Metastable two-component gel—exploring the gel–crystal interface

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This paper reports a two-component system in which molecular recognition rapidly leads to the formation of a homogeneous fibrillar gel that, over a period of hours, aggregates *via* fibre–fibre interactions to yield microcrystals—providing insight into the relationship between nanoscale gels and microscale crystals.

The assembly of molecular scale building blocks is a powerful tool for bottom-up fabrication of nanomaterials.[1] Self-assembly of low molecular weight compounds into gels has been of particular interest.[2] Most gels have fibrillar nanostructures, reminiscent of the fibrils formed from protein aggregation in Alzheimer's disease.[3] Two-component gels rely on the presence of two complementary components, which form a complex that subsequently assembles into a nanoscale gel-phase network.[4,5] Such two-component materials have exquisite tunability and it is possible to obtain a high degree of morphological control.[6] In this communication, we report a metastable two-component gel that slowly converts into a microcrystalline form—ideal for probing mechanisms of molecular aggregation and the gel–crystal interface. Gels are usually formed *via* slow cooling of a solution phase, and the relationship between gelation and crystallization is therefore particularly intriguing,[7] with direct observations of phase transitions being particularly valuable.[8] Although it is well known that gels are a kinetically trapped state of matter, there are few examples in which a gel spontaneously evolves into a more thermodynamically stable crystalline form over short timescales. In a key paper also published this year, Tang and co-workers claim the first example of spontaneous crystallisation during the storage of a two-component hydrogel[9]—in this paper we report microcrystallisation within a two-component organogel.

We have previously investigated gelators based on lysine,[10] and to extend this work, we synthesized compound **1** (Fig. 1), in which pyrene chromophores were appended to the amino groups of L-lysine methyl ester.† This compound did not, in its own right, form gels. However, inspired by work from the groups of Bag and Maitra in particular,[11] we mixed an acceptor molecule, trinitrofluorenone (**TNF**), with **1**, in order to form a donor–acceptor complex based on π–π interactions. On doing this, gelation of aromatic solvents occurred—the pale yellow solution turned into a bright red gel, consistent with the formation of a donor–acceptor complex between the pyrene units and **TNF**. The optimal molar ratio of the two components for gelation was 1 : 2 (**1** : **TNF**), corresponding to a donor : acceptor ratio of 1 : 1. Adding more **TNF** reduced the thermal stability of the gels, while samples containing less **TNF** were unable to induce macroscopic gelation.

We monitored the thermal stability of freshly made samples of the **1** : **TNF** (1 : 2) gel using simple, reproducible tube inversion methodology,[12] and generated a phase diagram in an apolar aromatic solvent (styrene–divinylbenzene, 90 : 10, Fig. 2).‡ On increasing the concentration of the two-component mixture, the T_{gel} value increased until a plateau value of *ca.* 34 °C was achieved at a concentration of *ca.* 20 mM (of **1**). This concentration corresponds to a total gelator loading, accounting for both components, of *ca.* 2.7% w/v. Interestingly, the red colour of the gel, which is associated with the donor–acceptor interaction[11] between **1** and **TNF** (Fig. 3C) persisted, even in the sol phase above the T_{gel} value (Fig. 3B). Indeed, the solution remained red until a temperature of *ca.* 55 °C, and only above this temperature did the solution become pale yellow (Fig. 3A), indicating the loss of donor–acceptor interactions. This demonstrates that donor–acceptor interactions exist even above the T_{gel} value, even though the aggregates at these elevated temperatures are clearly insufficiently extensive to fully support a gel-phase network.

On standing for longer periods of time (*ca.* 24 h), these gels were observed to be metastable. The previously homogeneous red transparent gels (Fig. 3C) became inhomogeneous, having dark/cloudy crystalline regions and beginning to leach solvent (Fig. 3D). We decided to investigate this further using field emission gun scanning electron microscopy (FEGSEM), in order to provide an insight into the nanoscale processes which underpin metastability. Within the aged gel, individual nanofibres, *ca.* 20 nm in diameter, were clearly observed (Fig. 4A). This kind of object usually underpins gelation.[2] However, it was also evident that these individual nanofibres were laterally aggregating into microscale fibres (Fig. 4A and 4B). Furthermore, Fig. 4C clearly demonstrates the presence of significant microcrystalline aggregates within the sample. These are presumably responsible for the cloudiness, and the loss of gel-phase properties in this aged sample. These images therefore

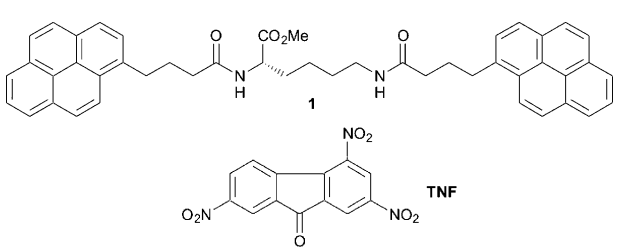

Fig. 1 Compound **1** and **TNF**—a two-component gelation system.

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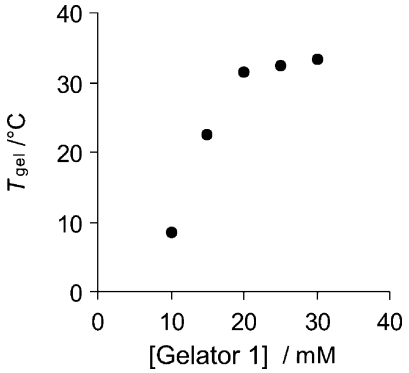

Fig. 2 T_{gel} values in styrene–divinylbenzene (DVB) (9 : 1) for gelation system **1** : **TNF** (1 : 2) estimated by tube-inversion methodology.

provide direct insight into the metastability of the gel—individual nanoscale fibres normally associated with gel-phase assemblies aggregate laterally *via* a kinetically slow process to form larger microscale fibres which ultimately crystallize into objects, incapable of supporting a sample-spanning network.

Clearly this process is related to Ostwald ripening, in which smaller kinetically favoured 'crystals' gradually aggregate into more thermodynamically favourable larger 'crystals'. Although Ostwald ripening is well known in crystallisation,[13] the generation of crystals directly from a gel is rare.[7c–e,11] It is noteworthy that, like us, Tang and co-workers also made use of a two-component gelation system to observe this phenomenon.[11]

Interesting analogies can be made with the crystallisation of polymers, which can be considered to occur from a metastable polymer melt, that evolves over a free energy barrier, the height of which controls the crystallisation rate.[14] We argue that similar factors should control the evolution of gels into crystals, because gels are actually supramolecular polymers.

To probe the molecular-level organization, ^{1}H NMR spectra were obtained for the gels freshly formed by the two-component complex with 1 : 2 stoichiometry, in d_6-benzene, at concentrations (of **1**) of 6, 12 and 18 mM. Clearly molecules bound fully within the gel network are NMR silent due to relaxation effects. The NMR resonances observed in such gels therefore reflect the fraction of mobile molecules and oligomers, and hence report on the molecular recognition pathways between molecules which ultimately underpin gelation itself.[15]

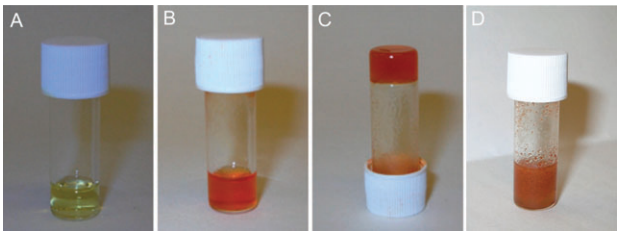

Fig. 3 Photographs of gelation system **1** : **TNF** (1 : 2) in styrene–DVB (9 : 1) at A, 80 °C; B, 50 °C; C, 20 °C; D, after 24 h at 20 °C.

The aromatic regions of the spectrum, although containing overlapping peaks, were strongly perturbed, with significant upfield shifts of the TNF protons and shifts of the aromatic pyrene protons on compound **1** (spectra not shown)—consistent with the proposed π–π donor–acceptor interactions. More interestingly, however, the N–H protons of **1** were also perturbed on increasing the concentration of the **1** : **TNF** complex (Fig. 5). The N–H peaks were observed to shift downfield as the concentration of complex increased, with one N–H peak shifting from *ca.* 4.5 ppm to 4.7 ppm, consistent with the involvement of the N–H proton in intermolecular hydrogen bond interactions. This was initially surprising, as no direct interaction was expected between **TNF** and the amide N–H groups of **1**. On complex formation, therefore, the N–H protons of **1** must become involved in intermolecular hydrogen bonding interactions with C=O groups. However, in their own right, these intermolecular hydrogen bond interactions are not sufficient to support gelation, as compound **1** alone was not able to form gels. We therefore propose that the donor–acceptor interactions between **1** and **TNF** organize the molecular building blocks such that hydrogen bonding molecular recognition pathways involving the amides are also switched on—reinforcing gelation.

In summary (Fig. 6), compound **1** forms a two-component gel with **TNF**. Donor–acceptor interactions drive complexation, between the building blocks, and gelation is subsequently reinforced by hydrogen bond interactions. This molecular recognition process occurs rapidly (minutes) leading to a homogeneous gel. Then over a period of hours, a kinetically slow process occurs in which the fibres aggregate laterally into microcrystalline domains and the homogeneity of the gel is lost. Intriguingly, it has been suggested that a similar mechanism occurs in amyloidogenesis, with rapid formation of

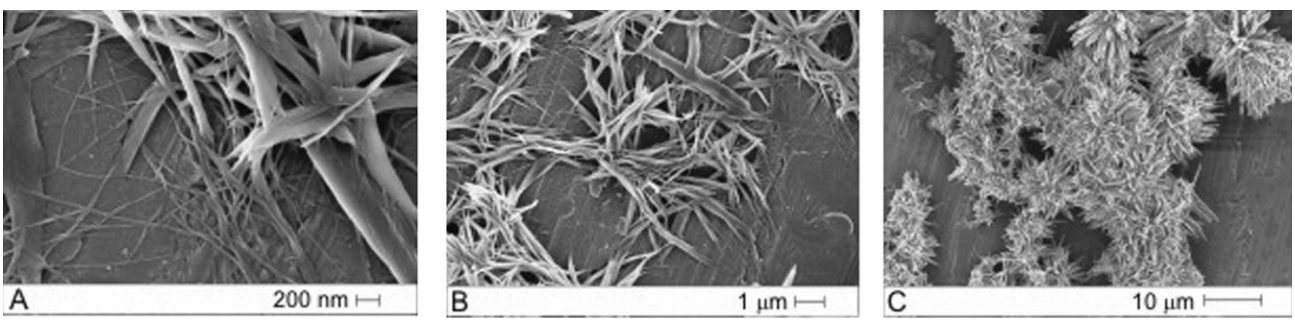

Fig. 4 FEGSEM images of **1** : **TNF** (1 : 2) dried from styrene–DVB (9 : 1) after ageing. (A) High magnification shows nanofibres merging to form microfibres. (B) Intermediate magnification shows microfibres and some nanofibres. (C) Low magnification shows microcrystalline deposits of microfibres.

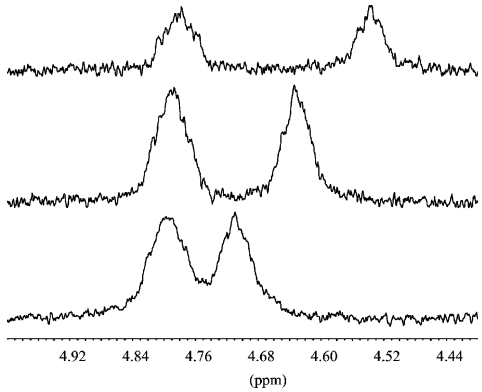

Fig. 5 ^{1}H NMR spectra in d_6-benzene of increasing concentrations of **1** : **TNF** complex. Top, **1** : **TNF** (6 : 12 mM); middle, **1** : **TNF** (12 : 24 mM); bottom **1** : **TNF** (18 : 36 mM).

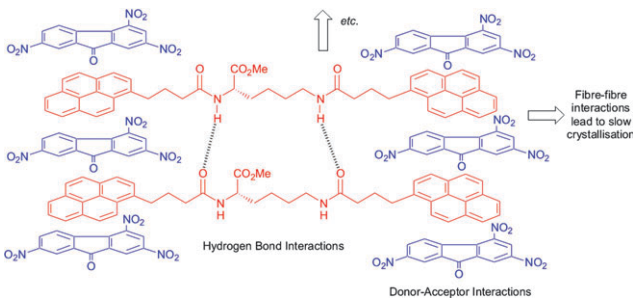

Fig. 6 Schematic outline diagram of mode of gelation of **1** : **TNF**.

protofibrils, followed by a slower process of their lateral aggregation.[3] As such, these preliminary results provide an insight into the interface between gel-phase nanoscale materials and crystalline microscale structures, and suggest how one may evolve into the other. It is anticipated that further detailed kinetic studies of metastable gels will provide deeper insights, which will be of relevance in biological soft matter science.[16]

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Notes and references

† Synthesis and characterisation of compound **1**. L-Lysine methyl ester dihydrochloride[17] (1.03 g, 4.30 mmol) was suspended in dichloromethane (40 ml). NEt_3 (1.26 ml, 8.93 mmol) was added, followed by 1-pyrenebutyric acid (1.45 g, 5.0 mmol). The mixture was stirred under a nitrogen atmosphere for 5 min. The mixture was cooled to 0 °C, then HOBt (1.21 g, 8.93 mmol) and DCC (1.84 g, 8.93 mmol) were added simultaneously as a mixture of solids. The reaction was allowed to return to room temperature and stirred for 16 h. The precipitate was removed by filtration and washed with DCM (50 ml) followed by MeOH (100 ml) to leave the pure product as a beige solid with a yield of 2.60 g (86% yield). Mp: 133–135 °C; α_D^{293} +4.0 (c = 1, CH_2Cl_2); R_f: 0.67 (CH_2Cl_2 : MeOH 90 : 10); m/z (ESI) $C_{47}H_{44}N_2O_4$ [M] requires 700.88; found 723.4 (100%, $[M + Na]^+$), 724.4 (45%), 701.3 (11% $[M + H]^+$); δ_H (400 MHz, $CDCl_3$) 7.50–7.21 (18H, m, Ar*H*), 7.06–7.03 (4H, t, J = 8.0 Hz, CH_2Ar), 5.78 (1H, d, J = 7.6 Hz, CON*H*), 5.20 (1H, t, J = 5.5 Hz, CON*H*), 3.84 (1H, m, COC*H*R), 2.59 (4H, m, NHCOCH_2), 2.56 (2H, m, CH_2NHCO), 1.57–0.54 (10H, m, CH_2); δ_C (400 MHz, $CDCl_3$) 173.8 (*C*O_2Me), 173.3 (*C*ONH), 173.2 (*C*ONH), 132.7, 130.7, 130.2, 130.0, 127.8, 127.4, 127.3, 127.0, 126.2, 126.2, 125.1, 125.0, 124.4, 123.9, 123.5, 123.0 (all pyrene), 52.5 (*C*OCHR), 51.7 (*C*O_2CH_3), 38.9 (CH_2CH_2NH), 36.8, 33.9, 31.9, 29.3, 26.2, 22.7 (all *C*H_2); ν_{max} (solid phase) 3319 m (NH), 2928 m, 2850 m (CH_3, CH_2), 1754 m (*C*OOCH_3), 1627 s (CONH), 1538 s (CONH). Trinitrofluorenone (TNF) was synthesised according to literature methodology and had data in agreement with those previously published.[18]

‡ This solvent was used in order to enable the study of polymer–gel hybrids, the results of which will be reported in detail elsewhere.

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