Effect of Self-Sorting and Co-Assembly on the Mechanical Properties of Low Molecular Weight Hydrogels

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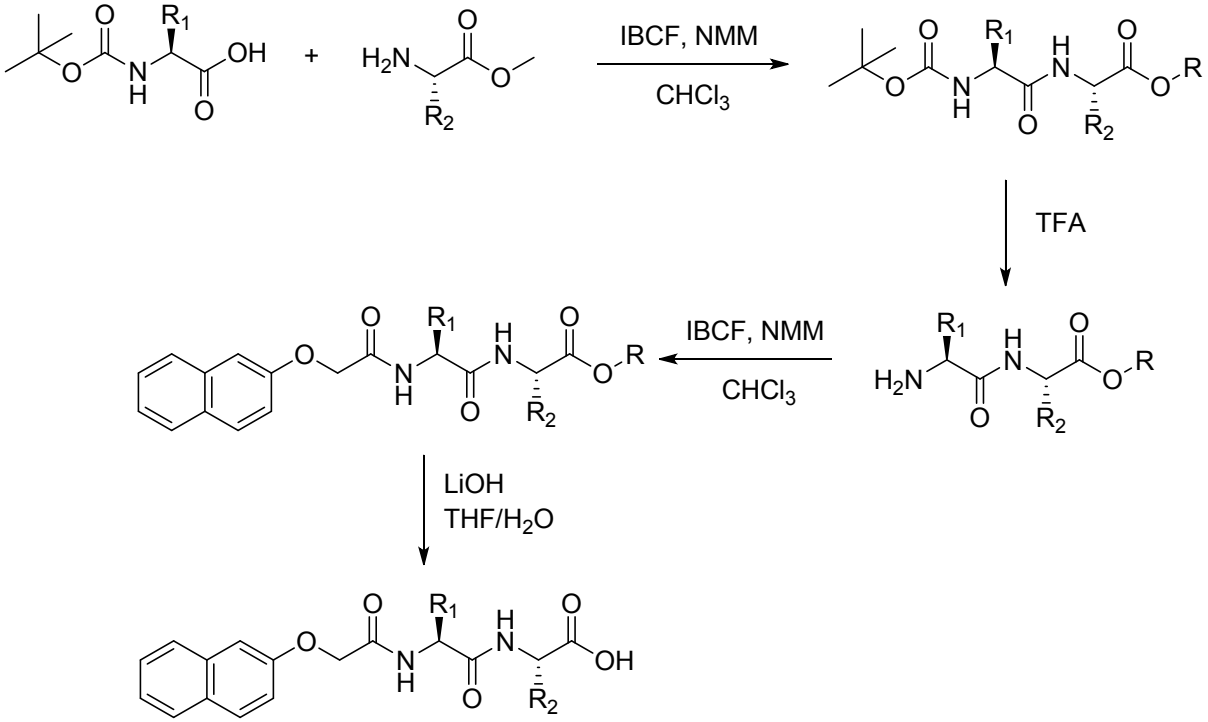
SUPPORTING INFORMATION

1. Materials.

All chemicals and solvents were purchased from Sigma-Aldrich and used as received. De-ionised water was used throughout. Dipeptides **1**, **2**, **3** and **4** were prepared as described previously.[1-3] Dipeptides **5** and **6** were prepared using analogous methods (Scheme S1).



Scheme S1. Synthesis of dipeptides. R = CH_3 (**3**) or CH_2CH_3 (**4**). IBCF = isobutylchloroformate; NMM = *N*-methylmorpholine.

Boc-IF-OEt: ^{1}H NMR ($CDCl_3$) 7.23 (m, ArH, 3H), 7.13 (m, ArH, 2H), 6.31 (bd, NH, 1H, J_{HH} = 6.4 Hz), 5.00 (bd, NH, 1H, J_{HH} = 7.4 Hz), 4.84 (dd, CHNH, 1H, J_{HH} = 7.8 Hz, J_{HH} = 6.0 Hz), 4.14 (q, CH_2, 2H, J_{HH} = 7.1 Hz), 3.93 (m, CHNH, 1H), 3.12 (d, CH_2Ph, 2H, J_{HH} = 5.7 Hz), 1.82 (m, CH, 1H), 1.49 (s, CH_3, 9H), 1.22 (t, CH_2CH_3, 3H, J_{HH} = 7.1 Hz), 1.10 (m, CH, 1H), 0.93 (m, 2 x CH_3, 6H) ppm. ^{13}C NMR ($CDCl_3$) 171.2, 155.6, 135.8, 129.3, 128.6, 127.1, 79.9, 69.7, 61.5, 59.2, 53.1, 38.1, 37.3, 30.9, 28.3, 24.7, 18.9, 18.8, 15.4, 14.1, 11.4 ppm. MS (ES) 429 ([M+Na]$^+$). Accurate mass calculated for $C_{22}H_{34}N_2O_5Na$: 429.2365. Found: 429.2375.

IF-OEt.TFA: ^{1}H NMR (DMSO) 8.88 (d, NH, 1H, J_{HH} = 7.1Hz), 8.11 (s, NH_3, 3H), 7.29 (m, ArH, 5H), 4.53 (dd, CHNH, 1H, J_{HH} = 7.6 Hz, J_{HH} = 7.1 Hz), 4.04 (q, CH_2CH_3, 2H, J_{HH} = 7.1 Hz), 3.68 (d, CHNH, 1H, J_{HH} = 5.2 Hz), 3.03 (m, CH_2Ph, 2H), 1.84 (m, CH, 1H), 1.47 (m, CH, 1H), 1.10 (t, CH=3, 3H, J_{HH} = 7.1 Hz), 0.92 (d, CH_3, 3H, J_{HH} = 6.9 Hz), 0.88 (t, CH_3, 3H, J_{HH} = 7.3 Hz) ppm. ^{13}C NMR (DMSO) 170.8, 168.1, 136.7, 129.0, 128.3, 126.7, 60.7, 56.4, 53.9, 36.5, 36.3, 23.5, 14.4, 13.9, 11.1 ppm. MS (ES) 307 ([M+H]$^+$). Accurate mass calculated for $C_{17}H_{27}N_2O_3$: 307.2022. Found: 307.2022.

2NapIF-OEt: ^{1}H NMR ($CDCl_3$) 7.79 (m, ArH, 2H), 7.73 (d, ArH, 1H, J_{HH} = 8.2Hz), 7.44 (t, ArH, 1H, J_{HH} = 8.2 Hz), 7.37 (t, ArH, 1H, J_{HH} = 8.2 Hz), 7.20 – 7.14 (m, ArH and NH, 6H),

7.09 (m, ArH, 2H), 6.30 (d, NH, 1H, J_{HH} = 7.9 Hz), 4.84 (dt, CHNH, 1H, J_{HH} = 7.9 Hz, J_{HH} = 6.2 Hz), 4.64 (d, OCH, 1H, J_{HH} = 14.9 Hz), 4.56 (d, OCH, 1H, J_{HH} = 14.9 Hz), 4.35 (dd, CHNH, 1H, J_{HH} = 8.9 Hz, J_{HH} = 7.0 Hz), 4.17 (q, CH_2, 2H), 3.11 (dd, CHPh, 1H, J_{HH} = 13.9 Hz, J_{HH} = 5.9 Hz), 3.02 (dd, CHPh, 1H, J_{HH} = 13.9 Hz, J_{HH} = 6.4 Hz), 1.88 (m, CH, 1H), 1.38 (m, CH, 1H), 1.24 (t, CH_3, 3H, J_{HH} = 7.1Hz), 0.95 (d, CH, 1H), 0.88 (d, CH_3, 3H, J_{HH} = 6.8Hz), 0.81 (t, CH_3, 3H, J_{HH} = 7.4 Hz) ppm. ^{13}C NMR ($CDCl_3$) 171.1, 170.1, 168.0, 154.9, 135.6, 134.3, 129.9, 429.5, 129.3, 128.6, 127.7, 127.2, 126.9, 126.8, 124.4, 118.2, 107.6, 67.2, 61.6, 57.2, 53.1, 37.8, 37.1, 30.9, 24.8, 15.3, 14.1, 11.2 ppm. MS (ES) 485 ($[M+Na]^+$). Accurate mass calculated for $C_{27}H_{30}N_2O_5Na$: 485.2052. Found: 485.2060.

2NapIFOH (Dipeptide 5): ^{1}H NMR (DMSO) 8.40 (d, NH, 1H, J_{HH} = 7.8 Hz), 7.90 (d, NH, 1H, J_{HH} = 9.2 Hz), 7.84 (m, ArH, 2H), 7.75 (d, ArH, 1H, J_{HH} = 8.1 Hz), 7.45 (t, ArH, 1H, J_{HH} = 6.9 Hz), 7.35 (t, ArH, 1H, J_{HH} = 6.9 Hz), 7.24 (m, ArH, 7H), 4.71 (d, OCH, 1H, J_{HH} = 14.7 Hz), 4.64 (d, OCH, 1H, J_{HH} = 14.7 Hz), 4.45 (m, CHNH, 1H), 4.32 (dd, CHNH, 1H, J_{HH} = 9.1 Hz, J_{HH} = 7.4 Hz), 3.06 (dd, CHPh, 1H, J_{HH} = 13.9 Hz, J_{HH} = 5.3 Hz), 2.89 (dd, CHPh, 1H, J_{HH} = 13.9 Hz, J_{HH} = 9.2 Hz), 1.74 (m, CH, 1H), 1.36 (m, CH, 1H), 0.98 (m, CH, 1H), 0.80 (d, CH_3, 3H, J_{HH} = 6.8 Hz), 0.75 (t, CH_3, 3H, J_{HH} = 7.3 Hz) ppm. ^{13}C NMR (DMSO) 172.7, 17.7, 157.1, 155.5, 137.4, 133.9, 129.4, 129.0, 128.7, 128.1, 127.5, 126.6, 126.5, 126.4, 123.8, 118.5, 107.3, 66.7, 53.1, 53.3, 36.9, 35.5, 23.9, 15.1, 10.8 ppm. MS (ES) 461 ($[M-H]^-$). Accurate mass calculated for $C_{27}H_{29}N_2O_5$: 461.2076. Found: 461.2070.

Boc-ML-OMe: ^{1}H NMR ($CDCl_3$) 6.56 (bd, NH, 1H, J_{HH} = 7.1 Hz), 5.18 (bd, NH, 1H, J_{HH} = 7.1 Hz), 4.61 (m, CHNH, 1H), 4.29 (m, CHNH, 1H), 3.73 (s, OCH_3, 3H), 2.60 (t, CH_2S, 2H, J_{HH} = 7.3 Hz), 2.12 (s, SCH_3, 3H), 2.06 (m, C$H_2$$CH_2$, 1H), 1.95 (m, C$H_2$$CH_2$, 1H), 1.66 (m, C$H_2CH(CH_3)_2$, 2H), 1.57 (m, C$H(CH_3)_2$, 1H), 1.44 (s, CH_3, 9H), 0.93 (d, CH_3, 6H) ppm. ^{13}C NMR ($CDCl_3$) 173.1, 171.3, 155.5, 80.2, 53.1, 52.3, 50.7, 41.4, 31.3, 30.1, 28.3, 24.8, 22.8, 21.8, 15.1 ppm. MS (ES) 399 ($[M+Na]^+$). Accurate mass calculated for $C_{17}H_{32}N_2O_5SNa$: 399.1930. Found: 399.1943.

ML-OEt.TFA: ^{1}H NMR (DMSO) 8.81 (d, NH, 1H, J_{HH} = 7.4 Hz), 8.20 (bs, NH_3, 3H), 4.3 (m, CHNH, 1H), 3.90 (t, CHNH, 1H, J_{HH} = 6.1 Hz), 3.65 (s, OCH_3, 3H), 2.51 (m, CH_2 and CH_2, 4H), 2.07 (s, SCH_3, 3H), 2.01 (m, $CH(CH_3)_2$, 1H), 1.57 (m, CH and CH_2, 3H), 0.92 (d, CH_3, 3H, J_{HH} = 6.5 Hz), 0.87 (d, CH_3, 3H, J_{HH} = 6.5 Hz) ppm. ^{13}C NMR (DMSO) 172.4, 168.4, 52.1, 51.4, 50.5, 30.9, 27.8, 24.1, 22.7, 21.6, 14.4 ppm. MS (ES) 277.2 ($[M+H]^+$). Accurate mass calculated for $C_{12}H_{25}N_2O_3S$: 277.1586. Found: 277.1581.

2Nap-ML-OMe: ^{1}H NMR ($CDCl_3$) 7.79 (m, ArH, 2H), 7.73 (d, ArH, 1H, J_{HH} = 8.2 Hz), 7.44 (t, ArH, 1H, J_{HH} = 6.9 Hz), 7.37 (m, ArH and NH, 2H), 7.19 (dd, ArH, J_{HH} = 8.9 Hz, J_{HH} = 2.6 Hz), 6.96 (d, ArH, 1H, J_{HH} = 2.6 Hz), 6.59 (bd, NH, 1H, J_{HH} = 8.1 Hz), 4.77 (m, CHNH, 1H), 4.66 (d, OCH, 1H, J_{HH} = 14.9 Hz), 4.62 (d, OCH, 1H, J_{HH} = 14.9Hz), 4.55 (m, CHNH, 1H), 3.74 (s, OCH_3, 3H), 2.60 (t, CH_2, 3H, J_{HH} = 7.1 Hz), 2.08 (m, $CH(CH_3)_2$ and CH, 4H), 1.56 (m, CH and CH_2, 3H), 0.91 (m, CH_3, 6H) ppm. ^{13}C NMR ($CDCl_3$) 172.9, 170.3, 168.1, 154.9, 134.2, 129.9, 129.5, 127.7, 126.9, 126.8, 124.4, 118.2, 107.5, 67.2, 52.4, 51.3, 51.3, 50.9, 41.1, 31.2, 29.9, 24.9, 22.7, 21.8, 14.9 ppm. MS (ES) 483.2 ($[M+Na]^+$). Accurate mass calculated for $C_{24}H_{32}N_2O_5Na$: 483.1930. Found: 483.1943.

2NapMLOH (Dipeptide 6): ^{1}H NMR (DMSO) 8.28 (d, NH, 1H, J_{HH} = 7.8 Hz), 8.22 (d, NH, 1H, J_{HH} = 8.2 Hz), 7.84 (m, ArH, 2H), 7.76 (d, ArH, 1H, J_{HH} = 8.2 Hz), 7.48 (t, AtH, 1H, J_{HH} = 8.2 Hz), 7.46 (t, ArH, 1H, J_{HH} = 8.2 Hz), 7.27 (d, ArH, 1H, J_{HH} = 2.4 Hz), 7.24 (dd, ArH, 1H, J_{HH} = 8.2 Hz, J_{HH} = 2.8 Hz), 4.70 (d, OCH, 1H, J_{HH} = 14.7 Hz), 4.66 (d, OCH, 1H, J_{HH} = 14.7 Hz), 4.50 (m, CHNH, 1H), 4.22 (m, CHNH, 1H), 2.40 (t, SCH_3, 3H, J_{HH} = 7.2 Hz), 1.80 (m, CH and CH_3, 4H), 1.51 (m, CH and CH_2, 3H), 0.87 (d, CH_3, 3H, J_{HH} = 6.5 Hz), 0.80 (d, CH_3, 3H, J_{HH} = 6.5 Hz) ppm. ^{13}C NMR (DMSO) 173.9, 170.8, 167.4, 155.5, 134.0, 129.3, 128.7, 127.5, 126.7, 126.4, 123.8, 118.5, 107.2, 66.7, 51.3, 50.3, 32.1, 29.3, 24.2, 22.8, 21.2, 14.6 ppm. MS (ES) 445 ([M-H]$^-$). Accurate mass calculated for $C_{23}H_{29}N_2O_5S$: 445.1797. Found: 445.1793.

2. Methods

Preparation of Dipeptide Solutions. Solutions were prepared by suspending the required molecules in D_2O, and then adding and equimolar amount of NaOD (0.1 M in D_2O). The solution was then stirred until all of the dipeptides had dissolved.

To prepare the gels, the solutions were transferred to a vial containing a pre-weighed amount of GdL, shaken gently to ensure dissolution of the GdL and then allowed to stand. The same stock solutions were used for all measurements when the pH, rheology and NMR spectroscopy data were to be compared.

pH Measurement and pK_a determination. A FC200 pH probe (HANNA instruments) with a (6 mm x 10 mm) conical tip was employed for the pH measurements with a stated accuracy of t ± 0.1. pH changes during the gelation process were recorded every 1 minute for 24 hours. All measurements were conducted at room temperature. Apparent pK_a measurements were determined via the additions of aliquots of DCl (0.1 M) to a solution of the gelator. pH measurements were recorded after each addition of DCl until a stable value was reached. To prevent a gel forming, the solutions were stirred continuously. A water bath was used to maintain the temperature at 25 ^{o}C. Alternatively, a solution of gelator was prepared as above and added to a pre-weighed aliquot of GdL. After swirling to ensure dissolution of the GdL, the sample was placed in a water bath and 25 ^{o}C and the pH measured continuously. In this case, the sample was not stirred.

Rheology. All rheological experiments were performed using an Anton Paar Physica MCR101 rheometer. Time sweeps were carried out at 25 ^{o}C using parallel plates, with a sandblasted 50 mm measuring plate used throughout. The solution was directly added to the bottom plate after GdL addition and mixing. The plate gap was 0.9 mm. After the top plate was lowered, mineral oil was applied around the plate as a solvent trap. The time sweeps were performed at an angular frequency of 10 rads^{-1} and at a strain amplitude of 0.5 % over a period of 17 hours. The shear moduli were quoted as a function of time.

NMR Spectrsoscopy. NMR spectra were recorded on a Bruker DPX-400 spectrometer, operating at 400 MHz for ^{1}H NMR. For NMR analysis with time, the gelator solution was mixed with GdL (as above) and then directly loaded into an NMR tube to gel. During this time, NMR spectra were collected ever 90 seconds for the first 70 acquisitions, and then

typically every 5 minutes for the remaining experiment time (typically 17 hours total). The experiments were carried out at 298 K. For the samples for NMR spectroscopy studies, an amount of ethanol (1 μL/mL) was added as an internal standard. An NMR spectrum of the solution was recorded prior to adding GdL to ensure that the amount of ethanol present was accurately known relative to the dipeptides. This ensured any slight variations in weighing were taken into account for each sample.

Single Crystal X-ray Diffraction Refinement Details. Single crystal X-ray data for **2**·H_2O was measured on a Rigaku MicroMax-007 HF rotating anode diffractometer (Mo-Kα radiation, λ = 0.71073 Å, Kappa 4-circle goniometer, Rigaku Saturn724+ detector). Empirical absorption corrections using equivalent reflections were performed with the program SADABS.[4] Structures were solved with SHELXD,[5] or by direct methods using SHELXS,[5] and reined by full-matrix least squares on F^2 by SHELXL,[5] interfaced through the programme OLEX2.[6] Unless stated, all non-H atoms were refined anisotropically and H atoms were fixed in geometrically estimated positions using the riding model.

SEM. Scanning electron microscopy (SEM) images were recorded using a Hitachi S-4800 FE-SEM at 3 kV. Glass cover slips were fixed onto the aluminium stubs with carbon tabs. The sample was added to the surface of the cover slip and then left to air dry overnight. The samples were gold coated for 3 minutes at 15 μA using a sputter-coater (EMITECH K550X) prior to imaging.

Small-Angle Neutron Scattering (SANS). Small-angle neutron scattering experiments were performed on the time-of-flight LOQ diffractometer at the ISIS pulsed Spallation Neutron Source, Rutherford Appleton Laboratory, Didcot, UK. Typically, a range defined by $Q = (4\pi/\lambda)\sin(\theta/2)$ between 0.005 and ≥ 0.3 $Å^{-1}$ is obtained by using neutron wavelengths (λ) spanning 2.2 to 10 Å (LOQ) or 1.75 to 16.5Å (SANS2d) with a fixed sample-detector distance of ~ 4m.

All scattering data were (a) normalized for the sample transmission, (b) background corrected using the empty quartz cell, and (c) corrected for the linearity and efficiency of the detector response using the Mantid software package.[7] A fuller description of the method can be found elsewhere.[8, 9]

3. Figures

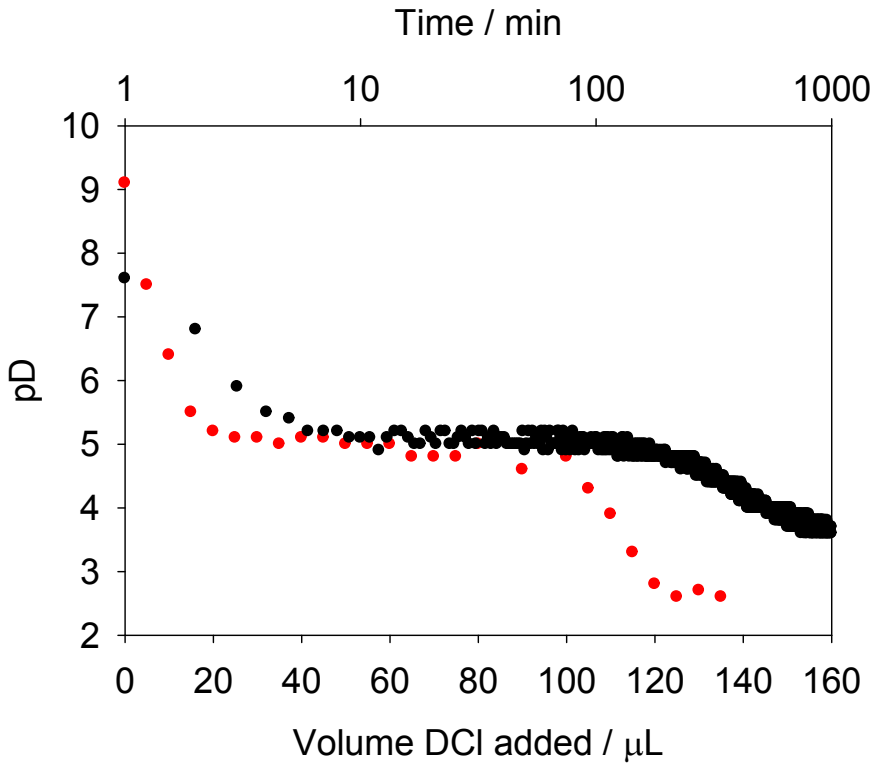


Figure S1. Example pH titration data for dipeptide **1** using aliquots of DCl (ꟷ, bottom axis) and the addition of GdL (ꟷ, top axis). Note the top axis is in time, since a single amount of GdL is added, which slowly hydrolyses.

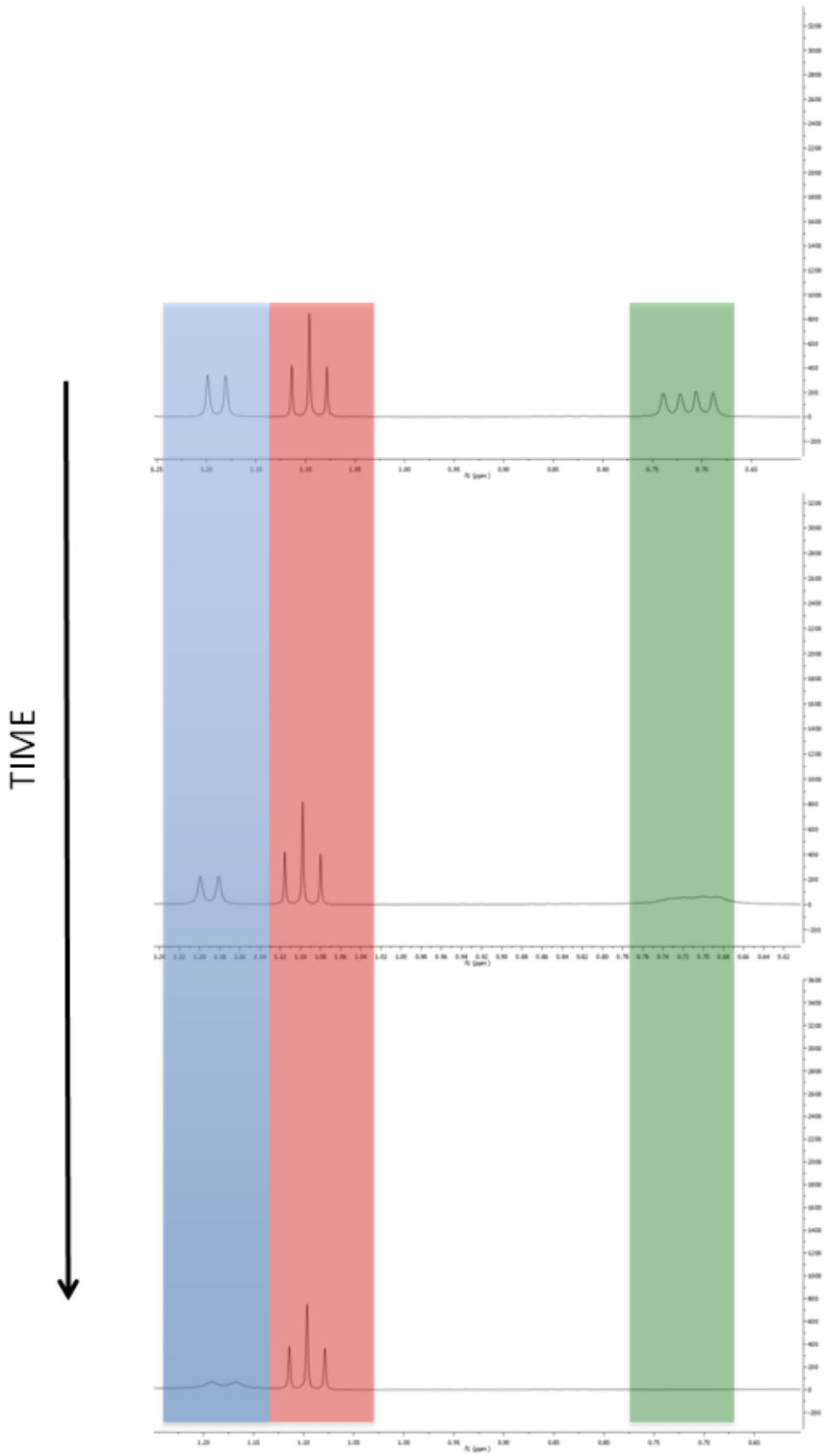


Figure S2. Example ^{1}H NMR data collected over time for a mixture of **1** and **2**. The doublet highlighted in blue is from one of the methyl groups on an alanine residue of **2**; the triplet highlighted in red is from the methyl group of ethanol, added as an internal standard; the two doublets highlighted in green are from the methyl groups on the valine residue of **1**. The peaks from **1** broaden and disappear significantly before the doublets associated with **2**. This shows the sequential assembly of the two gelators.

Crystal Data for 2·H_2O: Formula $C_{18}H_{22}N_2O_6$; M = 362.38 g·mol^{-1}; orthorhombic space group $P2_12_12_1$, colourless crystal; a = 5.7838(7), b = 8.613(1), c = 36.312(5) Å; V = 1809.0(4) Å^3; ρ = 1.331 g·cm^{-3}; μ = 0.101 mm^{-3}; F (000) = 768; crystal size = 0.18 × 0.15 × 0.02 mm^3; T = 100(2) K; 33375 reflections measured (2.24 < Θ < 30.50°), 5504 unique (R_{int} = 0.0365), 5261 ($I > 2\sigma(I)$); R_1 = 0.0290 for observed and R_1 = 0.0309 for all reflections; wR_2 = 0.0784 for all reflections; max/min residual electron density = 0.304 and -0.188 e·Å^{-3}; data/restraints/parameters = 5504/0/257; GOF = 1.075. CCDC # 1012303.

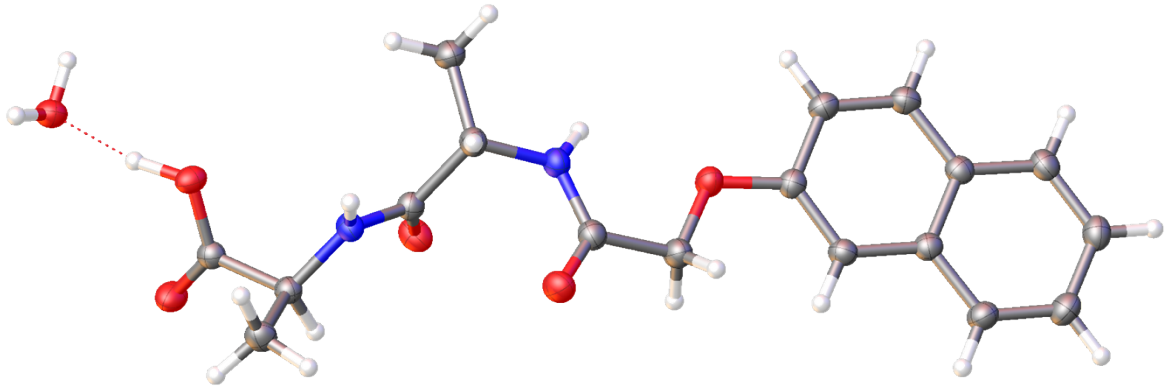


Figure S3. Displacement ellipsoid plot of the asymmetric unit from the single crystal structure **2**·H_2O. Ellipsoids displayed at 50 % probability level.

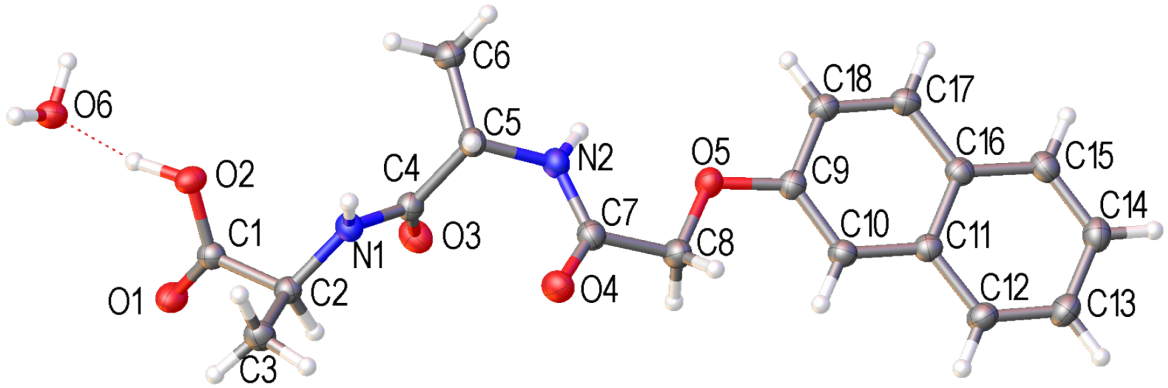


Figure S4. Labelled displacement ellipsoid plot of the asymmetric unit from the single crystal structure **2**·H_2O. Ellipsoids are displayed at 50 % probability level.

Table S1. Hydrogen bonds for **2**·H_2O [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
O(2)-H(2)...O(6)	1.02(2)	1.58(2)	2.585(1)	167(2)
N(1)-H(1)...O(1)^{i}	0.85(2)	2.12(2)	2.973(1)	176(1)
O(6)-H(6D)...O(3)^{ii}	0.89(2)	1.83(2)	2.715(1)	174(2)
O(6)-H(6E)...O(4)^{i}	0.91(2)	1.91(2)	2.813(1)	169(2)

Symmetry transformations used to generate equivalent atoms: i: -x+1,y+1/2,-z+1/2; ii: -x+2,y+1/2,-z+1/2

Crystal Data for 2·H_2O: Formula $C_{18}H_{22}N_2O_6$; M = 362.38 g·mol^{-1}; orthorhombic space group $P2_12_12_1$, colourless crystal; a = 5.8099(5), b = 8.6209(8), c = 36.256(3) Å; V = 1815.9(3) Å^3.

A crystal similarity search was performed between **2**·H_2O grown from a mixture of **1** and **2** with those from a gel of **2** only, which along with the close similarity in cell parameters reveals these conditions, afforded an isostructural single crystal phase (**Figure S5-7**)

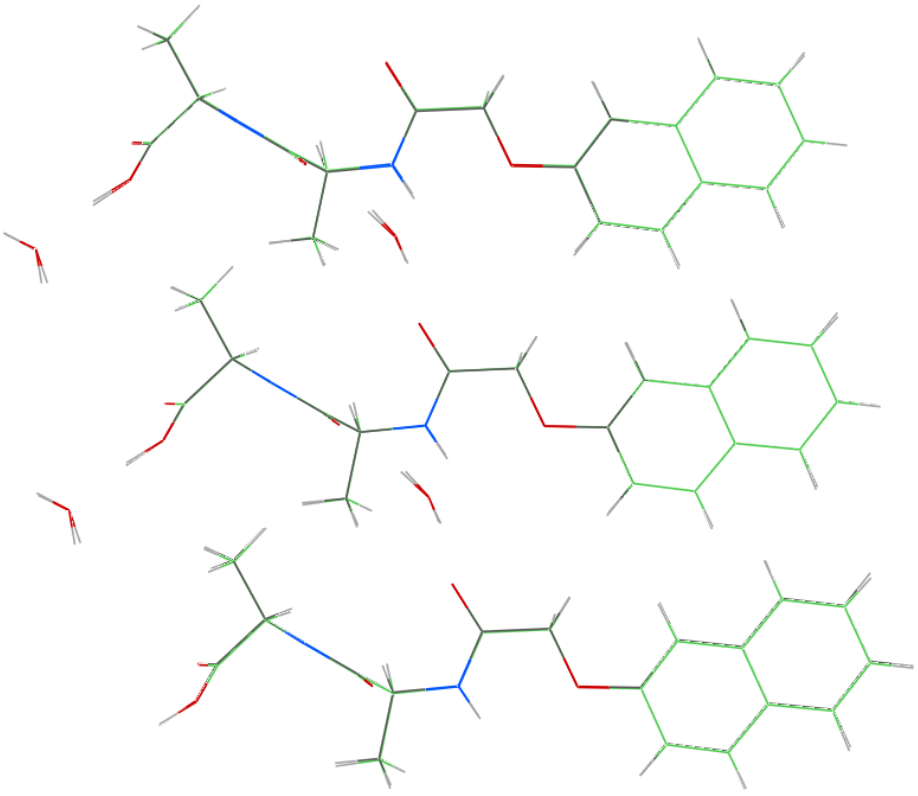


Figure S5. Crystal similarity search between crystal structures of **2**·H_2O grown from a mixture of **1** and **2** with those from a gel of **2** only.

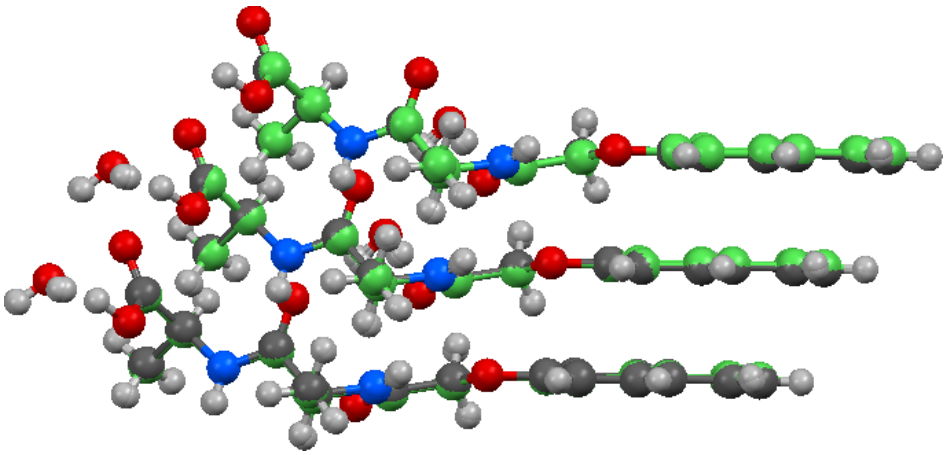


Figure S6. Crystal similarity search between crystal structures of **2**·H_2O grown from a mixture of **1** and **2** with those from a gel of **2** only.

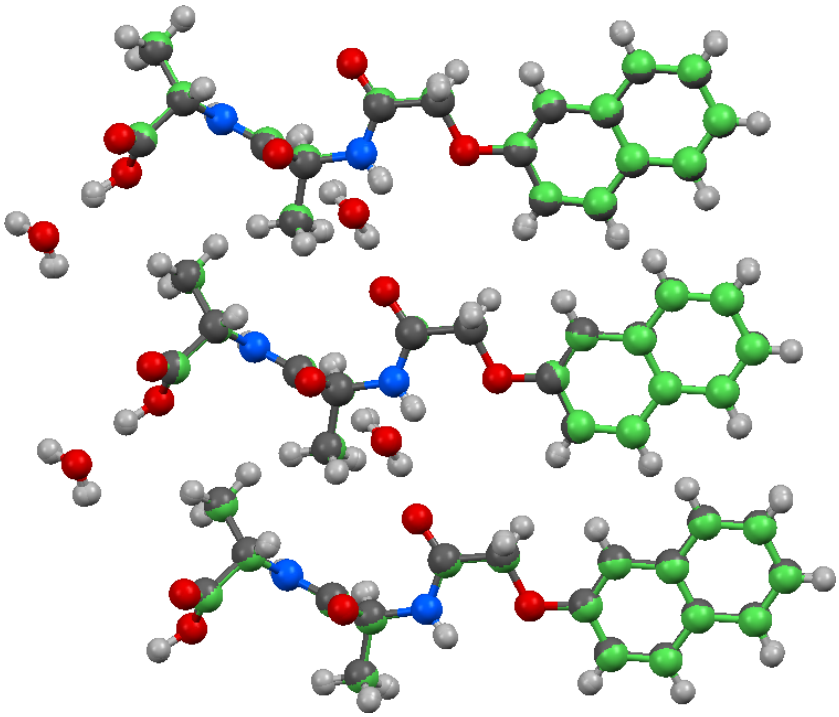


Figure S7. Crystal similarity search between crystal structures of **2**·H_2O grown from a mixture of **1** and **2** with those from a gel of **2** only.

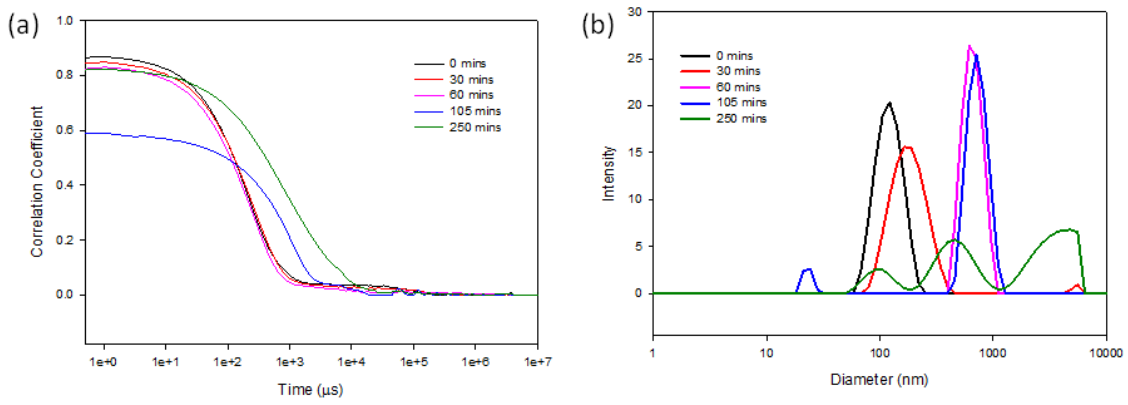


Figure S8. DLS data for a solution of **3** after adding GdL. The correlograms are shown in (a), recorded at different times after GdL is added. The intensity plots extracted from the correlograms are shown in (b). The data shows that there are colloidal structures in solution.

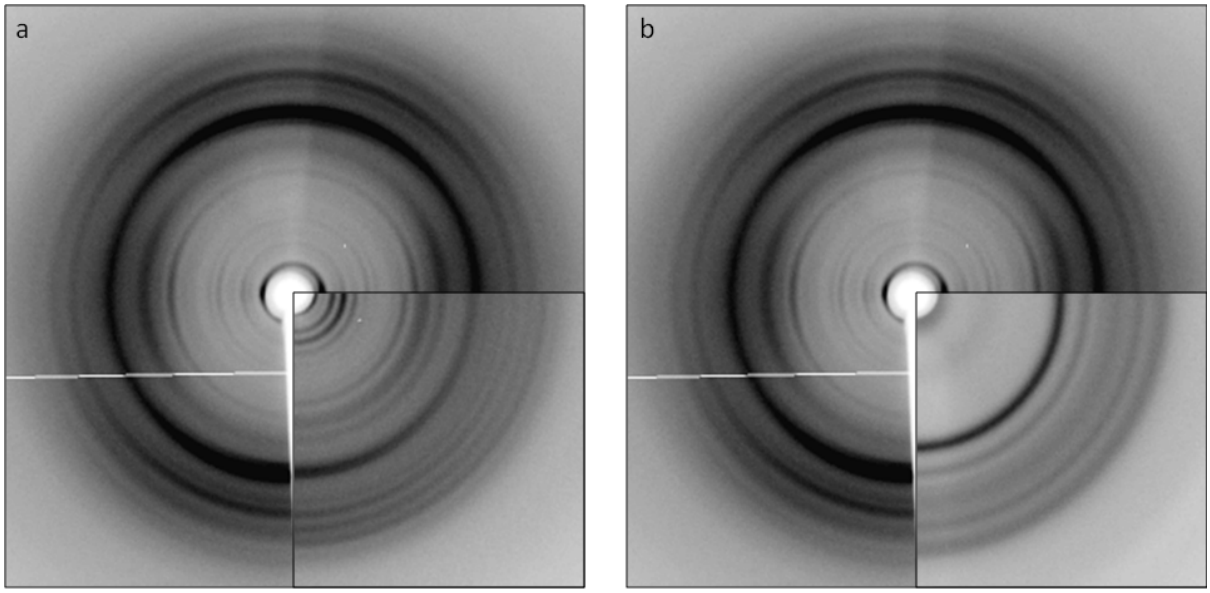


Figure S9. Comparison of the fibre X-ray diffraction from aligned fibres of mixed **1** and **3**, as insets compared to (a) **1** and (b) **3** alone.

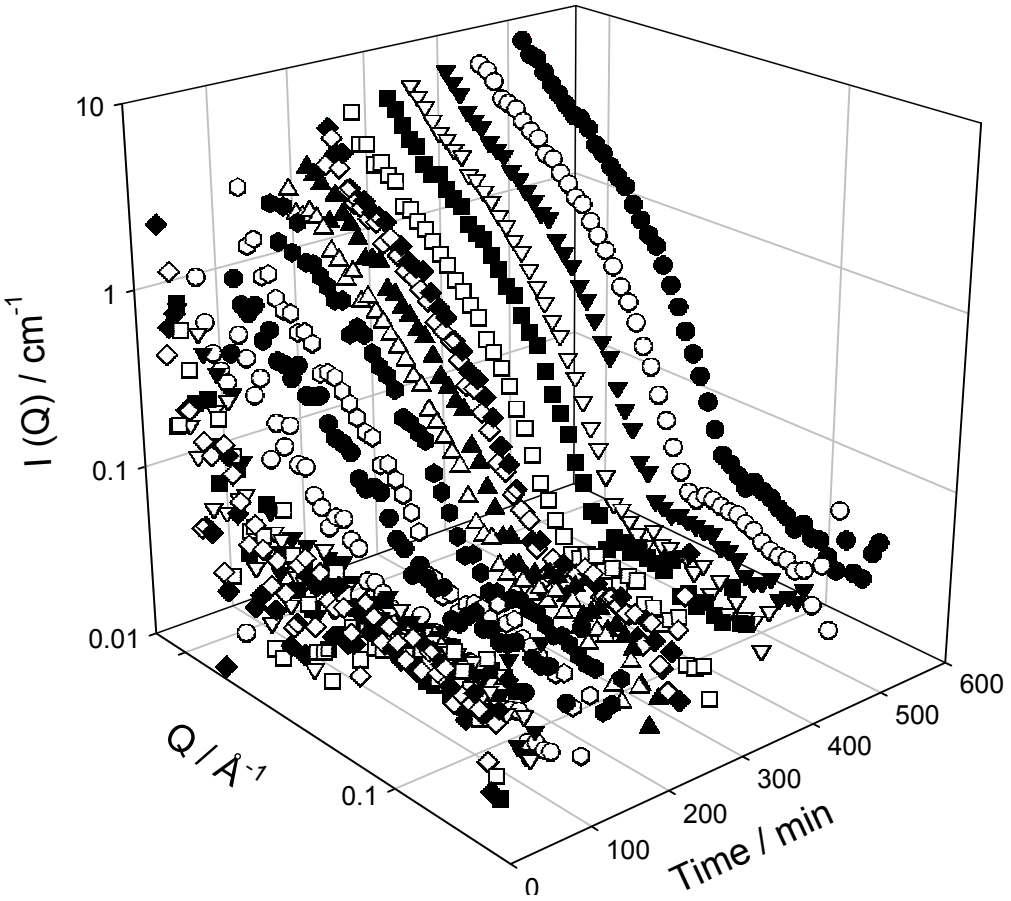


Figure S10. Evolution of gelation of mixture of **1** & **3** followed by small angle neutron scattering.

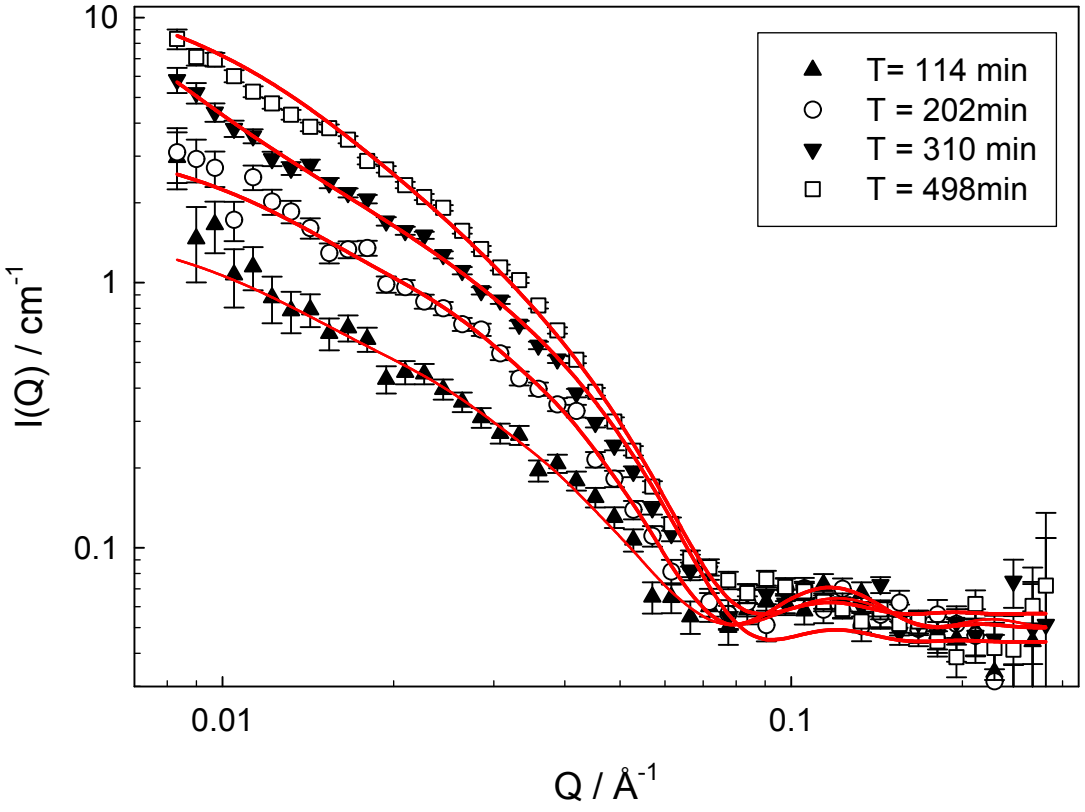


Figure S11. SANS for mixture of **1 &3** at range of times. Solid lines are fits to a hollow cylinder model (T=114min & T=202min) or fits to a flexible cylinder model (T=310min & T=498min).

References

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