Annealing Supramolecular Gels by a Reaction Relay

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Supporting Information

Supplementary Figures

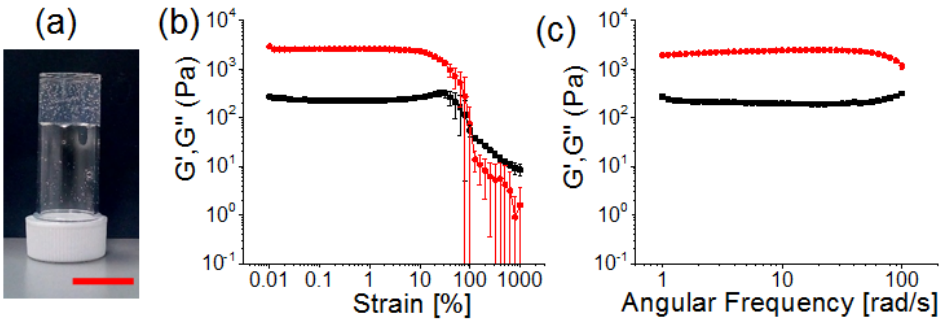

Figure S1. (a) Photograph of the DMSO-H_2O gel of **1** (the scale bar represents 1.7 cm). The white structures in the gel are air bubbles, not precipitation. (b) Strain and (c) frequency sweep experiments of the hydrogel of **1** (the red data represents G′ and the black data G″). In all cases, concentration of **1** is 2 mg/mL and solvent is 20/80 DMSO/water (v/v).

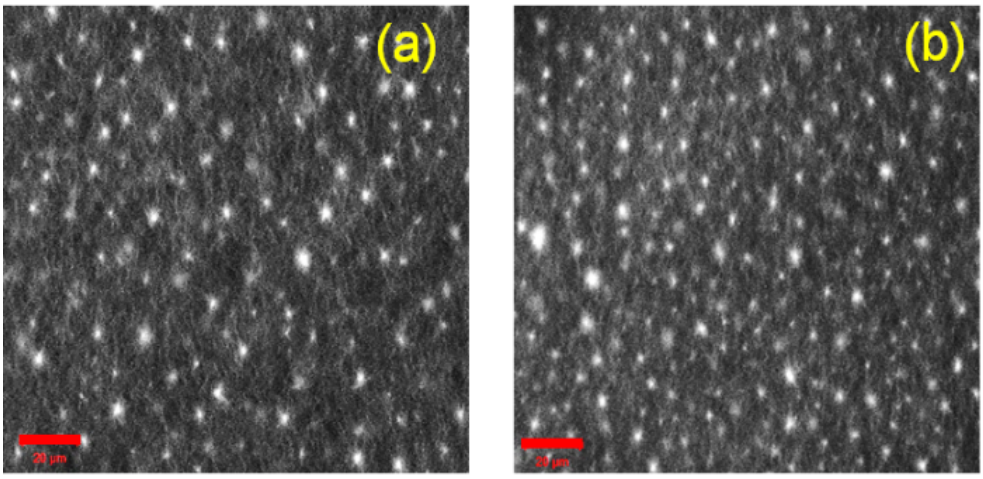

Figure S2. Confocal fluorescence microscopy images (the scale bars represent 20 μm) of the DMSO-H_2O gel of **1** (concentration = 2 mg/mL). Solvent is 20/80 DMSO/water (v/v).

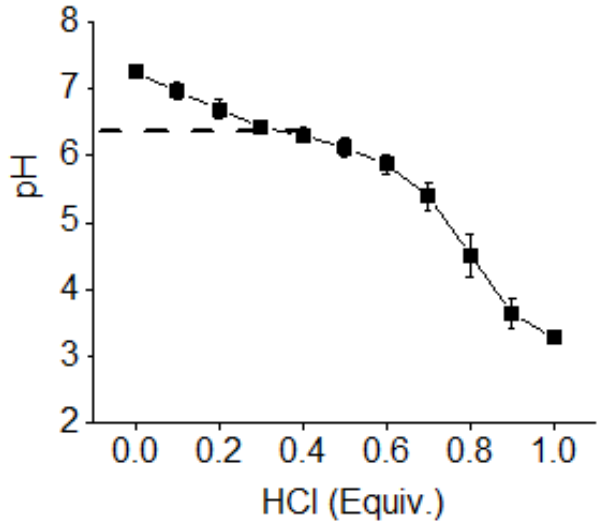

Figure S3. Determination of apparent pK_a of gelator **1** in 20/80 DMSO/water (v/v). The plateau is taken to represent the apparent pK_a value.

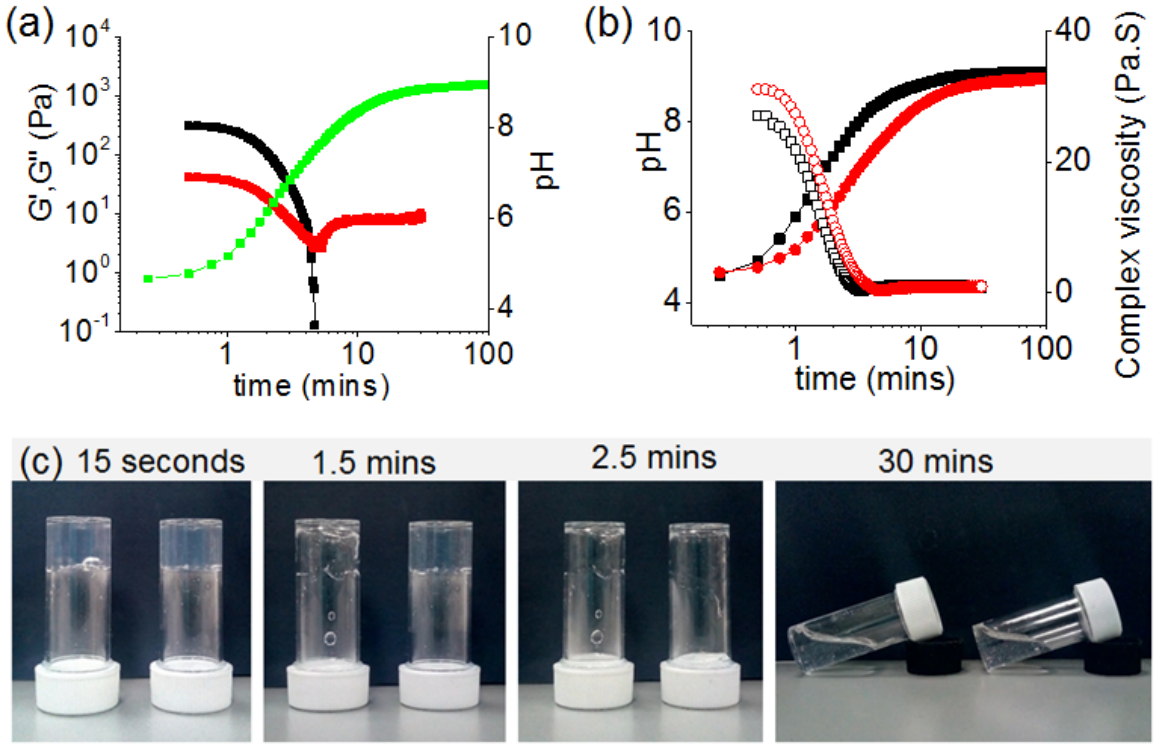

Figure S4. (a) Variation of pH (green), G′ (black), G″ (red) with time for **1** in presence of urea-urease reaction involving initial reaction conditions: [urease] = 0.2 mg/mL, [urea] = 0.01 M. (b) Variation of pH (closed symbol) and complex viscosity (open symbol) with time for **1** in presence of urea-urease reaction involving initial reaction conditions: [urease] = 0.2 mg/mL, [urea] = 0.02 M (black data); [urease] = 0.2 mg/mL, [urea] = 0.01 M (red data). (c) Photographs showing the phase change of **1** with time when aqueous solution of the enzyme was added to the mixture of **1** in DMSO and urea. In each photograph, from left to right: [urease] = 0.2 mg/mL and [urea] = 0.02 M, [urease] = 0.2 mg/mL and [urea] = 0.01 M. For (a)-(c) solvent is 20/80 DMSO/water (v/v) and [**1**] = 2 mg/mL.

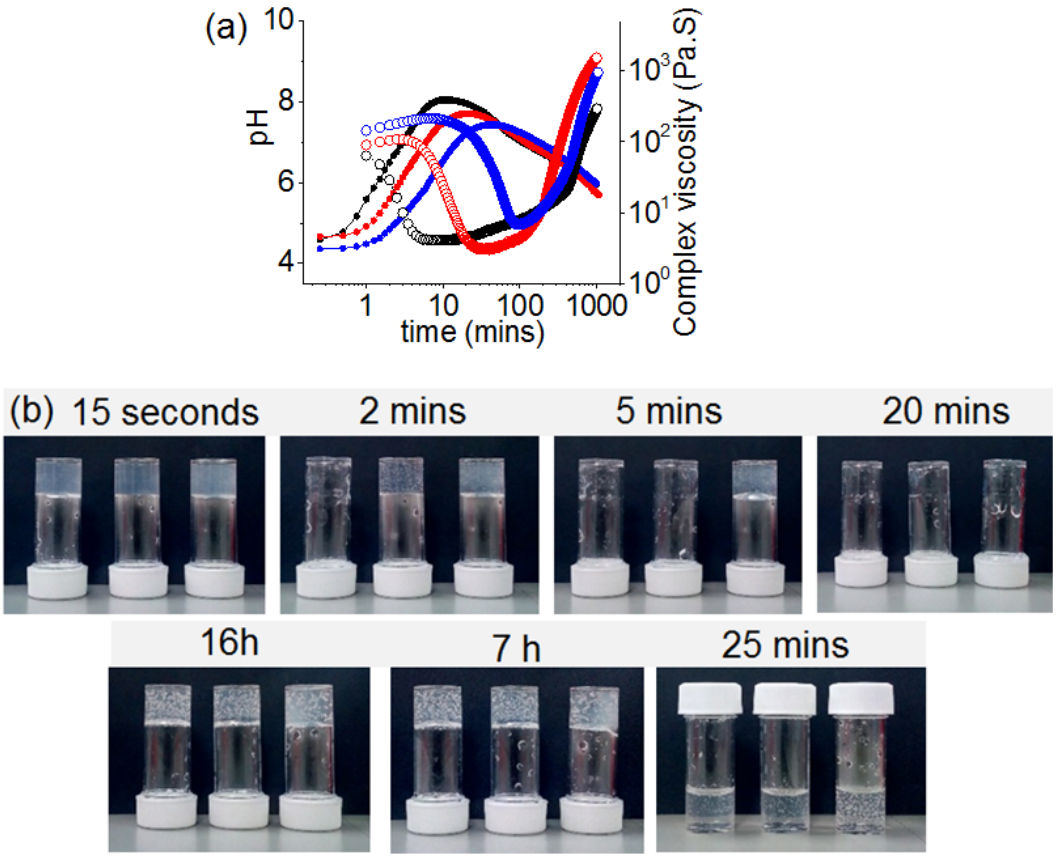

Figure S5. (a) Variation of pH (close symbol) and complex viscosity (open symbol) for **1** in presence of urea-urease reaction under different conditions: (black) [urease] = 0.2 mg/mL, [urea] = 0.02 M, volume of methyl formate = 100 μL; (red) [urease] = 0.2 mg/mL, [urea] = 0.01 M, volume of methyl formate = 100 μL; (blue) [urease] = 0.2 mg/mL, [urea] = 0.02 M, volume of methyl formate = 150 μL. (b) Photographs showing the phase change of **1** with time from the enzymatic reaction in presence methyl formate. In each photograph, from left to right: [urease] = 0.2 mg/mL, [urea] = 0.02 M, volume of methyl formate = 100 μL; [urease] = 0.2 mg/mL, [urea] = 0.01 M, volume of methyl formate = 100 μL; [urease] = 0.2 mg/mL, [urea] = 0.02 M, volume of methyl formate = 150 μL. For (a) and (b) solvent is 20/80 DMSO/water (v/v) and [**1**] = 2 mg/mL. The white structures in the gels are air bubbles, not precipitation.

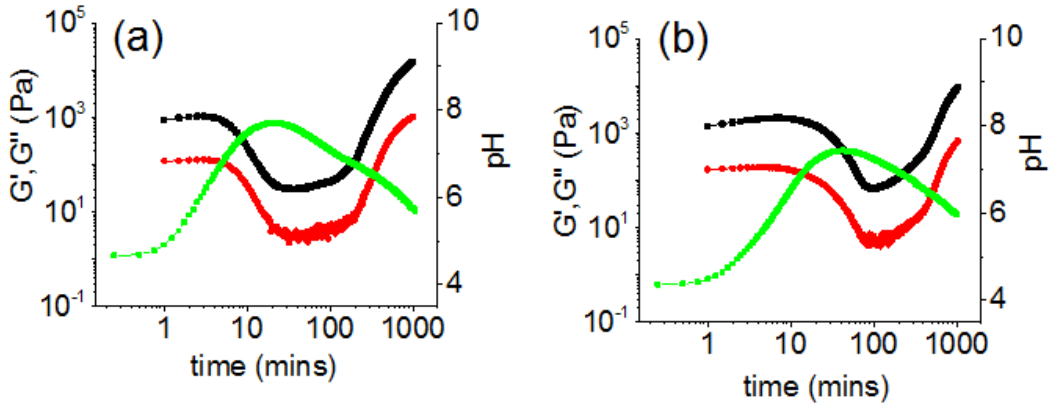

Figure S6. Variation of pH (green), G′ (black) and G″ (red) with time for **1** (2 mg/mL) from the enzymatic reaction in presence methyl formate. Initial reaction condition is: (a) [urease] = 0.2 mg/mL, [urea] = 0.01 M, volume of methyl formate = 100 μL; (b) [urease] = 0.2 mg/mL, [urea] = 0.02 M, volume of methyl formate = 150 μL. In both cases, solvent is 20/80 DMSO/water (v/v).

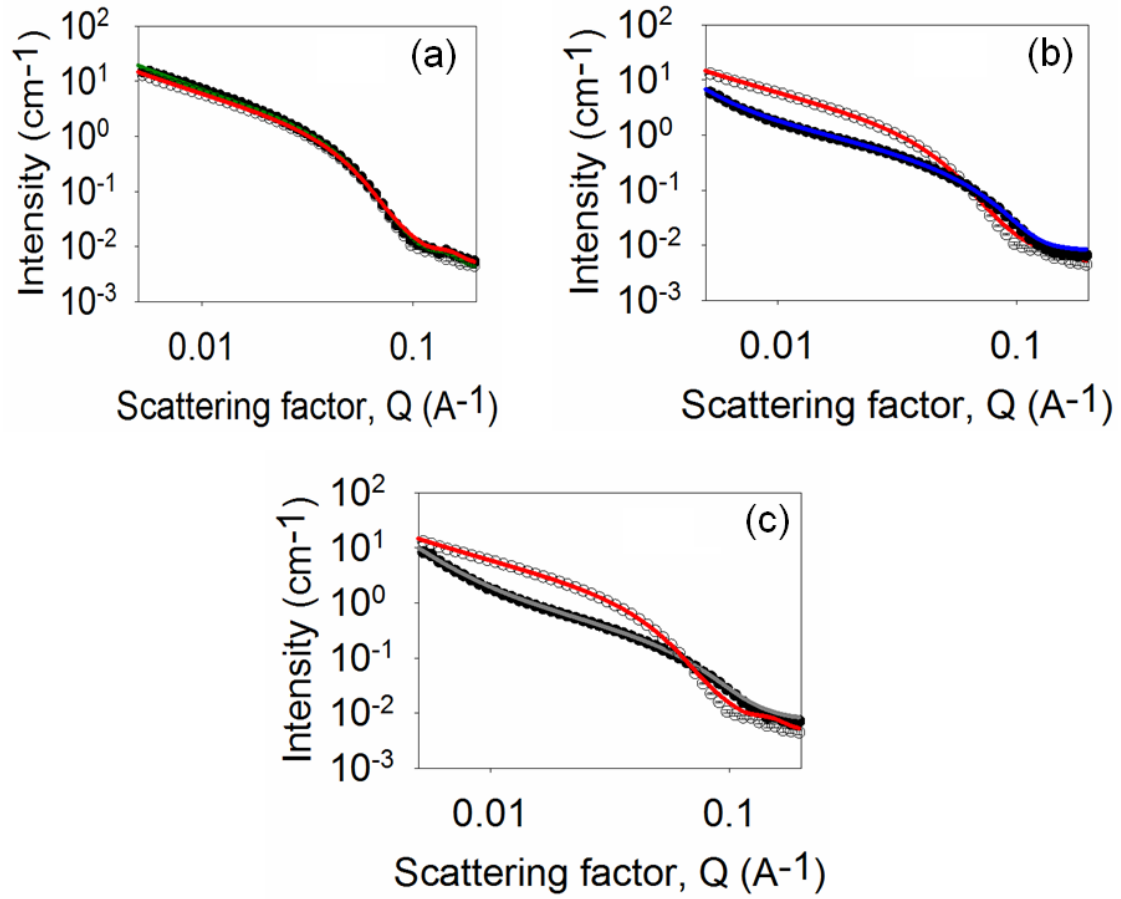

Figure S7. SANS scattering data for the hydrogels of **1** prepared under different conditions. (a) gels of **1** in absence (close symbol) and presence (open symbol) of urease (0.2 mg/mL). The lines represent the fit to the data of the gels prepared in absence (green) and presence (red) of urease. (b) Gels of **1** (in presence of urease) obtained before (open symbol) and after annealing (close symbol) involving initial conditions [urease] = 0.2 mg/mL, [urea] = 0.01 M, volume of methyl formate = 100 μL. The lines represent the fit to the data before (red) and after (blue) annealing. (c) Gels of **1** (in presence of urease) obtained before (open symbol) and after annealing (close symbol) involving initial conditions [urease] = 0.2 mg/mL, [urea] = 0.02 M, volume of methyl formate = 150 μL. The lines represent the fit to the data before (red) and after (gray) annealing. In all cases, concentration of **1** is 2 mg/mL and solvent is 20/80 DMSO/water (v/v).

Table S1. Summary of fits to the SANS data for gel **1** before and after annealing. Data set **S1** and **S3** correspond to the gel **1** before annealing in absence and presence of the enzyme respectively. The rest correspond to gel **1** after thermal (**S2**) and annealing involving pH cycle (**S4**, **S5** and **S6**). For pH cycle annealing, different annealing rates were tested: [urease] = 0.2 mg/mL, [urea] = 0.02 M, volume of methyl formate is 100 μL (**S4**), [urease] = 0.2 mg/mL, [urea] = 0.1 M, volume of methyl formate is 100 μL (**S5**) and [urease] = 0.2 mg/mL, [urea] = 0.02 M, volume of methyl formate is 150 μL (**S6**).

	S1	**S2**	**S3**	**S4**	**S5**	**S6**
Background_A (cm^{-1})	3.02E-03 ± 5.52E-04	6.48E-03 ± 2.39E-04	3.75E-03 ± 3.68E-04	8.20E-03 ± 2.52E-04	7.89E-03 ± 2.17E-04	8.01E-03 ± 2.84E-04
Scale_A	1.95E-03 ± 2.96E-05	5.72E-04 ± 8.64E-06	1.78E-03 ± 1.70E-05	1.40E-03 ± 1.59E-05	1.38E-03 ± 1.14E-05	1.26E-03 ± 2.03E-05
Radius minor_A (Å)	32.97 ± 0.33	38.00 ± 0.20	32.20 ± 0.22	18.90 ± 0.35	20.7 ± 0.30	17.4 ± 0.39
Axis ratio_A	1.85 ± 0.03	2.91 ± 0.04	1.82 ± 0.02	1.76 ± 0.05	1.75 ± 0.04	2.04 ± 0.07
Length_A (Å)	7324 ± 265	>10^6	6773 ± 64	10011 ± 131	5608 ± 111	5605 ± 125
Kuhn length_A (Å)		208.10 ± 4.80				
Scale_B	1.48E-05 ± 6.24E-06		2.83E-05 ± 6.58E-06	1.82E-08 ± 5.33E-9	3.46E-09 ± 1.44E-9	1.55E-07 ± 2.62E-08
Power_B	2.45 ± 0.08		2.25 ± 0.04	3.65 ± 0.06	3.92 ± 0.08	3.34 ± 0.03
χ^2	3.73	3.32	6.8	1.29	0.95	1.19

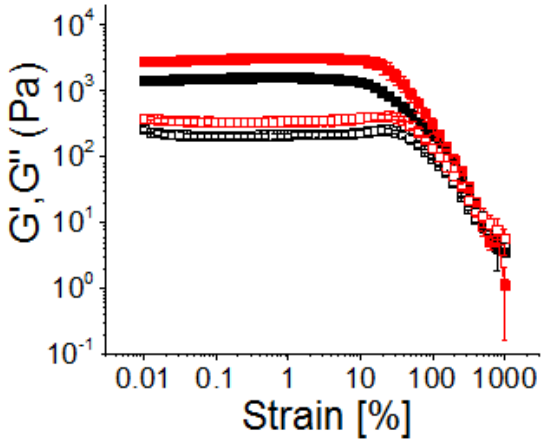

Figure S8. Strain sweep experiments of the hydrogels of **1** obtained after annealing involving urease-urea reaction under different conditions: (black data) [urease] = 0.2 mg/mL, [urea] = 0.02 M, volume of methyl formate = 100 μL; (red data) [urease] = 0.2 mg/mL, [urea] = 0.02 M, volume of methyl formate = 150 μL In In both cases, the closed symbols represent G′, the open symbols G″. Solvent is 20/80 DMSO/water (v/v) and [**1**] = 2 mg/mL.

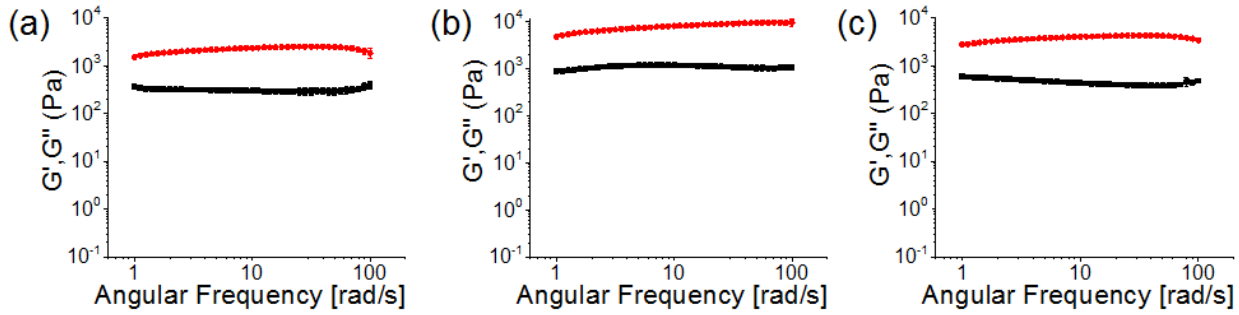

Figure S9. Frequency sweep experiments of the hydrogels of **1** (2 mg/mL) obtained from the enzymatic reactions involving initial conditions: (a) [urease] = 0.2 mg/mL, [urea] = 0.02 M, volume of methyl formate = 100 μL; (b) [urease] = 0.2 mg/mL, [urea] = 0.01 M, volume of methyl formate = 100 μL; (c) [urease] = 0.2 mg/mL, [urea] = 0.02 M, volume of methyl formate = 150 μL. In all cases, solvent is 20/80 DMSO/water (v/v). In all cases, the red data represents G′ and the black data G″.

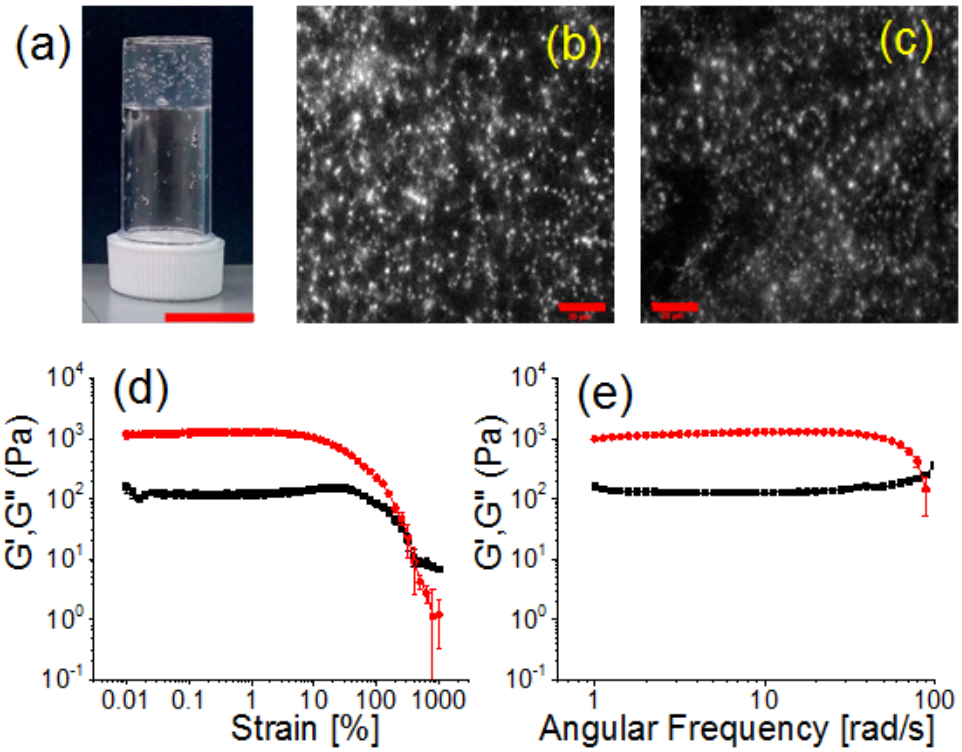

Figure S10. (a) Photograph representing the hydrogel of **1** prepared in presence of urease (scale bar is 1.7 cm). The white structures in the gel are air bubbles, not precipitation. Confocal fluorescence microscopy images (the scale bars represent 20 μm) [(b) and (c)], strain (d) and frequency (e) sweep experiments of the hydrogels of **1** prepared in presence of urease. For (b) and (c) the red data represents G′ and the black data G″. In all cases, concentration of **1** is 2 mg/mL, concentration of urease is 0.2 mg/mL and solvent is 20/80 DMSO/water (v/v).

Table S2: Comparison of rheological data of hydrogels of **1** prepared by different methods

Initial reaction conditions		**Conc. of urea (M)**	**volume of methyl formate (μL)**	**From frequency sweep**	**From strain sweep**	
				G′ (Pa) [at 10 rad/s]	**Critical strain (%)**	**%Strain at crossover point**
[1] = 2 mg/mL	Without annealing	-	-	1285	5	350
[urease] = 0.2 mg/mL Solvent is 20% DMSO in water	After annealing	0.02	100	2360	12	655
		0.01	100	8100	11	850
		0.02	150	4045	16	435

In general, storage modulus G′ is the measurement of a gel material to be stiff or soft. Higher G′ value indicates stiffer gel. At critical strain, the gel material starts to break. Thus, high gel strength indicates higher value of critical strain i.e. more capacity to resist an elastic deformation. The crossover point or the yield point represents maximum capacity of a material to maintain its viscoelastic nature before complete destruction. In our case, a decrease in rate of annealing resulted in ~2-4 times increase in stiffness (G′) of the gel. Importantly, irrespective of the rate of pH change, annealing resulted in significant increase in gel stiffness (~ 2-7 times stiffer material) and gel strength (>2-3 times increase in gel strength) as well as high crossover points in comparison to the gel directly prepared from **1** and urease.

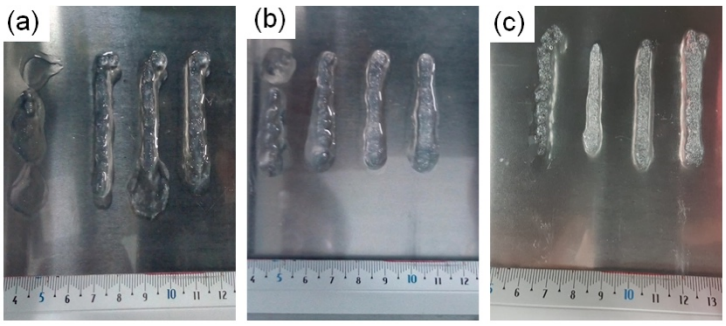

Figure S11. Photographs of optimised 3D printed lines of gels of **1** (2 mg/mL) prepared in absence (a) and (b), and presence (c) of urease (0.2 mg/mL). In each photograph, the length of the printed lines is 50 mm with applied shear rate (from left to right) 250, 500, 1K and 2K s^{-1}. Total volume of gel printed is 375 μL for (a) and (c), and 500 μL for (b). For (a)-(c), the gels were prepared in a syringe in 2 mL volume. Solvent is 20/80 DMSO/H_2O (v/v).

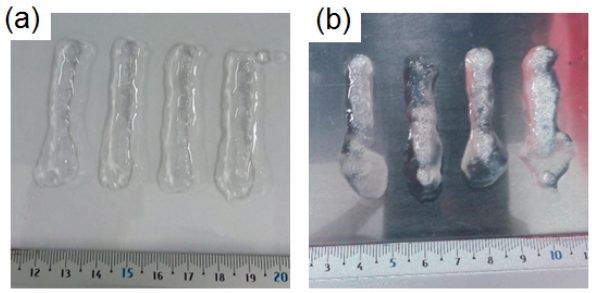

Figure S12. Photographs of optimised 3D printed lines of gels of **1** (2 mg/mL) obtained after annealing involving urease-urea reaction. Initial conditions are: [urease] = 0.2 mg/mL, [urea] = 0.02 M, volume of methyl formate = 100 μL for (a) and 150 μL (b). In both cases, the gels were prepared in a syringe in 2 mL volume. Solvent is 20/80 DMSO/H_2O (v/v). In each photograph, the length of the printed lines is 50 mm with applied shear rate (from left to right) 250, 500, 1K and 2K s^{-1}. Total volume of gel printed is 375 μL.

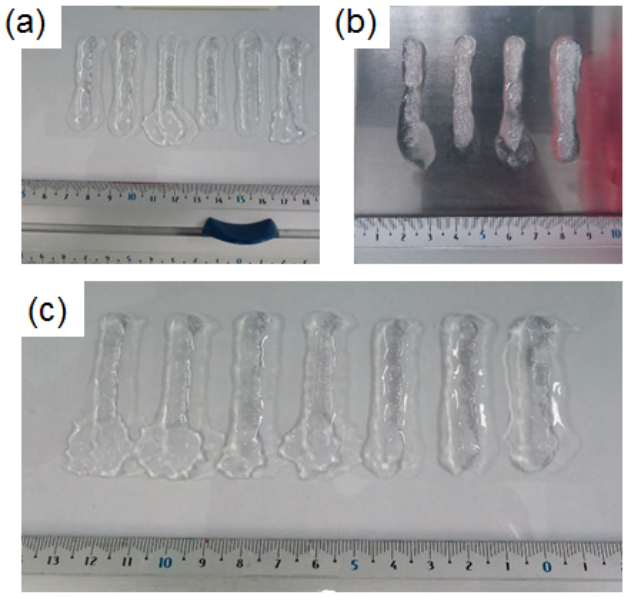

Figure S13. (a)-(c) Photographs of optimised 3D printed lines of gels of **1** (2 mg/mL) obtained after annealing involving urease-urea reaction. Initial conditions are: [urease] = 0.2 mg/mL, [urea] = 0.02 M, volume of methyl formate = 150 μL. In all cases, the gels were prepared in a syringe in 2 mL volume. Solvent is 20/80 DMSO/H_2O (v/v). In each photograph, the length of the printed lines is 50 mm. Total volume of gel printed is (a) 325, (b) 400 and (c) 425 μL. From left to right, the applied shear rate is (a) 250, 500, 1K, 2K, 3K, 4K s^{-1}; (b) 250, 1K, 2K, 3K s^{-1} and (c) 1K, 2K, 3K, 4K, 5K, 6K, 7K s^{-1}.

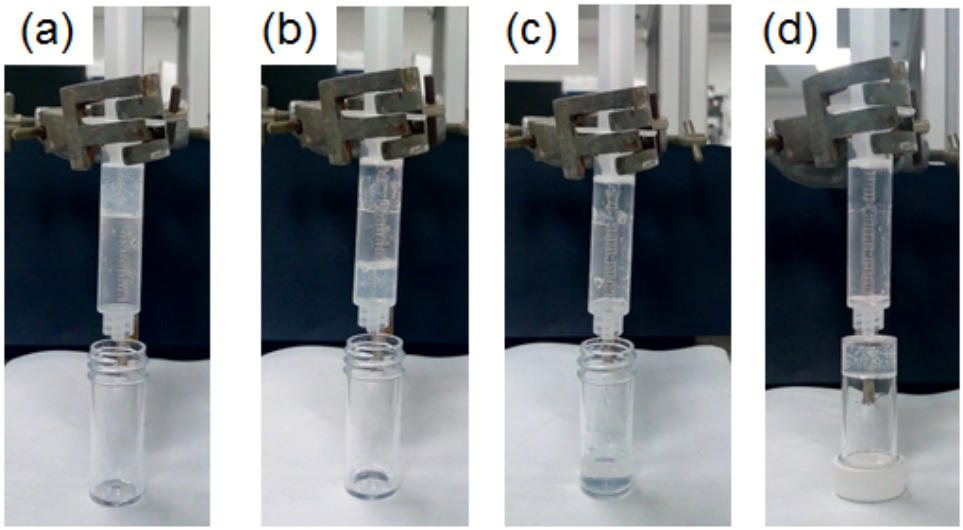

Figure S14. Experiment showing the temporal aspect of the materials. An initially formed gel (a) returns to a solution state (b). The solution state allows flow of the material from one container to a second under gravity (c), before re-gelling and immobilizing the solvent (d). Photographs taken after (a) 15 seconds, (b) 2 mins, (c) 10 mins, (d) 7 h. Note that a slight twist of the syringe barrel (with no downward force) is required at point (b) to allow air ingress and flow. Initial conditions are [**1**] = 2 mg/mL, [urease] = 0.2 mg/mL, [urea] = 0.02 M, volume of methyl formate = 100 μL. Solvent is 20/80 DMSO/H_2O (v/v).

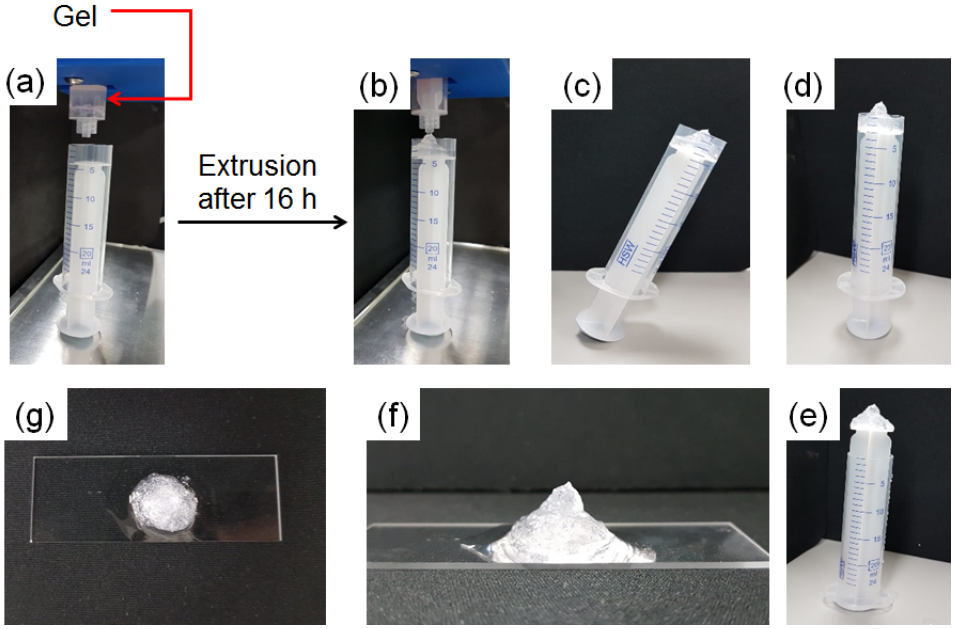

Figure S15. Photographs showing the moulding experiment of the DMSO/H_2O (20/80 (v/v)) gel of **1** (2 mg/mL). The gel was initially prepared in 2 mL volume in a syringe (a) and extruded after 16 h of preparation (b-d). (e)-(g) represent photographs of the moulded gel obtained after experiment.

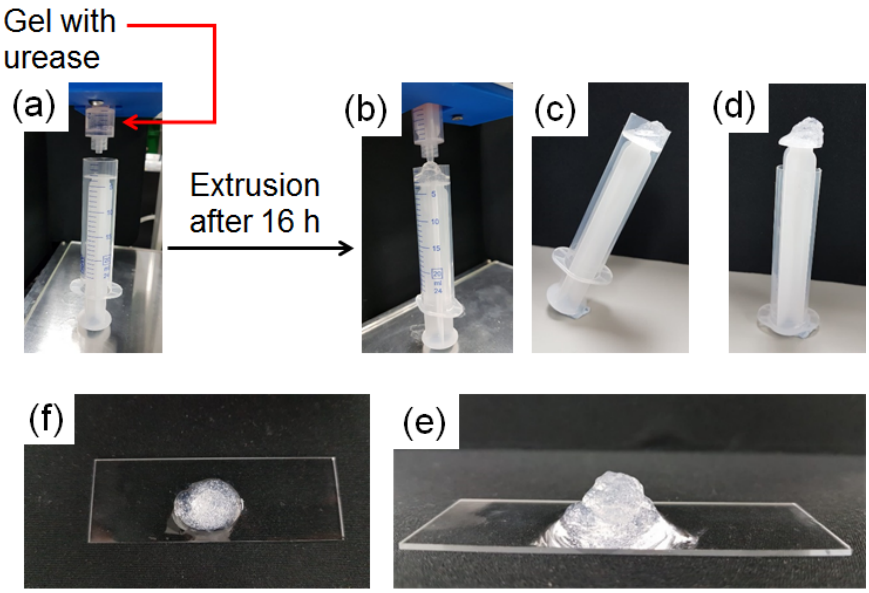

Figure S16. Photographs showing the moulding experiment of the DMSO/H_2O (20/80 (v/v)) gel of **1 (**2 mg/mL). The gel was initially prepared in 2 mL volume in a syringe in presence of urease (0.2 mg/mL) (a) and extruded after 16 h of preparation (b-c). (d)-(f) represent photographs of the moulded gel obtained after experiment.

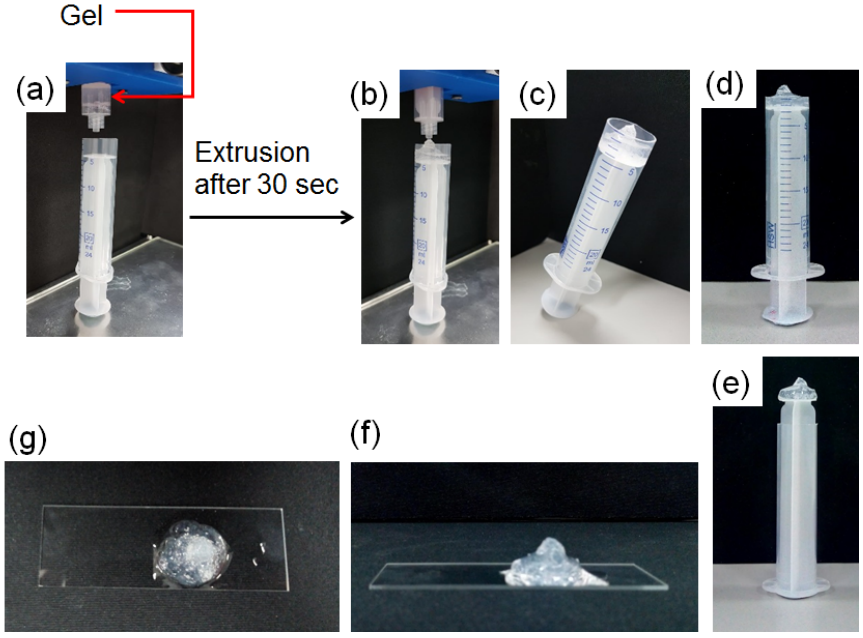

Figure S17. Photographs showing the moulding experiment involving the DMSO/H_2O (20/80 (v/v)) gel of **1 (**2 mg/mL). The gel was initially prepared in 2 mL volume in a syringe (a) and then immediately extruded after 30 sec of preparation (b-c). Then the system was left undisturbed for 16 h (d). (e)-(g) represent photographs of the moulded gel obtained after experiment.

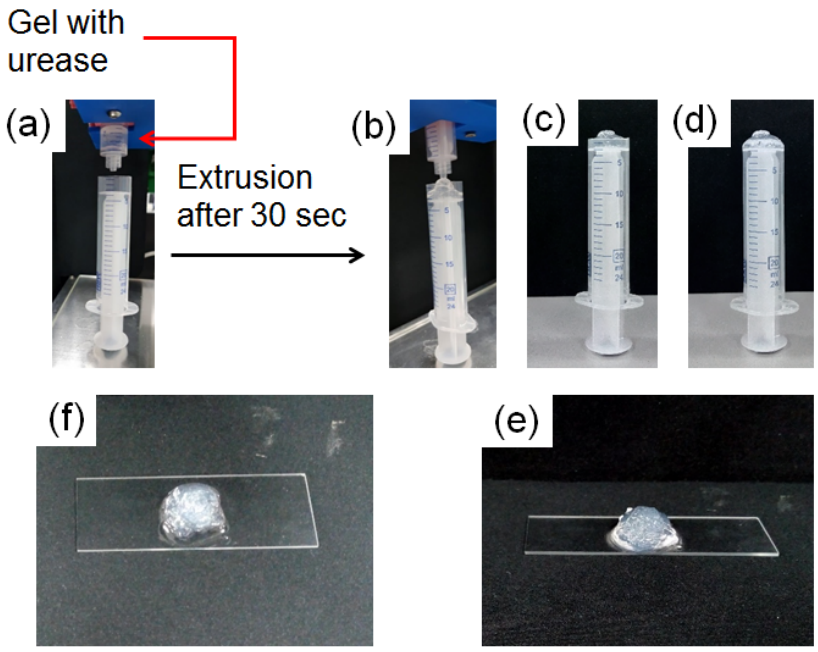

Figure S18. Photographs showing the moulding experiment of the DMSO/H_2O (20/80 (v/v)) gel of **1** (2 mg/mL). The gel was initially prepared in 2 mL volume in a syringe in presence of urease (0.2 mg/mL) (a) and then immediately extruded after 30 sec of preparation (b). Then the system was left undisturbed for 16 h (c). (d)-(f) represent photographs of the moulded gel obtained after experiment.

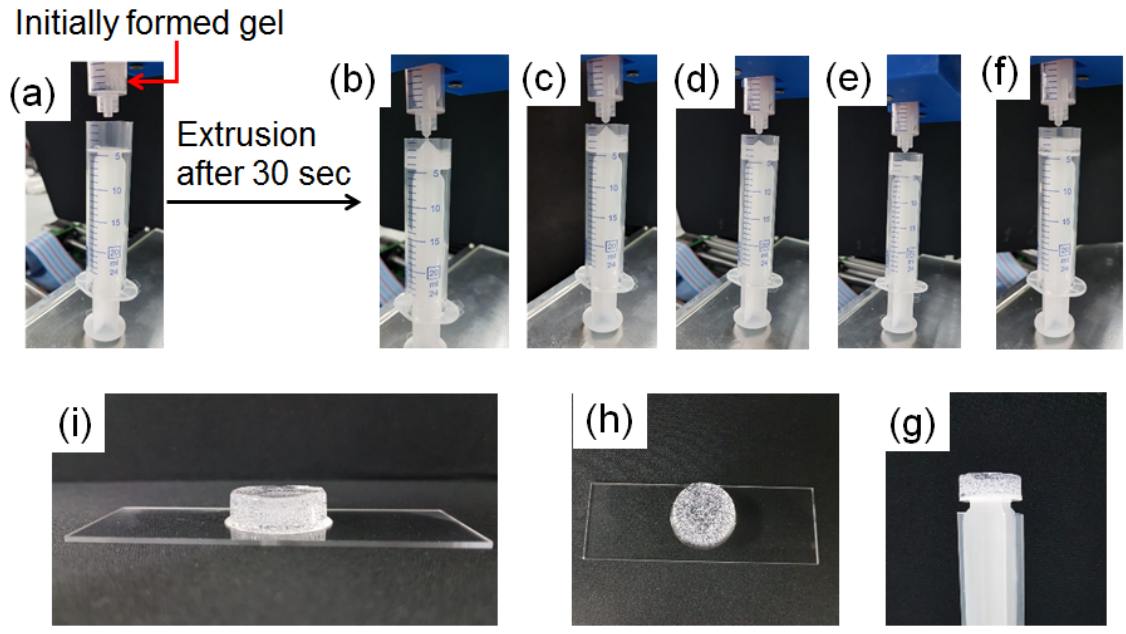

Figure S19. Experiment showing autonomous programming of homogeneous 'moulding and casting' of the hydrogel of **1** in time involving the initial conditions: [**1**] = 2 mg/mL, [urease] = 0.2 mg/mL, [urea] = 0.02 M, volume of methyl formate = 150 μL. Solvent is 20/80 DMSO/H_2O (v/v). An initially formed gel inside a syringe (a) was immediately extruded after 30 sec of preparation (b). With time the gel progressively converted into homogeneous solution and acquired the shape of the container (c-f). Photographs taken after 1 min (c), 5 mins (d), 10 mins (e) and 20 mins (f) of extrusion. Then the system was left undisturbed for 16 h. (g)-(i) represent photographs of the moulded gel obtained after experiment. The white structures in the gel are air bubbles, not precipitation.

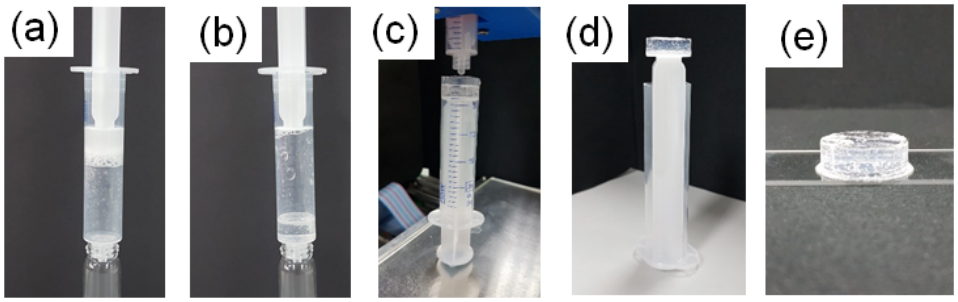

Figure S20. Experiment showing autonomous programming of homogeneous 'moulding and casting' of the hydrogel of **1** in time involving the initial conditions: [**1**] = 2 mg/mL, [urease] = 0.2 mg/mL, [urea] = 0.02 M, volume of methyl formate = 100 μL. Solvent is 20/80 DMSO/H_2O (v/v). An initially formed gel inside a syringe (a) was allowed to become a solution (b) and then extruded (c) and the system left undisturbed for 16h. With time the solution become thick and acquired the shape of the container (d-e). Photograph taken after 2 mins (b), 5 mins (c) and 16 h (d-e).

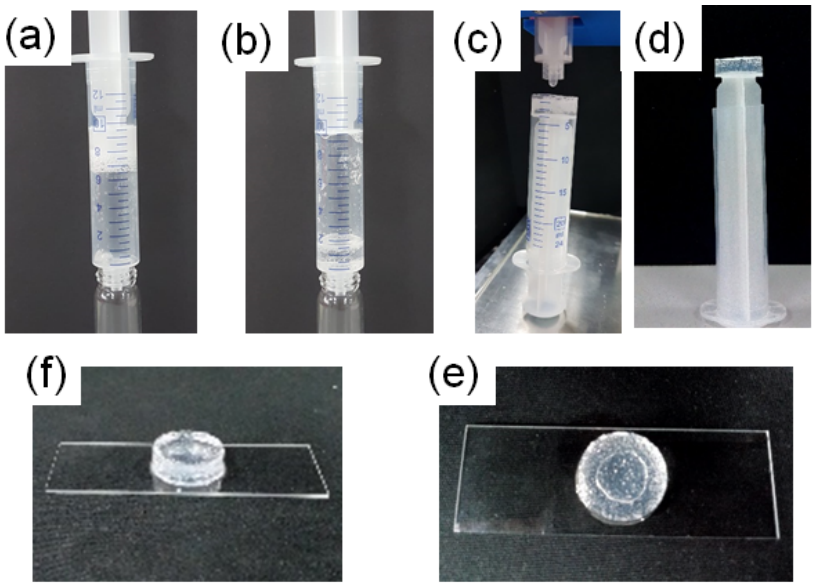

Figure S21. Experiment showing autonomous programming of homogeneous 'moulding and casting' of the hydrogel of **1** in time involving the initial conditions: [**1**] = 2 mg/mL, [urease] = 0.2 mg/mL, [urea] = 0.02 M, volume of methyl formate = 150 μL. Solvent is 20/80 DMSO/H_2O (v/v). An initially formed gel inside a syringe (a) was allowed to become a solution (b) and then extruded (c) and the system left undisturbed for 16h. With time the solution become thick and acquired the shape of the container (d-f). Photograph taken after 20 mins (b), 25 mins (c) and 16 h (d-f).

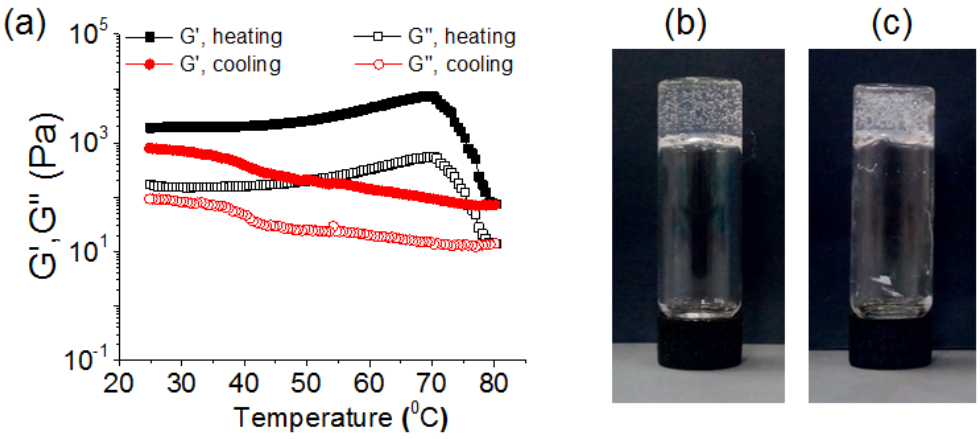

Figure S22. (a) Temperature sweep experiment of the hydrogel of **1** (2 mg/mL) prepared from DMSO-H_2O (20/80, v/v). Photograph of hydrogel of **1** (2 mg/mL) prepared from DMSO-H_2O (20/80, v/v) before (b) and after (c) thermal annealing. The white structures in the gels are air bubbles, not precipitation.

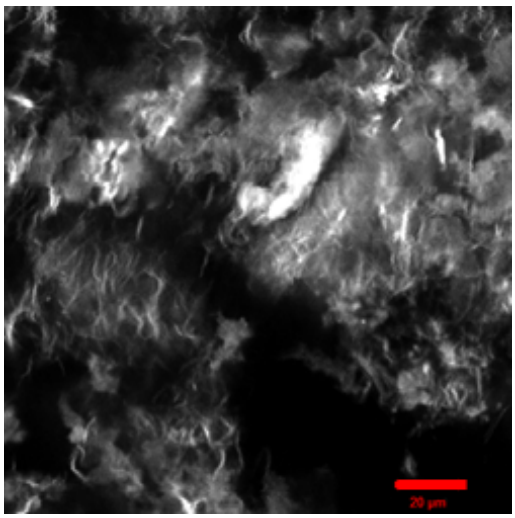

Figure S23. Confocal microscopy image of hydrogel of **1** obtained after thermal annealing (scale bar represents 20 μm). Solvent is 20/80 DMSO/water (v/v) and [**1**] = 2 mg/mL.

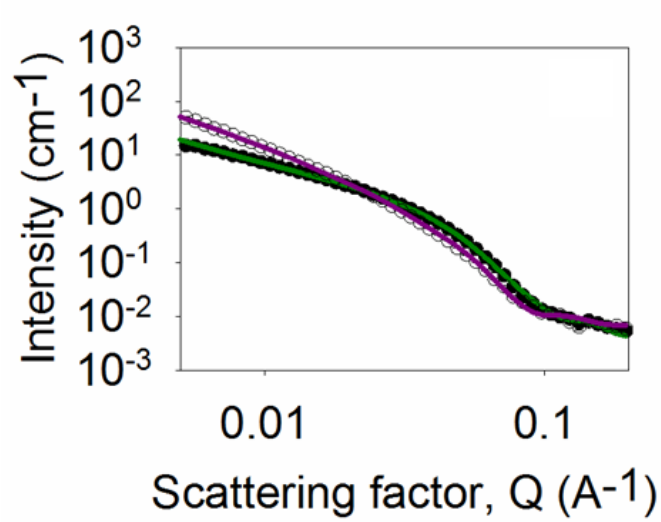

Figure S24. SANS scattering data for the hydrogels of **1** before (closed symbol) and after (open symbol) thermal annealing. The lines represent the fit to the data before (green) and after (purple) annealing. In both cases, concentration of **1** is 2 mg/mL and solvent is 20/80 DMSO/water (v/v).

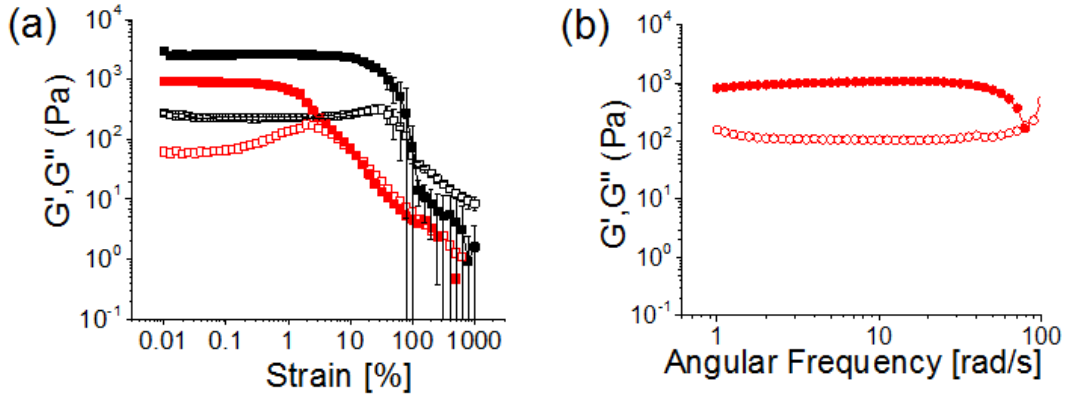

Figure S25. (a) Strain sweeps for hydrogels of **1** before (black data) and after (red data) thermal annealing. (b) Frequency sweep for hydrogel of **1** obtained after thermal annealing. In both cases, the closed symbols represent G′, the open symbols G″. Solvent is 20/80 DMSO/water (v/v) and [**1**] = 2 mg/mL.