Chem Soc Rev

RSC Publishing

TUTORIAL REVIEW

View Article Online
View Journal | View Issue

Cite this: *Chem. Soc. Rev.*, 2013, **42**, 5143

The importance of the self-assembly process to control mechanical properties of low molecular weight hydrogels

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Received 28th January 2013

DOI: 10.1039/c3cs60030k

www.rsc.org/csr

Hydrogels can be formed by the self-assembly of certain small molecules in water. Self-assembly occurs *via* non-covalent interactions. The self-assembly leads to the formation of fibrous structures which form the matrix of the gel. The mechanical properties of the gels arise from the properties of the fibres themselves (thickness, persistence length *etc.*), the number and type of cross-links and also how the fibres are distributed in space (the microstructure). We discuss here the effect of assembling the molecules under different conditions, *i.e.* the self-assembly process. There is sufficient literature showing that *how* the molecules are assembled can have a significant effect on the properties of the resulting gels.

Key learning points

- Hydrogels can be formed as certain small molecules self-assemble in water.
- A range of methods can be used to self-assemble the molecules, including heat–cool cycles, solvent-mediated approaches, pH switches and enzymatic cleavage of bonds depending on the gelator.
- The self-assembly process can strongly affect the mechanical properties of the resulting gels.
- The different mechanical properties arise from differences in nanostructure and/or microstructure of the gels.

Introduction

Hydrogels are widely useful materials, with applications in foods, cosmetics and medicine amongst others. Hydrogels can be formed from cross-linked polymer networks or by biopolymers such as gelatin or pectin.[1]

An alternative strategy to prepare hydrogels is *via* the self-assembly of small molecules (low molecular weight gelators, LMWG) into one-dimensional structures, which entangle to form the matrix of the gel (Fig. 1).[2–4] Supramolecular hydrogels formed from LMWG tend to have very different mechanical properties to those of polymeric or biopolymer gels. For example, the gels tend to break at relatively low strains.[5] The gels are also often reversible, for example melting and re-forming on warming and cooling respectively. These properties mean that hydrogels formed from LMWG have different advantages and disadvantages over polymeric or biopolymer hydrogels and there is significant current interest in these materials.

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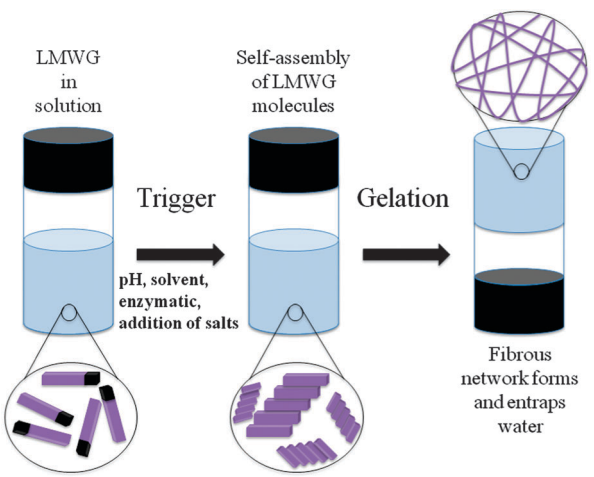

Fig. 1 Schematic showing the self-assembly of a gelator leading to a fibrous network and self-supporting gel.

Design of LMWG

There are a large number of LMWG that have been described. A number of reviews highlight the diversity in molecular

structure of LMWG.[2–4,6,7] Example LMWG include molecules from classes such as bile salts, functionalised sugars, oligopeptides, surfactant-like molecules and dendrimers. Chirality is a common feature.[8] LMWG that form hydrogels are generally composed of hydrophilic regions, which ensure compatibility with water, and hydrophobic regions which drive the self-assembly of the LMWG in water. Beyond this, despite this diversity in molecular structure, there is a limited understanding of the supramolecular design rules for LMWG. Many LMWG are discovered by accident and then further examples discovered by small changes in structure around known LMWG. However, it is common for very small changes in molecular structure to result in a LMWG becoming a non-gelator.[9] This is poorly understood, although there are recent reports linking the molecular structure to dissolution enthalpies.[10] Nonetheless, serendipity and library-based approaches are often used to discover new gelators.[9]

Gelation by LMWG

For gelation, a network of fibres needs to be formed. This occurs *via* multiple hierarchical assembly from molecule to network (Fig. 2). First, as mentioned above, the molecular interactions must drive self-assembly, which controls the nanostructure. This nanostructure must favour formation of one-dimensional fibres over other possible structures. The self-assembly into such one-dimensional structures, as opposed to crystallisation, is driven by a balance of forces. Assembly is driven by one-dimensional non-covalent interactions such as hydrogen-bonding and π-stacking. These interactions direct the assembly, but are of course weak in water. Assembly in water is therefore driven by hydrophobic forces. The packing of LMWG in the fibres is also a poorly understood area. Some examples exist where the packing has been suggested,[11,12] but it is generally difficult to access data due to the lack of scattering from the gel phase from many systems meaning that X-ray scattering approaches are often difficult. Most scattering data is collected from dried gel samples.

Gelation requires the formation of a continuous network structure. Hence, cross-links between the fibres are needed. These cross-links can either arise from entanglement of fibres or by branching of fibres. These cross-links are of course non-covalent in nature, which means that the gels are reversible.

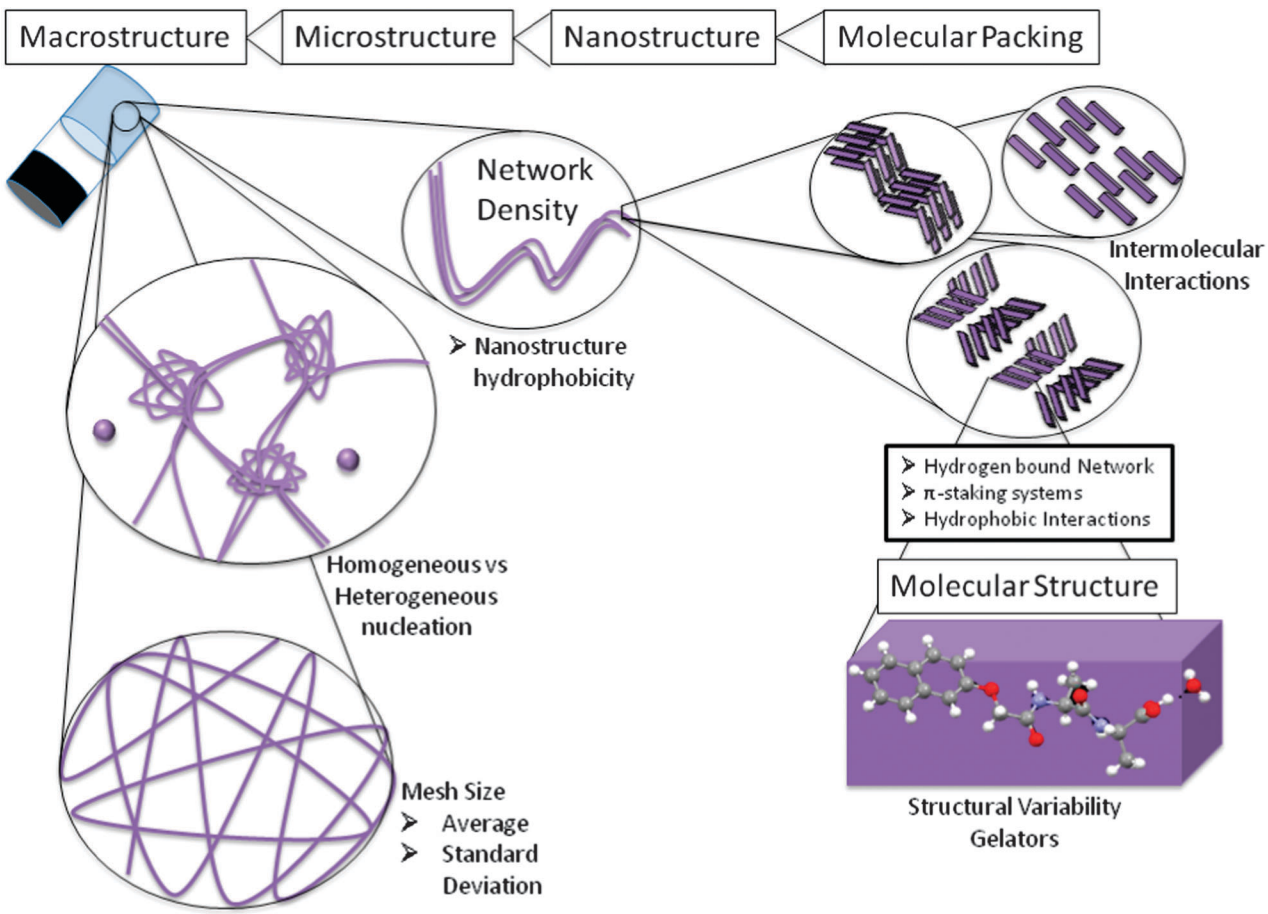

Fig. 2 Schematic showing the different levels of assembly leading to gelation. The process can affect all levels of the assembly. The molecular structure can be changed but the emergent phenomena resulting from those changes have been so far difficult to predict and control. The three different molecular packing models illustrate possible variability in packing of the molecules. Microstructure can be dramatically different even though the molecular packing may be very similar. The final dynamic equilibrium of the nanostructure and macrostructure properties can also be varied.

Key factors that determine the mechanical properties of a hydrogel are the average thickness and mechanical properties of the fibres themselves, the degree of branching (*i.e.* the distance between the cross-linking points), the type of cross-link and the microstructure (*i.e.* how the fibres are distributed at a larger length scale).[3] These properties are to some degree controlled by the molecular structure, since the primary assembly is governed by the intermolecular interactions programmed into the molecule. It is much less clear how to control the degree of cross-linking, although it has been shown that fibre branching can be controlled in related organogels by additives, which affect the number of nucleation sites.[13] Controlling the microstructure can have a significant effect on the properties of the gels, although again it is often unclear how to control this. Clearly the properties of the gels control how effective they are for specific applications. Rheology is an effective means of characterising gels.[14] However, there is a relative paucity of rheological characterisation of hydrogels formed from LMWG, with the ubiquitous image of an inverted vial being significantly more prevalent. This means that it is difficult to correlate mechanical properties of hydrogels with the molecular structure or even perhaps class of LMWG. As a result, linking the molecular structure of a LMWG to the mechanical properties of the resulting hydrogels is poorly understood.

Importance of process

However, there is possibly an even more important level of complexity which is in our opinion often ignored. This is the *process* by which the self-assembly of the LMWG is carried out to give the hydrogel. To prepare gels from LMWG, a trigger of some kind is required. Often this is simply a heat–cool cycle. Heating solubilises the LMWG. On cooling, the LMWG are no longer soluble and so self-assemble (instead of precipitating or crystallising as would most commonly be expected). This occurs due to one of the important parameters of a LMWG, a relatively high hydrophobicity, Other triggers have also been reported. These include (1) changes in pH (the LMWG have significantly higher solubility at one pH over another),[15] (2) a change in solvent polarity (the LMWG have a high solubility in a water-miscible organic solvent, but a low solubility in water)[16] and (3) the use of an enzyme to cleave a functional group from a pro-gelator, thereby significantly reducing the solubility of the molecule in water.[17] Fig. 3 schematically shows how a single precursor could have different process-dependant pathways of assembly. In all of this, the formation of fibres can be thought of as a consequence of inhibited crystallisation or precipitation[18] and, as such, may not represent the thermodynamic minimum, but rather a kinetically trapped structure, *e.g.* State 2b in Fig. 3.

In all of these cases, the process therefore is a means to go from a 'highly-soluble' environment to a 'poorly-soluble' environment and, as such, it is highly likely that this will be strongly kinetically dependent. For example, the process will control nucleation processes and growth rates, both of which will control the microstructure of the gel phase. As noted above,

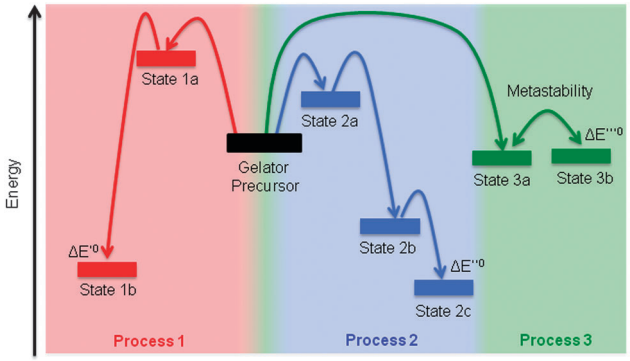

Fig. 3 Gelation can be thought of as a kinetically trapped state, rather than the thermodynamic minimum. The process of assembly may result in different pathways being followed.

this is expected to directly translate into differences in mechanical properties. It is less clear whether the process will control the nanostructure, which might be thought to be determined by the molecular structure, which should control the intermolecular interactions and hence drive the assembly. In some cases, the assembly in a gel phase has been described as being under thermodynamic control.[19] However, there are also reports showing that crystallisation from the gel phase of the gelator is possible, with the packing in the gel fibres and in the crystals being different.[20] It has also been shown that an enzymatic formation of a gel resulted in kinetically trapped states in some cases, which were able to convert to more thermodynamically favoured states after a further heat–cool cycle.[21] This clearly demonstrates that for some examples at least, the fibre phase is not the thermodynamic minimum.

In general, reports of individual LMWG tend to report the use of a single trigger or assembly protocol to form the gels. We contend that changing the self-assembly process for a specific LMWG will have a profound effect on the self-assembly pathway, the kinetics of assembly and as a result will directly affect the mechanical properties of the resulting hydrogels. Inherently, the process of assembly is potentially at least as important as the molecular structure of the LMWG. The mechanical properties of a hydrogel are clearly of paramount importance and can affect the suitability to a proposed application. For example, the mechanical strength of a gel has been shown to affect the survival of different cell types and growth of cultured cells.[22] The relevance of the mechanical properties is crucial not only for the cellular mechanical microenvironment stability, but also for quantifying and controlling the functionality of the microstructure, for example in stem cell engineering applications. Control over the density of available binding points in the hydrogel matrix represents a major milestone for stem cell differentiation.

In this tutorial review, we exemplify the importance of process on the supramolecular self-assembly of a small number of LMWG. As mentioned, there is a scarcity of rheological data for many of the gels reported in the literature (as an introduction, Yan and Pochan have written an excellent review detailing rheological analysis of hydrogels[23]). We have therefore focussed

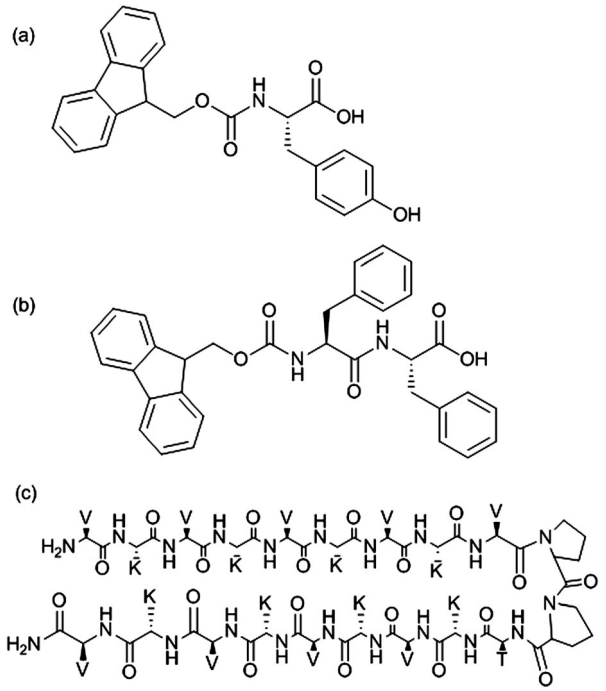

Fig. 4 Structures of the three gelators discussed in this review. (a) FmocY. (b) FmocFF. (c) MAX1, V = valine, K = lysine.

this tutorial review on three specific LMWG for which there have been a number of detailed reports (Fmoc-tyrosine (FmocY), Fmoc-diphenylalanine (FmocFF) and MAX1, Fig. 4).

We concentrate on discussing how the process affects the outcome of the self-assembly and additionally discuss what the direct consequences of these changes in process are on the mechanical properties of the gels. Due to the nature of this tutorial review, we cannot of course be completely comprehensive in describing all possible LMWG. However, we suggest that the broad observations that a change in process can affect the observed mechanical properties will be conceptually similar across all classes of LMWG. The importance of process has been highlighted for specific organogelators[13] and it is likely that many of the concepts discussed there are also important for hydrogels. We highlight that the importance of process on the outcome of self-assembly has been discussed for other unrelated systems such as block copolymer assembly, again a case where the process is a means to go from a 'highly-soluble' environment to a 'poorly-soluble' environment.[24]

Fmoc-tyrosine

FmocY is an interesting example of a LWMG and is a commercially available N-protected amino acid. There are two ways to form a hydrogel using FmocY (Fig. 5). The first is to dephosphorylate FmocY-phosphate. The second is to adjust the pH of a solution of FmocY from high pH to a lower pH. Both of these methods have been reported and it is clear from the results that the methods lead to gels at different pH and that the mechanical properties of the gels are different depending on how gelation is carried out.

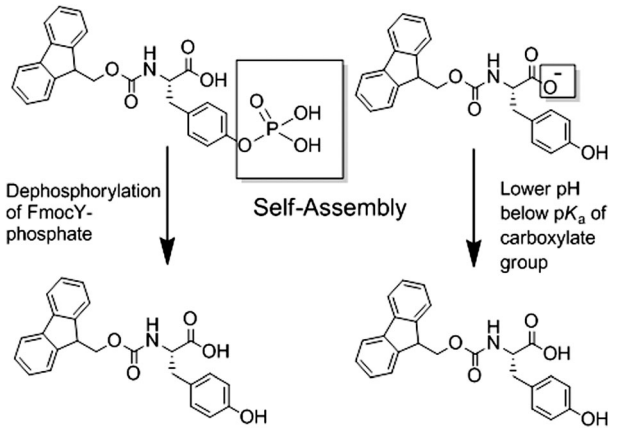

Fig. 5 FmocY can form a hydrogel by either (left) enzymatic formation of FmocY or (right) *via* a pH switch.

Dephosphorylation of FmocY-phosphate

FmocY was first shown to form gels in 2004 by Yang *et al.*[25] Here, the gelation was achieved by the addition of an enzyme to dephosphorylate FmocY-phosphate. This resulted in the initially soluble phosphate becoming insoluble and assembling into nanofibres. This gelation occurred at pH 9.6 at 37 °C. The turbid gel had a storage modulus (G') of approximately 1000 Pa and a loss modulus (G'') of approximately 300 Pa at FmocY concentrations of 1.9 wt%. Later work by the same group showed that the same process at pH 6 and 37 °C resulted in a gel with a G' of 4000 Pa and a G'' of 1000 Pa at a concentration of 1.55 wt%.[26] In light of later results, it is perhaps not surprising that the gels have higher moduli at pH values closer to the pK_a of FmocY (see below). Interestingly, the circular dichroism and fluorescence data also are slightly different at the two different pH values. Circular dichroism (CD) is often used to monitor the secondary structure of peptides. However, for these systems, the chiral orientation of the Fmoc groups are the main contributor to the CD due to the high extinction coefficients of these groups. The CD signals are stronger at the lower pH and there are slight changes in the peak intensities. More specifically, there is a significantly stronger peak at 275 nm at lower pH. Fluorescence has been used to monitor the stacking the Fmoc groups in these and similar systems. At pH 9.6, a slight red shift was observed on gelation, with a low intensity broad peak at 450 nm observed, attributed to stacking of multiple fluorenyl groups. At lower pH, there were more pronounced broad peaks centered at 420 and 450 nm, implying that multiple fluorenyl groups aggregate *via* π-stacking. This suggests that there may be differences in packing at the molecular level at the different pH values that perhaps translate directly into differences in the mechanical properties.

Thornton *et al.* used the same dephosphorylation process to form gels from FmocY at pH 7.4, again at 37 °C and a concentration of 1.55 wt% (Fig. 5).[27] They showed that varying the amount of enzyme added affected the rate of dephosphorylation and hence the rate of gelation. This directly translated

into differences in rheological properties. Using 10 U μL^{-1}, the gelation time was 60–120 minutes. G' was found to be 34 ± 13 kPa. Using 1 U μL^{-1}, the gelation time was 120–240 minutes, with the resulting gels having lower moduli ($G' = 9 \pm 8$ kPa). For these systems at pH 7.4, the CD data appears similar to that at pH 6, but the fluorescence data is more similar to that at pH 9.6. However, by Scanning Electron Microscopy (SEM), the fibre diameters were similar in all cases, implying that the rheological properties were not due to differences in the assembled structures themselves, but presumably due to differences in the degree of cross-linking.

Schnepp *et al.* prepared gels at pH 9.6 and 37 °C at a concentration of 1.55 wt%.[28] These gels had higher moduli than reported previously ($G' > 10^5$ Pa). It is not clear why this is the case, but may be due to the kinetics of gelation as discussed above. No CD or fluorescence data is available to compare to the previously described data for molecular level packing. Passive diffusion of calcium ions into the gels resulted in mineralisation and further increases in rheological properties. Later, Mann's group showed that cerium oxide nanoparticles could be used as catalysts to dephosphorylate the phosphate group.[29] This was carried out at pH 10.2, but at room temperature (Fig. 6).

The rheological properties were again high, $G' = 2 \times 10^4$ Pa, $G'' = 4 \times 10^3$ Pa. Interestingly, there were a number of differences between the gels formed by enzyme dephosphorylation and those where cerium oxide nanoparticles were used. The minimum concentration of FmocY needed to form a gel was significantly lower when the nanoparticles were used as catalyst. The enzyme-induced hydrogels were found to be softer and less viscous; they also displayed a considerable degree of structural deformation at much lower shear stress values than the gels formed using the nanoparticles. Finally, the melting points for the gels formed using enzymes were 40 °C lower than those formed using the nanoparticles. It was suggested that this may be due to the nanoparticles becoming entrapped in the network or could be due to the assembly mechanism itself. Using the nanoparticles, flat, ribbon-like structures were imaged in the gel as opposed to the fibres found for the enzyme-triggered gels. Also, the gels were found to be transparent when the nanoparticles were used, implying a different size range of self-assembled structures when compared to the turbid enzyme-triggered gels.

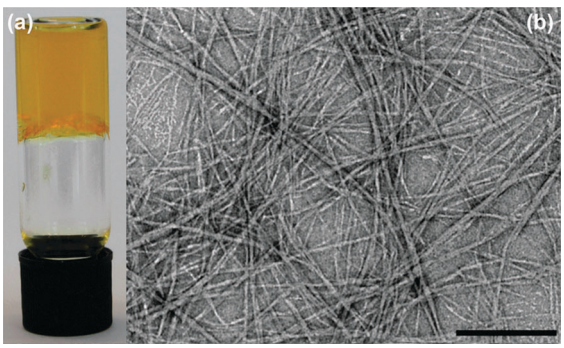

Fig. 6 (a) Self-supporting hydrogel formed by the dephosphorylation of FmocY-phosphate using cerium oxide nanoparticles. (b) Unstained Transmission Electron Microscopy (TEM) micrographs of FmocY hydrogel network of semi-rigid nanofilaments. Scale bar = 500 nm. Figure from ref. 29.

pH triggered gelation of FmocY

In all of the above, the gelator is always described as being FmocY. However, for the majority of the examples above, the solution pH is above the expected pK_a of the terminal carboxylic acid. Although not specifically reported to the best of our knowledge, a pH titration suggests that the pK_a is approximately 5.5.[30] Whilst the pK_a is likely to be affected by the concentration of FmocY used, the presence of salts and temperature (as shown for other systems),[31] it is likely that the gelator is the carboxylate salt of FmocY in at least some cases described above. Hydrogels can also be prepared from FmocY using a pH-trigger (Fig. 5). Here, a solution at high pH (where the FmocY is solubilised by the deprotonated carboxylate group) is initially prepared. Interestingly, this might be expected to give a gel from the results of the enzyme-triggered gels above. The fact that FmocY forms a free-flowing solution under these conditions as opposed to a gel may be due to the presence of the buffers which are used for the enzyme and nanoparticle catalysed gelations. Recent results have shown that the presence of salts can induce gelation in solutions of similar molecules.[32,33] We showed that the addition of a range of salts can result in gel formation for naphthalene–dipeptide LMWG systems which have the appropriate worm-like micelle structure.[34] Roy *et al.* have shown for Fmoc-tyrosine-leucine (FmocYL) that the degree of order and rheological properties of the gels formed by dispersion of FmocYL at pH 8 are strongly affected by the addition of salts.[33] The properties could be linked to the Hofmeister series, with different salts resulting in the formation of different nanostructures. Fibres were formed in the presence of kosmotropes (water-structuring salts) whilst spherical structures were formed with the chaotropes (water-breaking salts). Later work from the same group reported the enzymatic formation of gels from FmocYL.[21] Again, the presence of salts was shown to be important, not only affecting the assembly of the gelator, but also the enzyme performance. Hence, the effect of salts is in our opinion an important point that has been insufficiently discussed. Nonetheless, to induce a gel by pH change, the pH of the FmocY solution is then lowered to give a gel. This was first reported by Thornton *et al.* where the pH was reduced from 9.6 to 7.4, again at 1.55 wt%.[27] This pH is above the apparent pK_a,[30] but as noted above, this may be due to the higher concentration used (the pK_a was determined at approximately 1 wt%). Gelation occurred quickly (in 5 minutes). However, the rheological properties ($G' = 0.6 \pm 0.1$ kPa) were lower than for the enzyme-mediated gelation discussed above. This quick gelation therefore did not lead to improved rheological properties (this is opposite to what was found with the enzyme, see above). However, here the fibre widths were found to be greater than for the enzyme-triggered gels, implying that the local self-assembly is different.

Later work also discussed the use of a pH-trigger. Sutton *et al.* used a lower concentration of FmocY (0.97 wt%) and adjusted the pH from 8.2 to 4.7.[35] To adjust the pH, glucono-δ-lactone (GdL) was used, which hydrolyses to gluconic acid. This results in a significantly slower pH change as compared to mineral acid which can lead to profound changes in gel properties.[36] As at higher pH, long fibrillar structures were formed, resulting this time in a transparent self-supporting gel. The rheological properties were higher than for the gels prepared at higher pH, with $G' = 2 \times 10^4$ Pa and $G'' = 3 \times 10^3$ Pa, despite the overall concentration of FmocY being lower. These gels were found to break however at low strain (about 0.01%). Aufderhorst-Roberts *et al.* later examined this process on the micron-scale using microrheology.[30] They showed that the addition of GdL resulted in assembly below the pK_a of FmocY. At this stage, clusters started to form, with gelation occurring as the cluster size increased *via* a percolation transition. Their data implies that the final overall degree of network heterogeneity is low in these systems.

Fmoc-diphenylalanine

A related LMWG to FmocY is FmocFF. This LMWG has been widely reported and gelation can be induced again in a number of ways. Firstly, again a pH-trigger can be used. Second, a change in the solvent quality has been used. Finally, the addition of certain salts to a solution of FmocFF at high pH results in gel formation. FmocFF has been shown to form gels at physiological pH. Again, it is clear from the published data that the method of assembly and the exact conditions used have a profound effect on the mechanical properties of the resulting gels.

pH triggered gelation of FmocFF

As for FmocY, gels can be prepared from FmocFF solutions by adjusting the pH of the solution. The first report describes increasing the pH of a solution to above pH 8.0, followed by the addition of concentrated hydrochloric acid to reduce the pH again.[37] It is important to note that mixing was used as the HCl was added in aliquots (see below for a discussion around mixing effects). The mechanical properties of the resulting gels were not reported, although gels prepared at concentrations of between 0.22 and 1.07 wt% were described as "weak, flowing" gels. Later work from the same group reported a transparent gel at a concentration of 1.07 wt% formed by the same method.[11] The rheological properties were reported, with the gel having a G' of 10^4 Pa and G'' being approximately one order of magnitude less, as expected for a gel. A model for the self-assembly was built from IR, CD, X-ray scattering and fluorescence data suggesting β-sheets formed between the dipeptides and π-stacking between the Fmoc groups being important. In 2009, Tang *et al.* reported that FmocFF has two apparent pK_a values, with the self-assembled structures formed and resulting properties of the system being strongly pH dependent.[38]

A modified method was used to assemble FmocFF here. After HCl addition, the samples were heated to 75–80 °C for one minute, homogenised and then stored at 4 °C for 12 hours. Heating and cooling cycles were also used during the pK_a measurements. The two reported apparent pK_a values were approximately 9.9 and 5.7 (Fig. 7, top) and are related to defined structural transitions resulting from peptide self-assembly. At pH values above the highest pK_a value, thin fibres were observed by TEM (we later suggested that these are actually worm-like micelles[39] on the basis of the shear-thinning behaviour at high pH). Below this first pK_a, an entangled network was imaged. At pH 9.0, the rheological properties were very low, with G' being approximately 1 Pa (Fig. 7, bottom). At lower pH (7.6), the rheological properties were even weaker. The authors attributed the discrepancy between this data and their previous reports[11] to the presence of kinetically trapped aggregates giving higher moduli. The hypothesis was that the heating of the samples and the storage at 4 °C resulted in significantly more homogeneous systems. At lower pH, below

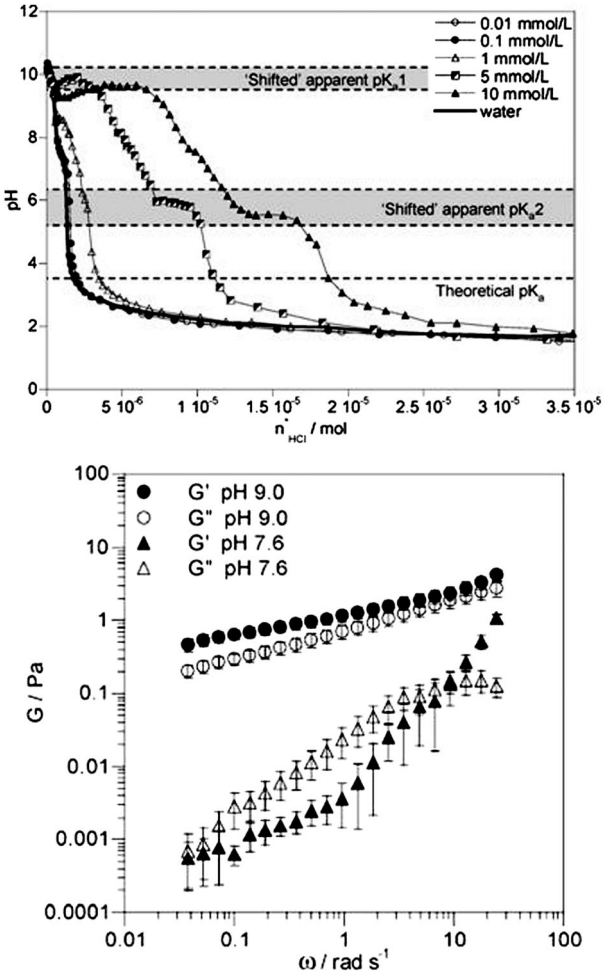

Fig. 7 (top) Titration curves of FmocFF at 0.01, 0.1, 1, 5, and 10 mmol L^{-1}. (bottom) Rheological data for FmocFF samples at 10 mmol L^{-1} at pH 9.0 (below apparent pK_a 1) and at pH 7.6 (above apparent pK_a). Reprinted with permission from ref. 38. Copyright 2009 American Chemical Society.

the second apparent pK_a, rigid, rod-like ribbons were formed, leading to precipitation and phase separation.

As noted for FmocY, the presence of salts may well lead to differences in assembled structure. In fact, it has been reported that the addition of calcium salts to a solution of FmocFF at high pH results in gel formation (G' = 10.5 kPa).[32] FmocFF has been examined for cell culturing, where the presence of salts is necessary. Jayawarna *et al.* described one method of forming the gels in the presence of cell culturing medium by preparing a solution of FmocFF at high pH with sonication and vortexing used to mix the solutions.[40] Diffusion of standard cell medium (Dulbecco's Modified Eagle Medium, DMEM) through a semi-permeable membrane was found to lower the pH to 7.5 and result in gel formation. At a concentration of 1.07 wt% and a temperature of 25 °C, FmocFF was found to form a gel with a G' of 21.2 kPa, with G'' being 4.8 times lower. These data were shown to be reproducible *via* repeat measurements. Under these conditions, FmocFF was reported to form fibres with a thickness of 54 ± 19 nm, which differs from that reported in water at the same pH.[38] This implies that the presence of the salts is affecting the assembled structure of FmocFF. In line with this, the IR data showed differences compared to the data[11] in the absence of salts. With no salt, the IR showed strong peaks at 1625 cm^{-1} and 1687 cm^{-1}.[11,38] In the presence of the buffer, the IR showed a broad peak between 1630 and 1640 cm^{-1}, with a lower intensity peak at 1690 cm^{-1}.

Another report centred around cell culturing describes the use of a pH adjustment from 10.0 to 7.2 to form a gel.[41] For rheological analysis, FmocFF solutions were mixed with DMEM. The reported G' was 1.9 kPa, lower than for the previous report.[40] This may be due to the reported fast gelation as opposed for the slow diffusion method used before.[40] Again, slight differences in the IR data was observed, with peaks of approximately equal intensity at around 1635 cm^{-1} and 1690 cm^{-1}. All the IR data suggests that the different methods of self-assembly and the presence of salts may be affecting the secondary structure of the assembled aggregates.

The effect of mixing method on the assembly and mechanical properties of the gels has been shown to be marked.[42] Gels were formed using a pH adjustment to pH 6.0 and then the samples heated to 37 °C. On adding HCl to adjust the pH, two different mixing methods were used. First, a 'high-shear' approach was used where mixing was carried out using a vortex mixer. Alternatively, a 'low-shear' approach was used where mixing was carried out by hand or using an orbital mixer. The mechanical properties were found to be very dependent on the mixing used. The values of G' were lower for the high-shear mixed samples and the values of tan δ (defined as G''/G') higher. Differences were also found by creep studies. The rheological data implies that the high-shear samples have a lower density of cross-links or a different type of interconnection between fibres. AFM and nanoindentation studies showed that the high-shear samples contained a large amount of fibrils and stiff, wide ribbons, whereas the low-shear contained softer, less polydisperse ribbons. The authors hypothesised that low shear altered the mode of aggregation. This is a clear demonstration of how the process of assembly can affect the outcome, with the authors highlighting that ''mixing/agitation procedures are likely to be largely depend on the operator'' and hence the rheological data presented were qualitative.

We later investigated the rheological properties of FmocFF gels by a number of methods at a concentration of 0.5 wt%.[39] Our data implies that FmocFF has a single pK_a, rather than the two reported previously. This single pK_a value is indicative of a different assembly process to the situation above, where two distinct apparent pK_as were observed, and is possibly due to their being only one distinct structural re-arrangement. This difference may be due to the fact that heat–cool cycles were not used during the measurements. We showed that gels were formed below this apparent pK_a. Using HCl to adjust the pH, self-supporting gels were formed below a pH of 7.0. Rheological analysis showed that the gels were weak until a pH of 6.0, although significantly higher G' and G'' were found as compared to the results of Tang *et al.* where a heat–cool cycle was used.[38] G' increased further between pH 6.0 and pH 5.0, before becoming approximately constant at 10^4 Pa. In line with previous work, mixing was shown to be really important. If no mixing was used (*i.e.* a FmocFF solution simply added to a solution of HCl), the gels had higher moduli than when vortex mixing was used. However, this was only really noticeable for the stiffer gels; above pH 6.0, the values of G' and G'' were very similar for both mixing methods. As discussed above, the slow hydrolysis of GdL can also be used to adjust the pH. We also examined this method to form gels from FmocFF. Here, a similar profile was observed to an unmixed HCl system, with weak gels ($G' < 1000$ Pa) formed between pH 7.0 and pH 6.0, whereupon the moduli increased until becoming relatively constant at approximately 10^4 Pa below pH 5.0.

A pH switch method with GdL has been used by Berillo *et al.* to form macroporous cryogels from FmocFF.[43] Here, the GdL was added to slowly adjust the pH, and the sample then immediately placed at −12 °C. This results in freeze-concentration of the gelator, with ice crystals concentrating the solute in the non-frozen phase. The self-assembly of the FmocFF occurs in the thin non-frozen sections of the sample, resulting in the formation of a macroporous cryogel (Fig. 8A). A salt (K_2SO_4) was added in some cases to depress the freezing point of water, thus increasing the volume available for the gelation. The final pH was 3.0–4.0. Rheologically, the cryogels were found to have a higher G' of 800–900 Pa as compared to a hydrogel (formed at room temperature as opposed to −12 °C), which had a G' of approximately 700 Pa. However, the cryogels failed at a lower strain than the hydrogels. The cryogels are macroporous due to the presence of the ice crystals during the self-assembly process, with the pore walls being formed from densely packed fibres of FmocFF (Fig. 8B).

Another method to self-assemble FmocFF in ultra-thin films was described by Braun and Cardoso.[12] Here, FmocFF was self-assembled in liquid marbles and also at a liquid–air aqueous interface. In the former case, the self-assembled membrane was formed at the liquid–air interface on a liquid marbles stabilised by *L. clavatum* spores. In the second case, a TEM copper grid

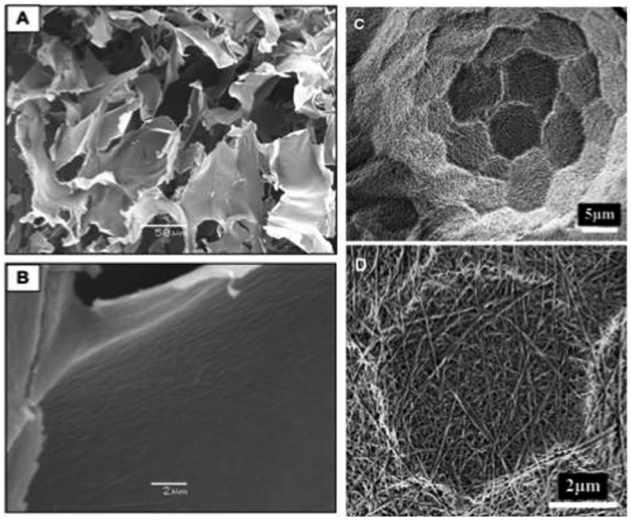

Fig. 8 (A) SEM image of dried cryogel formed from FmocFF after a process of freeze-drying. (B) A higher magnification SEM of (A). (C) SEM image of an ultra-thin membrane (50 nm) self-assembled at the liquid–air interface of spore indentations. (D) A higher magnification image of (C) showing one indentation in a spore with visual observation of highly dense individual FmocFF ribbons fibres. (A) and (B) were modified from ref. 43. Copyright 2012 with permission from Elsevier. (C) and (D) were modified from ref. 12. Copyright 2012 with permission from Elsevier.

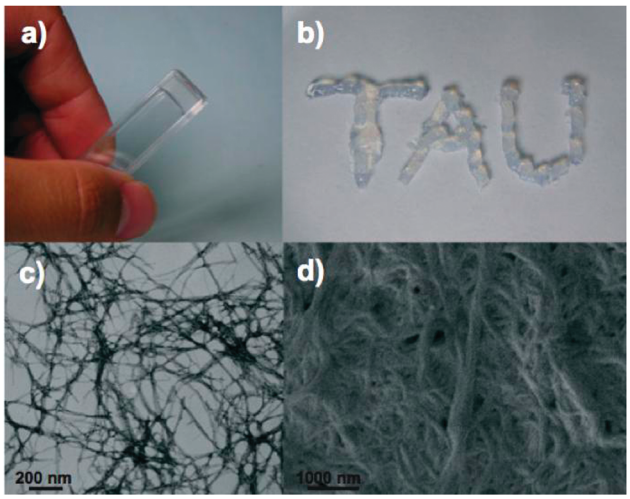

Fig. 9 (a) Hydrogel formed from FmocFF by adding water to a solution of FmocFF in HFIP. (b) The hydrogel can be extruded through a needle and maintain structure. (c) TEM of fibrils which form the gel matrix. (d) HR-SEM of fibrils forming the matrix. Figure modified from ref. 16 with permission from John Wiley and Sons.

was used to probe and characterise the ultra-thin membrane and test the mechanism of liquid–air interfacial triggered gelation. The pH change leading to self-assembly was induced by using an atmosphere of carbon dioxide. Using a gas to change the pH resulted in gelation occurring in a confined environment at the liquid–air interface. The resulting microstructure was the formation of a thin membrane (Fig. 8C). The thickness of the membrane could be controlled by the time of exposure to carbon dioxide and the relative humidity. As for where the cryogel described above, the confinement of a hydrogel membrane consisted of densely packed fibres (Fig. 8D). This method allowed a further understanding of the crystallographic structure of the FmocFF fibres by using electron diffraction experiments on almost a single FmocFF fibre. A typical packing of 4.8 Å was found along the fiber axis and an additional 23 Å in the perpendicular axis was assigned to the fiber thickness, conflicting with the previously proposed helical model[11] and leading to a alternative molecular packing model of the FmocFF system, a columnar packing model. This data, along with the IR data described above may imply that the molecular packing is affected by the process of assembly.

Solvent-mediated gelation of FmocFF

Gels can also be prepared from FmocFF by adjusting the solvent quality. When water on an aqueous buffer is added to a solution of FmocFF in a suitable organic solvent, a gel can be formed in some cases. This was first reported in 2006 by Mahler *et al.*[16] Transparent, self-supporting gels were formed by the addition of water to a solution of FmocFF in hexafluoroisopropanol (HFIP) (Fig. 9a). Two concentrations of FmocFF in HFIP were examined (100 mg mL^{-1} and 25 mg mL^{-1}), which translates into gels with different ratios of water to HFIP. These gels were reported to be stable across a range of temperatures, pH and in the presence of urea and guanidinium hydrochloride. These gels could also be injected through a needle and maintain structure (Fig. 9b). Fibrils with diameters ranging from 10 to 100 nm were imaged, forming the gel matrix. Interestingly, the IR data differed from that reported for gels formed by the pH-triggered method. A significant peak at 1658 cm^{-1} was observed, which is not present for the pH trigger.[38] The data was still interpreted as arising from β-sheets formed between the dipeptides. The rheological data showed G' being approximately 2×10^4 Pa for a concentration of 0.5 wt%, although the amount of HFIP present was not reported. Similar data were obtained if acetone was used in place of HFIP.

Liebmann *et al.* later showed that DMSO could be used in place of HFIP.[44] Phosphate-buffered saline (PBS) was used in place of water. No rheological data was given, but it was reported that the amount of DMSO in the final materials affected stability. When a solution of FmocFF in DMSO at a concentration of 100 mg mL^{-1} was diluted with PBS to give a final FmocFF concentration of 0.5 wt% (*i.e.* a DMSO : H_2O ratio of 5 : 95), the material was unstable. However, a soft gel was formed when a solution in DMSO at 25 mg mL^{-1} was diluted to the same FmocFF concentration. Hence, an increased amount of DMSO results in a more stable gel. Replacing PBS with another buffer (minimum essential medium, MEM) however resulted in an unstable material.

Orbach *et al.* also showed that gels could be formed on addition of water to a solution of FmocFF in DMSO.[45] FmocFF gels formed in this way were reported to be stable from pH 3.0 to pH 12.0. The gels form quickly, with the rheological properties being stable after approximately 700 seconds ($G' = 10^4$ Pa). Fibre diameters were reported to be 10–30 nm in diameter.

An interesting observation was that there was a transition in the optical properties as the gel formed. On water addition, a turbid solution was formed, which then clarified to result in a transparent hydrogel. This is very different to what occurs when a pH switch is used where no such transition occurs. This shows that the aggregation process must be different between the pH switch and solvent-switch approaches. Later, the same group showed that the kinetics of this transition was both concentration- and temperature-dependent.[46] The authors hypothesised that this turbidity change was due to a restructuring of an initial self-assembled structure from many irregular aggregates to highly ordered structures. We showed for a closely related LMWG (FmocLG, Fmoc-leucine-glycine) that a similar turbidity change occurs.[34] Here, the turbid solution was found to contain highly regular spherical aggregates. It did not appear that the fibres nucleated from these spheres, but the exact nature of these structures is unclear.

We later showed that the rheological properties of gels formed from FmocFF by addition of aqueous solutions to a DMSO solution of the gelator were dependent both on the pH of the medium and the volume fraction of DMSO.[39] Adding water to a solution of FmocFF resulted in a gel with a pH of 4.0, presumably due to the weak acidity of FmocFF. As for the gels formed *via* a pH switch method (see above), the rheological properties were low at pH 7 (achieved by adding a buffer). At pH 5 and below, significantly stiffer gels ($G' > 10^4$ Pa) were formed, similar to the data achieved with a pH-switch. As suggested by the work of Liebmann *et al.*,[44] the rheological data was dependent on the buffer used, with variations observed between buffers. We also showed that the volume fraction of DMSO in the gels was an important factor. G' was found to be 17 kPa at a volume fraction of DMSO of 0.05 (*i.e.* 5 : 95 DMSO : H_2O). On increasing the volume fraction to 0.25, G' increased to approximately 24 kPa for a constant concentration of FmocFF. Tan δ was below 0.12 for all these gels. A dip in G' was observed at a volume fraction of 0.35 before increasing again. Above a volume fraction of 0.50, G' steadily decreased until no gels could be formed above a DMSO volume fraction of 0.64. Interestingly, tan δ steadily increased across this volume fraction range to approximately 0.23 at a volume fraction of 0.64. Recovery after shear is an important parameter if gels are to be injected for example.[47] As noted above, Mahler *et al.* reported that FmocFF gels formed from HFIP on water addition could be passed through a needle.[16] We found that the ability to recover after shear was strongly dependent on the volume fraction of DMSO in the gels. After applying a constant high shear to disrupt the gels, the rheological properties were monitored with time after cessation of the high shear. Gels formed at low volume fractions of DMSO (<0.35) were found to recover well. Above a volume fraction of 0.35, recovery was relatively poor. These differences were found to correlate with the microstructure, with gels prepared at low DMSO volume fractions being found to contain spherulitic domains of fibres (such a microstructure is very different to that found when gels are formed by a pH switch[39]). This seems key for the preparation of gels which can recover after shear.[47] We have shown similar results for FmocLG,[34] where interestingly the nanostructure of the fibres (as measured by cryo-TEM and small angle neutron scattering) was found not to be significantly different whether or not the gel could recover after shear; it was the microstructure that was found to be key.

Effect of additives on the gelation of FmocFF

Along with the choice of buffer and absolute pH, other additives have also been shown to affect the mechanical properties of gels prepared from FmocFF. For gels formed by the addition of water to a solution of FmocFF in DMSO, the presence of polymeric additives in the aqueous solution affects the self-assembly and the properties of the resulting gels.[48] A buffered solution was used to remove the effect of pH on the assembly. Using dextrans of different molecular weights as additives, it was found that the viscosity of the aqueous solution was a critical parameter (Fig. 10).

For solutions with viscosities of 8 mPa s and below, the mechanical properties were very similar (G' was approximately 20–25 kPa as compared to 29 kPa in the absence of any additive, with tan δ being between 0.10 and 0.20). G' then decreased with increasing viscosity above this value. Using confocal microscopy, it was demonstrated that the reduced mechanical

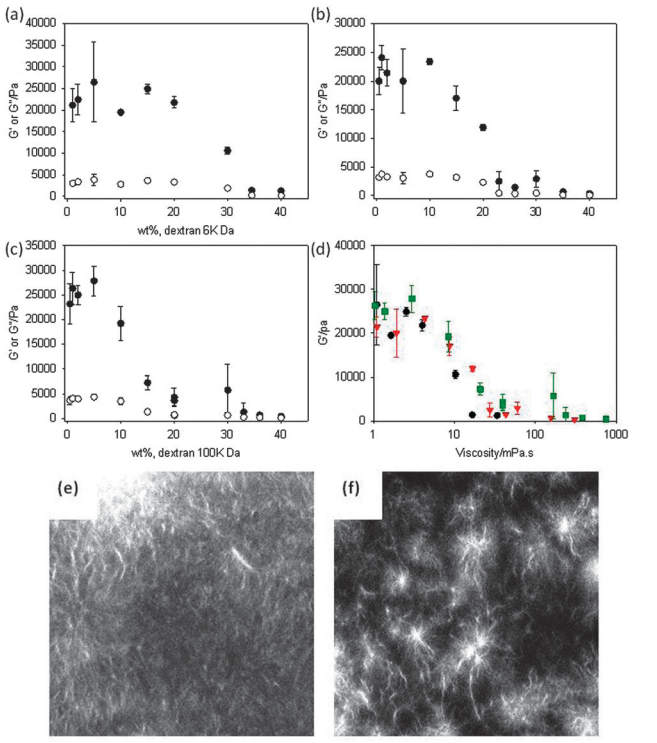

Fig. 10 Effect of adding dextran of different molecular weight on the mechanical properties of gels formed from FmocFF. Dextran molecular weights are (a) 6000 Da, (b) 40 000 Da, (c) 100 000 Da, (d) shows the data plotted as a function of the viscosity of the dextran solutions (black circles: 6000 Da, red triangles: 40 000 Da, green squares: 100 000 Da dextran). (e) Confocal image of FmocFF gel in the absence of additive. (f) Confocal image of FmocFF gel in the presence of 20 wt% dextran (40 000 Da). Scale bars represent 20 μm. Figure from ref. 48.

properties were as a result of smaller spherulitic domains being formed as compared to in the absence of additive, with a lower degree of connectivity between spherulites. Other polymeric additives were also examined. For example, the mechanical properties were found to be very sensitive to the presence of polyethylene oxides, implying that the effect of additives will be additive-specific. Elsewhere, the effect of adding a different polysaccharide, konjac glucomannan, on the mechanical properties of FmocFF gels was examined.[49] Gels were prepared again from addition of an aqueous solution of the polysaccharide to a DMSO solution of FmocFF. The presence of the polysaccharide slowed down the gelation process, taking several hours as opposed to minutes in the absence of additive. The gels were found to have improved mechanical properties as compared to those in the absence of additive (G' = 13.7 kPa as compared to 7.5 kPa in the absence of additive). The gels were also found to be significantly less sensitive to frequency. SEM showed that the FmocFF fibres were thinner in the presence of additive. SEM also showed that the FmocFF fibres were interpenetrated and interwoven with the polysaccharide chains.

From all of this data, it is clear that the mechanical properties of FmocFF gels are extremely sensitive to the absolute pH, the presence of salts and temperature as well as the mixing methodology used. It is less clear how the apparent changes in molecular packing suggested by IR, CD and fluorescence data affect the mechanical properties of the resulting gels. Deconvoluting molecular packing, the number of cross-links, changes in persistence length and microstructure is extremely complex.

MAX1

Our third case study is an unrelated LMWG MAX1 which is a 20 amino acid β-hairpin molecule developed by the groups of Pochan and Schneider.[50–55] This gelator consists of two strands with an alternating sequence of valine (V) and lysine (K) residues that are connected with a tetrapeptide type II' turn sequence (–V^{D}PPT–) (Fig. 11). Upon folding, this molecule becomes facially amphiphilic, with the non-polar V residues and the K residues making up each face on the hairpin respectively. When the peptide folds, self-assembly occurs as a result. Therefore, conditions that favour β-hairpin formation will favour self-assembly and gelation. MAX1 is known to fold and consequently self-assemble under specific pH, temperature and/or salt concentrations. At neutral to low pH, coupled with low ionic strength and temperature (~20 °C), the lysine residues of MAX1 are mainly positively charged with charge repulsion rendering peptide folding energetically unfavourable.

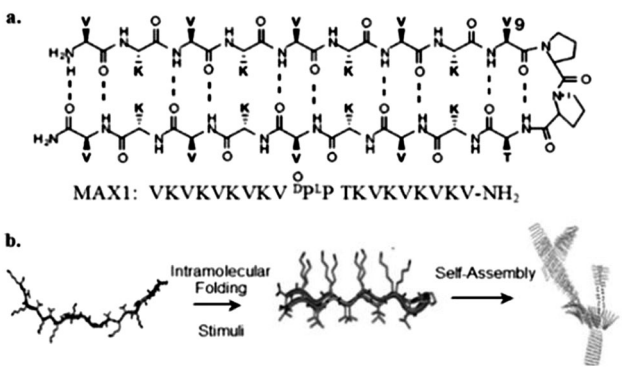

Fig. 11 (a) 20 amino acid sequence and folded β-hairpin of MAX1. (b) Proposed model for the self-assembly of MAX1. Reprinted with permission from ref. 54. Copyright 2004 American Chemical Society.

CD data collected for gels of MAX1, with varying assembly conditions, have shown that β-hairpin formation always precedes self-assembly. β-sheet formation is monitored by measuring the mean residue ellipticity at 216 nm. Under assembly conditions, the data show that β-sheets are present and folding is triggered.[51] Similar data has been collected for all assembled states for MAX1 indicating that the assembly trigger has no effect on the type of molecular packing adopted.[50–52,54–56] Furthermore, both cryo-TEM and TEM data show that on the nanometre level of the self-assembled MAX1, fibril widths of ~3 nm were measured.[56] Gels prepared with NaCl as the salt in the trigger displayed such fibril widths,[51,54] which were in agreement with MAX1 gels that used DMEM buffer as the salt in the trigger.[52] The concentration of gelator slightly varies in these cases, but as the assembly conditions are also different it can be deduced that intramolecular folding at the nanoscale is unaffected by the trigger used. Manipulation of the assembly conditions allows folding and subsequent self-assembly to occur with differences in the mechanical properties of the resulting gels.

At low pH, MAX1 remains unfolded and cannot form gels. This is largely due to electrostatic repulsion between positively charged lysine residues. When the pH is increased above the pK_a of the lysines, repulsion is diminished and intramolecular folding occurs. This is reversible. Acidification can lead to unfolding and hence hydrogel dissolution. The first reported MAX1 gels by Pochan and Schneider were prepared at pH 9.0.[50] At this pH, intramolecular folding occurs, followed by self-assembly which is facilitated *via* both H-bonding between β-hairpins and hydrophobic association of valine-rich faces of folded peptide. These two factors contribute to lateral and facial assembly, respectively (Fig. 11). For gels prepared with 2 wt% peptide material in pH 9 buffer (125 mM borate and 10 mM NaCl) at 25 °C, rheological data was collected. After 30 minutes, G' was greater than G'', indicative of gel-like properties. A final G' of ~1200 Pa was measured for such gels. These gels also displayed the ability to recover after shear cessation with 100% recovery of G' (after 30 minutes) after application of 1000% strain for 180 seconds.[50] As discussed for the FmocFF gels above, mixing is important. Schneider *et al.* report that more uniform gels are formed where agitation of the samples is minimised.

Using the conditions for the gels at pH 9, no gelation occurs if the pH is lowered. MAX1 forms random coils in solution at pH values lower than 9.0 under these solution conditions.[50] If the pH of the solutions is altered to pH 9.7, even more of the lysine residues are deprotonated than before, allowing folding and gelation to occur at a lower temperature. Conversely, lowering the pH with an increase in temperature does not produce gels.

The peptide is incapable of folding under these conditions as lysine residues are likely to be fully protonated (at pH 8.0).[51] However, MAX1 gels have been reported at and around physiological pH by altering the temperature or salt conditions of the system. MAX1 can form gels at pH 7.4 using bis-trispropane buffer.[47,51,54] At pH 7.4, MAX1 has a net positive charge. Increasing the concentration of NaCl in the buffer solution results in screening of the positive charges and thus allows folding to proceed. These gels have G' of $\sim$2900 Pa (Fig. 12a) and also have shown an ability to recover after shear cessation with 95% recovery after 2 hours after 1000% strain. This recovery profile differs for the same concentration gels at pH 9.0, however the salt concentration differs in each case. Interestingly, MAX1 gels at pH 7.4 were studied with different shear times/rates and both studies showed that increased shear time (40 seconds) and shear rate (1000% strain used) individually decreased the percentage recovery of G' after shear cessation.[47]

Increased salt concentration (and therefore ionic strength) drives hydrophobic association of fibrils with faster hydrogelation kinetics. This has been studied using rheology. A 2 wt% MAX1 gel prepared at pH 7.4 with 20 mM NaCl showed no significant increase in G' in the first 50 minutes of the gelation process (Fig. 12b). Increasing the salt concentration to 150 mM decreases the lag time by half, while further increasing the salt concentration to 400 mM shows no lag time in G' evolution during gelation. The 20, 150 and 400 mM salt concentrations give final G' values of $\sim$100, $\sim$300 and $\sim$3000 Pa at 20 °C, respectively (Fig. 12a).[54] Higher salt concentrations provide faster gelation kinetics, resulting in stronger gels. Ozbas *et al.* attribute this to the formation of a more highly cross-linked network at higher rates of assembly. Similarly, the rate of gel formation at 37 °C is higher than the rate of gelation at 20 °C. This higher rate of gelation directly relates to a stiffer gel.

The salts used in these systems can affect the mechanical properties of the hydrogels produced, not just the concentration. Whilst MAX1 hydrogelation is commonly triggered using NaCl in a variety of concentrations, with potential biological applications in mind, cell culture media has been used.[52,53] MAX1 gels (2 wt%) using serum-free DMEM, which contained 165 mM of salt produced gels at pH 7.4 and 37 °C. Kinetic rheological data for gelation showed that after 30 minutes $G' > 1800$ Pa and after 120 minutes, the $G' > 2500$ Pa (Fig. 13a).[53] After 24 hours, G' had increased further to approximately 10 kPa.[54] The 2 wt% gels containing DMEM also showed good recovery from shear (1000%). Interestingly, after shear, the increase in G' in the recovery phase was quicker than in the initial gelation.[54] MAX1 gels were also prepared in cell culture media but were additionally loaded with 10% calf-serum.[53] After 24 hours, a G' value of $\sim$10 kPa was also obtained for these gels. In comparison to gels prepared under the same conditions (2 wt% MAX1, pH 7.4, 37 °C) minus serum, the G' values barely differ (Fig. 13b). MAX1 gels containing calf-serum display a slight increase in G' but not a significant increase. This shows that the additional material within a gel matrix does not appear to strongly affect the fibril network formation since the mechanical properties are generally unaffected.

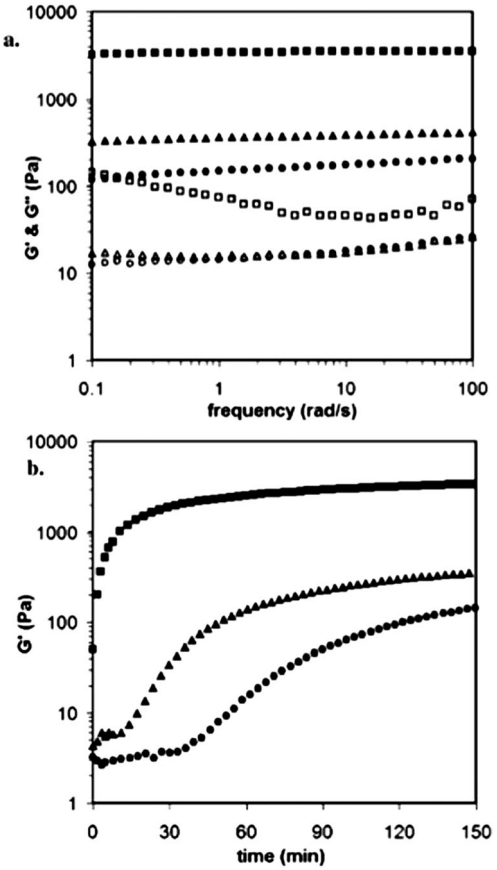

Fig. 12 (a) Frequency sweep rheological data of 2 wt% MAX1 gels, pH 7.4 with 20 mM (G', ●; G'', ○), 150 mM (G', ▲; G'', △), and 400 mM NaCl (G', ■; G'', □). (b) Time sweep data of 2 wt% MAX1 gels with 20 mM (G', ●), 150 mM (G', ▲) and 400 mM NaCl (G', ■). Reprinted with permission from ref. 54. Copyright 2004 American Chemical Society.

The complexation of borate anions with MAX1 has an effect on the rheological properties of MAX1 gels.[57] Boric acid, $B(OH)_3$ has a trigonal planar structure in solution and is a weak Lewis acid in equilibrium with the tetrahedral borate anion, $B(OH)_4$. MAX1 gels containing this boric acid/borate additive were prepared under basic conditions. As such, any complexation between additive and gelator is between the borate anion and MAX1 (boric acid has a pK_a of 9.2). As mentioned previously, solutions containing MAX1 must surpass a transition temperature, above which β-hairpin folding and intermolecular self-assembly occurs. This is a reversible process, with unfolding occurring as the temperature is lowered. However, in the presence of boric acid, this folding and assembly process was found to be irreversible. Gels were prepared (0.5 wt% MAX1 at pH 9.1, 10 mM NaCl, 40 °C) with 125 mM boric acid added.[57] Heat and cool cycles run on a rheometer show the reversibility of the gelation system for MAX1 as expected. However, in the presence of boric acid, decreasing the temperature below the assembly temperature resulted in an increase in G'. Ozbas *et al.* attributed this stiffening transition to complexation between borate anions and the lysine side chains of MAX1. With boric acid and borate having 3 and 4 interactions sites per molecule,

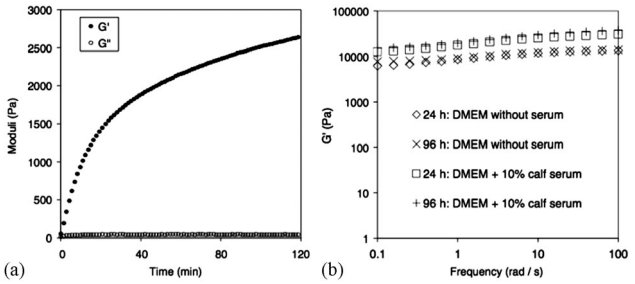

Fig. 13 (a) Time-dependent rheology of 2 wt% MAX1 hydrogel formed upon addition of DMEM to an aqueous solution of MAX1. Closed symbols represent the storage modulus (G') and open symbols represent the loss modulus (G''). (b) Frequency sweep rheology monitoring G' of hydrogels under cell culture conditions in the presence and absence of 10% calf serum. Figure from ref. 53. Copyright 2005 with permission from Elsevier.

respectively, physical crosslinking between MAX1 molecules can occur. This is in addition to any physical fibrillar crosslinks already present in a MAX1 network above the transition temperature. When a heat–cool cycle was run (Fig. 14), an increase in temperature from 5–40 °C resulted in an initial increase in G' (due to folding and self-assembly). G' reached a plateau at 600 Pa after 2 hours. When the temperature was decreased back to 5 °C (1 °C min^{-1}), G' remained constant until ~21 °C, where it began to increase and continued to do so to 5 °C (G' of 2000 Pa). In the absence of borate, this second in increase in gel strength was not observed. The transition between the two gel phases was reversible. An increase in temperature back to 40 °C saw the original G' restored. CD has shown that in the presence of boric acid, β-sheets are observed both above and below the transition temperature. Conversely, below the self-assembly transition temperature (before gelation), random coils are observed. This was also the case when the temperature was lowered after gelation in the absence of borate. The stiffening transition observed is not due therefore to conformational changes at the molecular level. Despite a clear increase in gel strength due to these ion complexation interactions, the gel structure below the transition temperature is much more brittle than a gel under "normal" conditions (40 °C). A strain sweep on a gel at 5 °C showed that the G' deviated from linearity at 1% strain, but for a gel at 40 °C it was observed at 40% strain. A further additive to this boric acid–MAX1 system was shown to compete with MAX1 for the complexation interactions with boric acid/borate anion. When 5 mM of glucose was added, the stiffening process was still observed but when an amount of glucose that could sufficiently consume borate was added (25 and 50 mM glucose were tested), hydrogel stiffening did not occur.[57]

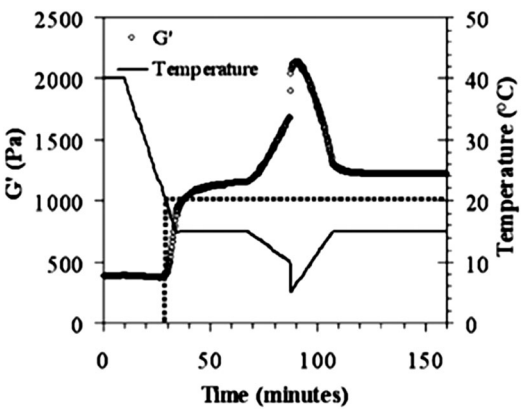

Fig. 14 Dynamic time sweep rheological measurement of 0.5 wt% MAX 1 at pH 9.1 with 10 mM NaCl and 125 mM boric acid. Hydrogel was initially formed at 40 °C. Reprinted with permission from ref. 57. Copyright 2007 American Chemical Society.

As for FmocY and FmocFF, it is clear that the conditions of the trigger of self-assembly have an effect on the final mechanical properties of MAX1 gels. No differences are generally observed on a molecular level, with β-hairpins always forming upon folding of MAX1. Additionally, these β-hairpins assemble to form fibrils with homogenous cross sections of ~3 nm. The differences in mechanical properties must therefore be the result of the self-assembly process on a longer length-scale. The mechanical properties may consequently be governed by microstructure arrangement of the fibres and/or the degree of inter-fibril branching and entanglements.

Conclusions

LMWG are useful molecules that can be used to form gels for a range of applications. There is currently significant interest for example in cell culturing in such hydrogels, as well as potential applications in drug delivery and energy materials. Many LMWG exist. Currently, many LMWG are discovered by accident or by molecular iteration around an already known example. However, it is also clear that sometimes small changes in molecular structure can result in a gelator becoming a non-gelator. As a result, designing a gelator from first principles is extremely difficult.

The mechanical properties of the gels are key for applications. The mechanical properties arise from the properties of the fibres into which the LMWG assemble, the number of cross-links and the microstructure of the fibrous network. The interactions leading to self-assembly are of course 'programmed' into the gelator structure and hence it might be expected that the gel properties are dependent on this information. However, it is clear from the examples that we have discussed above that the mechanical properties are dependent not just on the molecular structure of the LMWG, but also critically on the process by which self-assembly occurs.

The process is a means to go from a 'highly-soluble' environment to a 'poorly-soluble' environment. This can include heat–cool cycles (with of course the rate of heating and cooling being additional variables), changes in pH, changes in solvent quality or the enzymatic removal of a solubilising group. Further differences in process include the presence of salts. Each of the processes has a certain kinetic history. As described above, the kinetics of the assembly affects fibre formation and growth, as well as the number of cross-links in the gel. It is also possible to change the microstructure fundamentally by changing the method of assembly. This can

translate directly into different properties, for example the ability to re-heal after shear. A challenge is clearly preparing gels with reproducible properties. To some degree, since it is clear that even subtle changes in the process can result in different assembly (for example the two apparent pK_as for FmocFF if one titration process is used,[38] and only one if the heat–cool cycle between acid additions is removed[39]), it is likely that reproducibility comes from very careful control over the process. A corollary of this process dependence of the mechanical properties is that it is possible to prepare gels with different properties from a single gelator. Hence, in light of the difficulties in designing gelators from first principles, it may be more fruitful to examine changes in process if a specific set of properties are required as opposed to varying the molecular structure.

Another challenge in this area is that rheological data is often not reported and in some cases where it is, there are significant discrepancies in the results, which are not always discussed fully in comparison to previous reports. This may be due to the mechanical properties being measured in different ways, for example the geometry used for rheological measurements may be parallel plates, vane or cone and plate. Other differences may arise from whether the gel was prepared on the rheometer or transferred after gelation, which can lead to damage to the gel.

In conclusion, we have highlighted how altering the process of assembly for three specific LMWG can translate directly into the formation of gels with different properties. This review is of course not exhaustive, but we anticipate that these observations are likely to be universal and encourage all those in the field to investigate the effect of the process as an additional parameter for controlling the properties of gels prepared from LMWG.

References

1 N. A. Peppas, P. Bures, W. Leobandung and H. Ichikawa, *Eur. J. Pharm. Biopharm.*, 2000, **50**, 27–46.
2 P. Terech and R. G. Weiss, *Chem. Rev.*, 1997, **97**, 3133–3159.
3 L. A. Estroff and A. D. Hamilton, *Chem. Rev.*, 2004, **104**, 1201–1217.
4 M. de Loos, B. L. Feringa and J. H. van Esch, *Eur. J. Org. Chem.*, 2005, 3615–3631.
5 F. M. Menger and K. L. Caran, *J. Am. Chem. Soc.*, 2000, **122**, 11679–11691.
6 C. Tomasini and N. Castellucci, *Chem. Soc. Rev.*, 2013, **42**, 156–172.
7 *Molecular Gels: Materials with Self-Assembled Fibrillar Networks*, ed. R. G. Weiss and P. Terech, Springer, Dordrecht, 2006.
8 A. Brizard, R. Oda and I. Huc, in *Low Molecular Mass Gelators: Design, Self-Assembly, Function*, ed. F. Fages, 2005, vol. 256, pp. 167–218.
9 J. H. van Esch, *Langmuir*, 2009, **25**, 8392–8394.
10 M. L. Muro-Small, J. Chen and A. J. McNeil, *Langmuir*, 2011, **27**, 13248–13253.
11 A. M. Smith, R. J. Williams, C. Tang, P. Coppo, R. F. Collins, M. L. Turner, A. Saiani and R. V. Ulijn, *Adv. Mater*, 2008, **20**, 37–41.
12 H. G. Braun and A. Z. Cardoso, *Colloids Surf., B*, 2012, **97**, 43–50.
13 J. L. Li and X. Y. Liu, *Adv. Funct. Mater.*, 2010, **20**, 3196–3216.
14 C. Chassenieux and L. Bouteiller, in *Supramolecular Chemistry: From Molecules to Nanomaterials*, ed. P. A. Gale and J. W. Steed, John Wiley & Sons, 2012, vol. 2, pp. 517–528.
15 Z. M. Yang, G. L. Liang, M. L. Ma, Y. Gao and B. Xu, *J. Mater. Chem.*, 2007, **17**, 850–854.
16 A. Mahler, M. Reches, M. Rechter, S. Cohen and E. Gazit, *Adv. Mater.*, 2006, **18**, 1365–1370.
17 Z. Yang, G. Liang and B. Xu, *Acc. Chem. Res.*, 2008, **41**, 315–326.
18 A. Brizard, M. Stuart, K. van Bommel, A. Friggeri, M. de Jong and J. van Esch, *Angew. Chem., Int. Ed.*, 2008, **47**, 2063–2066.
19 R. J. Williams, A. M. Smith, R. Collins, N. Hodson, A. K. Das and R. V. Ulijn, *Nat. Nanotechnol.*, 2009, **4**, 19–24.
20 K. A. Houton, K. L. Morris, L. Chen, M. Schmidtmann, L. C. Serpell, G. O. Lloyd and D. J. Adams, *Langmuir*, 2012, **28**, 9797–9806.
21 S. Roy, N. Javid, J. Sefcik, P. J. Halling and R. V. Ulijn, *Langmuir*, 2012, **28**, 16664–16670.
22 D. E. Discher, P. Janmey and Y. L. Wang, *Science*, 2005, **310**, 1139–1143.
23 C. Q. Yan and D. J. Pochan, *Chem. Soc. Rev.*, 2010, **39**, 3528–3540.
24 R. C. Hayward and D. J. Pochan, *Macromolecules*, 2010, **43**, 3577–3584.
25 Z. M. Yang, H. W. Gu, D. G. Fu, P. Gao, J. K. Lam and B. Xu, *Adv. Mater.*, 2004, **16**, 1440–1444.
26 Z. M. Yang and B. Xu, *Chem. Commun.*, 2004, 2424–2425.
27 K. Thornton, A. M. Smith, C. L. R. Merry and R. V. Ulijn, *Biochem. Soc. Trans.*, 2009, **37**, 660–664.
28 Z. A. C. Schnepp, R. Gonzalez-McQuire and S. Mann, *Adv. Mater.*, 2006, **18**, 1869–1872.
29 A. J. Patil, R. K. Kumar, N. J. Barron and S. Mann, *Chem. Commun.*, 2012, **48**, 7934–7936.
30 A. Aufderhorst-Roberts, W. J. Frith and A. M. Donald, *Soft Matter*, 2012, **8**, 5940–5946.
31 L. Chen, S. Revel, K. Morris, L. C. Serpell and D. J. Adams, *Langmuir*, 2010, **26**, 13466–13471.
32 L. Chen, G. Pont, K. Morris, G. Lotze, A. Squires, L. C. Serpell and D. J. Adams, *Chem. Commun.*, 2011, **47**, 12071–12073.
33 S. Roy, N. Javid, P. Frederix, D. A. Lamprou, A. J. Urquhart, N. T. Hunt, P. J. Halling and R. V. Ulijn, *Chem.–Eur. J.*, 2012, **18**, 11723–11731.
34 L. Chen, J. Raeburn, S. Sutton, D. G. Spiller, J. Williams, J. S. Sharp, P. C. Griffiths, R. K. Heenan, S. M. King, A. Paul, S. Furzeland, D. Atkins and D. J. Adams, *Soft Matter*, 2011, **7**, 9721–9727.
35 S. Sutton, N. L. Campbell, A. I. Cooper, M. Kirkland, W. J. Frith and D. J. Adams, *Langmuir*, 2009, **25**, 10285–10291.
36 D. J. Adams, M. F. Butler, W. J. Frith, M. Kirkland, L. Mullen and P. Sanderson, *Soft Matter*, 2009, **5**, 1856–1862.
37 V. Jayawarna, M. Ali, T. A. Jowitt, A. E. Miller, A. Saiani, J. E. Gough and R. V. Ulijn, *Adv. Mater.*, 2006, **18**, 611–614.

38 C. Tang, A. M. Smith, R. F. Collins, R. V. Ulijn and A. Saiani, *Langmuir*, 2009, **25**, 9447–9453.
39 J. Raeburn, G. Pont, L. Chen, Y. Cesbron, R. Levy and D. J. Adams, *Soft Matter*, 2012, **8**, 1168–1174.
40 V. Jayawarna, S. M. Richardson, A. R. Hirst, N. W. Hodson, A. Saiani, J. E. Gough and R. V. Ulijn, *Acta Biomater.*, 2009, **5**, 934–943.
41 M. Zhou, A. M. Smith, A. K. Das, N. W. Hodson, R. F. Collins, R. V. Ulijn and J. E. Gough, *Biomaterials*, 2009, **30**, 2523–2530.
42 W. Helen, P. de Leonardis, R. V. Ulijn, J. Gough and N. Tirelli, *Soft Matter*, 2011, **7**, 1732–1740.
43 D. Berillo, B. Mattiasson, I. Y. Galaev and H. Kirsebom, *J. Colloid Interface Sci.*, 2012, **368**, 226–230.
44 T. Liebmann, S. Rydholm, V. Akpe and H. Brismar, *BMC Biotechnol.*, 2007, **7**, 88–92.
45 R. Orbach, L. Adler-Abramovich, S. Zigerson, I. Mironi-Harpaz, D. Seliktar and E. Gazit, *Biomacromolecules*, 2009, **10**, 2646–2651.
46 R. Orbach, I. Mironi-Harpaz, L. Adler-Abramovich, E. Mossou, E. P. Mitchell, V. T. Forsyth, E. Gazit and D. Seliktar, *Langmuir*, 2012, **28**, 2015–2022.
47 C. Yan, A. Altunbas, T. Yucel, R. P. Nagarkar, J. P. Schneider and D. J. Pochan, *Soft Matter*, 2010, **6**, 5143–5156.
48 G. Pont, L. Chen, D. G. Spiller and D. J. Adams, *Soft Matter*, 2012, **8**, 7797–7802.
49 R. L. Huang, W. Qi, L. B. Feng, R. X. Su and Z. M. He, *Soft Matter*, 2011, **7**, 6222–6230.
50 J. P. Schneider, D. J. Pochan, B. Ozbas, K. Rajagopal, L. Pakstis and J. Kretsinger, *J. Am. Chem. Soc.*, 2002, **124**, 15030–15037.
51 K. Rajagopal, M. S. Lamm, L. A. Haines-Butterick, D. J. Pochan and J. P. Schneider, *Biomacromolecules*, 2009, **10**, 2619–2625.
52 L. Haines-Butterick, K. Rajagopal, M. Branco, D. Salick, R. Rughani, M. Pilarz, M. S. Lamm, D. J. Pochan and J. P. Schneider, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 7791–7796.
53 J. K. Kretsinger, L. A. Haines, B. Ozbas, D. J. Pochan and J. P. Schneider, *Biomaterials*, 2005, **26**, 5177–5186.
54 B. Ozbas, J. Kretsinger, K. Rajagopal, J. P. Schneider and D. J. Pochan, *Macromolecules*, 2004, **37**, 7331–7337.
55 D. J. Pochan, J. P. Schneider, J. Kretsinger, B. Ozbas, K. Rajagopal and L. Haines, *J. Am. Chem. Soc.*, 2003, **125**, 11802–11803.
56 T. Yucel, C. M. Micklitsch, J. P. Schneider and D. J. Pochan, *Macromolecules*, 2008, **41**, 5763–5772.
57 B. Ozbas, K. Rajagopal, L. Haines-Butterick, J. P. Schneider and D. J. Pochan, *J. Phys. Chem. B*, 2007, **111**, 13901–13908.

