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Catalytic Hydrogelators

Synthesis of a Double-Network Supramolecular Hydrogel by Having One Network Catalyse the Formation of the Second

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Abstract: Self-assembly of biomolecules catalytically controls the formation of natural supramolecular structures, giving highly ordered complex materials. Such desirable hybrid systems are very difficult to design and construct synthetically. A hybrid double-network hydrogel with a maximum storage modulus (G'_{\max}) of up to 55 kPa can be synthesized by using a self-assembled hydrogel that catalyses the formation of another kinetically arrested hydrogel network. Tuning of the catalytic efficiency of the first network allowed spatiotemporal control over the evolution of the second network and the resulting mechanical properties. The distribution of active catalytic sites was optimal for catalytic fibres prepared at the minimum gelation concentration (MGC) to give the double-network hydrogel with highest storage modulus. This approach could be very useful in preparing complex hierarchical structures with tailor-made properties.

In nature, orthogonal existence of different self-assembled systems contributes to the formation of complex hierarchical structures with functional properties.^[1] By virtue of non-covalent interactions, microtubules and actin filaments can undergo orthogonal assembly/disassembly, allowing the cytoskeleton to have tuneable mechanical properties against deformations.^[1,2] In sponges, the fibres of self-assembled silicatein molecules catalyse the formation of another inorganic silica network to form a biosilica skeleton. The intertwined double-network system in biosilica provides it with materials properties (mechanical, optical, self-healing) far above those of current human made materials.^[3] Currently, these strategies are un-

available for synthetic materials. Such multicomponent hybrid systems can be used to make new advanced materials with tailor-made properties.^[3,4] However, construction of hybrid orthogonal systems based solely on non-covalent interactions delicately depends on the extent of recognition of same kind of molecules to self-assemble in a mutually exclusive way. Moreover, other parameters, such as temperature, pH, stimuli, and solvents, which are hard to control, present problems in the design and characterisation of such systems. Owing to these limitations, very few successful examples of non-covalent hybrid systems have been reported.^[4] Thus it is essential to develop new methods for construction of such hybrid systems. One of the possible ways would be to follow the strategy adopted by nature, where one supramolecular system can trigger or direct the formation of another system, allowing spatiotemporal control over the formation of complex hierarchical architectures and their properties.

Following the same lines, herein we present the formation of a hybrid double-network (DN) hydrogel by using a hydrogel made of **1** to catalytically control the formation of a second hydrogel of **4** (Scheme 1). The organisation of the second network could be modulated by controlling the kinetics of the formation of the second network (**4**) by varying the concentration of the catalytic hydrogelator. The double-network hydrogel showed high mechanical stiffness depending on the rate of formation of the second network and the synergistic effect of the two orthogonally assembled fibres present in the same system.

Catalytic control over formation and self-assembly of hydrogelator molecules of **4** allows to control and fine-tune the properties of their self-assembled structures by kinetically arresting them in metastable states.^[5,6] Formation and self-assembly of a trishydrazone hydrogelator **4** from the non-assembling trishydrazide **2** and benzaldehyde **3** building blocks through the formation of a hydrazone bond has been described.^[6,7] The spatial and temporal control over the hydrogel network formation and its mechanical properties has also been reported.^[8] In aqueous environments, the rate of formation of **4** could be enhanced by using either acid or aniline catalysis, resulting in pronounced effects on the rate of gelation, fibre morphology, and macroscopic gel properties.^[7-9]

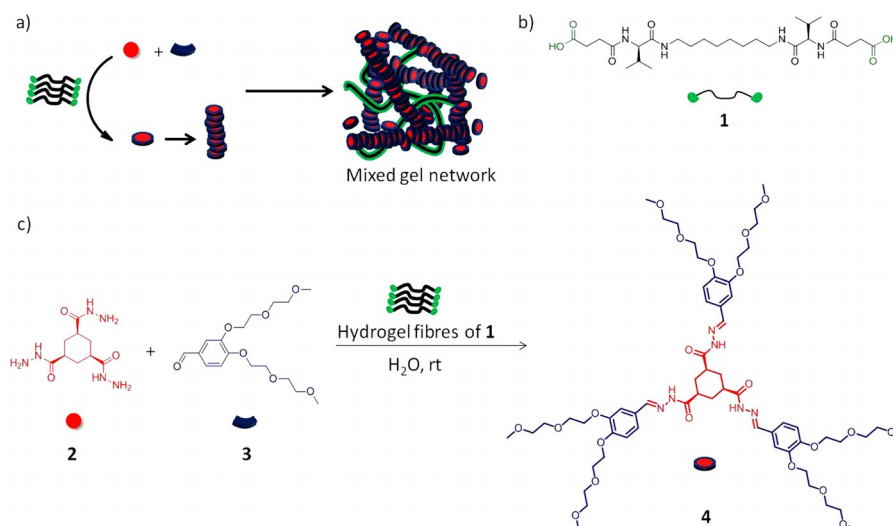
On the other hand, hydrogelators functionalized with catalytic groups can exhibit enhanced enzyme like catalytic efficiency upon self-assembly, owing to high local concentration of the catalytic groups and formation of hydrophobic pockets.^[10] The fibres of bola-amphiphilic hydrogelator **1** were re-

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Scheme 1. a) Representation of the formation of hydrogel **4** by using hydrogel **1** as a catalyst, and subsequent orthogonal gelation of two hydrogel networks; b) chemical structure of **1**; c) formation of **4** from its precursors **2** and **3** using hydrogel **1** as a catalytic hydrogelator.

cently reported as an effective acid catalyst for deacetalisation of dimethylacetal benzaldehyde to benzaldehyde.^[11] In the current work, we chose to use fibres of **1** as an acid catalyst for the formation of gelator **4**. Bola-amphiphilic hydrogelator **1** was dissolved in pure water by gentle heating, followed by slow cooling to ambient temperature, leading to the formation of a transparent hydrogel with a minimum gelation concentration (MGC) of 6.0 mM. Vortexing the hydrogel of **1** at 1200 RPM for one minute gave a viscous solution with dispersed fibres of **1**, which do not turn into self-supporting gels for at least three hours. In contrast, mixing the soluble precursors **2** (0.25 mL, 10 mM) and **3** (0.25 mL, 60 mM) with the dispersed fibres of **1** (0.5 mL), resulted in a self-supporting turbid gel in around 10 minutes. Mixing **2** and **3** in the same amount in absence of **1** led to the formation of a turbid suspension only after 100 minutes.

These observations indicate that the fibres of **1** could be acting as an effective catalyst for the formation of the hydrogel of **4**. In pure water, the solubility of compound **1** is 2.5 mM, resulting in a mixture with a pH around 5.5. At this concentration, compound **1** catalyses the reaction of **2** and **3** to give a self-supporting gel tested by inverted vial method in about 50 minutes (Supporting Information, Figure S1 a). Between $[1]=2.5$ and 6.0 mM (the MGC), suspensions of **1** contain fibres but do not gelate. Within this range, the pH is constant at 5.5. Using suspensions made with $[1]=2.5$ –6.0 mM to catalyse gel formation from **2** and **3** resulted in a reduction in gelation time with increasing concentration of **1**, down to 7 minutes when approaching the MGC. We speculate that, at concentrations of **1** above 2.5 mM, the formed fibres of **1** have acidic interfaces resulting in a decrease in the pH locally near the surface of the fibres but not contributing to the bulk pH of the solution. The decrease of the gelation times suggest that the high local concentration of acid groups on the surface of the fibres of **1** catalyse the formation of **4**, leading to accelerated hydrogel formation.

After observing the catalytic effect of fibres of **1** on the formation of hydrogel of **4**, different microscopic techniques were used to study the morphology of the fibrillary network. Confocal laser scanning fluorescence microscopy (CLSM) was used to observe the networks in the mixed system. A fluorescein-labelled aldehyde was incorporated into the hydrogel network of **4**,^[6,7] while Nile red was used to visualize the fibre network of **1**. We could observe the evolution of the fibre network of **4** over time, superimposed on the network of **1**. The formation of hydrogel **4** was mainly seen around the network of the hydrogel **1** and not in the bulk where the fibres of **1** were absent. This indicates that the high catalytic conversion carried out by fibres of **1** allows rapid organisation of the molecules of **4** in their vicinity (Figure 1).

Atomic force microscopy (AFM) and transmission electron microscopy (TEM) images revealed straight fibres of 1 μm in length and 6 nm in diameter for compound **4**. Compound **1** shows twisted fibres which were several micrometres in length and around 6 nm in diameter (Figure 2a,b). The mixed system clearly showed a network of the two different kinds of fibres (Figure 2c,d; Supporting Information, Figures S2–S6). Twisted fibres several micrometres in length were assigned to the hydrogel of **1** (Figure 2) and the straighter fibres were assigned to compound **4** (Figure 2). With the help of confocal microscopy, TEM, and AFM, we could thus confirm the formation of a network consisting of two different types of fibres, which strongly suggest that orthogonal self-assembly of **1** and **4** into a double, interpenetrating network has occurred. The co-existence of the two types of fibres was also confirmed by wide angle X-ray diffraction (WAXD). The WAXD pattern of the hydrogel of **1** was amorphous, whereas that of **4** showed main reflections at 27.0 and 15.9 \AA . The pattern of the mixed system was an overlap of the two individual systems without any emergence of new peaks (Supporting Information, Figure S7).

After confirming the formation of a double-network consisting of individual fibres of **1** and **4**, we decided to investigate

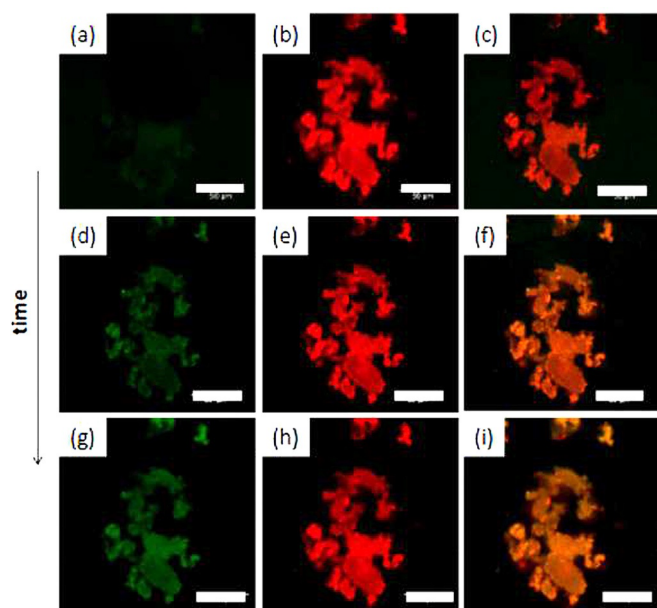


Figure 1. Confocal micrographs (Scale bar: 50 μm) of in situ evolution of hydrogel 4 network ($\lambda_{\text{exc}} = 488 \text{ nm}$): a),d),g) around the network of hydrogel 1 ($\lambda_{\text{exc}} = 543 \text{ nm}$): b),e),h) at the minimum gelation concentration (MGC). c),f),i) Overlaid images for the two hydrogelators ($\lambda_{\text{exc}} = 488 \text{ nm}$ and 543 nm).

the effect of formation of the second network on the mechanical properties of the system. The mechanical behaviour of the DN hydrogels was investigated by rheology. The maximum G' for hydrogel 1 at its MGC and that of a fibre network of 4 (in absence of catalyst at pH 7) was 0.3 kPa and 4.0 kPa, respectively. The G'_{max} (10 kPa) and gelation time for hydrogel 4 formed at pH 5.5 in the absence of catalyst was comparable to that in presence of compound 1 at 2.5 mM (Supporting Information, Figure S1). However, the observed maximum storage modulus (G'_{max}) of the DN hydrogel increased with increasing initial concentration of 1 (until MGC) as seen from the rheology data, while the gelation time for the hybrid system decreased (Figure 3; Supporting Information, Figure S8).

The maximum G' for the DN hydrogel was 55 kPa when fibres of hydrogel 1 were used at MGC for the formation of hydrogel 4. Interestingly, when hydrogels of 1 above the MGC (12 mM $>$ [1] $>$ 6.0 mM) were used for catalysis, even though the gelation time was similar to that of DN hydrogel with com-

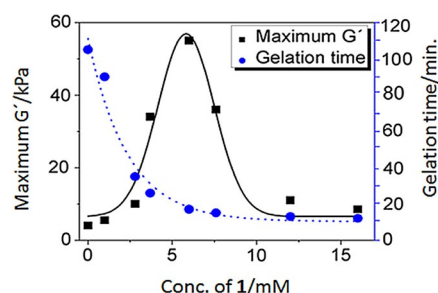


Figure 3. Maximum storage modulus (G') and gelation time at different concentrations of 1. The applied concentration of 2 and 3 was 10 mM and 60 mM, respectively, for all of the experiments. The gelation time was defined as the moment that G' measures 95% of the plateau value (Supporting Information, Figure S9). The lines are a guide to the eye.

ound 1 at MGC, the final G'_{max} values were lower. After reaching a plateau value during formation, the G'_{max} values of the DN hydrogels remained unchanged even after 24 hours, suggesting that the self-assembled materials are kinetically trapped (Supporting Information, Figure S9). No changes in the rate, conversion, or the fibre morphology of 4 was observed by NMR studies, and AFM and TEM images when fibres of 1 prepared at 6 mM and 12 mM were used for catalysis, so possibly the crowding by the fibres of 1 at high concentrations might hinder the uniform proliferation and organisation of the fibres of 4, resulting in lower overall mechanical strength (Supporting Information, Figures S12a, S2–S5, S15). However, the catalytic turnover number for aldehyde consumption was measured as $1.0 \times 10^{-2} \text{ s}^{-1}$ with hydrogel 1 prepared at 6 mM and $0.5 \times 10^{-2} \text{ s}^{-1}$ for hydrogel 1 prepared at 12 mM (Supporting Information, Figure S12a). This suggested that increase in the concentration of 1 did not result in proportional increase in the active sites on the surface of the fibres. When a similar experiment was performed using different volumes of hydrogel 1 prepared at MGC, the turnover rate was higher in systems with higher amount of catalytic fibres (employing same final concentrations of the precursors 2 and 3; Supporting Information, Figure S12b). For different volumes of hydrogel 1 at MGC (0.25 mL, 0.30 mL, and 0.40 mL), the catalytic turnover rates were $1.0 \times 10^{-2} \text{ s}^{-1}$, $1.5 \times 10^{-2} \text{ s}^{-1}$, and $3.8 \times 10^{-2} \text{ s}^{-1}$, respectively. This suggests that the number of catalytic sites exposed on the surface of hydrogel 1 is optimal at its MGC. Using TEM we

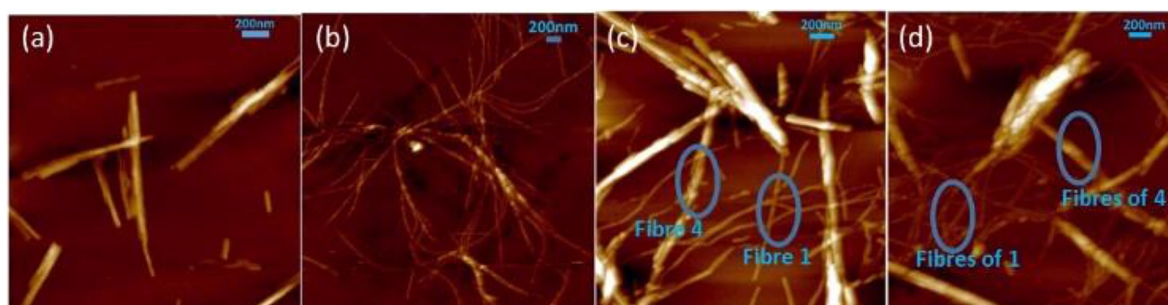


Figure 2. AFM images of the gel fibres. a) Straight fibres for 4 (scale bar: 200 nm); b) twisted fibres for 1 at MGC (scale bar: 200 nm); c),d) presence of two co-existing fibres in the DN system (scale bar: 200 nm).

investigated possible changes occurring in the morphology of the supramolecular structures at higher concentrations of compound **1**, effecting the availability of active sites. The average diameter of fibres at 6 mM of **1** was found to be around 6–8 nm whereas thicker fibres of diameter around 10 nm were observed for 12 mM samples (Supporting Information, Figures S13, S14). This difference in morphology could result in the masking of the catalytic groups of compound **1** at 12 mM. Hence it could be said that the distribution of the active sites on the surface of the fibres are optimal for compound **1** at MGC.

In conclusion, we show how orthogonal assembly of two supramolecular gel networks into a double-network material can be achieved by having one network catalyse the formation of the other. We have demonstrated the use of a catalytic hydrogelator to attain controlled spatiotemporal organisation of a double-network hydrogel. The final DN hydrogel showed high mechanical strength which could be modulated by changing the initial concentration of the catalytic fibres. The catalytic fibres of **1** at MGC tend to have an optimal distribution of catalytic sites for the formation of **4** to give a DN hydrogel with a storage modulus of 55 kPa. This approach can prove to be effective in formation of spatially and temporally controlled hybrid materials with complex hierarchical architecture and desired properties.

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