 and 4, one can write:

\[ \sqrt{N^3 c^5} k_B T l_{p,0}^2(T) \]  
(S16)

and

\[ \sigma_c(T) \propto c^2 k_B T l_{p,0}(T) \]  
(S17)

Note that eqs. S16 and S17 contain the assumption that no cross-over from the tight to the loose bundle regime is expected, also at higher bundle numbers. If, as has been observed for actin filaments, this would be the case, the dependence of \( G_0 \) and \( \sigma_c \) on \( N \) will change significantly, however, not their dependence on \( c \) and \( l_{p,0} \). In the loose bundle limit, where

\[ l_{p,B} = l_{p,0} N \]  
(S18)

eqs. S16 and S17 change to:

\[ \sqrt{N^{-1} c^5} k_B T l_{p,0}^2(T) \]  
(S19)

and

\[ \sigma_c(T) \propto N^{-1} c^2 k_B T l_{p,0}(T) \]  
(S20)

which show that in this regime the bundling is no longer effective to increase either the gel modulus or its critical stress.

References and Notes:

