

Supporting Information
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Using Rheo-SANS to understand how functionalised dipeptides form gels

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SUPPORTING INFORMATION

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Experimental Details

Materials. The gelators used in this study were synthesised as reported previously.^{1, 2} All other chemicals were purchased from Sigma Aldrich. D₂O was used throughout unless otherwise stated.

Stock Solutions. 2NapFF and 2NapVG gelator solutions were prepared at 10 mg/mL, and BrNapAV at 5 mg/mL. All were prepared by dissolving the pre-weighed gelator by the addition of D₂O to the desired concentration and the addition of 1 molar equivalent of NaOD (0.1 M).. The solutions were stirred overnight to ensure complete dissolution of the gelator. Next, the pH of each solution was measured and adjusted, if required, to pH 11 ± 0.1 with 1 M NaOD.

Gelation. To induce gelation a pH switch method was used. This was done by the addition of the stock solution to a pre-weighed amount of glucono- δ -lactone (GdL). For all gelators this was 8 mg/mL. The solutions were then gently swirled until the GdL had dissolved. This could then be transferred into the rheometer for the rheo-SANS experiments or into a Sterilin vial for the pH measurements.

pH Measurements. All pH measurements were performed using a FC200 pH probe (HANNA Instruments) with a 6 mm x 10 mm conical tip. The accuracy of the pH measurements is quoted as ± 0.1. 2 mL of gelator stock (10 mg/mL) was added to 16 mg of GdL in a 7 mL Sterilin vial. This was gently swirled to ensure the complete dissolution of GdL. A pH probe was added to the mixture and was placed in a controlled temperature water bath at 29°C pH measurements were recorded over 16 hours with a reading taken every 30 seconds.

***In Situ* Rheo-SANS.**

Rheo-SANS experiments for 2NapFF and 2NapVG were carried out at the ISIS Neutron and Muon source of the STFC Rutherford Appleton Laboratory (Didcot, UK), experiment number RB1910193. Scattering data were collected with an 8 mm incident beam, a sample-to-detector distance of 4.1 m and a wavelength range of 2.2-10 Å; resulting in a wave vector range of $0.007 \leq q \leq 1.5 \text{ \AA}^{-1}$, $q = \frac{4\pi}{\lambda} \sin(\theta/2)$, with λ the neutron wavelength and θ the scattering angle.³ Rheology was collected on a Anton-Paar Physica MCR 501 rheometer with a customised titanium concentric cylinder (ME49-1.16-60/108.5/T, d=0.053 mm) and a temperature controller (TC-30), in the radial direction with a gap of 1 mm.

For 2NapVG, 2NapFF and BrNapAV gelation was measured over time at a set strain of 0.5% and 10 rad/s (within the linear viscoelastic region of the gel) at 29°C.

The 2NapVG gel structural changes were investigated under 5 shear rates (0.1, 1, 10, 100 and 1000%) at room temperature (29°C).

Raw scattering data were corrected for sample transmission, detector efficiency and solvent background scattering (as described in detail in Heenan et al.³) using the Mantid Software, and then converted to absolute scattering cross section ($I(q) / \text{cm}^{-1}$) using the scattering from a standard sample (comprising a solid blend of hydrogenous and perdeuterated polystyrene) in accordance with established procedures.⁴

Rheo-SANS for the BrNapAV was carried out at the Institut Laue Langevin, Grenoble, France on the D11 beamline, experiment 9-11-1801. A neutron beam, with a fixed wavelength of 6 Å and divergence of $\Delta\lambda/\lambda = 9\%$, allowed measurements over a large range in Q [$Q = 4\pi\sin(\theta/2)/\lambda$] range of 0.001 to 0.3 Å⁻¹, using two sample-detector distances of 1.75 m and 8 m. For the kinetic experiments, the 8 m detector distance was used over a Q range of 0.0084 to 0.086 Å⁻¹. The data were then reduced to 1D scattering curves of intensity vs. Q using the facility provided software. The electronic background was subtracted, the full detector images for all data were normalized and scattering from the empty cell was subtracted. The scattering from D₂O was also measured and subtracted from the data.

Rheology was collected on a Anton-Paar Physica MCR 501 rheometer with a customised titanium concentric cylinder (ME49-TI, d=0.08 mm) and a temperature controlled (at 25°C) in the radial direction with a gap of 1 mm.

Supplementary Figures

Fitting the Small Angle Neutron Scattering Data

All SANS data were fitted using SASView. To fit the SANS data, the following scattering length densities were used using the software available from NIST⁵ using an estimated density of 1.53 g/mL.

2NapVG: $2.436 \times 10^{-6} \text{ \AA}^{-2}$

2NapFF: $2.730 \times 10^{-6} \text{ \AA}^{-2}$

BrNapAV: $2.077 \times 10^{-6} \text{ \AA}^{-2}$

Data for 2NapVG

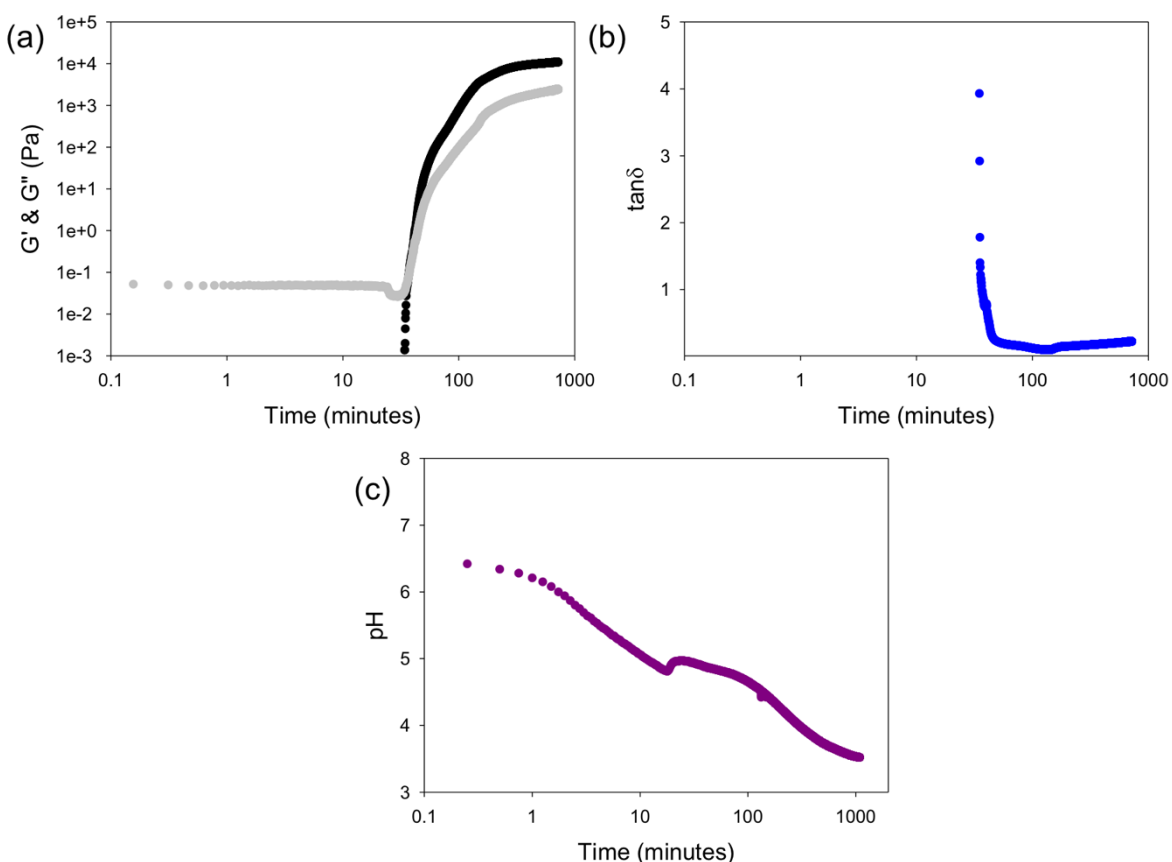


Figure S1. Monitoring gelation of 2NapVG over time (a) Rheology, grey and black data represent G' and G'' respectively collected at 0.5% strain and 10 rad/s. (b) $\tan\delta$ (c) pH. Data collected at 29°C

The data for the 2NapVG were fitted using the simultaneous fitting option available in SASView. Initial fits were carried out to the 12-hour data, with the flexible elliptical cylinder model giving the best fit to the data (other cylinder-based models were tried on the basis of previous data,⁶⁻⁸ but the flexible elliptical cylinder provided the best fit even when polydispersity was included in the other models). The output from this fit was inputted into the 12-hour data for the simultaneous fit. All data fitted well to this model apart from the 0.5-hour and 1-hour data, which

gave large errors for many of the parameters. These two data sets were then fitted manually to a flexible cylinder model, constraining the length and radius on the basis of the data from later fits. The output from the fits to the models are shown in Tables S1-S3, with the fits to the data shown in Figures S2-S3.

	0.5 hours (FC)	0.5 hours (FEC)	1 hours (FC)	1 hours (FEC)	2 hours
Background / cm⁻¹	0.018	0.018	0.018	0.018	0.018
Scale	$4.23 \times 10^{-5} \pm 5.22 \times 10^{-5}$	$0.00013 \pm 9.40 \times 10^{-4}$	$0.00011 \pm 4.38 \times 10^{-4}$	$0.00013 \pm 5.10 \times 10^{-4}$	$0.00029 \pm 6.27 \times 10^{-6}$
Length / Å	1000	999 ± 2304	1000	986 ± 1023	991 ± 206
Kuhn Length / Å	52 ± 122	97 ± 482	85 ± 161	85 ± 161	88 ± 35
Radius / Å	27	41.7 ± 96	27	43 ± 76	27.1 ± 4.3
Axis Ratio		0.43 ± 2.1		0.8 ± 2.6	1.76 ± 0.6
Chi squared	0.9495	0.9819	1.1214	1.0288	1.0147

Table S1. Fitting parameters for SANS data collected for 2NapVG gels at different times. All data were fitted to a flexible cylinder model as discussed above. The data for 0.5 hours and 1 hour were fitted to both the flexible cylinder (FC) and flexible elliptical cylinder (FEC) models as denoted in the headings. For the fitting to the flexible cylinder model, the fits were constrained to a radius of 27 and a length of 1000 Å.

	3 hours	4 hours	5 hours	6 hours	7 hours
Background / cm⁻¹	0.018	0.018	0.018	0.018	0.018
Scale	$0.00046 \pm 2.19 \times 10^{-6}$	$0.00066 \pm 6.33 \times 10^{-6}$	$0.00095 \pm 6.65 \times 10^{-6}$	$0.00098 \pm 5.36 \times 10^{-5}$	$0.00097 \pm 7.19 \times 10^{-5}$
Length / Å	992 ± 256	975 ± 120	1000 ± 130	1000 ± 137	1000 ± 32
Kuhn Length / Å	74 ± 31	83 ± 18	108 ± 26	105 ± 26	100 ± 9
Radius / Å	26.4 ± 2.24	29.5 ± 1.9	29.9 ± 1.5	29.5 ± 1.1	27.5 ± 1.1

Axis Ratio	1.87 ± 0.4	1.68 ± 0.25	1.63 ± 0.20	1.75 ± 0.2	2.00 ± 0.14
Chi squared	1.1478	1.0086	1.0467	0.93905	1.0475

Table S2. Fitting parameters for SANS data collected for 2NapVG gels at different times. All data were fitted to a flexible elliptical cylinder model as discussed above.

	8 hours	9 hours	10 hours	11 hours	12 hours
Background / cm⁻¹	0.018	0.018	0.018	0.018	0.018
Scale	0.0011 ± 4.60x10 ⁻⁵	0.0011 ± 4.96x10 ⁻⁵	0.0011 ± 5.45x10 ⁻⁵	0.0011 ± 3.17x10 ⁻⁵	0.0012 ± 2.96x10 ⁻⁵
Length / Å	1000 ± 120	1000 ± 152	999 ± 202	1022 ± 121	1014 ± 119
Kuhn Length / Å	113 ± 25	117 ± 32	133 ± 51	141 ± 33	150 ± 36
Radius Å	28.7 ± 0.8	28.0 ± 0.9	27.3 ± 1.2	27.4 ± 0.8	28.0 ± 0.8
Axis Ratio	1.92 ± 0.13	2.15 ± 0.13	2.37 ± 0.18	2.38 ± 0.1	2.434 ± 0.12
Chi squared	1.0003	1.2215	0.8753	1.0295	1.1761

Table S3. Fitting parameters for SANS data collected for 2NapVG gels at different times. All data were fitted to a flexible elliptical cylinder model as discussed above.

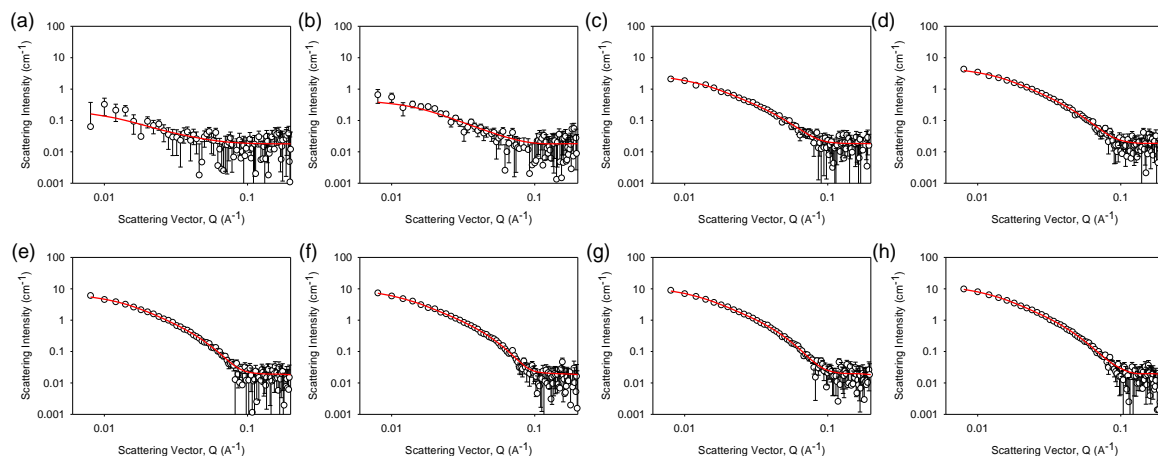


Figure S2. SANS data (open circles) and fits (red lines) to a flexible elliptical cylinder model for 2NapVG with time. Data and fits are shown for (a) 0.5 hours; (b) 1 hour; (c) 2 hours; (d) 3 hours; (e) 4 hours; (f) 5 hours; (g) 6 hours; (h) 7 hours.

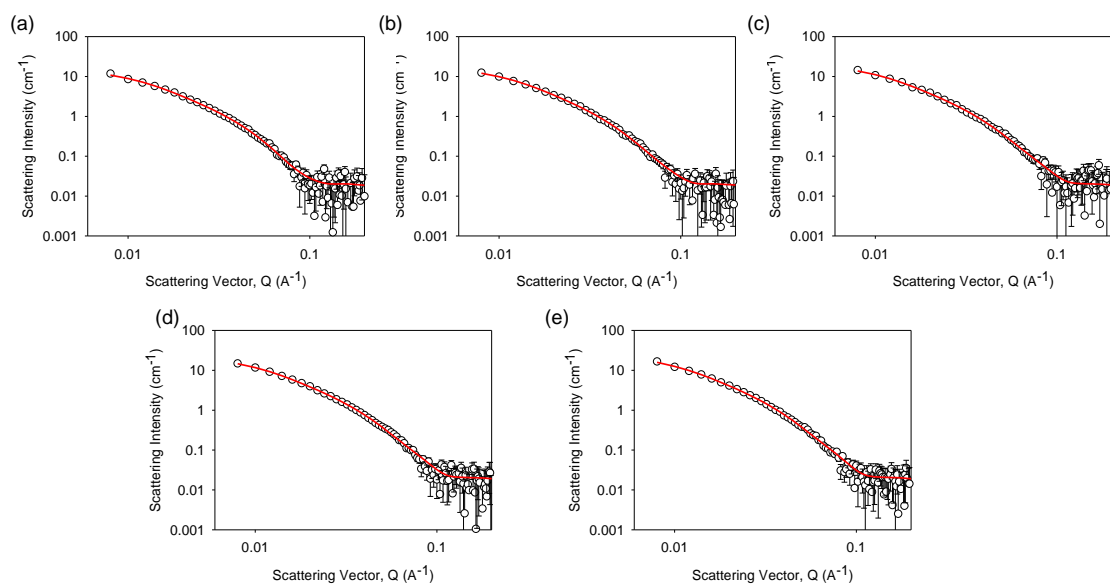


Figure S3. SANS data (open circles) and fits (red lines) to a flexible elliptical cylinder model for 2NapVG with time. Data and fits are shown for (a) 8 hours; (b) 9 hours; (c) 10 hours; (d) 11 hours; (e) 12 hours.

Data for 2NapFF

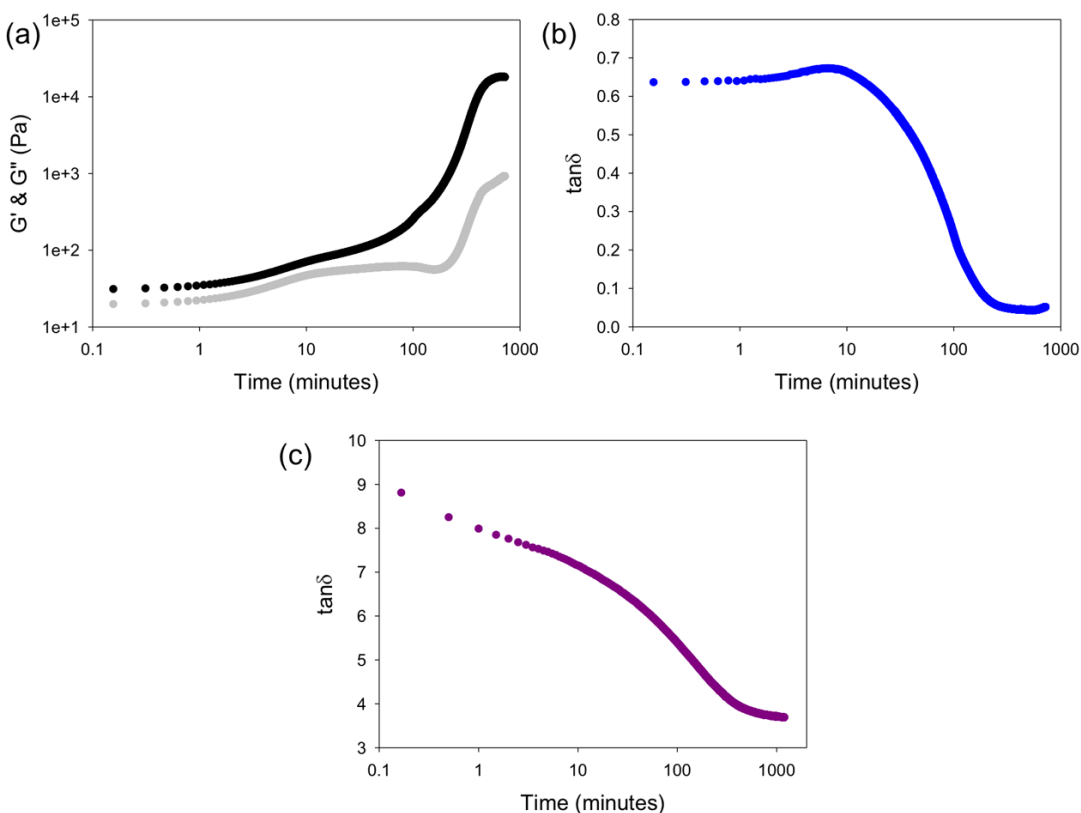


Figure S4. Monitoring gelation of 2NapFF over time (a) Rheology, grey and black data represent G' and G'' respectively collected at 0.5% strain and 10 rad/s. (b) $\tan\delta$ (c) pH. Data collected at 29°C

The data were fitted by initially fitting the data at 12 hours. The flexible elliptical cylinder model and the elliptical cylinder models gave good fits to the data at later times. The data at earlier times however were best fit with the elliptical cylinder model. On the basis of this, the elliptical cylinder model was used throughout. The outputs from this model are shown below in Tables S4-S6.

	0.5 hours	1 hours	2 hours
Background / cm^{-1}	0.014	0.014	0.014
Scale	$0.0041 \pm 7.17 \times 10^{-5}$	$0.0044 \pm 7.09 \times 10^{-5}$	$0.00045 \pm 6.58 \times 10^{-5}$
Length / Å	415 ± 42	378 ± 31	404 ± 36
Radius / Å	25.5 ± 0.9	26.8 ± 1.0	28.2 ± 1.1
Axis Ratio	1.8 ± 0.1	1.5 ± 0.1	1.5 ± 0.1
Chi squared	0.88634	0.99448	0.88385

Table S4. Fitting parameters for SANS data collected for 2NapFF gels at different times. All data were fitted to an elliptical cylinder model as discussed above.

	3 hours	4 hours	5 hours	6 hours	7 hours
Background / cm⁻¹	0.014	0.014	0.014	0.014	0.014
Scale	0.0047 ± 6.50x10 ⁻⁵	0.0048 ± 6.45x10 ⁻⁵	0.0050 ± 7.02x10 ⁻⁵	0.0050 ± 6.43x10 ⁻⁵	0.0050 ± 6.04x10 ⁻⁵
Length / Å	>2000	>2000	>2000	>2000	>2000
Radius / Å	26.6 ± 0.7	25.5 ± 0.6	23.7 ± 0.5	24.7 ± 0.5	25.1 ± 0.4
Axis Ratio	1.8 ± 0.1	2.2 ± 0.1	2.7 ± 0.1	2.8 ± 0.1	3.1 ± 0.10
Chi squared	0.95431	0.84746	1.1846	1.1413	1.4341

Table S5. Fitting parameters for SANS data collected for 2NapFF gels at different times. All data were fitted to an elliptical cylinder model as discussed above.

	8 hours	9 hours	10 hours	11 hours	12 hours
Background / cm⁻¹	0.014	0.014	0.014	0.014	0.014
Scale	0.0041 ± 6.17x10 ⁻⁵	0.0051 ± 5.85x10 ⁻⁵	0.0051 ± 5.59x10 ⁻⁵	0.0051 ± 5.36x10 ⁻⁵	0.0050 ± 4.93x10 ⁻⁵
Length / Å	>2000	>2000	>2000	>2000	>2000
Radius / Å	24.7 ± 0.4	25.3 ± 0.4	26.4 ± 0.4	26.4 ± 0.4	28.0 ± 0.4
Axis Ratio	3.5 ± 0.09	3.65 ± 0.10	3.5 ± 0.09	3.90 ± 0.09	3.8 ± 0.11
Chi squared	1.4361	1.5455	1.4463	1.2755	1.3144

Table S6. Fitting parameters for SANS data collected for 2NapFF gels at different times. All data were fitted to an elliptical cylinder model as discussed above.

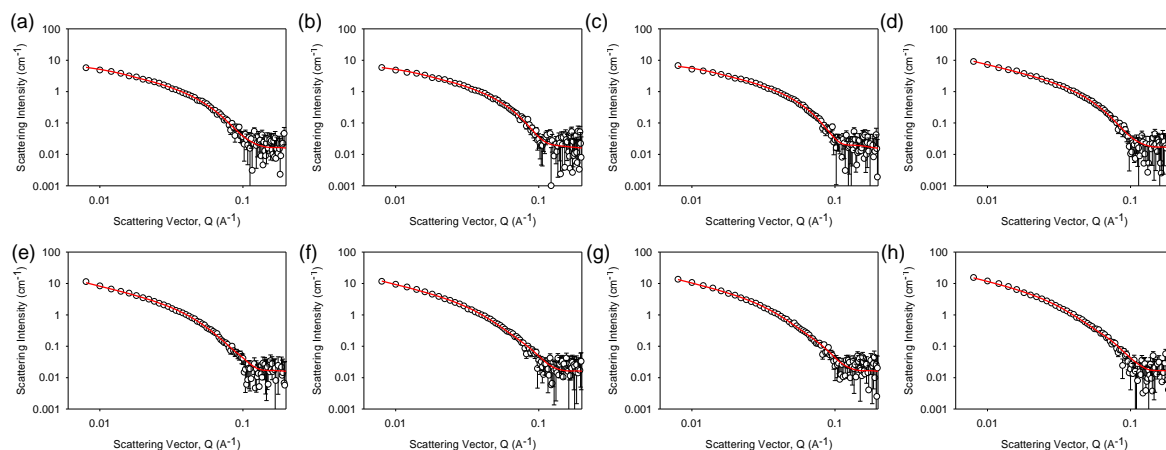


Figure S5. SANS data (open circles) and fits (red lines) to an elliptical cylinder model for 2NapFF with time. Data and fits are shown for (a) 0.5 hours; (b) 1 hour; (c) 2 hours; (d) 3 hours; (e) 4 hours; (f) 5 hours; (g) 6 hours; (h) 7 hours.

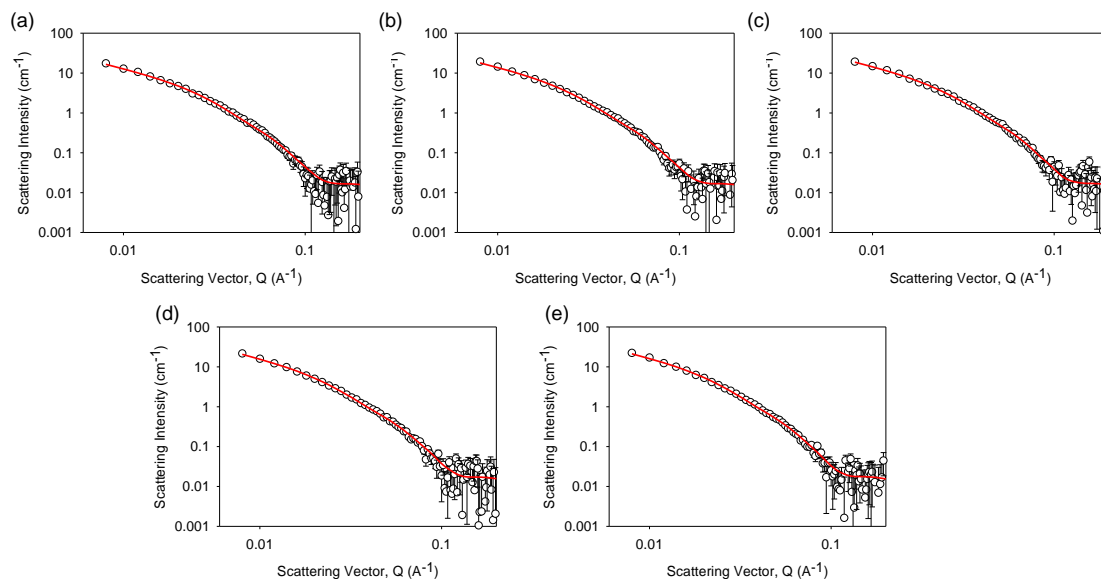


Figure S6. SANS data (open circles) and fits (red lines) to an elliptical cylinder model for 2NapFF with time. Data and fits are shown for (a) 8 hours; (b) 9 hours; (c) 10 hours; (d) 11 hours; (e) 12 hours.

Data for BrNapAV

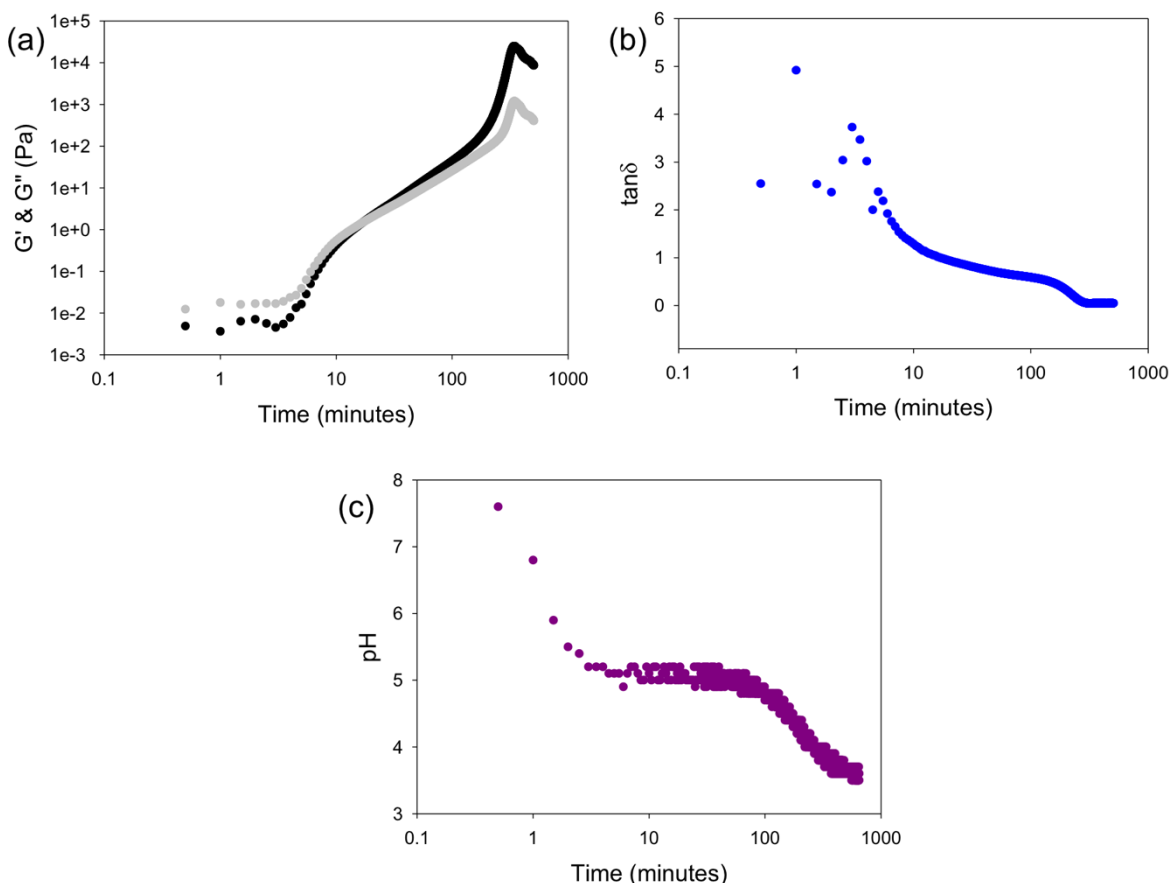


Figure S7. Monitoring gelation of BrNapAV over time (a) Rheology, grey and black data represent G' and G'' respectively collected at 0.5% strain and 10 rad/s. (b) $\tan\delta$ (c) pH. Data collected at 29°C

The data collected after 5 minutes fitted well to a power law. For the gel formed after 8 hours, data was collected at 8 m (as for the kinetic samples) and also at 1.75 m, meaning data were collected over a wider Q range. These data were fitted to a flexible elliptical cylinder model (other models such as the flexible cylinder alone required a significant polydispersity of >0.3 to fit the data, and the fit as determined by the chi squared value was worse than that for the flexible elliptical cylinder without polydispersity). Using this fit to fit the data accessed only by the 8 m camera length resulted in a good fit as expected. Re-fitting over this restricted range gave similar results as long as the background was fixed to that determined from the fit to the wider Q range (over the shorter Q range, the data do not plateau at high Q). This background was then fixed for fitting the data for earlier times.

	5 minutes (PL)	8 hours (wide Q range) (FEC)	8 hours (lower Q range) (FEC)	1 hours (lower Q range) (FEC)	2hours (lower Q range) (FEC)
Background / cm ⁻¹	0.071 ± 0.00055	0.06 ± 8.62x10 ⁻⁴	0.06*	0.06*	0.06*
Scale	1.21x10 ⁻⁶ ± 2.41x10 ⁻⁷	0.00075 ± 2.48 x10 ⁻⁶	0.00082 ± 3.94 x10 ⁻⁶	0.00037 ± 3.91x10 ⁻⁶	0.00059 ± 6.13x10 ⁻⁶
Power Law	2.79 ± 0.05				
Length / Å		3932 ± 158	3757± 208	1568 ± 99	1471 ± 36
Kuhn Length / Å		53 ± 3	58 ± 5	248 ± 9	147 ± 1
Radius / Å		21.7 ± 0.3	23.3 ± 0.6	28.2 ± 0.2	26.9 ± 0.2
Axis Ratio		2.44 ± 0.3	2.25 ± 0.5	2.46 ± 0.04	2.36 ± 0.03
Chi squared	1.3044	2.9971	2.7841	1.3896	2.6396

Table S7. Fitting parameters for SANS data collected for BrNapAV gels at different times. All data were fitted to a power law (PL) or a flexible elliptical cylinder (FEC) model as discussed above and noted in the header to the column.

	3 hours (lower Q range) (FEC)	4 hours (lower Q range) (FEC)	5 hours (lower Q range) (FEC)	6 hours (lower Q range) (FEC)	7 hours (lower Q range) (FEC)	8 hours (lower Q range) (FEC)
Background / cm ⁻¹	0.06*	0.06*	0.06*	0.06*	0.06*	0.06*
Scale	0.00074 ± 5.84 x10 ⁻⁶	0.00079 ± 5.75 x10 ⁻⁶	0.00087 ± 5.63 x10 ⁻⁶	0.00089 ± 5.36 x10 ⁻⁶	0.00086 ± 5.11 x10 ⁻⁶	0.00082 ± 3.94 x10 ⁻⁶
Power Law						
Length / Å	1547± 19	2360± 279	2655 ± 261	2931± 254	3121± 250	3757± 208
Kuhn Length / Å	118 ± 3	61 ± 10	61± 8	61 ± 7	61 ± 7	58 ± 5
Radius / Å	26.2 ± 0.2	24.5 ± 0.9	24.8 ± 0.8	24.5 ± 0.8	23.7 ± 0.8	23.3 ± 0.6
Axis Ratio	2.33 ± 0.3	2.09 ± 0.4	2.04 ± 0.4	2.06 ± 0.5	2.19 ± 0.6	2.25 ± 0.5
Chi squared	3.3465	2.583	3.5691	2.9555	2.8966	2.7841

Table S8. Fitting parameters for SANS data collected for BrNapAV gels at different times. All data were fitted to a power law (PL) or a flexible elliptical cylinder (FEC) model as discussed above and noted in the header to the column.

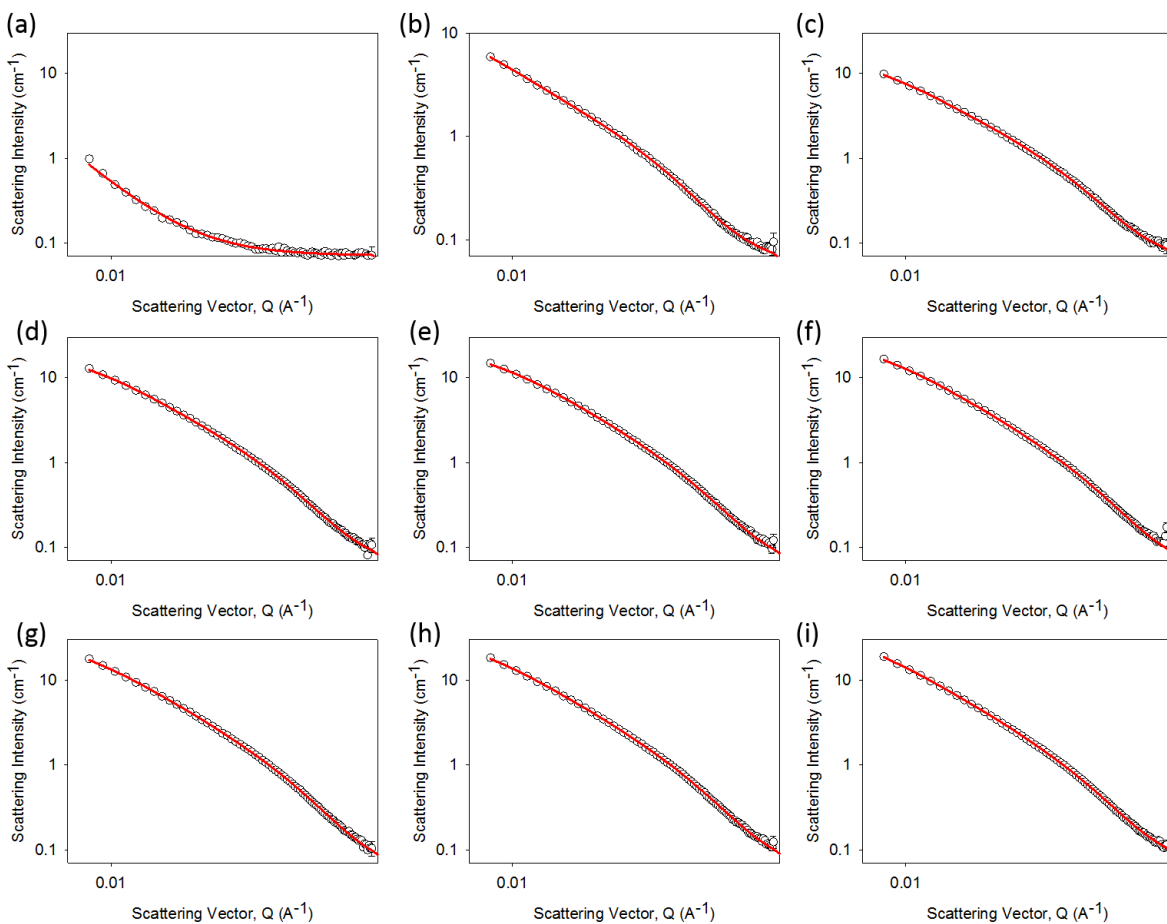


Figure S8. SANS data (open circles) and fits (red lines) to a power law or flexible elliptical cylinder model for BrNapAV with time. Data and fits are shown for (a) 5 minutes; (b) 1 hour; (c) 2 hours; (d) 3 hours; (e) 4 hours; (f) 5 hours; (g) 6 hours; (h) 7 hours; (i) 8 hours.

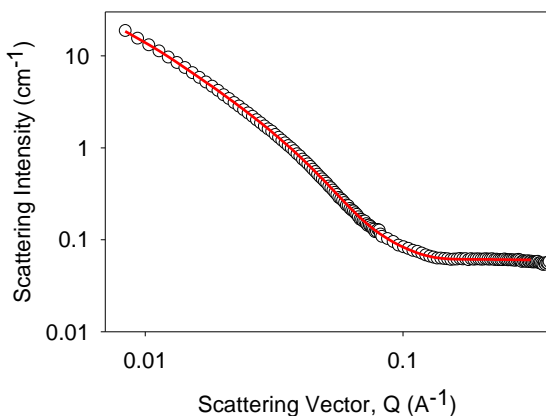


Figure S9. SANS data (open circles) and fit (red lines) to a flexible elliptical cylinder model for BrNapAV after 8 hours over an extended Q range (see discussion above).

Strain data of 2NapVG

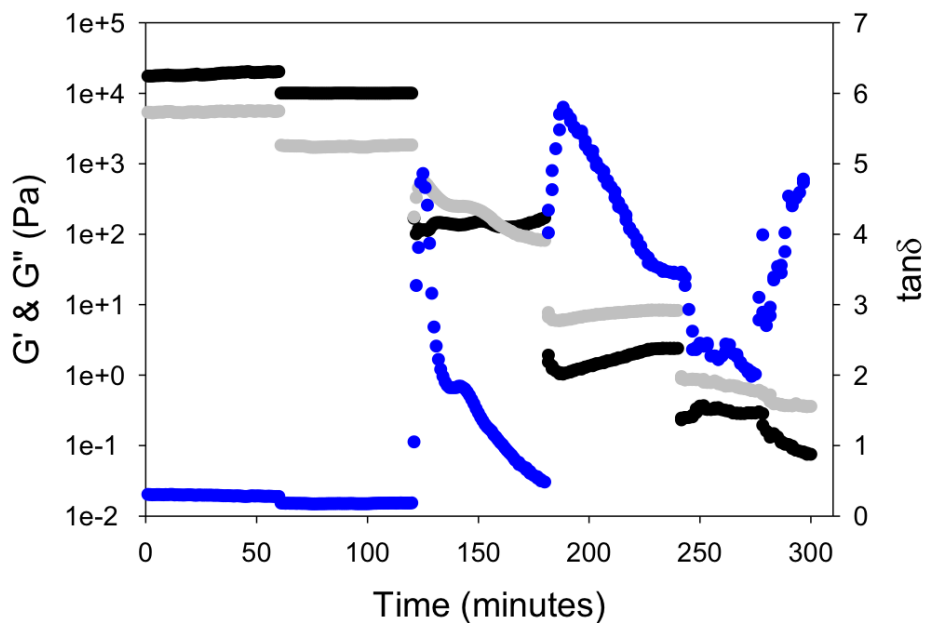


Figure S10. Strain sweep of 2NapVG. Grey and black data represent G' and G'' respectively collected at 10 rad/s. Blue data represents $\tan\delta$. Data collected at 29°C at 10 rad/s.

	0.1% strain	1% strain	10% strain	100% strain	1000% strain
Background / cm^{-1}	0.018	0.018	0.018	0.018	0.03
Scale	$0.0011 \pm 1.44 \times 10^{-5}$	$0.0011 \pm 1.42 \times 10^{-5}$	$0.0011 \pm 1.33 \times 10^{-5}$	$0.0011 \pm 1.51 \times 10^{-5}$	$0.0009 \pm 1.35 \times 10^{-5}$
Length / Å	1075 ± 50	1084 ± 50	1168 ± 53	1058 ± 42	887 ± 39
Kuhn Length / Å	151 ± 5	153 ± 5	152 ± 5	148 ± 5	144 ± 6
Radius / Å	27.8 ± 0.5	28.2 ± 4	32.1 ± 0.4	38.3 ± 0.4	42.6 ± 0.4
Axis Ratio	2.5 ± 0.1	2.6 ± 0.1	2.7 ± 0.1	2.5 ± 0.1	2.8 ± 0.1
Chi squared	1.0887	1.0934	0.9874	0.99635	1.3293

Table S9. Fitting parameters for SANS data collected for 2NapVG gels at different applied strains. All data were fitted to a flexible elliptical cylinder model as discussed above.

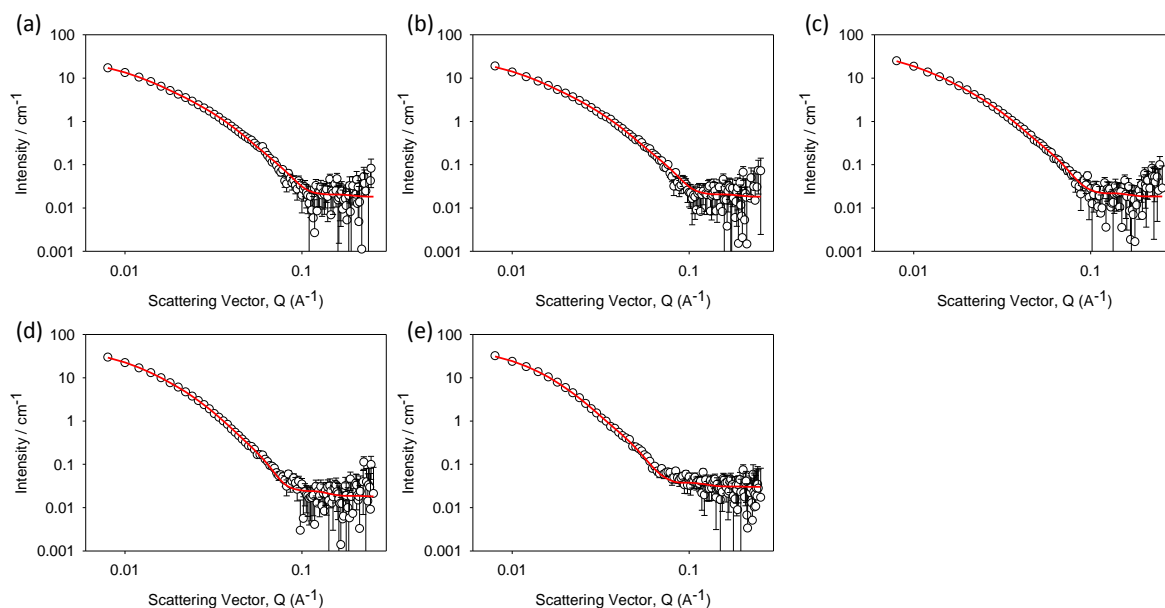


Figure S11. SANS data (open circles) and fits (red lines) to a flexible elliptical cylinder model for gels formed from 2NapVG under different applied strain. Data and fits are shown for (a) 0.1% strain; (b) 1% strain; (c) 10% strain; (d) 100% strain; (e) 1000% strain.

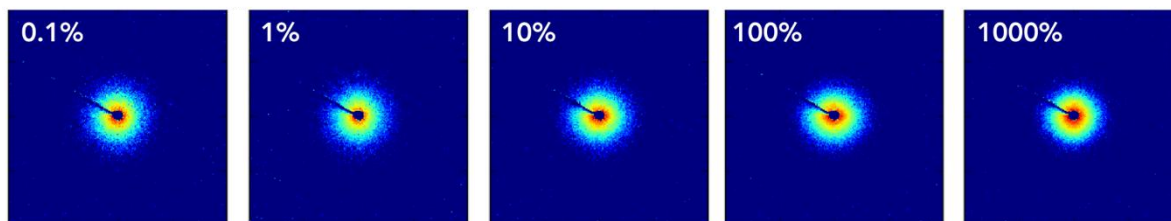


Figure S12. SANS patterns of 2NapVG at different strains showing no alignment

References

1. K. A. Houton, K. L. Morris, L. Chen, M. Schmidtman, J. T. A. Jones, L. C. Serpell, G. O. Lloyd and D. J. Adams, *Langmuir*, 2012, **28**, 9797-9806.
2. L. Chen, S. Revel, K. Morris, L. C. Serpell and D. J. Adams, *Langmuir*, 2010, **26**, 13466-13471.
3. R. K. Heenan, J. Penfold and S. M. King, *Journal of Applied Crystallography*, 1997, **30**, 1140-1147.
4. G. D. Wignall and F. S. Bates, *Journal of Applied Crystallography*, 1987, **20**, 28-40.
5. <https://www.ncnr.nist.gov/resources/activation/>.

6. M. C. Nolan, A. M. Fuentes Caparrós, B. Dietrich, M. Barrow, E. R. Cross, M. Bleuel, S. M. King and D. J. Adams, *Soft Matter*, 2017, **13**, 8426-8432.
7. E. R. Draper, M. Wallace, R. Schweins, R. J. Poole and D. J. Adams, *Langmuir*, 2017, **33**, 2387-2395.
8. A. Z. Cardoso, A. E. Alvarez Alvarez, B. N. Cattoz, P. C. Griffiths, S. M. King, W. J. Frith and D. J. Adams, *Faraday Discussions*, 2013, **166**, 101-116.