Electronic Supplementary Material (ESI) for Soft Matter.

Controlling the Network Type in Self-Assembled Dipeptide Hydrogels

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SUPPORTING INFORMATION

Materials

The 2NapFF was prepared as described elsewhere.[1] The NaOD was purchased from Sigma Aldrich as a 40 wt% solution in D_2O and diluted with D_2O to provide a 0.1 M solution.

Gel Formation

Ca-triggered Gels: A stock solution of 2NapFF in D_2O at high pH (12.6) was prepared using NaOD (0.1 M). Solutions at different concentrations were prepared (0.5 to 10 mg/mL) using D_2O and the pH was adjusted to 12.6 using NaOD (1 M). A 2 mL aliquot

of this solution was transferred into a 7 mL bijou (Sterilin) vial and $Ca(NO_3)_2$ (200 mg/mL) was added at a molar ratio of 2:1 Ca^{2+}:COO^-. Samples were gently mixed by hand for 2 seconds and left to gel untouched at room temperature for 18 hours.

Acid-triggered Gels: A stock solution of 2NapFF in D_2O (10 mg/mL) at high pH (12.3) was prepared using NaOD (0.1 M). Solutions at different concentrations were prepared (0.5 to 10 mg/mL) using D_2O and the pH was adjusted to 12.3 using NaOD (1 M). A 2 mL aliquot of each solution was transferred into a 7 mL bijou (Sterilin) vial containing GdL (1.6 mg per mg of 2NapFF). Samples were gently mixed by hand for 2 seconds and left to gel untouched at room temperature for 18 hours.

Solvent-triggered Gels: Stock solutions were prepared by dissolving 2NapFF at 5 mg/mL in DMSO-d_6 at φ_{DMSO} from 0.05 to 0.8. φ_{DMSO} is on a volume per volume basis, such that a φ_{DMSO} of 0.10 refers to a situation with 0.2 mL of DMSO and 1.8 mL of D_2O. Aliquots were transferred into 7 mL bijou (Sterilin) vials and D_2O was added to make up to a final volume of 2 mL. Mixing was achieved via the addition of D_2O in one aliquot and solutions were left to gel at room temperature for 18 hours untouched. For the work at a φ_{DMSO} of 0.1, stock solutions of 2NapFF at concentrations of 0.5 to 10 mg/mL were prepared and gelled as above. In all cases, after gelling, the pH was found to be 3.9.

Rheology All rheological measurements were performed using an Anton Paar Physica MCR101 rheometer. For the frequency sweeps and strain sweeps, a cup and vane system was used. Frequency sweeps were carried out from 1 rad/s to 100 rad/s at 0.5 % strain. Strain amplitude sweeps were performed within the linear viscoelastic region at 10 rad/s from 0.1 % to 1000 % strain. All measurements were performed at 25 °C. All samples were left 18 hours before being measured.

Confocal Microscopy The gels prepared as described were imaged with confocal microscopy using a Zeiss 880 on an Examiner Z1(Zeiss, Jena, Germany) with a Plan-APOCHROMAT 40x NA 1.0 DIC. The excitation source used was a 633 nm Helium Neon laser and the signal was acquired with an Airyscan detector. Images were collected and analysed using the ZEN (2.1) software (Zeiss, Jena, Germany).

Scanning electron microscopy (SEM) SEM images were obtained using a Hitachi S-4800 FE-SEM at 0.5 to 2 keV in deceleration mode at a height of 3 mm. Gels were

prepared as above and a small section of gel was air-dried directly onto microscope cover slips and attached to stubs using carbon disks. Two fresh samples of each were prepared and imaged to ensure each was representative. No metal was sputtered on to the sample before analysis; to minimise charging issues, deceleration mode was used.

Transmission Electron Microscopy 30 µL of each sample (prepared as above) prior to gelation was pipetted onto parafilm in a petri dish and a Formvar/carbon film coated, 400 mesh copper grid (Agar Scientific) was placed carbon side down onto each sample droplet. The petri dish was kept in a damp, sealed box overnight to allow the samples to gel. The grids were washed with 4µL 0.2 µm milliQ-filtered water and blotted dry after which 4µL 2% (w/v) uranyl acetate was added to the grid and immediately blotted dry. All grids were examined using a JEOL JEM1400-Plus TEM at 120kV and the images were captured using a Gatan OneView 4K camera.

Small Angle Neutron Scattering (SANS) The solutions were prepared as described above. The gels were prepared in UV spectrophotometer grade, quartz cuvettes (Hellma) with a 2 mm path length. These were housed in a temperature controlled sample rack during the measurements. SANS measurements were performed using the D11 instrument (Institut Laue Langevin, Grenoble, France). A neutron beam, with a fixed wavelength of 10 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 $Å^{-1}$, by using three sample-detector distances of 1.2 m, 8m, and 39 m.

The data were 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 instrument-independent data were then fitted to the models discussed in the text using the SasView software package.[2]

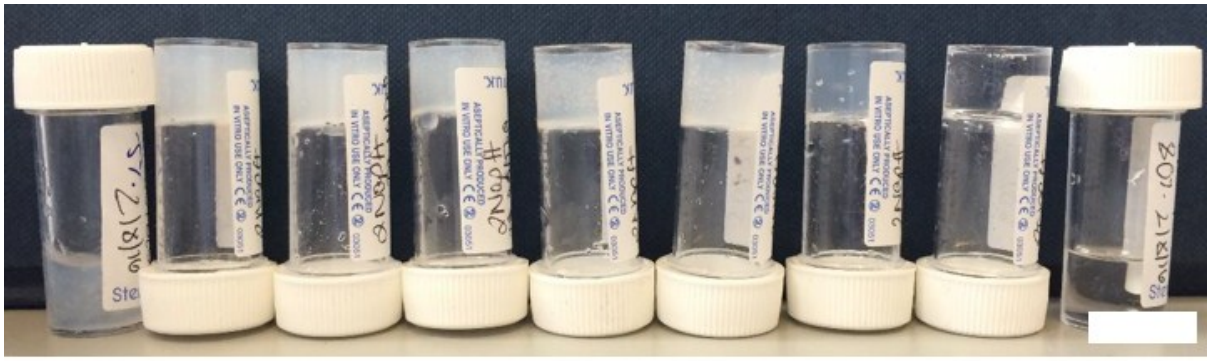

Figure S1. Optical images of the resulting hydrogels at φDMSO of 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.55, 0.6 and 0.8 (left to right) showing gelation over a range of values. The gels at a φDMSO of 0.05 initially formed a weak gel, but became a liquid after ~30 hours. The scale bar represents 1.5 cm.

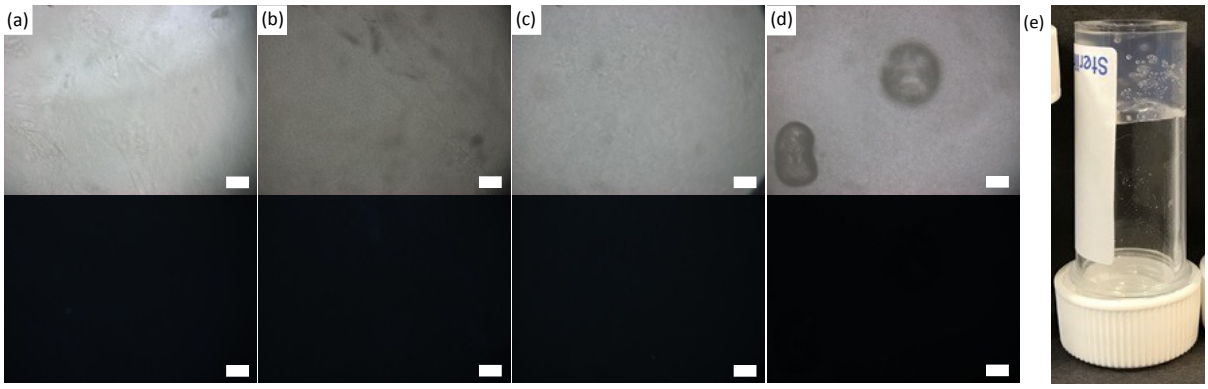

Figure S2. Microscope images showing images of gels formed using a solvent-switch at a φDMSO of 0.10 (top) and the same areas of the gels under cross-polarised light (bottom). (a) Shows a gel at a concentration of 2NapFF of 10 mg/mL; (b) shows a gel at 5 mg/mL; (c) shows a gel at 1 mg/mL; (d) shows another section of the gel at 1 mg/mL, where a bubble can be seen in the gel. In all cases, the scale bars represent 100 μ. (e) shows a magnified photograph of a gel at a concentration of 1 mg/mL, showing that the objects seen in the gel are small bubbles, not crystals.

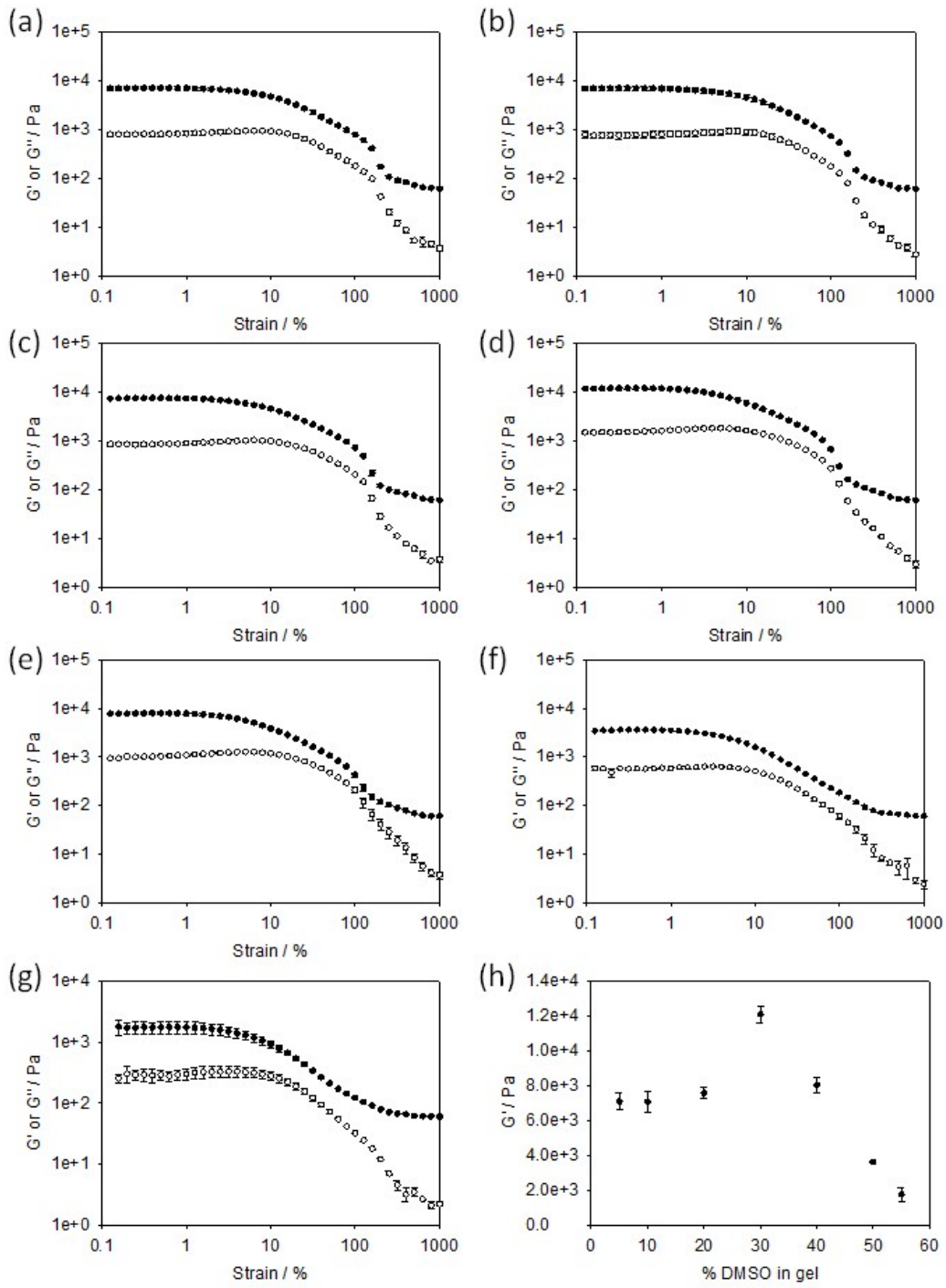

Figure S3. Rheological data for solvent-triggered gels. (a) – (g) show strain sweeps for gels formed by adding water to a solution of 2NapFF dissolved in DMSO such that the concentration of 2NapFF is 10 mg/mL, and the final ratio of φDMSO is (a) 0.05; (b) 0.10; (c) 0.20; (d) 0.30; (e) 0.40; (f) 0.50; (g) 0.55. In all cases, closed symbols represent G′ and open symbols represent G″. (h) shows a plot of G′ at a strain of 0.5 % against φDMSO. All data are the average of three repeat samples, with the error bars being the standard deviation between samples.

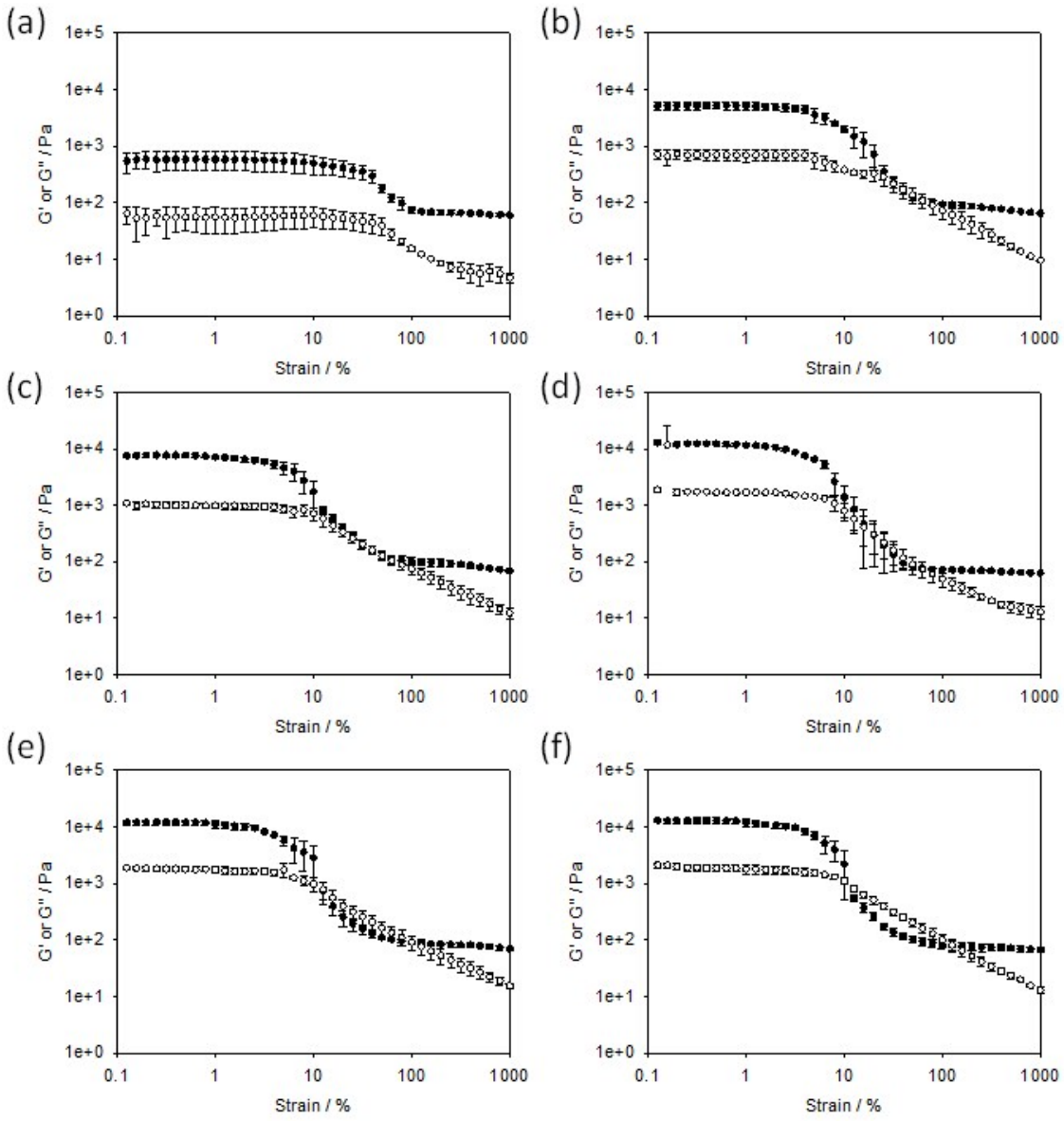

Figure S4. Strain sweeps for the Ca-triggered gels at a concentration of (a) 1 mg/mL; (b) 2 mg/mL; (c) 3 mg/mL; (d) 4 mg/mL; (e) 5 mg/mL; (f) 6 mg/mL. In all cases, closed symbols represent G′ and open symbols represent G″. All data are the average of three repeat samples, with the error bars being the standard deviation between samples.

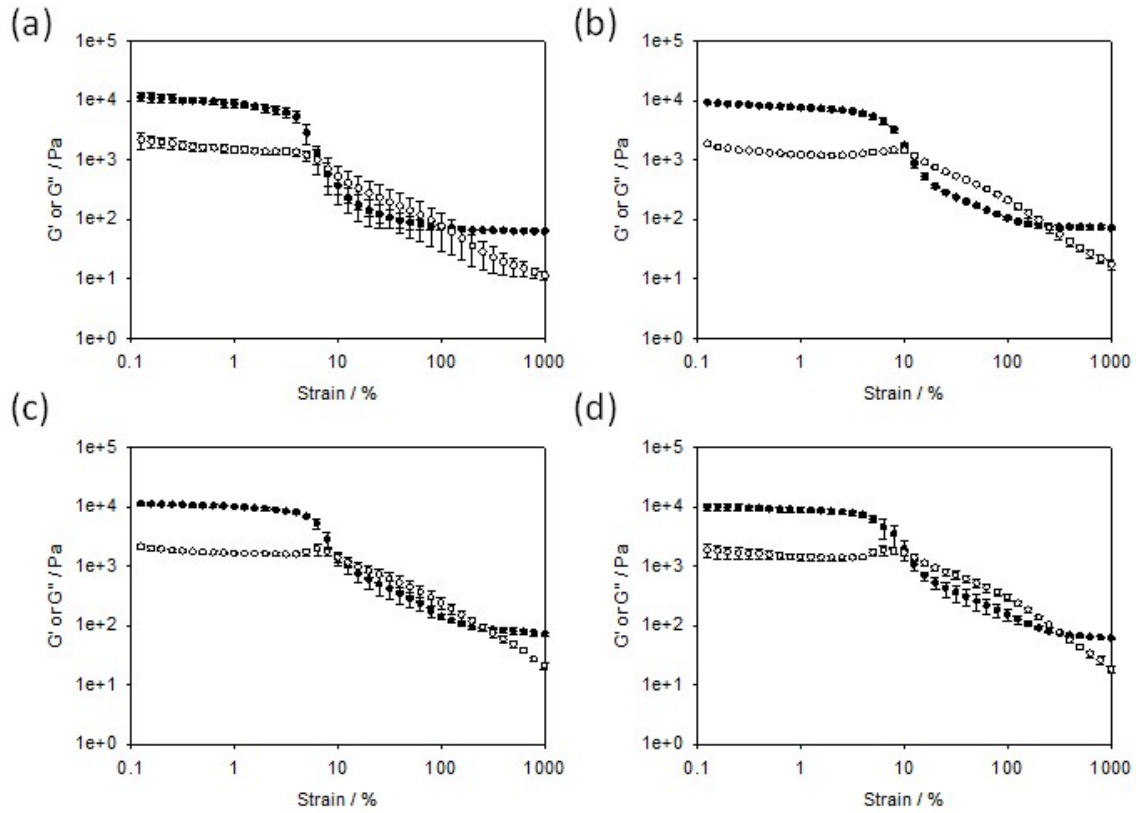

Figure S5. Strain sweeps for the Ca-triggered gels at a concentration of (a) 7 mg/mL; (b) 8 mg/mL; (c) 9 mg/mL; (d) 10 mg/mL. In all cases, closed symbols represent G′ and open symbols represent G″. All data are the average of three repeat samples, with the error bars being the standard deviation between samples.

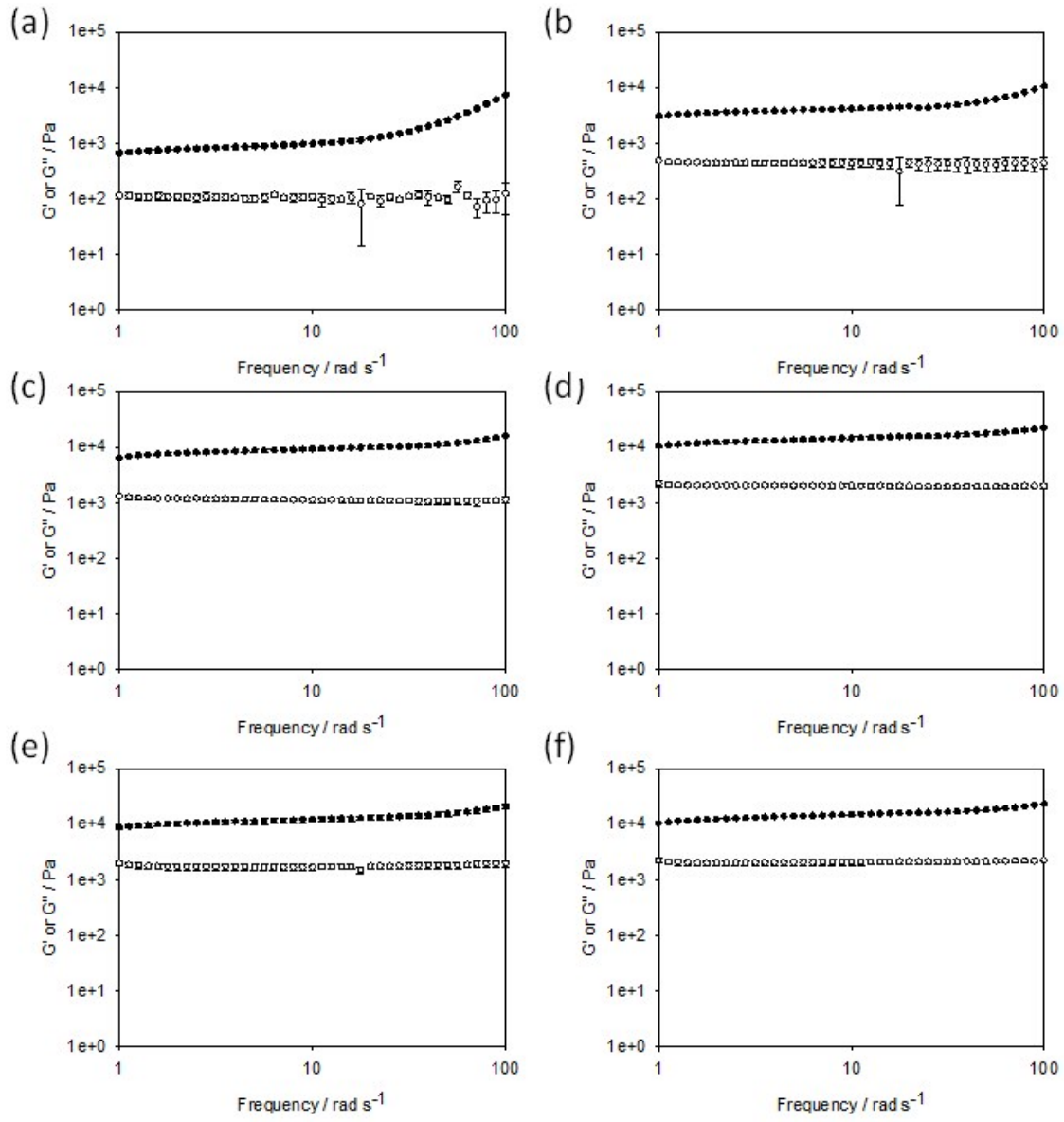

Figure S6. Frequency sweeps for the Ca-triggered gels at a concentration of (a) 1 mg/mL; (b) 2 mg/mL; (c) 3 mg/mL; (d) 4 mg/mL; (e) 5 mg/mL; (f) 6 mg/mL. In all cases, closed symbols represent G′ and open symbols represent G″. All data are the average of three repeat samples, with the error bars being the standard deviation between samples.

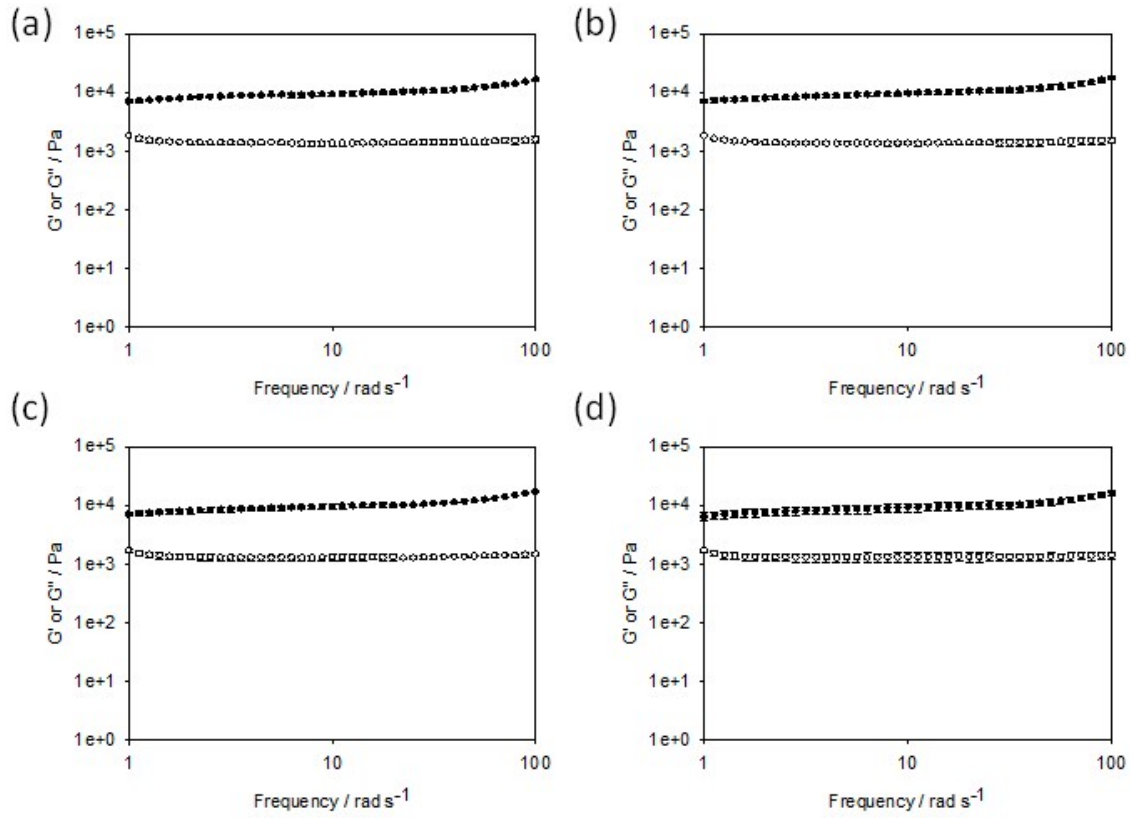

Figure S7. Frequency sweeps for the Ca-triggered gels at a concentration of (a) 7 mg/mL; (b) 8 mg/mL; (c) 9 mg/mL; (d) 10 mg/mL. In all cases, closed symbols represent G′ and open symbols represent G″. All data are the average of three repeat samples, with the error bars being the standard deviation between samples.

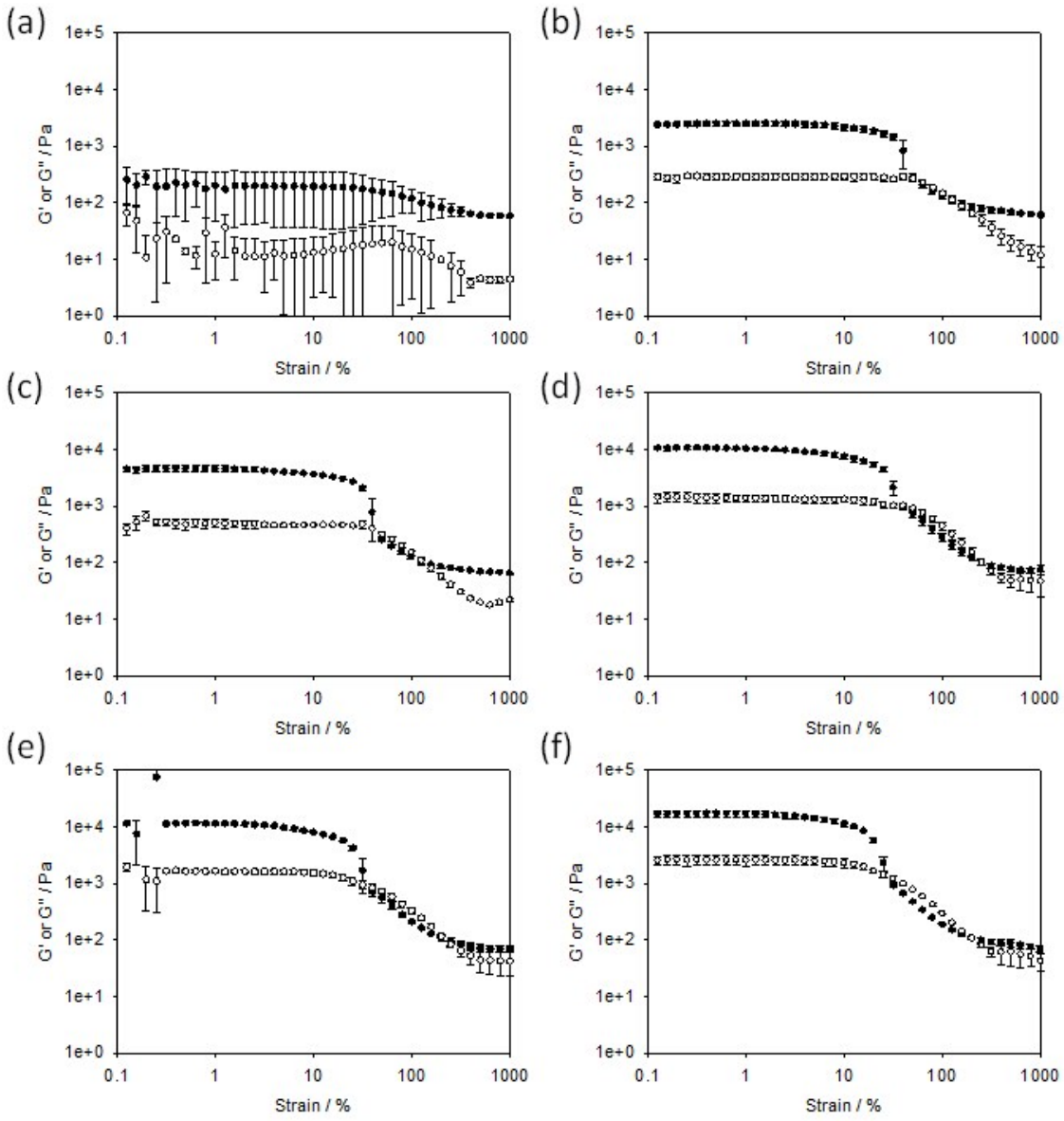

Figure S8. Strain sweeps for the acid-triggered gels at a concentration of (a) 1 mg/mL; (b) 2 mg/mL; (c) 3 mg/mL; (d) 4 mg/mL; (e) 5 mg/mL; (f) 6 mg/mL. In all cases, closed symbols represent G′ and open symbols represent G″. All data are the average of three repeat samples, with the error bars being the standard deviation between samples.

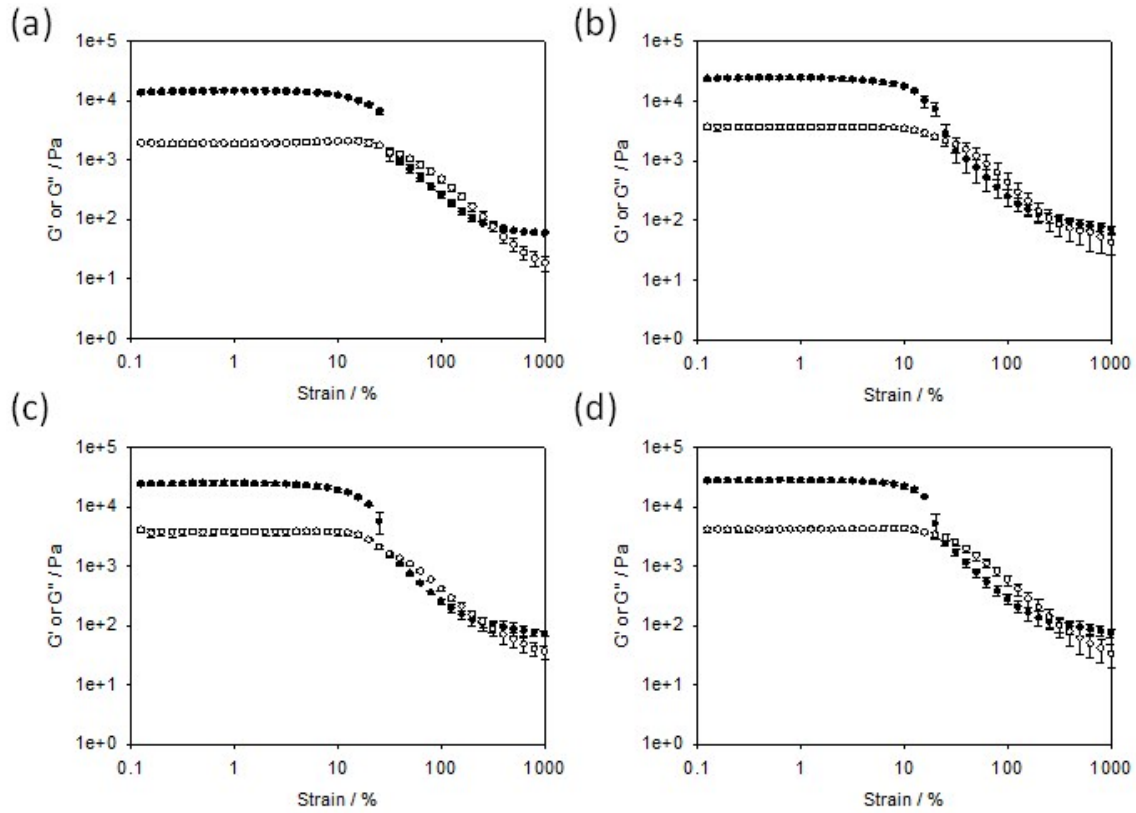

Figure S9. Strain sweeps for the acid-triggered gels at a concentration of (a) 7 mg/mL; (b) 8 mg/mL; (c) 9 mg/mL; (d) 10 mg/mL. In all cases, closed symbols represent G′ and open symbols represent G″. All data are the average of three repeat samples, with the error bars being the standard deviation between samples.

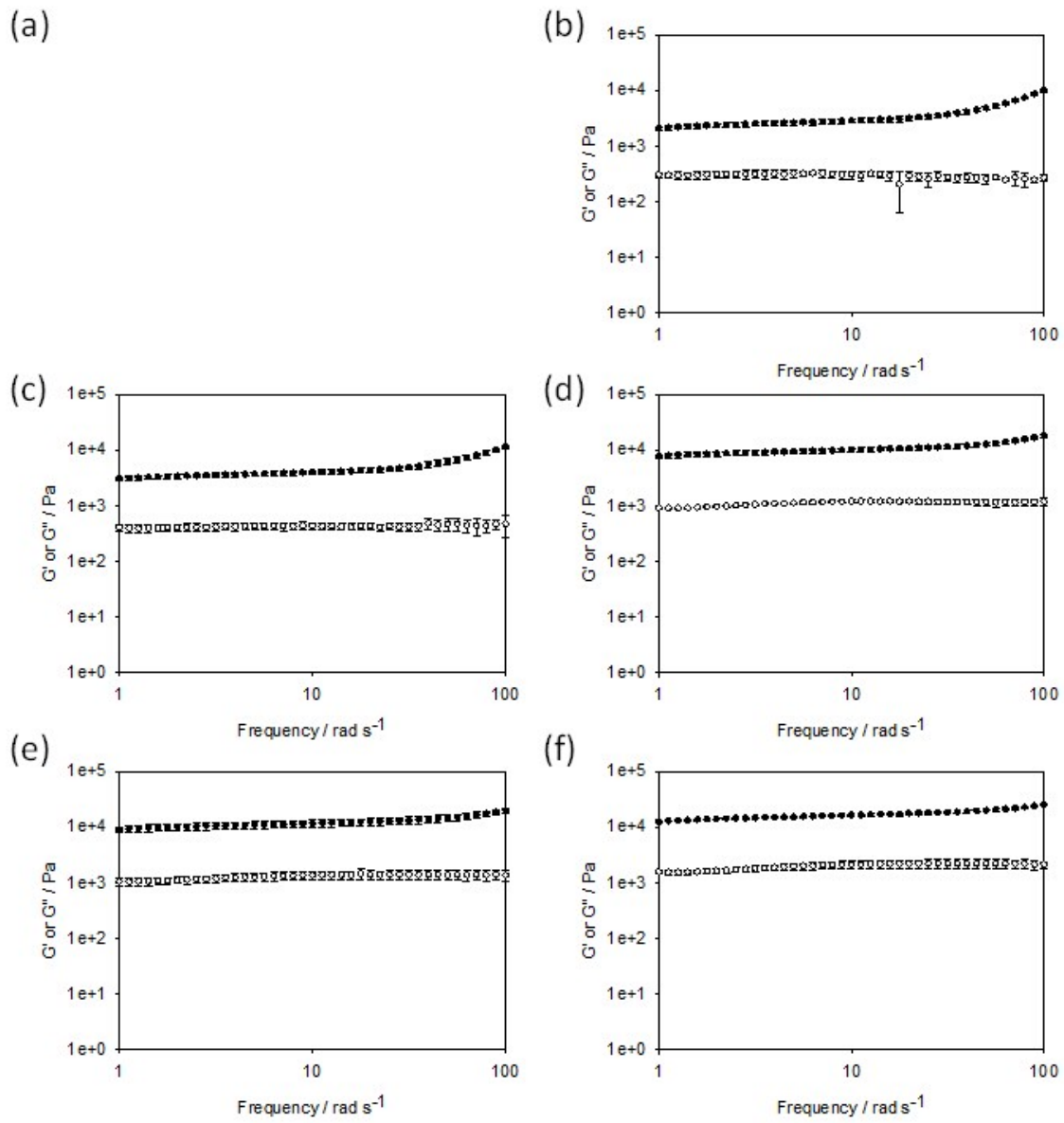

Figure S10. Frequency sweeps for the acid-triggered gels at a concentration of (a) 1 mg/mL – note, no data are shown as only very weak materials were formed at this concentration (see strain sweep above, Fig S6a) and so no data could be meaningfully collected; (b) 2 mg/mL; (c) 3 mg/mL; (d) 4 mg/mL; (e) 5 mg/mL; (f) 6 mg/mL. In all cases, closed symbols represent G′ and open symbols represent G″. All data are the average of three repeat samples, with the error bars being the standard deviation between samples.

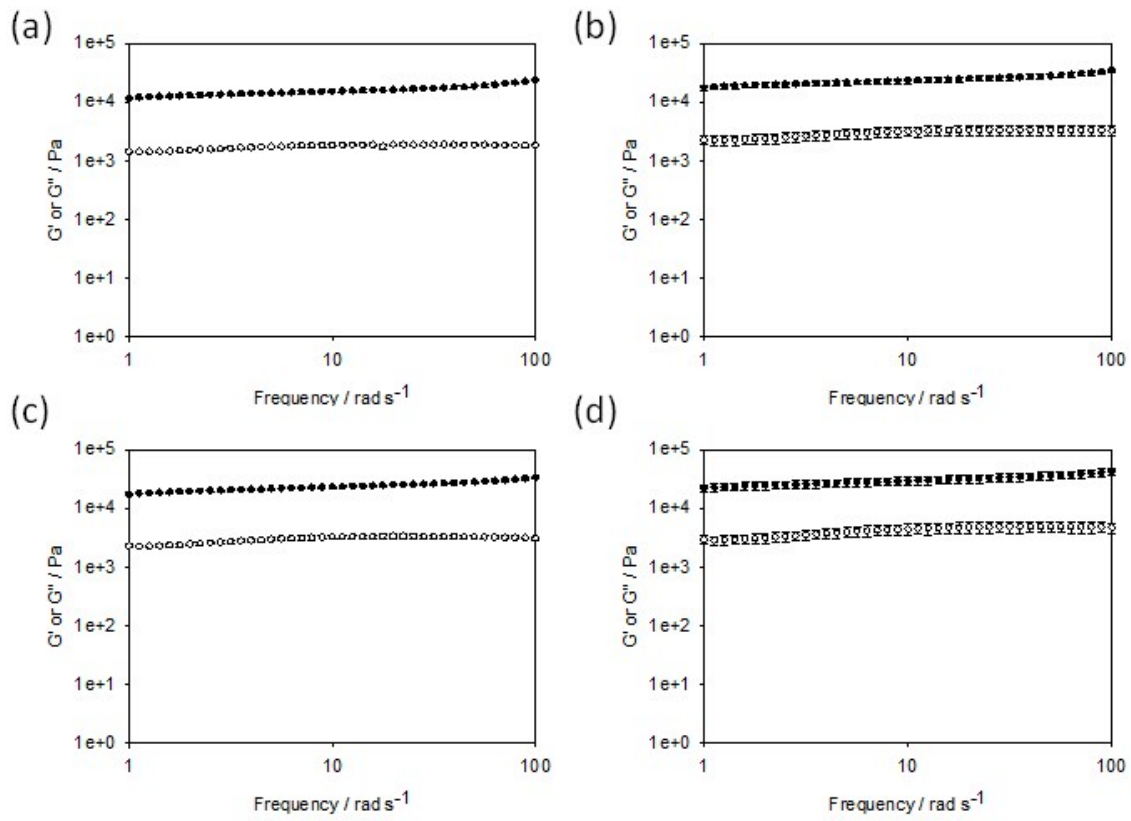

Figure S11. Frequency sweeps for the acid-triggered gels at a concentration of (a) 7 mg/mL; (b) 8 mg/mL; (c) 9 mg/mL; (d) 10 mg/mL. In all cases, closed symbols represent G′ and open symbols represent G″. All data are the average of three repeat samples, with the error bars being the standard deviation between samples.

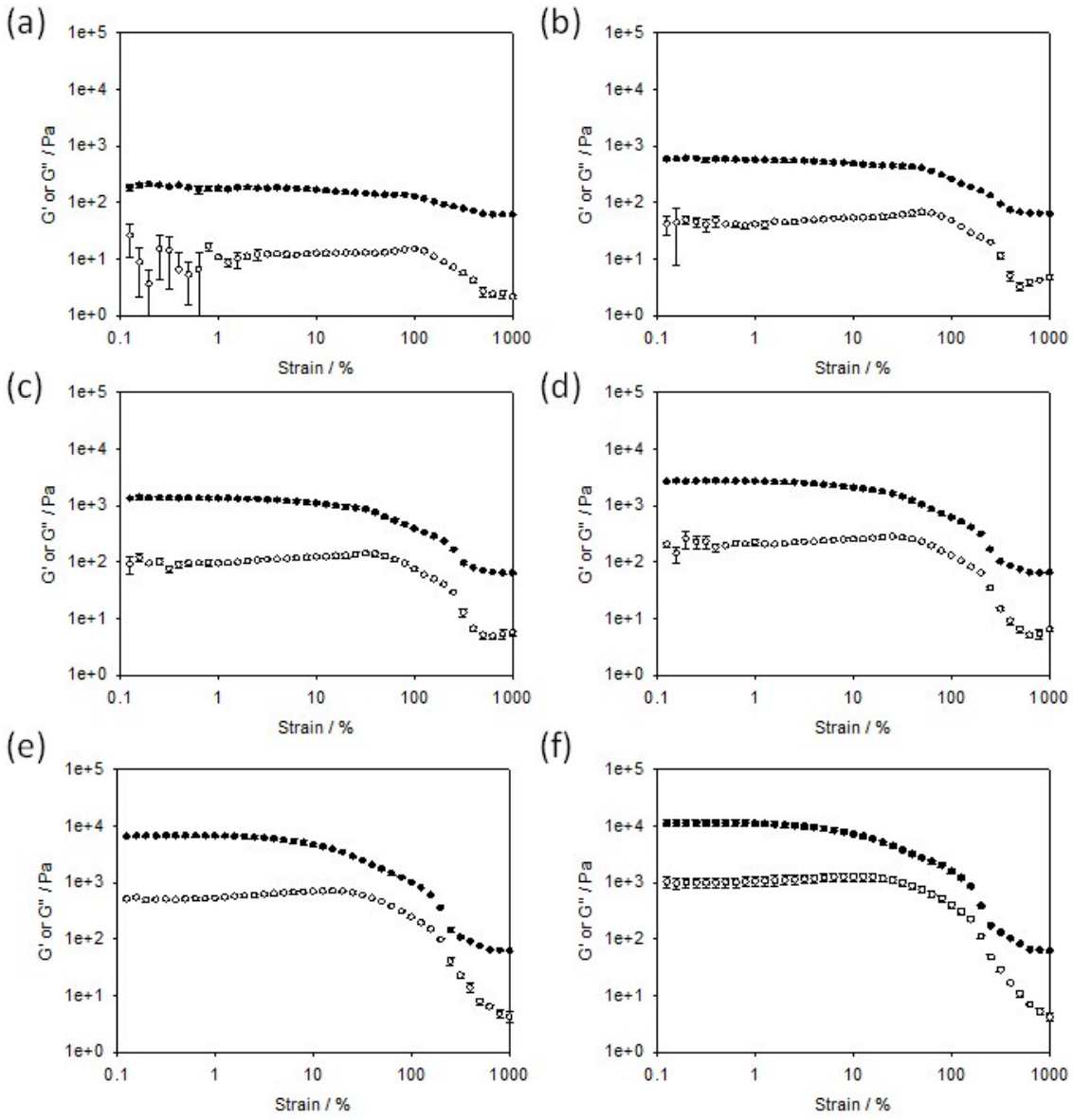

Figure S12. Strain sweeps for the solvent-triggered gels at a concentration of (a) 1 mg/mL; (b) 2 mg/mL; (c) 3 mg/mL; (d) 4 mg/mL; (e) 5 mg/mL; (f) 6 mg/mL. In all cases, closed symbols represent G′ and open symbols represent G″. All data are the average of three repeat samples, with the error bars being the standard deviation between samples.

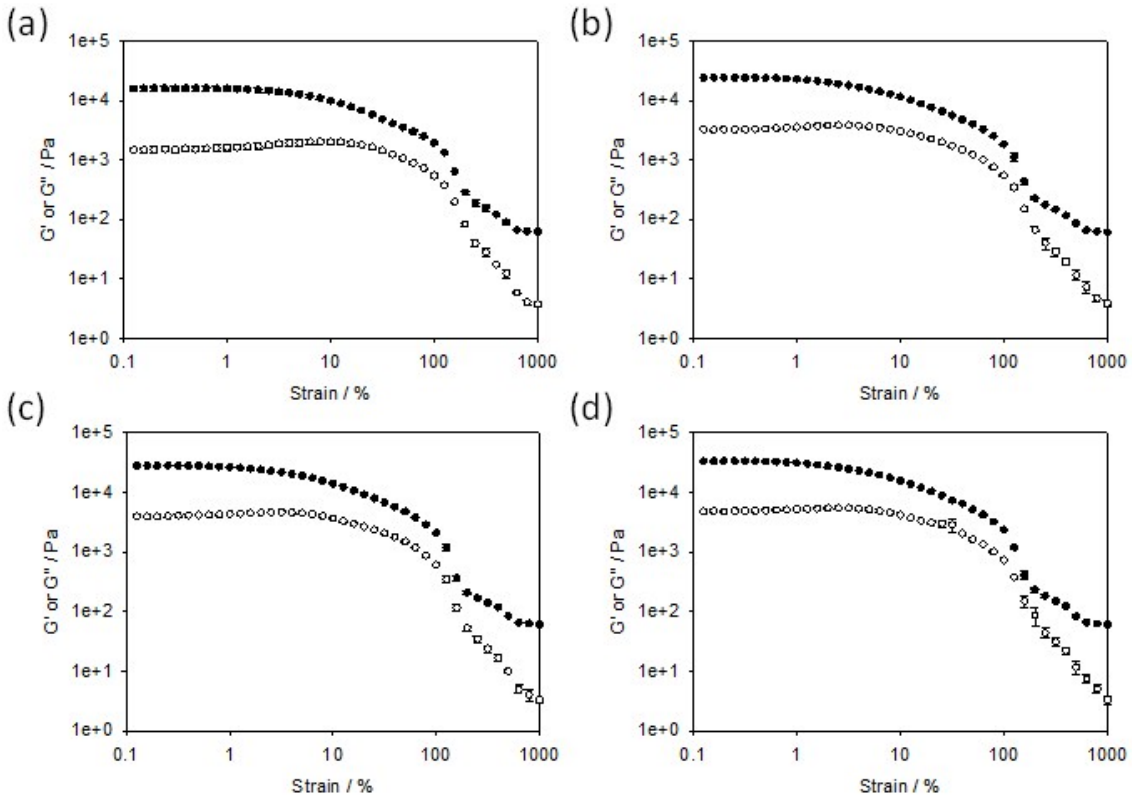

Figure S13. Strain sweeps for the solvent-triggered gels at a concentration of (a) 7 mg/mL; (b) 8 mg/mL; (c) 9 mg/mL; (d) 10 mg/mL. In all cases, closed symbols represent G′ and open symbols represent G″. All data are the average of three repeat samples, with the error bars being the standard deviation between samples.

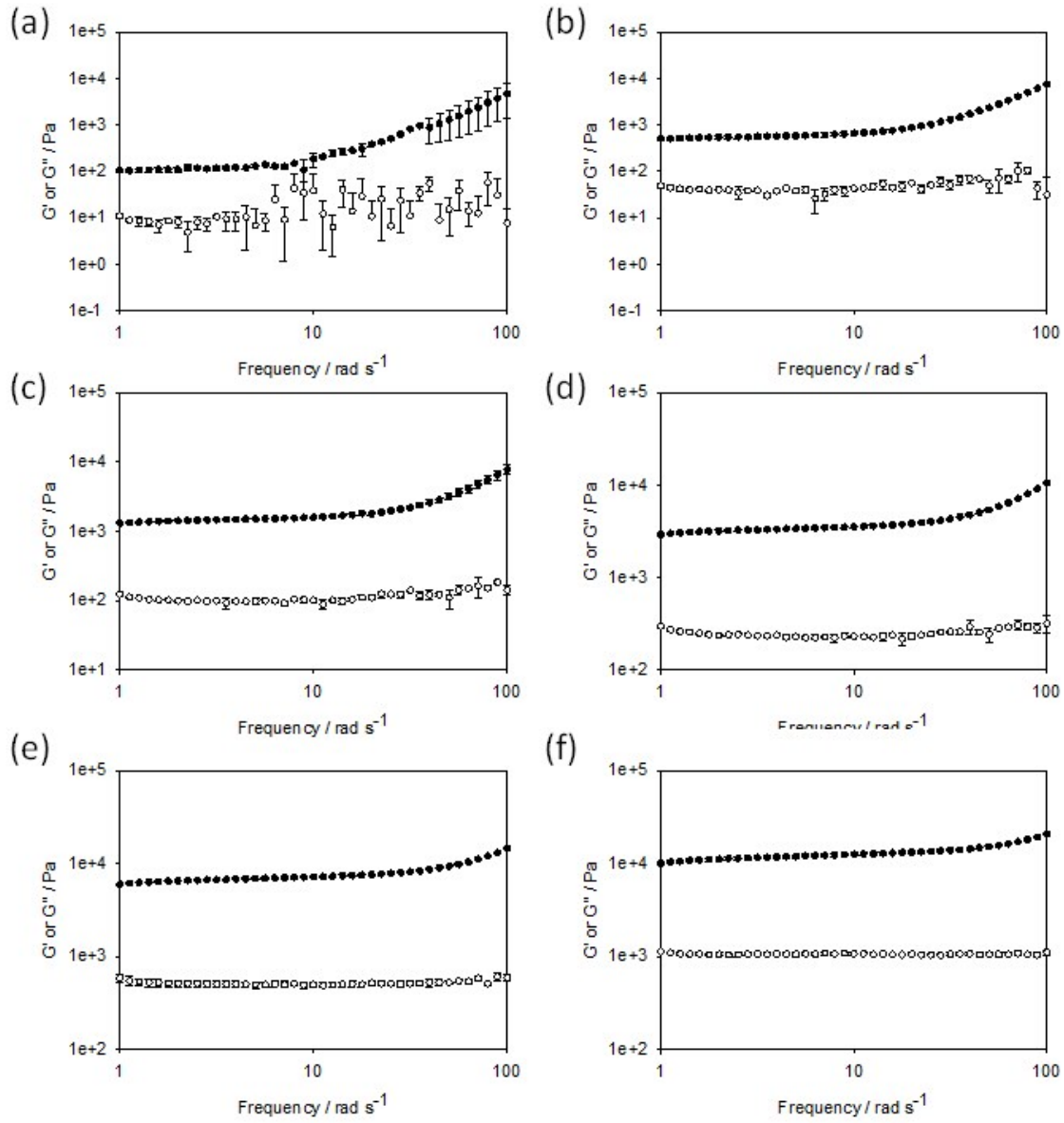

Figure S14. Frequency sweeps for the solvent-triggered gels at a concentration of (a) 1 mg/mL; (b) 2 mg/mL; (c) 3 mg/mL; (d) 4 mg/mL; (e) 5 mg/mL; (f) 6 mg/mL. In all cases, closed symbols represent G′ and open symbols represent G″. All data are the average of three repeat samples, with the error bars being the standard deviation between samples.

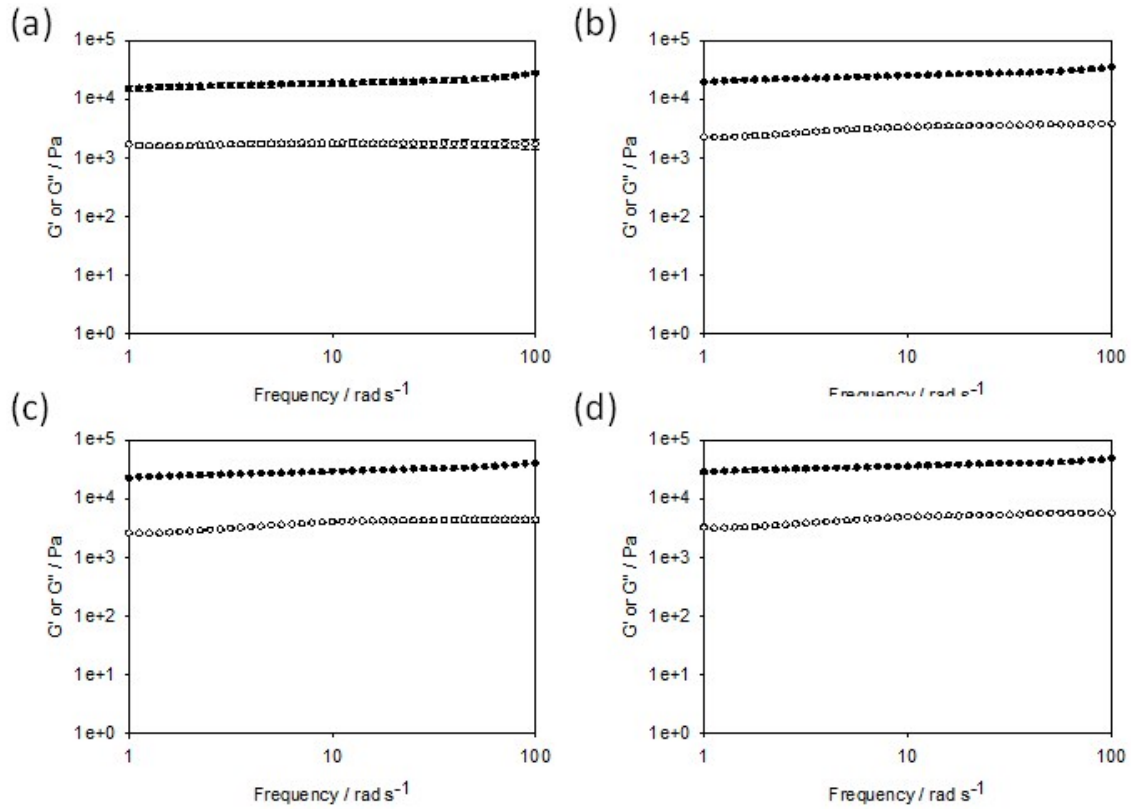

Figure S15. Frequency sweeps for the solvent-triggered gels at a concentration of (a) 7 mg/mL; (b) 8 mg/mL; (c) 9 mg/mL; (d) 10 mg/mL. In all cases, closed symbols represent G′ and open symbols represent G″. All data are the average of three repeat samples, with the error bars being the standard deviation between samples.

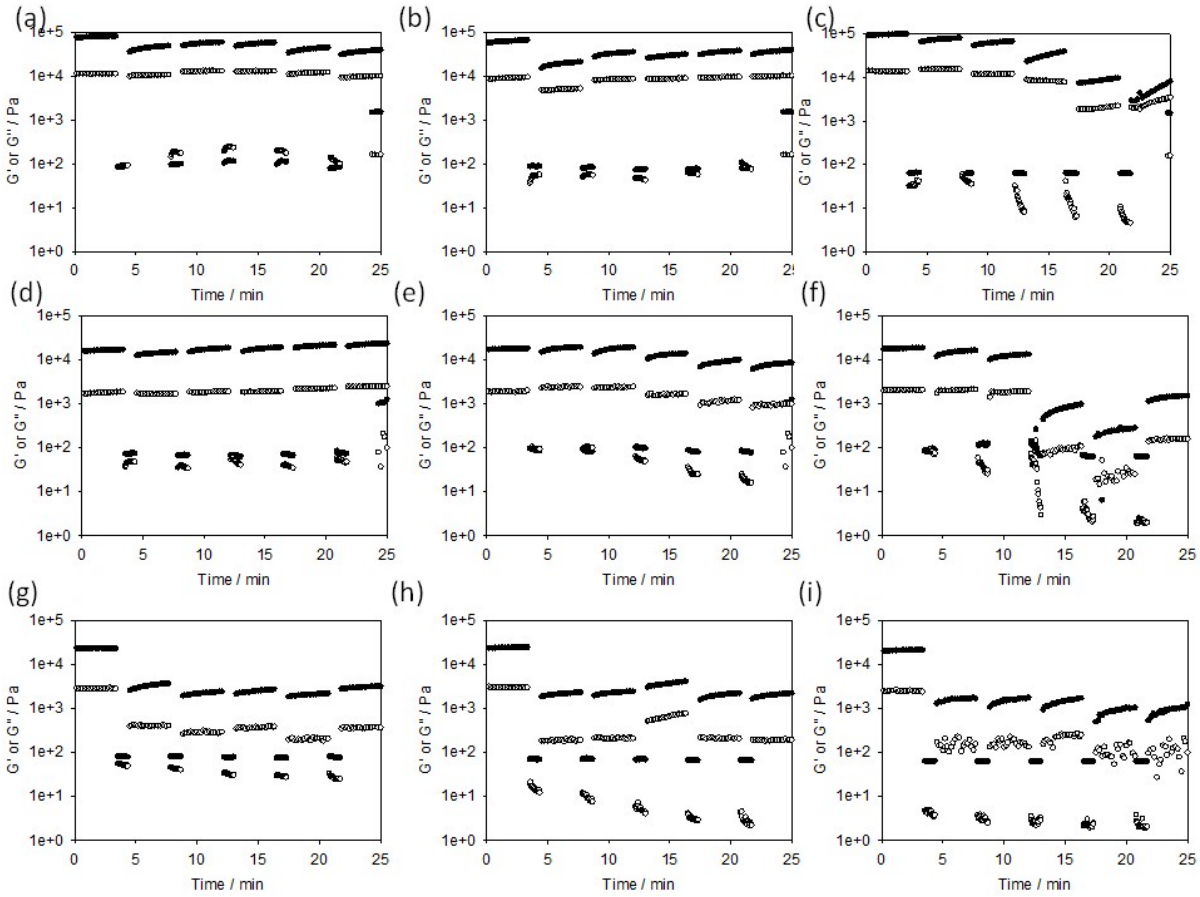

Figure S16. Recovery tests for the gels. In all cases, the gels were subjected to a constant frequency of 10 rad/s and strain of 0.5% for 200 seconds, followed by a higher strain for 60 seconds. Restoration of the gel was monitored in the subsequent time sweep (again at a frequency of 10 rad/s and strain of 0.5%). These cycles were repeated five times. The Ca-triggered gels are shown in (a), (b), and (c) where the strains used to deform the gels were 300%, 500%, and 1000% respectively. The acid-triggered gels are shown in (d), (e), and (f) where the strains used to deform the gels were 300%, 500%, and 1000% respectively. The solvent-triggered gels are shown in (g), (h), and (i) where the strains used to deform the gels were 300%, 500%, and 1000% respectively. In all cases, a concentration of 2NapFF of 10 mg/mL was used to prepare the gels.

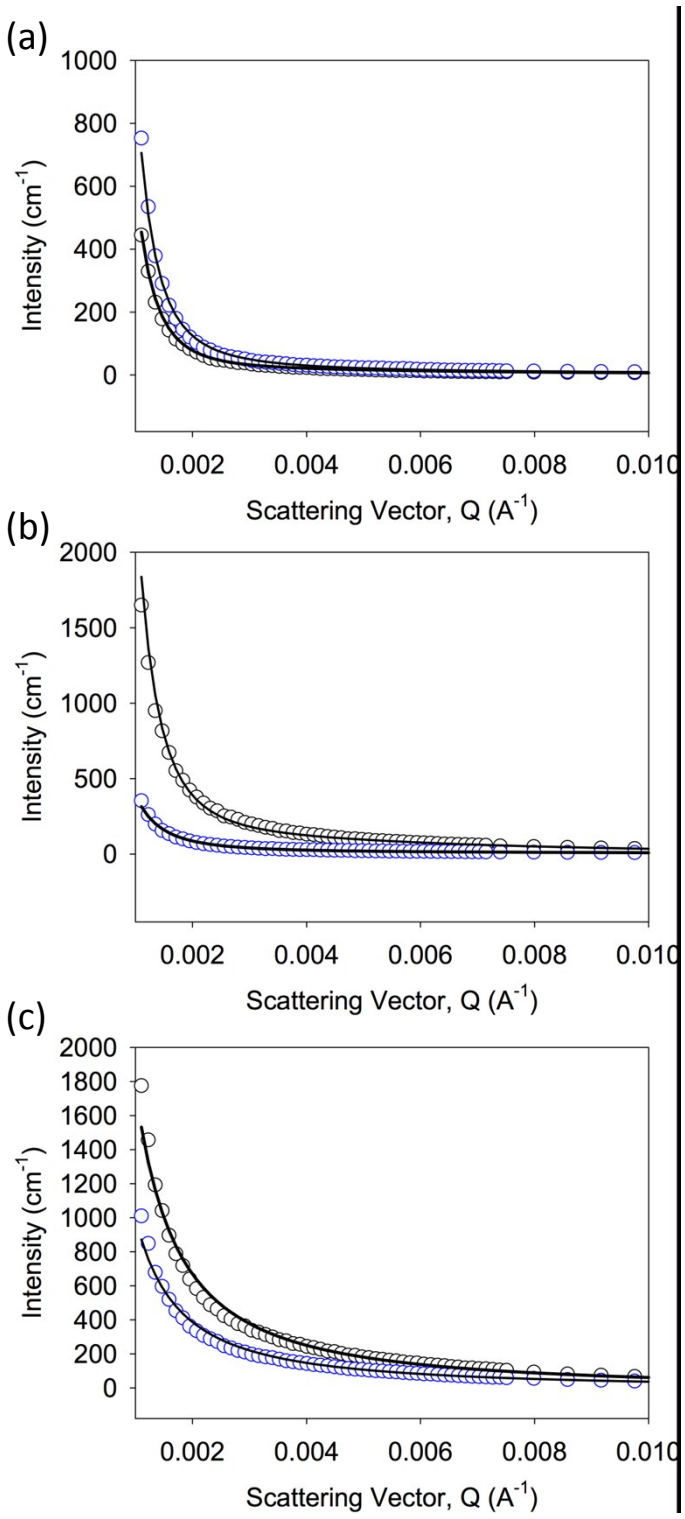

Figure S17. Plot of SANS data for (a) Ca-triggered gels; (b) Acid-triggered gels; (c) Solvent-triggered gels. The data at low Q are shown on a linear-linear plot to show the quality of the fits at low Q.

References

[1] L. Chen, S. Revel, K. Morris, L. C. Serpell, D. J. Adams, *Langmuir* **2010**, *26*, 13466-13471.
[2] www.sasview.org