

Supplementary Information

Emergence of Plasticity through Inter-Fibril Sliding in a Supramolecular Hydrogel.

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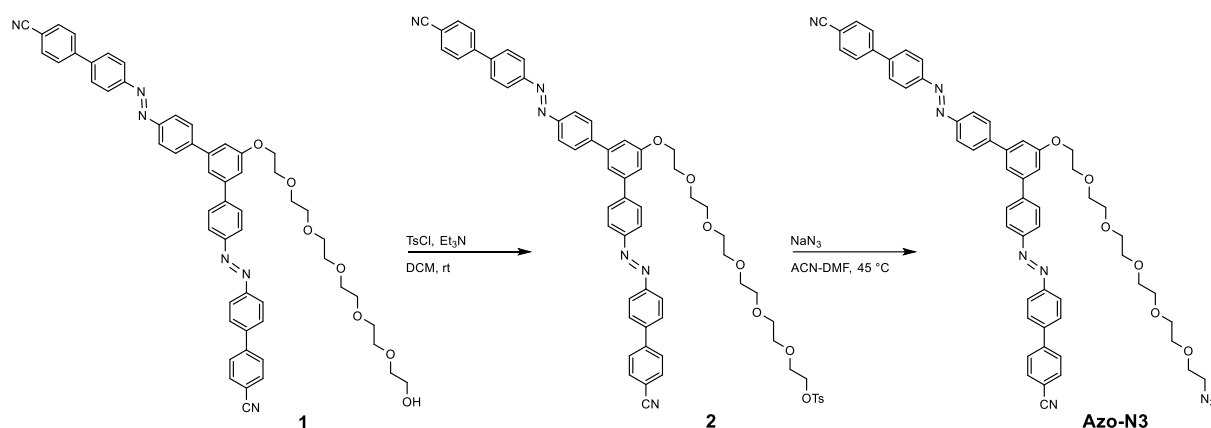
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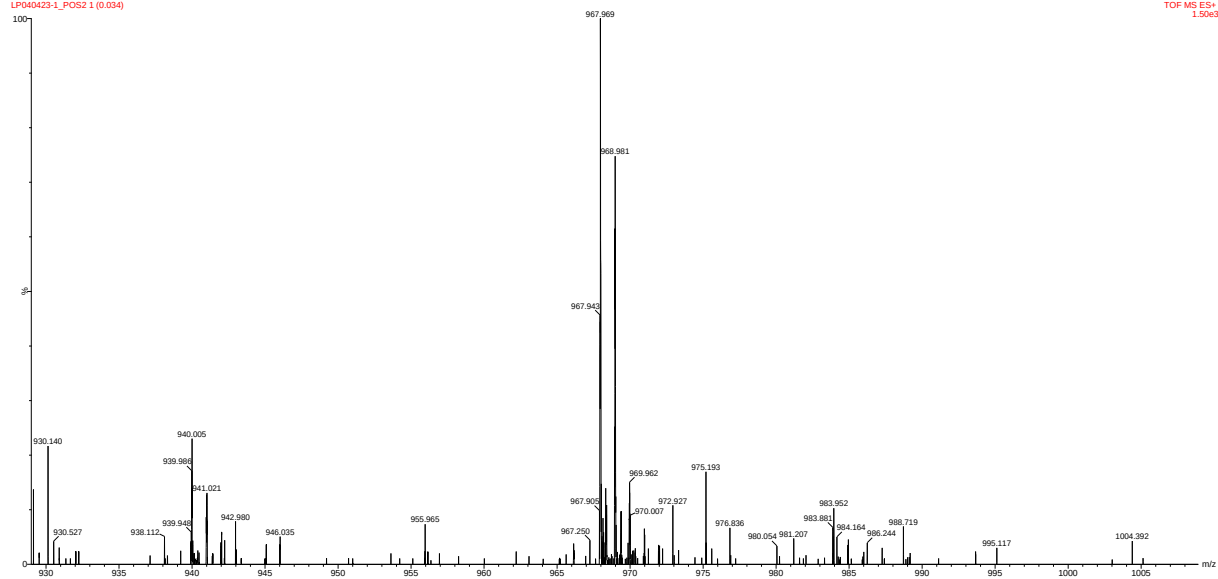
Synthesis and characterization of compounds.

The monomer was prepared according to the previously reported procedure by Fredy et al.²⁴.

Reagents. The commercial grade reagents and solvents were used without further purification unless stated otherwise. Compound 1 was prepared according to the previously reported procedure by Xiu et al.⁵¹. Thin-layer chromatography (TLC) was performed on aluminum-baked silica plates (60 F254, Merck). UV light (254 nm) was used for visualizing. Column chromatography was carried out using silica gel (Silicagel 60, 230-400 mesh, Merck). ¹H and ¹³C NMR spectra were recorded at 400 and 101 MHz, respectively. Chemical shifts (δ) are reported in ppm relative to the residual protonated solvent signals of CDCl₃ (¹H-NMR: δ = 7.26 ppm). ESI-MS spectra were obtained for samples dissolved in DCM-MeOH at low μ M concentrations.



Experimental Procedure. Compound 1 (29 mg, 0.03 mmol, 1 equiv) and tosyl chloride (19 mg, 0.10 mmol, 3 equiv) were dissolved in DCM (4 mL). Triethylamine (20 μ L, 0.14 mmol, 5 equiv) was added to the solution and the reaction mixture was stirred at room temperature overnight. The solvent was evaporated under reduced pressure and the residue was purified using silica gel column chromatography (DCM/MeOH = 30:1) to obtain product 2 (31 mg, 93%) as an orange paste which was immediately used in the next synthetic step. Compound 2 (31 mg, 0.03 mmol, 1 equiv) was dissolved in the mixture of acetonitrile (4 mL) and DMF (1mL). Sodium azide (10 mg, 0.15 mmol, 5 equiv) was added to the solution and the reaction mixture was stirred at 45 °C overnight. The solvent was evaporated under reduced pressure and the residue was purified using silica gel column chromatography (DCM/MeOH = 50:1) to obtain product Azo-N₃ (24 mg, 89%) as an orange paste. ¹H NMR (400 MHz, CDCl₃): δ 8.06 (dd, J = 8.6, 2.2 Hz, 8H), 7.83 (d, J = 8.6 Hz, 4H), 7.80 – 7.73 (m, 12H), 7.55 (s, 1H), 7.27 – 7.25 (m, 2H), 4.32 (t, J = 4.7 Hz, 2H), 3.95 (t, J = 4.8 Hz, 2H), 3.80 – 3.76 (m, 2H), 3.74 – 3.71 (m, 2H), 3.69 – 3.62 (m, 14H), 3.37 (t, J = 5.0 Hz, 2H). ¹³C-¹H time NMR (101 MHz, CDCl₃): δ 159.78, 152.60, 152.02, 144.62, 143.70, 142.19, 141.46, 132.72, 128.04, 128.00, 127.79, 123.66, 123.58, 121.36, 111.50, 70.92, 70.68, 70.62, 70.59, 70.02, 67.81, 50.67. ESI MS m/z , ([M+Na]⁺): calcd. for C₅₆H₅₁N₉O₆Na: 968.386, found: 967.969.



Expressions for $\tilde{\kappa}$ of fibres

Although fibres are not solid rods, a value for $\tilde{\kappa}$ can be derived if they are considered to be a bundle of solid rods/fibrils and the strength of interaction between the fibrils is known. Here we show the derivation of the expressions for $\tilde{\kappa}$ for a bundle of N fibrils in the weakly- and strongly-coupled limit based on a theory presented by Heussinger *et al.*³⁶ Whether a fibre is in the weakly or strongly coupled regime is set by the parameter

$$\alpha = \frac{k_x l^2}{k_s \delta^2} , \quad (1)$$

with k_x the resistance to shearing the fibrils and k_s the resistance to stretching of the fibrils over a lengthscale δ , the distance between inter-fibril crosslinkers, i.e. $k_s = \mu/\delta$. The fibre is in the weakly crosslinked limit if $\alpha \ll 1$ and in the strongly crosslinked limit if $\alpha \gg N$.

In between these limits the bending rigidity is described by the following wavelength dependent formula,

$$\kappa_n = N\kappa \left[1 + \left(\frac{12\hat{\kappa}}{N-1} + (q_n \lambda)^2 \right)^{-1} \right] . \quad (2)$$

Which includes a wavenumber $q_n \sim n/l$, a local dimensionless bending stiffness $\hat{\kappa} = \kappa/(k_s \delta b^2)$ and a length scale $\lambda = (1/\sqrt{\alpha})\sqrt{M\hat{\kappa}/(M-1/2)}$, where b is the distance between fibrils and $M = \sqrt{N}/2$.

The formula can be simplified significantly for a fixed wavelength (e.g. $n = 1$ and $l = L_0$) in the weakly crosslinked and strongly crosslinked limit. Furthermore, if we assume that N is large, we can simplify the length scale to $\lambda \approx (l/\sqrt{\alpha})\sqrt{\hat{\kappa}}$.

Derivation for weakly cross-linked fibres

In the weakly crosslinked limit $\alpha \ll 1$. Therefore,

$$\kappa_{n,\text{weak}} \approx N\kappa[1 + (\hat{\kappa}/\alpha)^{-1}] \approx N\kappa . \quad (3)$$

Therefore the bending rigidity of a fibre in the weakly coupled limit is $\kappa_{\text{fibre}} \approx N\kappa_{\text{fibril}}$. We can assume the stretching rigidity scales as $\mu_{\text{fibre}} \sim N\mu_{\text{fibril}}$ such that $\tilde{\kappa}_{n,\text{weak}} \sim (\kappa_{\text{fibril}}/\mu_{\text{fibril}})(1/L_0^2)$, with $\kappa_{\text{fibril}}/\mu_{\text{fibril}}$ a constant, so that $\tilde{\kappa}_{n,\text{weak}} \sim 1/L_0^2$.

Derivation for strongly cross-linked fibres

In the strongly crosslinked limit $\alpha \gg N$, so that,

$$\kappa_{n,\text{strong}} \approx N\kappa \left[1 + \left(\frac{12\hat{\kappa}}{N} \right)^{-1} \right] = N\kappa + \frac{\kappa N^2}{12\hat{\kappa}} \approx N\kappa + \frac{k_s \delta b^2 N^2}{12} = N\kappa + \frac{\mu b^2 N^2}{12} . \quad (4)$$

Therefore the bending rigidity of a fibre in the strongly coupled limit is $\kappa_{\text{fibre}} \approx N\kappa_{\text{fibril}} + N^2 \mu_{\text{fibril}} b^2 / 12$. The inter-fibril distance in the fibres is approximately four times the radius of hexameric ring, thus $b = 4a$. Furthermore, if the fibrils are treated as continuous rods $\kappa_{\text{fibril}} = \mu_{\text{fibril}} a^2 / 2$. Therefore we can approximate the bending rigidity of a strongly crosslinked fibre as $\kappa_{\text{fibre}} \approx N\kappa_{\text{fibril}} + \frac{2}{3} N^2 \kappa_{\text{fibril}}$.

We can assume the stretching rigidity scales as $\mu_{\text{fibre}} \sim N\mu_{\text{fibril}}$ such that $\tilde{\kappa}_{n,\text{strong}} \sim N(\kappa_{\text{fibril}}/\mu_{\text{fibril}})/L_0^2$ with $N \sim D_b^2$ and $\kappa_{\text{fibril}}/\mu_{\text{fibril}}$ a constant, so that $\tilde{\kappa}_{n,\text{strong}} \sim D_b^2/L_0^2$.

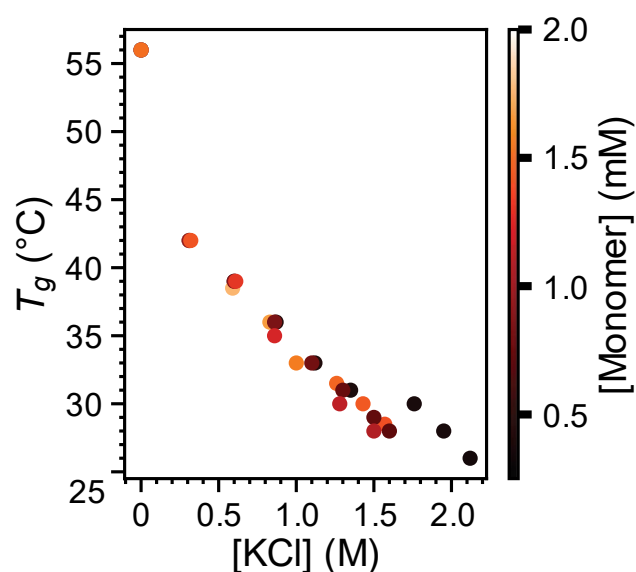


Figure S1. Gelation temperature as a function of KCl concentration.

Most of the points follow a very narrow distribution along a single linear decrease, regardless of monomer concentration. Very high KCl concentrations and low monomer concentrations make measurements more difficult. Due to inhomogeneities in the sample present turbidity before heating. Similarly, there seems to be a qualitative difference in the behaviour of gels with 0 M KCl. These gels also tend to necessitate more than one successive strain sweep before they start exhibiting strain-stiffening (see Supplementary Figure on the Compilation of phase diagram data).

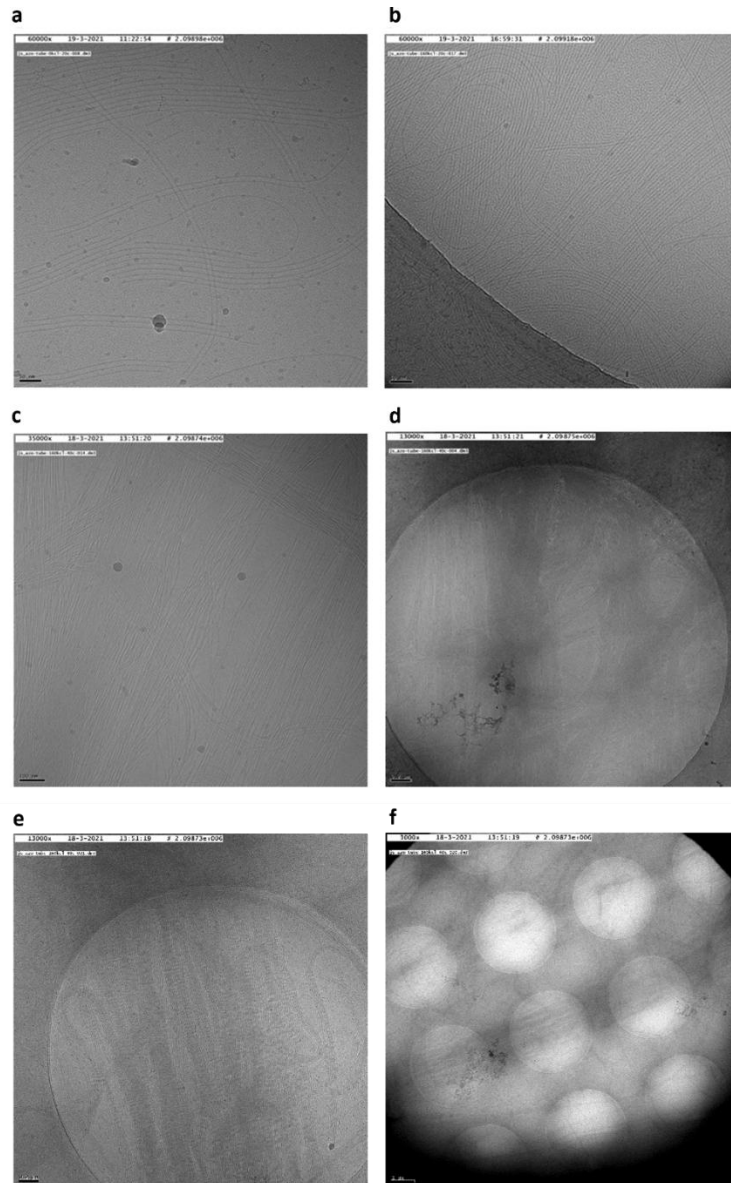


Figure S2. Cryo-TEM micrographs of a network containing 0.78 mM monomer.

a. Before addition of KCl, some alignment is visible, attributed to the blotting step during cryo-TEM sample preparation. Scale bar is 50 nm. b. After addition of 1.1 M KCl to the solution. Alignment is more pronounced. The mixture is still at a solution stage. This points towards weak interactions even though the tubules are more closely packed than in the previous image. Scale bar is 50 nm. c. After gelation at 40 oC, i.e. 5 oC above gelation temperature. The bundles are now more 3-dimensional even though they still appear flattened. Scale bar is 100 nm. d. Lower magnification image of the same sample, where larger structures are visible. Contrast increased to outline these structures. Scale bar is 200 nm. e. Same sample and magnification, another area. All the bundles observed here seem to be aligned. f. A lower magnification image of the same area reveals that they are all part of a larger bundle, similar to the ones observed under the fluorescence optical microscope. Scale bar is 1 μ m.

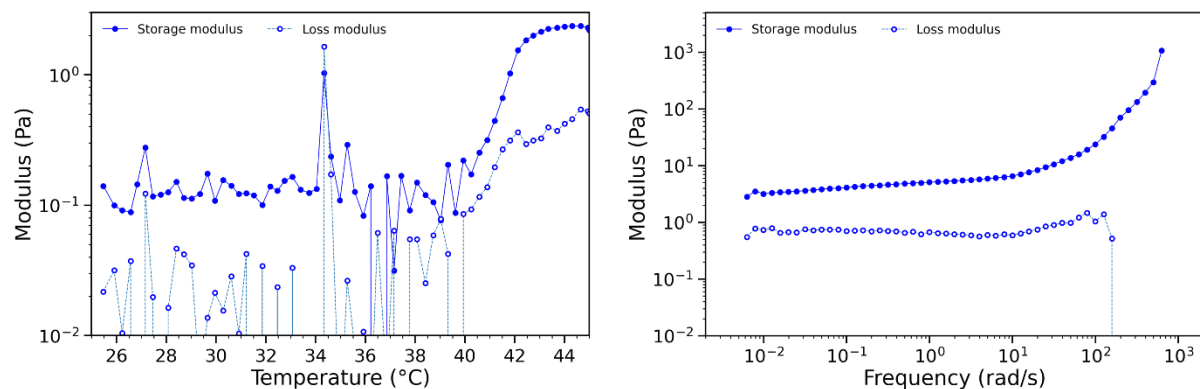


Figure S3. Basic rheological properties.

Left: Evolution of storage and loss moduli during thermo-gelation of a hydrogel network with $[\text{monomer}] = 0.93 \text{ mM}$ and $[\text{KCl}] = 0.31 \text{ M}$. The sigmoidal storage modulus increase marks the gelation temperature. Right: Frequency dependence of storage and loss moduli in the same network at 45°C. The lower limit of the linear plateau is not visible because of excessive measurement times, which puts it way below our deformation frequency of 1 Hz. There is therefore no unbinding / rebinding of fibres and nodes during our experiments, and the only rearrangements taking place are due to strain-induced plasticity instead of network dynamics.

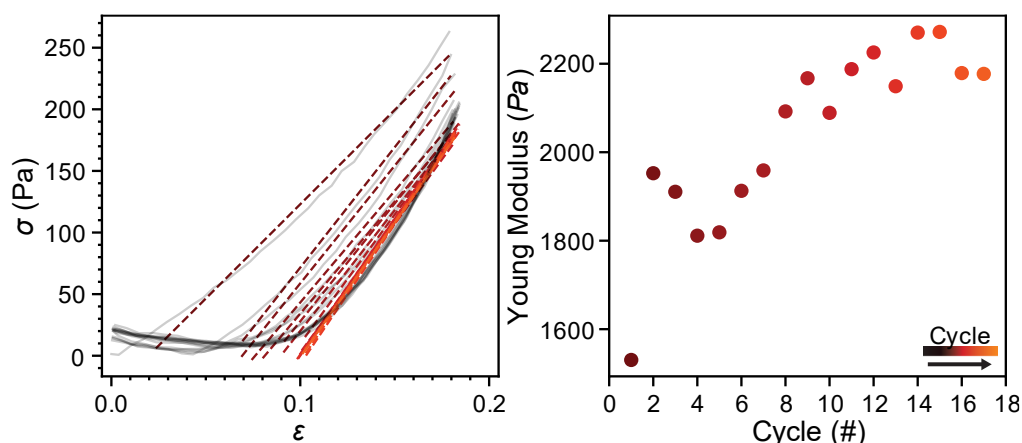


Figure S4. Shifted elastic response and strengthening for single fibre cyclic-loading.

Correlated analysis to Figure 3a (Main Text) of a single fibre caught in the optical traps under cyclic-loading from a sample of 666 μM monomer concentration, and 1 M KCl at 37 $^{\circ}\text{C}$ fluorescently labelled using 50:1 Azo-N3 incorporated into the fibril with 0.9 molar equivalents to the azide of DBCO-Cy5 with a maximum deformation of 1 μm above the initial length. The Young Modulus is measured by taking the linear slope of the elongational stroke starting at the zero-stress strain (left) demonstrating the lack of fibre weakening, towards stretching while plastically deforming (right).

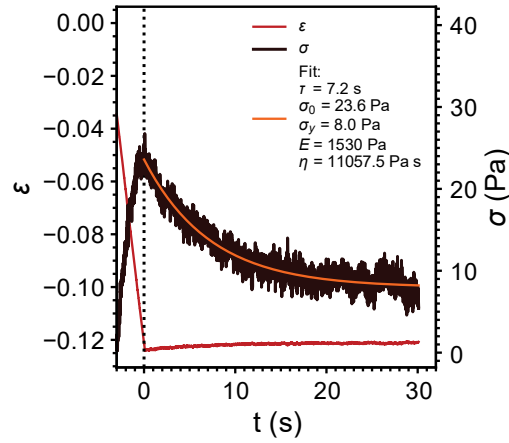


Figure S5. Compressional plasticity for a single fibre.

Stress relaxation measurement of a single fibre of 666 μM monomer concentration, 1 M KCl, at 37 $^{\circ}\text{C}$ fluorescently labelled using 50:1 Azo-N3 incorporated into the fibril with 0.9 molar equivalents to the azide of DBCO-Cy5 caught in the optical traps. Stress-relaxation is performed at a strain maximum of 1 μm maximum elongation from the initial length and a pause of 30 seconds. Consistent with the timescale of inter-fibrillar sliding for the elongational plasticity, compressional plasticity occurs on a similar timescale for both single fibres as for a gel network (Main Figure 3b; SI Figure 7). Fitting was performed using the Maxwell model for visco-elasticity, see methods section for more information.

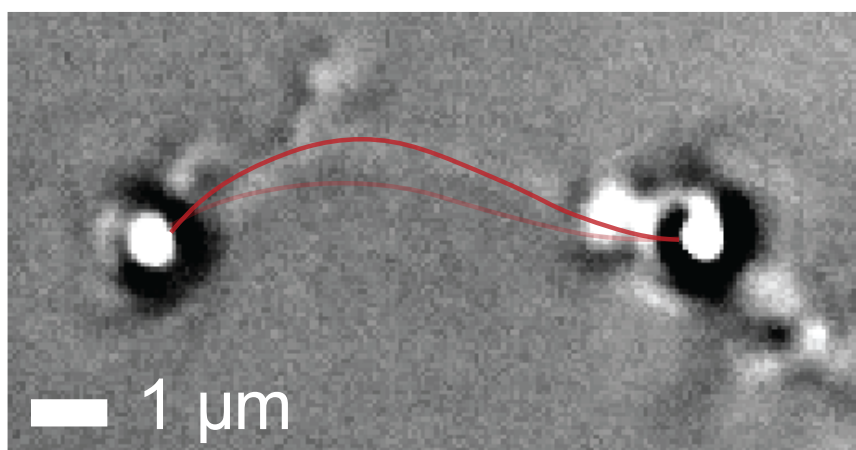


Figure S6. Brightfield analysis of buckling fibre on the compressive stroke over time.

63x magnification brightfield micrograph of a single fibre (666 μM; 1 M KCl; 37 °C; fluorescently labelled using 50:1 molar Azo-N3 and 0.9 molar equivalents of DBCO-Cy5 to the azide) caught in two optical traps in the optical tweezers set-up using two amine-coated fluorescent polystyrene particles of 1 μm in diameter (white spots with black circle). The coloured overlay trace the amount of buckling on the first compressive stroke and the subsequent compressive stroke. Indicating that due to the plastic inter-fibril sliding and the resulting lengthening the fibre has to successively buckle more a the same trap distance.

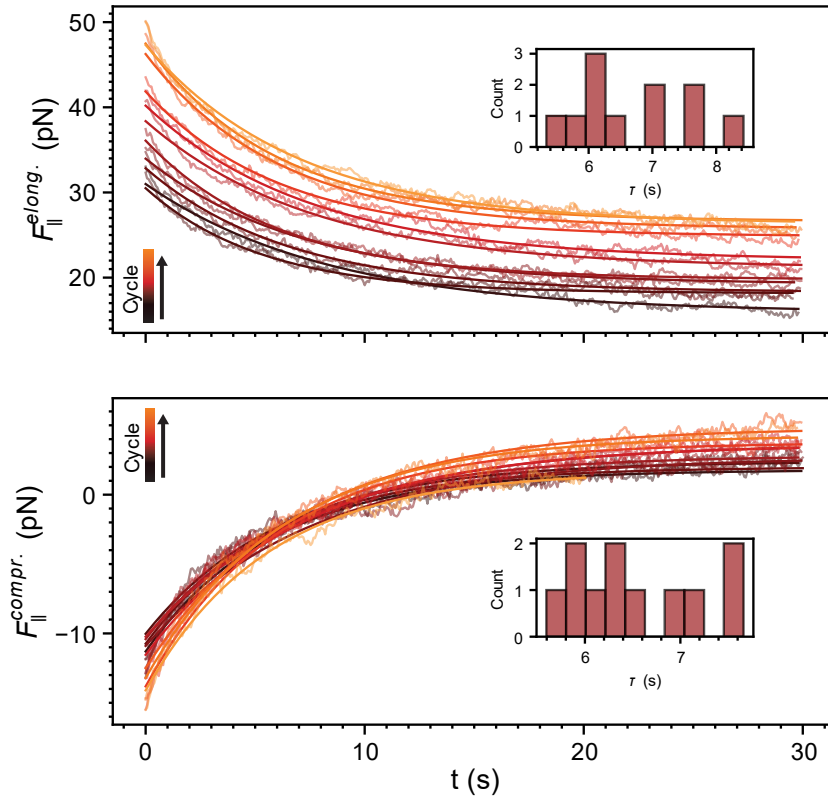


Figure S7. Fitted elongational and compressional plasticity for a gel network using Maxwell model.

Stress-relaxation experiment on a piece of hydrogel at 666 μM monomer concentration, 1 M KCl at 37 $^{\circ}\text{C}$ caught in the optical traps fluorescently labelled using 50:1 Azo-N3 incorporated into the fibrils with 0.9 molar equivalents to the azide of DBCO-Cy5. Stress-relaxation is performed at a strain maximum of 1 μm maximum elongation from the initial length and a pause of 30 seconds. One of the optical traps is moved while the force in the same direction (x-axis) is recorded. The data is split into elongational (top-) and compressive (bottom) stress-relaxation curves. Interestingly, the elongational plasticity seems consistent albeit shifted to higher stress (indicative of strain-stiffening) and the compressive plasticity sees little such effects (perhaps limited by buckling modes). The curves are fit with a Maxwell model (see Methods for more information) and the inter-fibril sliding timescale distribution is plotted in the inset histograms; consistent with measurements of a single fibre for both elongational and compressional plasticity.

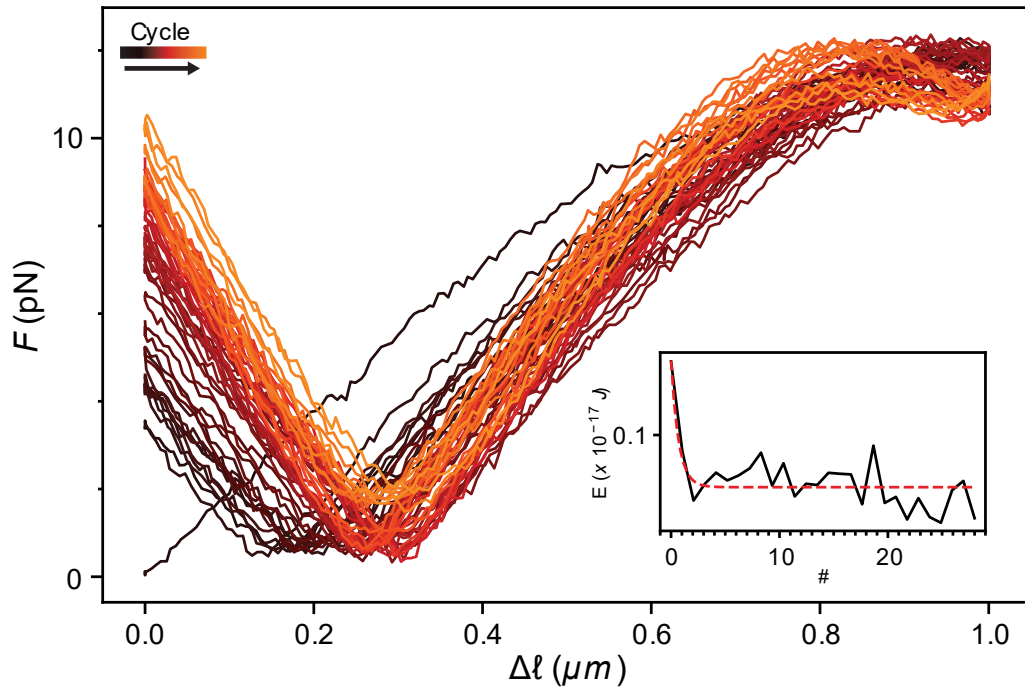


Figure S8. Probing gel network nodes with a point stress.

A sample of 0.5 mM monomer concentration, and 1 M KCl was co-incubated with amine-coated fluorescent polystyrene beads of 1 μm in diameter. The sample was loaded into the optical tweezers set-up and an oscillatory strain was applied to a bead attached in a node of the network. The zero-stress shifts to higher distance as a function of successive oscillations indicating the network relaxing plastically towards the repetitive strain. Inset: the energy (calculated from the elastic hysteresis between cycles) is plotted as a function of cycle and indicates a rapid relaxation of the material towards the applied strain.

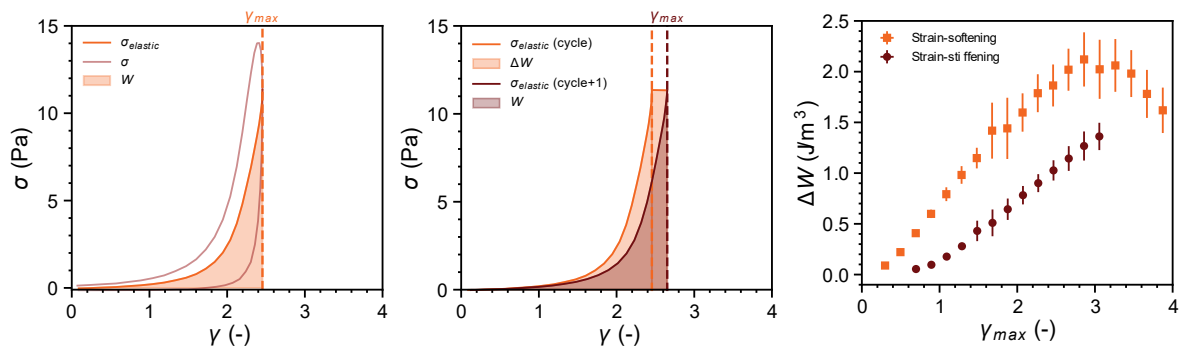


Figure S9. Quantification of energy dissipation from repeated strain cycles.

Left, For each step in the strain cycle the elastic stress $\sigma_{elastic}$ is calculated from the measured mechanical response as described in the methods section and plotted against the strain γ . As long as energy dissipation is limited, the orange area under this curve is equal to the work W required to deform the network up to a strain γ_{max} assuming that the majority of the energy dissipation occurs in the first step, we can thus estimate W for each cycle. Middle, An increase in γ_{max} the subsequent cycle (dark orange) leads to permanent changes in the mechanical response. If we extend the previous stress-strain curve (light orange), by assuming that on the first step it follows a straight line up to the current maximum strain, we can calculate the energy that is dissipated between these cycles ΔW is the difference in the area of the two curves. Right, In this way we can find ΔW as a function of γ_{max} for each network type. Error bars are calculated through error propagation of the standard deviation of work, stress, or strain, calculated over the 9 last steps of each 10-step cycle. The strain-softening sample has [monomer] = 0.93 mM, [KCl] = 0.31 M. The strain-stiffening sample has [monomer] = 0.78 mM and [KCl] = 1.1 M.

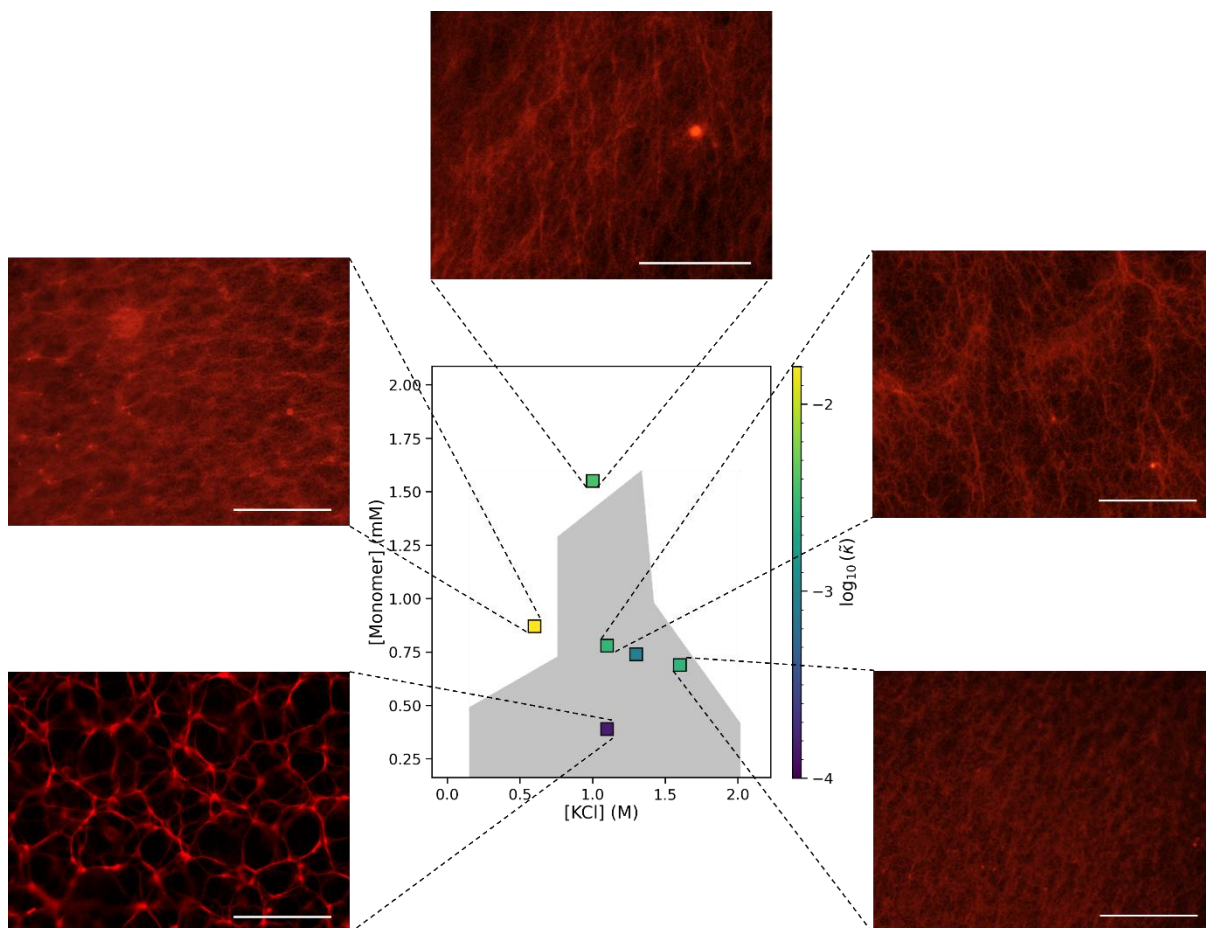


Figure S10. Correlation between $\tilde{\kappa}$, gel morphology, and strain-stiffening.

Phase diagram showing the gel networks presented in Figure 3, as a function of KCl and monomer concentrations. The colourmap indicates their respective $\tilde{\kappa}$ values. The grey area denotes the parameter space in which networks ostensibly strain-stiffen upon the first strain sweep. Scale bars are 20 μm for the topmost image and 50 μm for the other images.

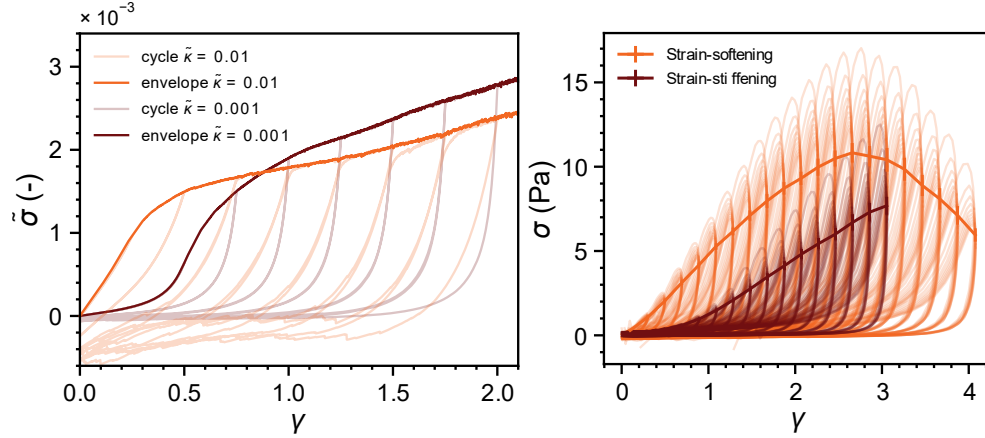


Figure S11. Comparison between low- $\tilde{\kappa}$ (dark orange, strain-stiffening) and high- $\tilde{\kappa}$ (light orange, strain-softening) networks.

Left. In the context of our simulation. Light orange curve: same as Figure 2c. Dark orange curve: high- $\tilde{\kappa}$ alternative. Note that unlike in the experiments, negative stresses build up at high- $\tilde{\kappa}$ due to compression induced buckling of lengthened fibres. Right: In experiments. The strain-softening sample has [monomer] = 0.93 mM, [KCl] = 0.31 M. The strain-stiffening sample has [monomer] = 0.78 mM and [KCl] = 1.1 M. Raw experimental curves are included with lower opacity, and the solid lines show the envelope of the elastic response.

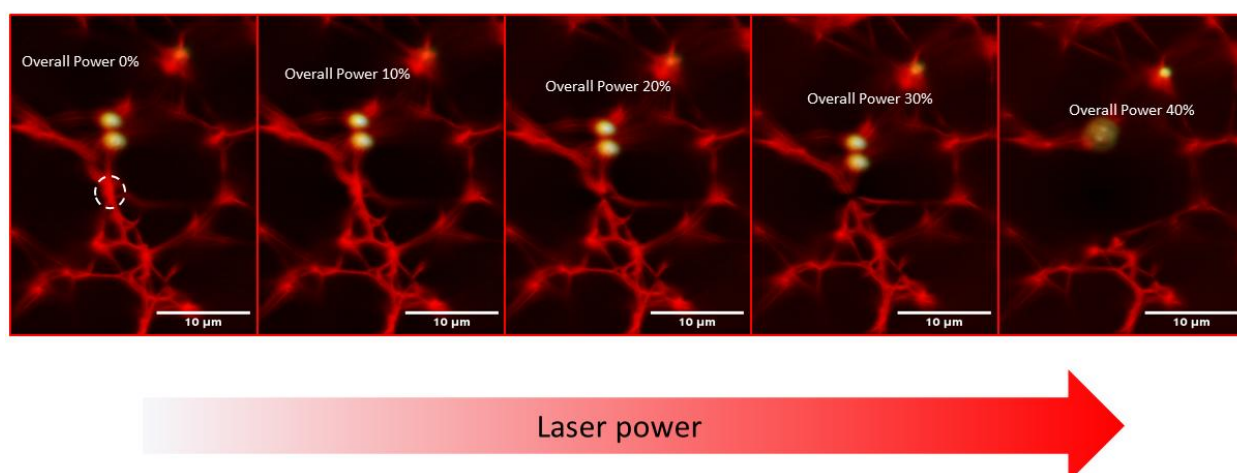
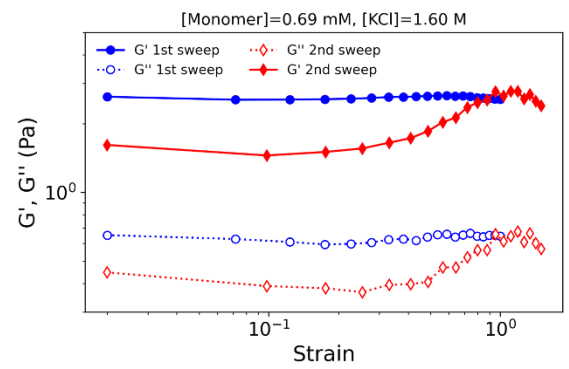
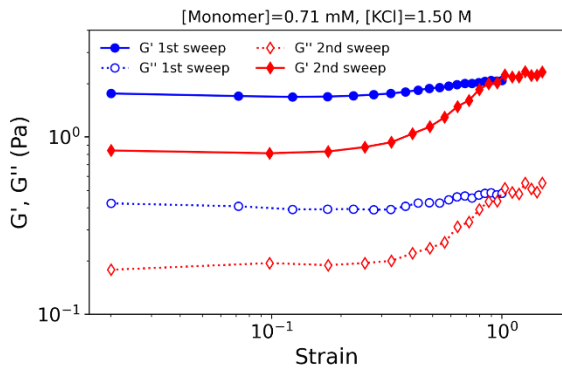
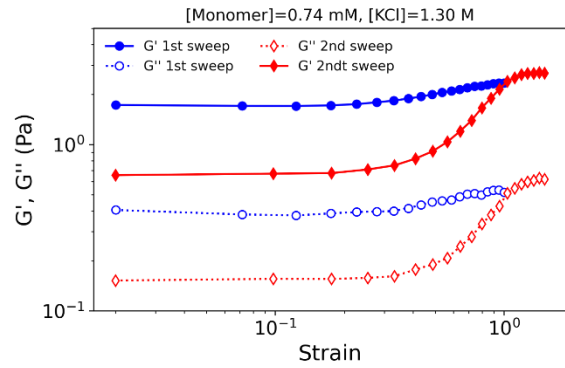
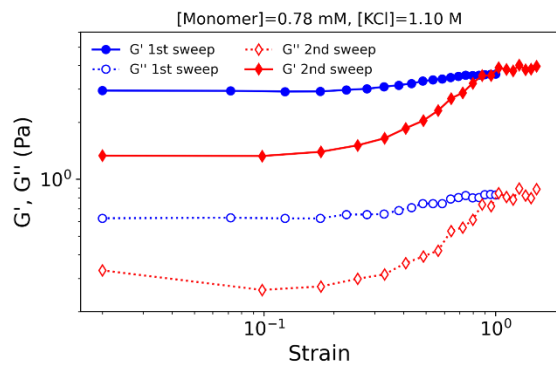
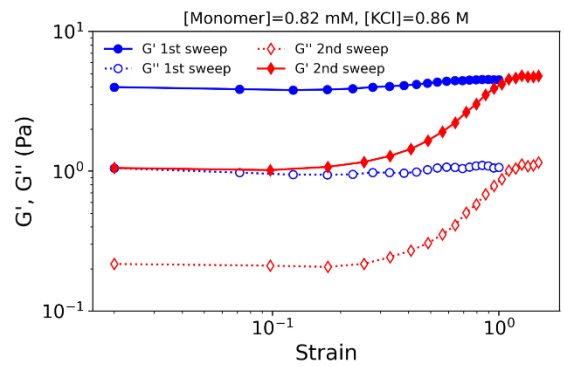
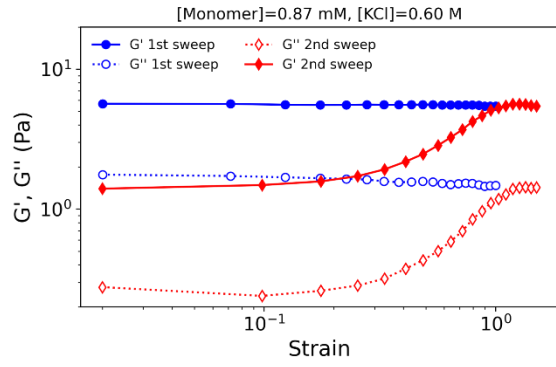
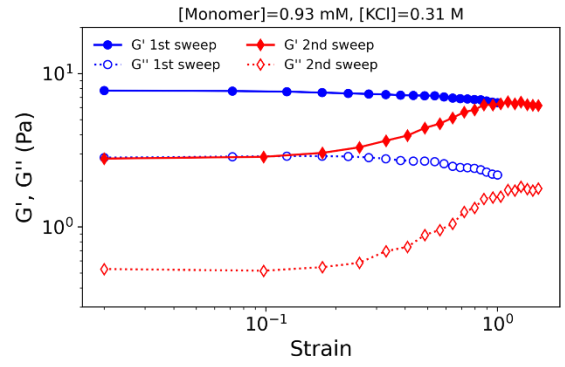
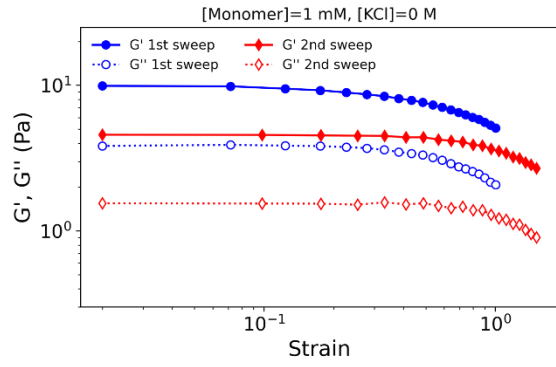
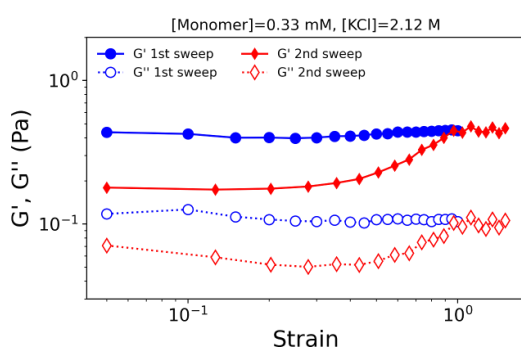
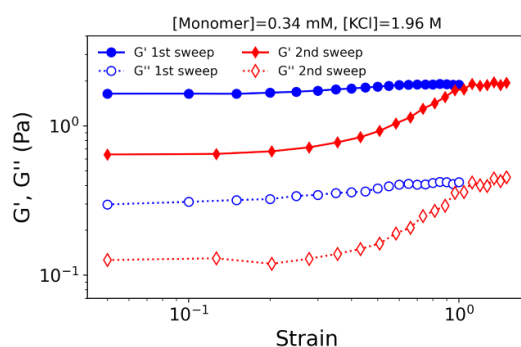
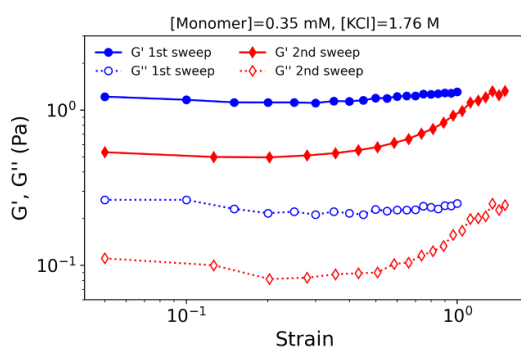
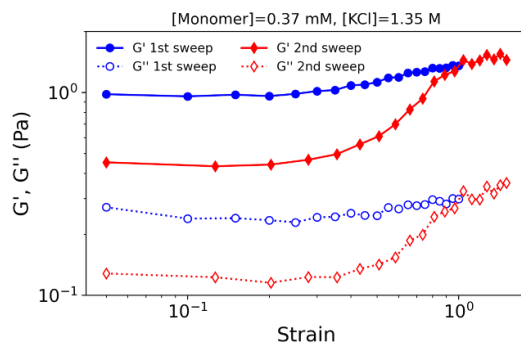
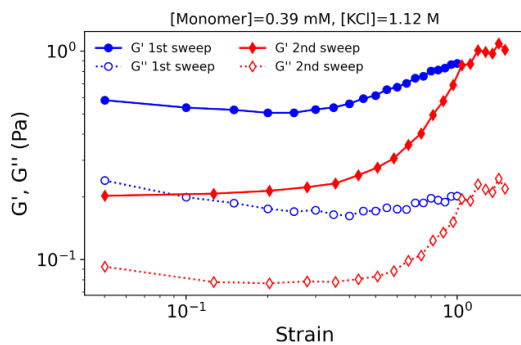
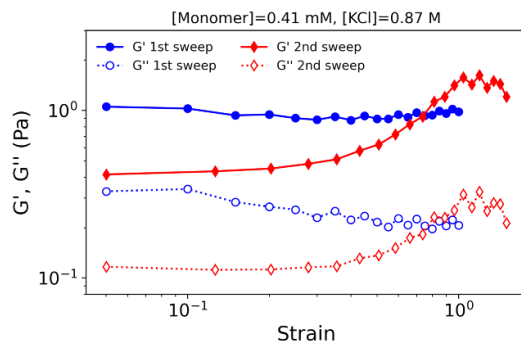
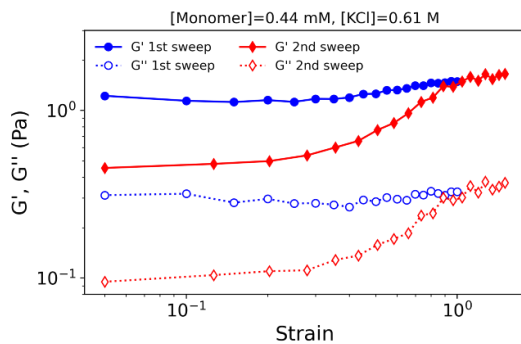
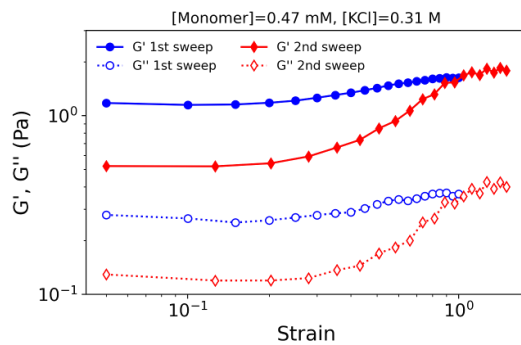
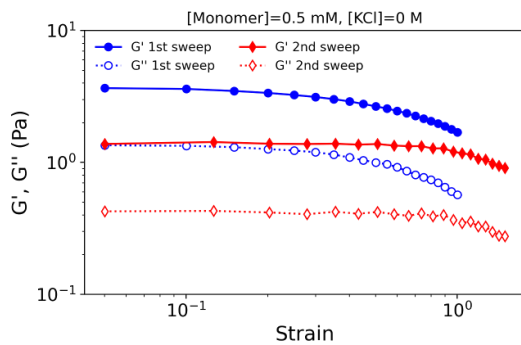
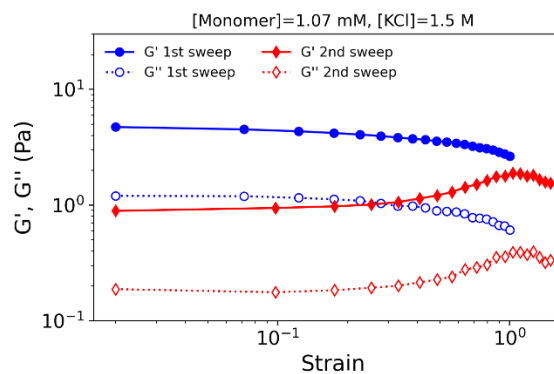
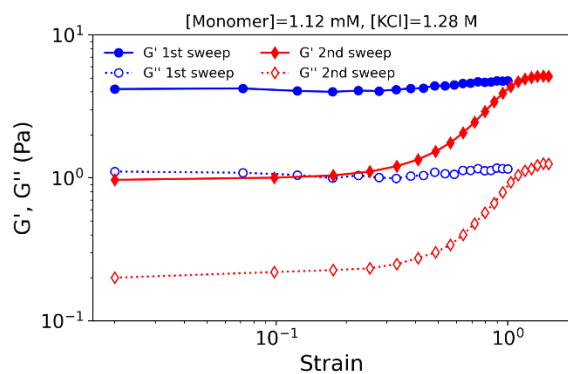
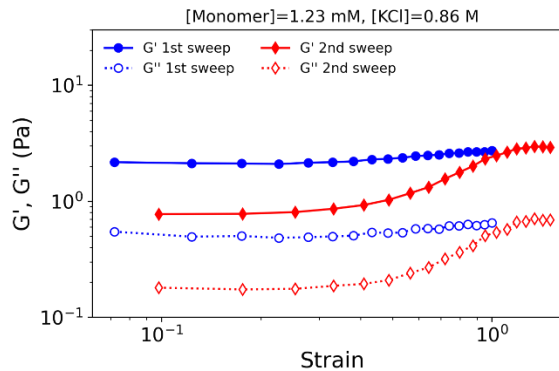
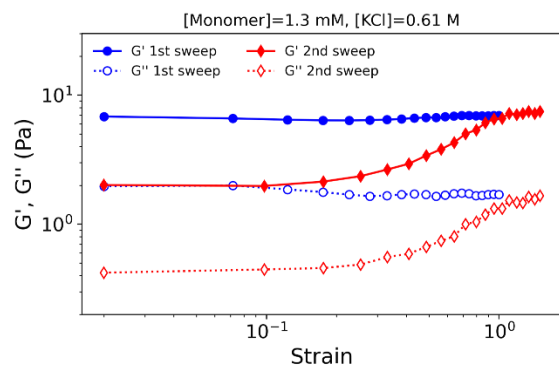
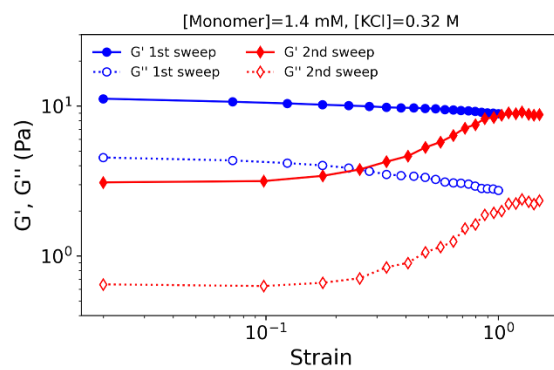
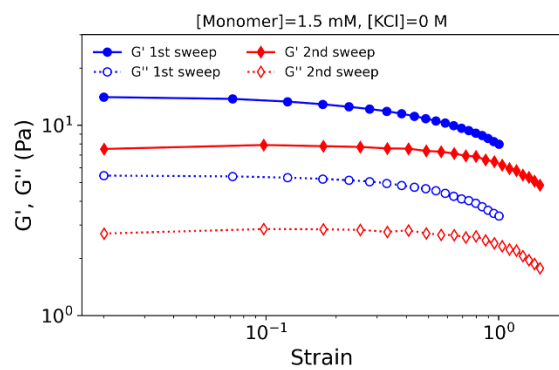


Figure S12. Determination of maximum laser power.

Testing the influence of laser irradiation using confocal scanning microscopy (638 nm, between 1 – 50 μ W) on the network of a 500 μ M monomer concentration sample with 1 M KCl at 37 °C fluorescently labelled using 50:1 Azo-N3 incorporated into the fibril with 0.9 molar equivalents to the azide of DBCO-Cy5. A laser power of 10% or less results in no visible damage to the network and was used as the maximum laser power throughout all measurements.







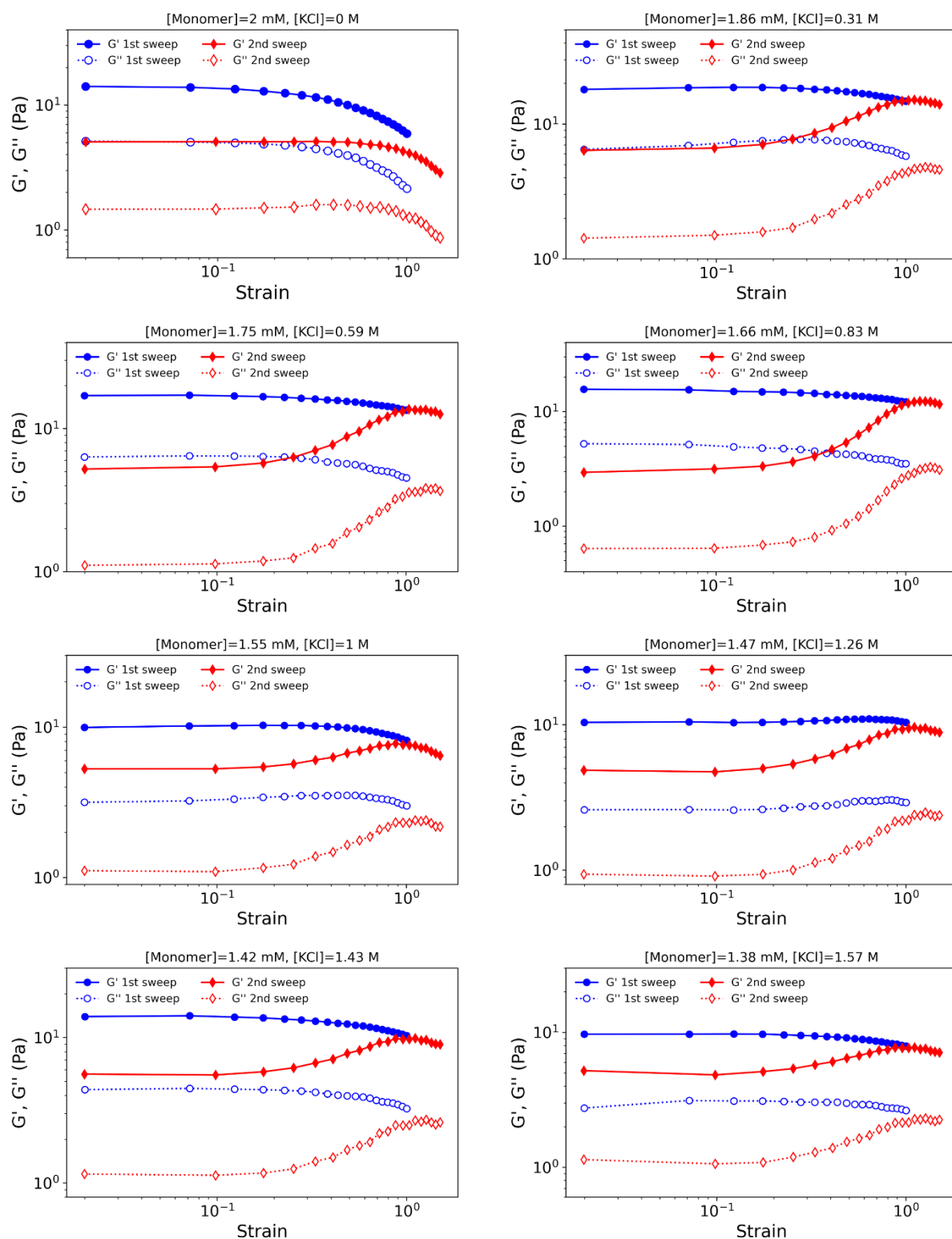


Figure S13. Compilation of phase diagram data for Figure 4A; organised by increasing monomer concentration, then by increasing KCl concentration. Two successive sweeps were performed on each sample (blue then red). Loss moduli are included as dotted lines for reference.

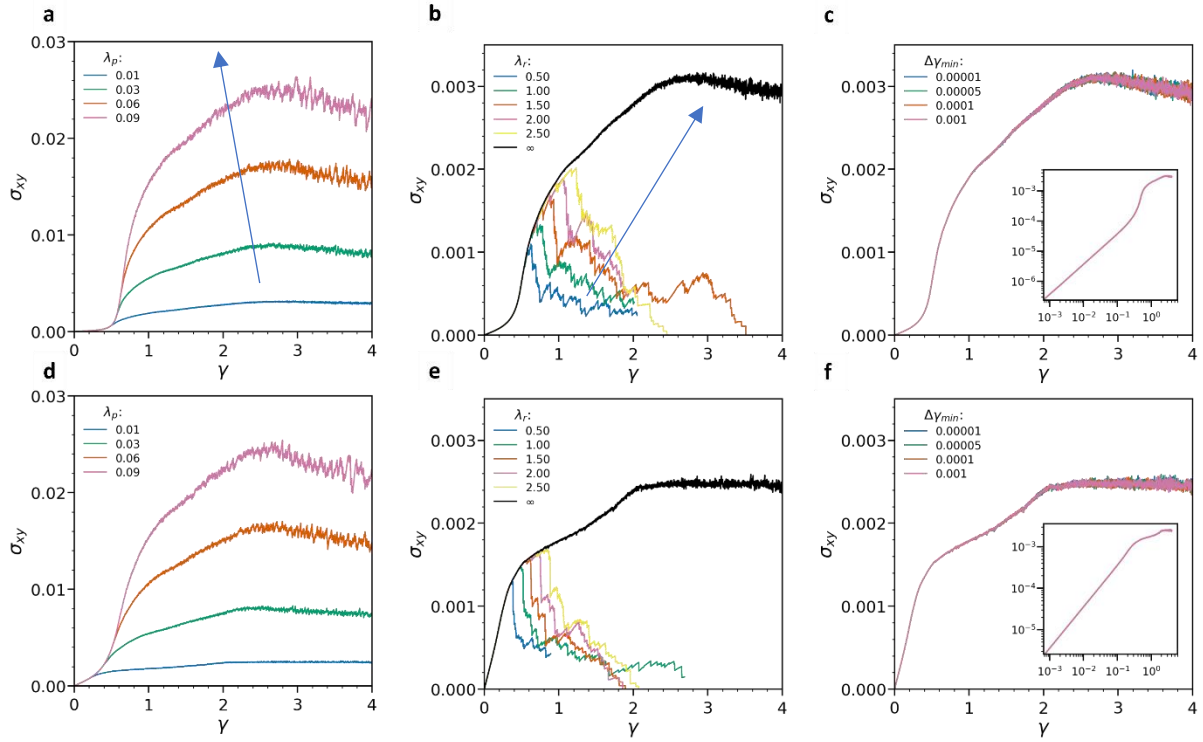


Figure S14. Effect of fibre plasticity and fibre breaking on stress-strain response.

a. d. Effect of the plasticity threshold λ_p for $\tilde{\kappa}=0.001$ a. and $\tilde{\kappa}=0.01$ d.. **b, e.** Effect of the rupture threshold λ_r for $\tilde{\kappa}=0.001$ b. and $\tilde{\kappa}=0.01$ e.. The introduction of a rupture threshold allows global failure of the networks, but does not affect the onset of plasticity. The networks have a significant plastic regime as long as $\lambda_p \ll \lambda_r$. The deviations of the model from the experimental model at high strains is likely caused by fibre rearrangement in the experimental system that are not captured by the model. **c., f.** Effect of the minimum strain step size $\Delta\gamma_{min}$ for $\tilde{\kappa}=0.001$ c. and $\tilde{\kappa}=0.01$ f.. The insets are log-log plots of the same response. Although the fluctuations in the stress-strain curve are not identical, indicating that $\Delta\gamma_{min}$ influences the order in which bonds lengthen, the global response is identical for all $\Delta\gamma_{min}$.

Property	Our Hydrogel	Collagen (1, 20-23)	Actin (1, 9-12)	Vimentin (1, 16-19)	Keratin (1, 13-15)
Fibre Persistence Length L_p (μm)	22	0.01	45	1	0.48
Fibril diameter θ_f (nm)	10	1.5	8	10	10
Fibre diameter θ_F (nm)	1000	155	350	10	120
Mass per unit length m/L (Da/nm)	20533	1000	16000	50500	37000
Plasticity	True	True	True	True	True

Table S1. Material properties of our hydrogel and relevant biological fibrillar systems across various conditions.

Data collected from various sources referenced in table heading, data used for the spider-diagram in main text Figure 1B. While the properties of the biological materials and that of our hydrogel vary across conditions these were selected to provide some context into common material properties and length scales.

[Monomer] (mM)	[KCl] (M)	Bundle diameter (μm)	Mesh size (μm)	$\tilde{\kappa}$
0.87	0.6	0.48 ± 0.13	0.89 ± 0.33	0.02
0.78	1.1	0.91 ± 0.21	4.23 ± 1.83	0.003
0.74	1.3	0.70 ± 0.19	5.84 ± 2.67	0.0009
0.69	1.6	0.40 ± 0.13	1.9 ± 0.5	0.003
0.39	1.1	0.68 ± 0.15	14.03 ± 4.98	0.0001
1.55	1.0	0.47 ± 0.12	1.94 ± 0.81	0.004

Table S2. Structural properties for different monomer and KCl concentrations.

Structural parameters extracted from epi-fluorescence micrographs of hydrogel samples at varying concentrations of monomer and potassium chloride used for populating the phase-diagram with values for the reduced bending rigidity. The bundle diameter, mesh size and bending rigidity are measured and calculated, respectively. Demonstrating the combined effect of monomer and salt concentration on the magnitude of the reduced bending rigidity $\tilde{\kappa}$.

Movie S1.

Tensile strain applied to a hydrogel (3.33 mM Monomer, 1M KCL) demonstrating plastic deformation and fibre alignment and deformation in line with network simulation predictions. Scalebar 1 mm. The material starts of in the solution phase at 20 °C. After which it was gelated at 37 °C indicated by the colour change.

Movie S2.

Correlated Brightfield timelapse of cyclic loading on a single fibre.

Movie S3.

Correlated Brightfield timelapse of cyclic loading on a hydrogel.

Movie S4.

Correlated Brightfield timelapse of point-stress deformations on a hydrogel.