Electronic Supplementary Material (ESI) for Chemical Communications.

Gel to gel transitions by dynamic self-assembly

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Supporting Information

Experimental Methods

Materials. Compound **1** was synthesised as described previously.[27] Urease (J61455 Urease, Jack Beans, minimum 45.0 units/mg solid) and urea (ultrapure 99%) were obtained from Alfa Aesar. Deionised water was used throughout all experiments. $CaCl_2$ (granular) was obtained from Fisher Scientific.

Preparation of solutions. Stock solutions of gelator **1** were prepared in DMSO at a concentration of 10 mg/mL by stirring. The enzyme, urea and $CaCl_2$ were highly soluble in H_2O and therefore did not require stirring. The stock solutions of the enzyme was prepared at concentrations of 0.254 mg/mL and 0.127 mg/mL in H_2O. The enzyme concentration in the stock solution was determined from the mass (in mg) dissolved in known volume of H_2O. Stock solutions of the urea and $CaCl_2$ were prepared in H_2O in the concentration of 2 M and 1 M, respectively. Solutions of gelators, urease, urea and $CaCl_2$ were prepared freshly before each experiment. Molar equivalents of Ca^{2+} were calculated with respect to the molar concentration of **1**. The enzyme-catalyzed reactions involving gelator **1** were performed either in presence and absence of Ca^{2+}. For the enzymatic reactions in absence of $CaCl_2$, 1.580 mL of the respective urease solution was transferred to the vial containing 0.40 mL of the gelator solution and urea (either 20 µL of urea or a mixture of 10 µL of urea and 10 µL of H_2O) and left undisturbed. Throughout these experiments a total volume of 2 mL was maintained in which the final ratio of DMSO and H_2O was 20:80. Therefore, for the enzymatic reactions, the concentration of **1** was 2 mg/mL, the concentration of urease was 0.2 mg/mL and 0.1 mg/mL as required, and the initial concentration of urea was 0.02 M and 0.01 M as required. A similar method was used to perform the enzymatic reactions in presence of $CaCl_2$. In this case, urease solution was added to the mixture of **1**, urea and $CaCl_2$ (either 4 µL or 16 µL) and the same solutions of **1**, urease and urea in respected volumes were used as above.

Hydrogel Formation. Hydrogels of **1** were prepared using different triggers involving different reaction conditions. For the solvent switch method, 1.6 mL of water was added to 0.4 mL of DMSO solution of **1** to form the hydrogel. Hydrogels were also prepared in the presence of $CaCl_2$ and/or the enzyme. The final gelator concentration was 2 mg/mL.

For the Ca^{2+}-triggered gels, two different methods were used. Firstly, the gelation experiments were carried out by adding urease solutions to the mixture of **1**, urea and Ca^{2+} as mentioned above. The samples were then left to stand overnight to allow gelation. These resulted in formation of homogeneous hydrogels (gelator concentration was 2 mg/mL).

Secondly, inhomogeneous Ca^{2+}- gels were prepared by separate addition of 16 µL of $CaCl_2$ (1 M in H_2O) to an alkaline solution of **1**. These alkaline solutions were prepared either by adding a molar equivalent of NaOH (0.1 M) to the DMSO solution of **1** followed by addition of

H_2O, or by performing the enzymatic reaction in absence of Ca^{2+} ion. For the enzymatic reaction, the initial reaction conditions were [urease] = 0.1 mg/mL, [urea] = 0.01 M. $CaCl_2$ (aqueous) was added to the solution after ~16 hours of performing the enzymatic reaction. In both cases a total volume of 2 mL of the reaction mixture was maintained in which the ratio of DMSO and H_2O was 20:80 and the gelator concentration was 2 mg/mL. In both cases, aqueous solution $CaCl_2$ was added on the top of the alkaline solutions and then the samples were left undisturbed for ~16h to allow gelation.

For all Ca^{2+}-gels, the molar equivalent of Ca^{2+} was calculated with respect to the molar concentration of **1**.

pH measurements. A FC200 pH probe from HANNA instruments with a 6 mm x 10 mm conical tip was used for pH measurements. The stated accuracy of the pH measurements is ±0.1. For the urea-urease reaction involving the gelator, the reaction mixtures were prepared as described above at a 2 mL volume in a 7 mL Sterilin vial and the pH change was monitored with time. The temperature was maintained at 25 °C during the measurement by using a circulating water bath.

pK_a determination was carried out by recording the pH values after each addition of HCl (0.1M) to the solution of **1** containing 1 molar equivalents of NaOH (0.1 M) in 20% DMSO in H_2O. During the titration, to prevent any gel formation, the solution was stirred continuously. The experimental temperature was 25 °C.

Rheological and viscosity measurements. All rheological measurements were undertaken on an Anton Paar Physica MCR 301 or MCR 101 rheometer at 25 °C. Strain, frequency and time sweeps were performed using a vane and cup geometry. Strain sweeps were performed at 10 rad/s from 0.01 % to 1000 % strain. Frequency sweeps were carried out from 1 rad/s to 100 rad/s at 0.5 % strain. All gels were left ~16 hours before being measured. Time sweeps were performed at an angular frequency of 10 rad/s and with a strain of 0.5%. For all experiments, gels were prepared as mentioned earlier in 2 mL volume in a 7 mL Sterilin vials.

A cone and plate measuring system was used to perform viscosity measurements of the solutions. A solution of **1** was prepared either by adding molar equiv. of NaOH (0.1 M) to the DMSO solution of **1** followed by addition of H_2O, or by performing the enzymatic reaction in absence of Ca^{2+} ion (as described above). Viscosity measurements of the enzyme triggered solutions were carried out after ~16 hours of performing the enzymatic reaction. In all cases, the gelator concentration was 2 mg/mL and the ratio of DMSO and H_2O was 20:80. 0.9 mL of the respective solutions were transferred onto the plate for measurement. The viscosity of each solution was recorded under the rotation shear rate varying from 0.1 to 1000 s^{-1}.

Confocal microscopy. A Zeiss LSM510 on a Zeiss Observer Z1 (Zeiss, Jena, Germany) was used for imaging. The samples were prepared as mentioned above containing Nile blue (2 μL/mL of a 0.1 wt% solution) in CELLview Culture dishes (35 mm diameter) and were excited at 633 nm and detected with a Zeiss Meta detector. Data were captured using Zeiss Zen software (Zeiss, Jena, Germany) and analysed using Zeiss LSM image browser (version 4.2.0.121).

UV-Vis measurements. Absorption spectra of **1** under different conditions were recorded on Agilent Technologies Cary 60 UV-Vis spectrophotometer using a 0.1 mm path length quartz cuvette. All the samples were prepared in Sterilin vials using the same methodology as described before and were immediately transferred to the cuvette for measurement.

Fluorescence spectroscopy. Emission spectra of **1** under different conditions were recorded on Agilent Technologies fluorescence spectrofluorophotometer. Samples were prepared in 2 mL volume in a PMMA cuvette with a path length of 1 cm by following the same procedure as mentioned before. In all cases, the excitation wavelength was 325 nm. Both the excitation and emission slit widths were 5 nm.

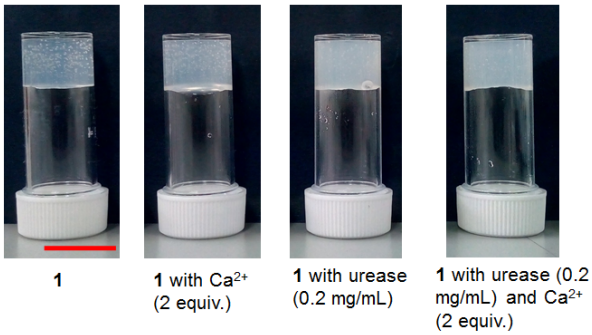

Figure S1. Photograph representing the hydrogels of **1** (concentration = 2 mg/mL) in 20/80 DMSO/water (v/v) prepared under different conditions (the white structures in the gels are air bubbles, not precipitation). The scale bar represents 1.7 cm.

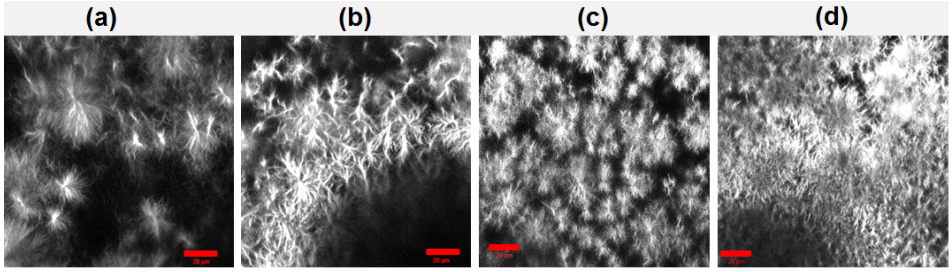

Figure S2. Confocal fluorescence microscopy images of the DMSO-H_2O gel of **1** (concentration = 2 mg/mL) formed under different conditions: (a) **1**, (b) **1** with Ca^{2+} (2 equivalents with respect to **1**), (c) **1** with urease (0.2 mg/mL) and (d) **1** with urease (0.2 mg/mL) and Ca^{2+} (2 equivalents with respect to **1**). In all cases, the scale bar represents 20 μm. Concentration of **1** is 2 mg/mL and solvent is 20/80 DMSO/water (v/v).

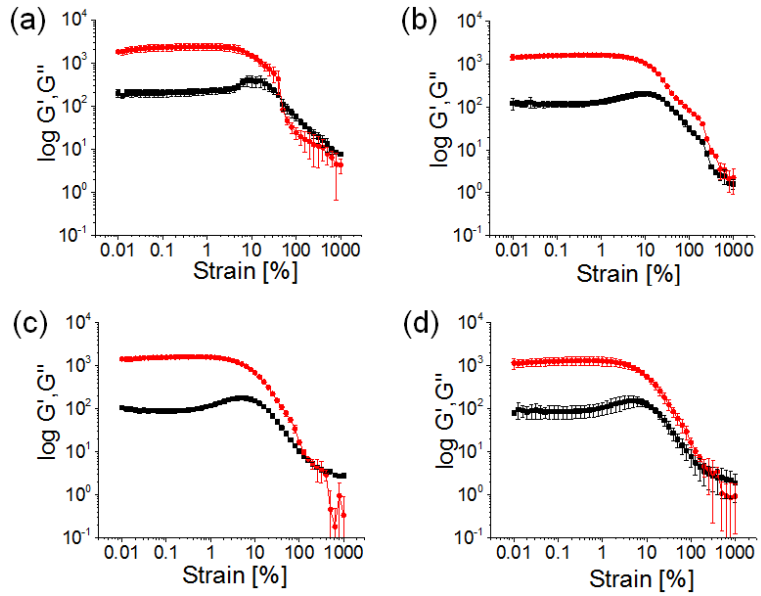

Figure S3. Strain sweep experiments of the hydrogels of **1** formed under different conditions: **1**, (b) **1** with Ca^{2+} (2 equivalents with respect to **1**), (c) **1** with urease (0.2 mg/mL) and (d) **1** with urease (0.2 mg/mL) and Ca^{2+} (2 equivalents with respect to **1**). In all cases, concentration of **1** is 2 mg/mL and solvent is 20/80 DMSO/water (v/v). The red data represents G′ and the black data G″.

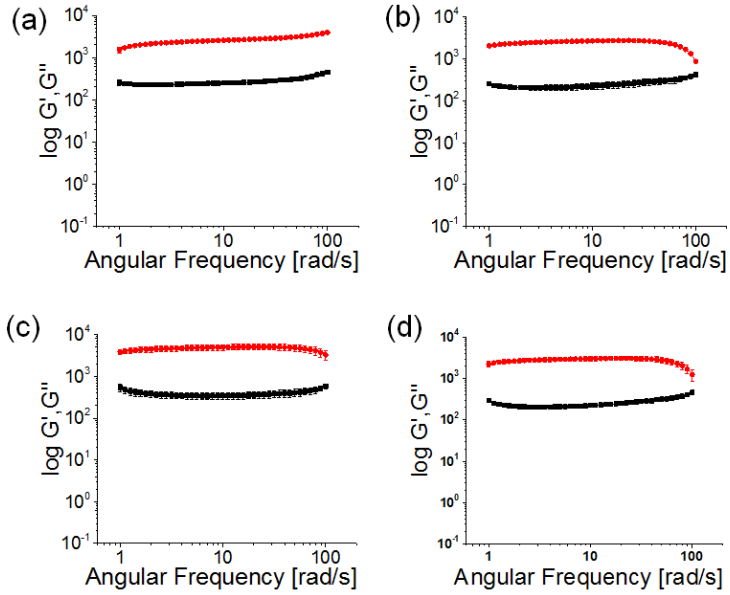

Figure S4. Frequency sweep experiments of the hydrogels of **1** formed under different conditions: (a) **1**, (b) **1** with Ca^{2+} (2 equivalents with respect to **1**), (c) **1** with urease (0.2 mg/mL) and (d) **1** with urease (0.2 mg/mL) and Ca^{2+} (2 equivalents with respect to **1**). In all cases, concentration of **1** is 2 mg/mL and solvent is 20/80 DMSO/water (v/v). The red data represents G′ and the black data G″.

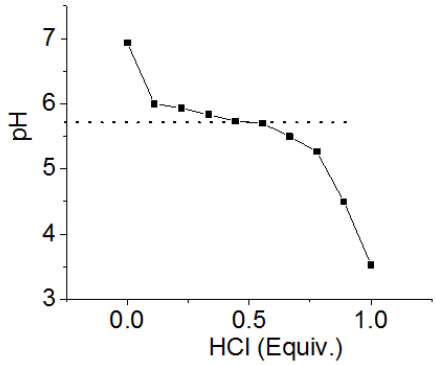

Figure S5. Determination of apparent pK_a of gelator **1** in 20/80 DMSO/water (v/v).

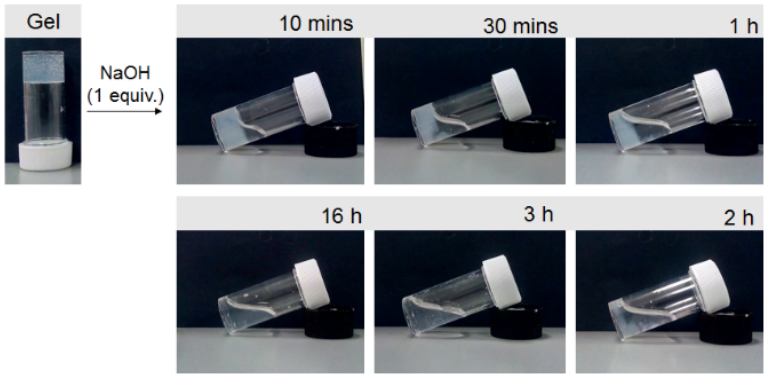

Figure S6. Photographs representing the phase change of **1** with time on addition of equimolar amount of NaOH (0.1 M). Concentration of **1** is 2 mg/mL and solvent is 20/80 DMSO/water (v/v).

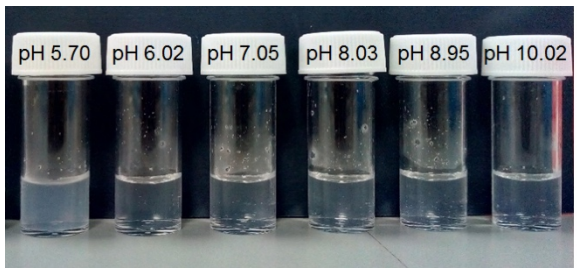

Figure S7. Photograph of solutions of **1** at different pH. The solutions are prepared by adding aqueous NaOH to the DMSO solution of **1**. In all cases, concentration of **1** is 2 mg/mL and solvent is 20/80 DMSO/water (v/v).

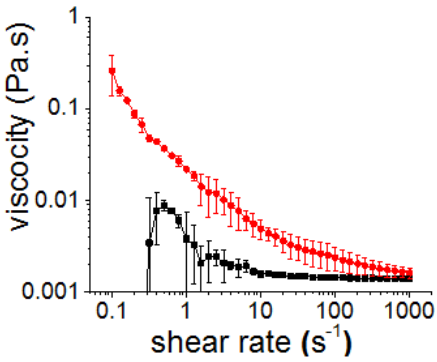

Figure S8. Viscosity measurements for the solutions of **1** (concentration = 2 mg/mL) obtained from (black) NaOH (molar equiv.) and (red) enzymatic reaction involving initial conditions: [urease] = 0.1 mg/mL; [urea] = 0.01 M. In both cases solvent is 20/80 DMSO/water (v/v).

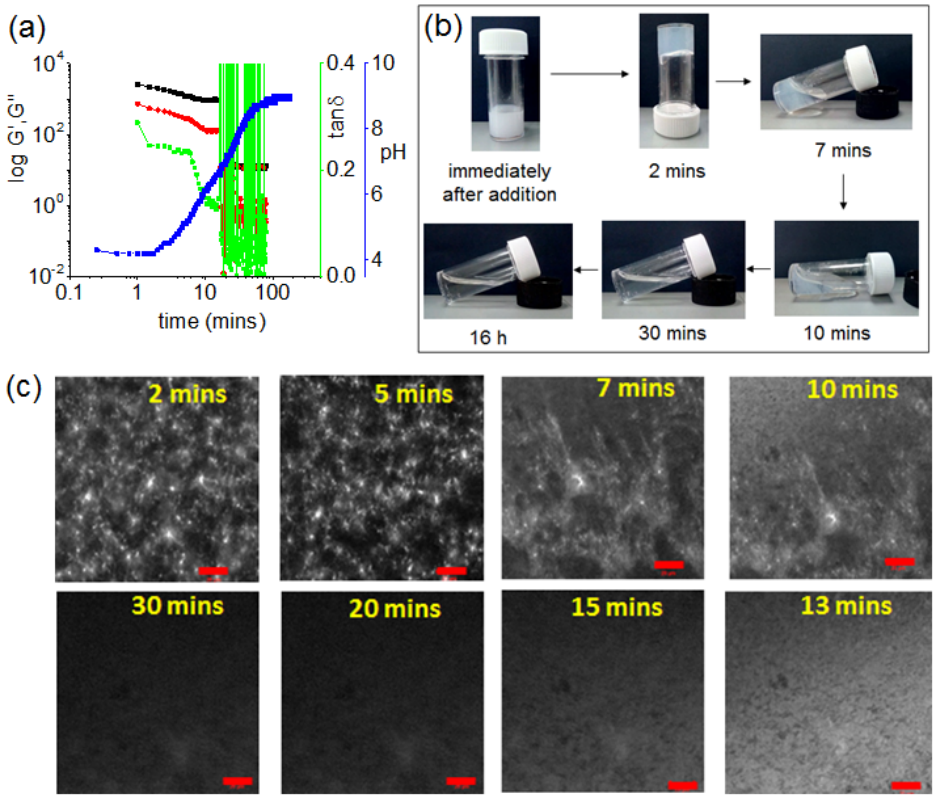

Figure S9. (a) Variation of G′ (black), G″ (red), tanδ (green) and pH (blue) with time for **1** in presence of urea-urease reaction. (b) Photographs showing the phase change of **1** with time in presence of urea-urease reaction. (c) Time dependent confocal microscopy images of **1** in presence of urea-urease reaction (scale bar is 20 μm). In all cases, solvent is 20/80 DMSO/H_2O (v/v) and initial reaction conditions are [**1**] = 2 mg/mL, [urease] = 0.2 mg/mL and [urea] = 0.02 M.

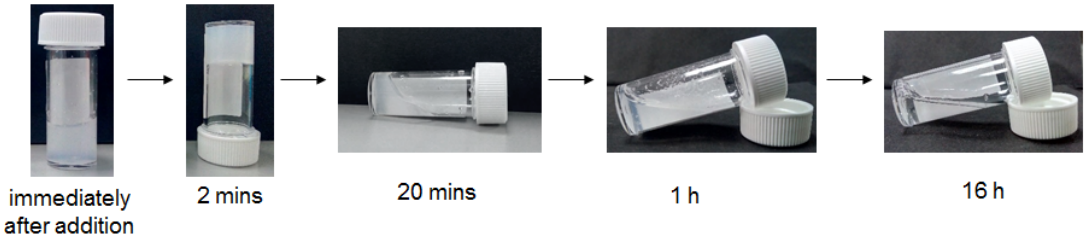

Figure S10. Photographs showing the phase change of **1** with time in presence of urea-urease reaction involving initial conditions [urease] = 0.1 mg/mL; [urea] = 0.02 M. Concentration of **1** is 2 mg/mL, solvent is 20/80 DMSO/H_2O (v/v).

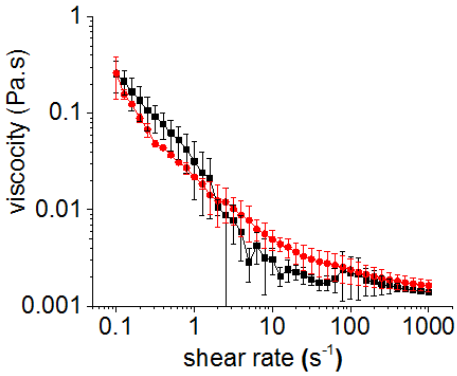

Figure S11. Viscosity measurements for the solutions of **1** (concentration = 2 mg/mL) in DMSO/water obtained from urea-urease reaction involving initial conditions (black data) [urease] = 0.2 mg/mL; [urea] = 0.02 M; (red data) [urease] = 0.1 mg/mL; [urea] = 0.01 M.

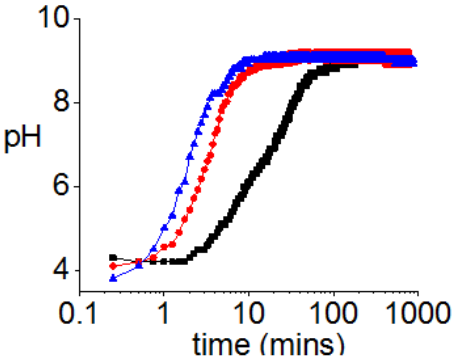

Figure S12. Change in pH with time for **1** from the urease–urea reaction in presence of Ca^{2+} involving initial reaction conditions [urease] = 0.2 mg/mL, [urea] = 0.02 M. In all cases, [**1**] = 2 mg/mL, solvent is 20/80 DMSO/water (v/v). The black data is for no Ca^{2+}, the red data for 0.5 equivalents of Ca^{2+} and the blue data for 2 equivalents of Ca^{2+}.

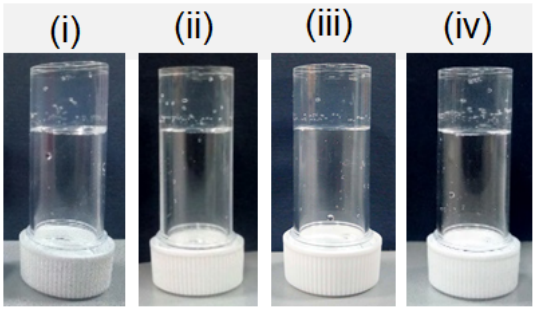

Figure S13. Photograph representing the Ca^{2+}-triggered [0.5 equivalents with respect to **1** for (i) and (iii), and 2 equivalents with respect to **1** for (ii) and (iv)] hydrogels of **1** obtained from the enzymatic reactions involving initial conditions: (i) and (ii) [urease] = 0.2 mg/mL, [urea] = 0.02 M; (iii) and (iv) [urease] = 0.1 mg/mL, [urea] = 0.01 M. In all cases, concentration of **1** is 2 mg/mL and solvent is 20/80 DMSO/water (v/v). The white structures in the gels are air bubbles, not precipitation.

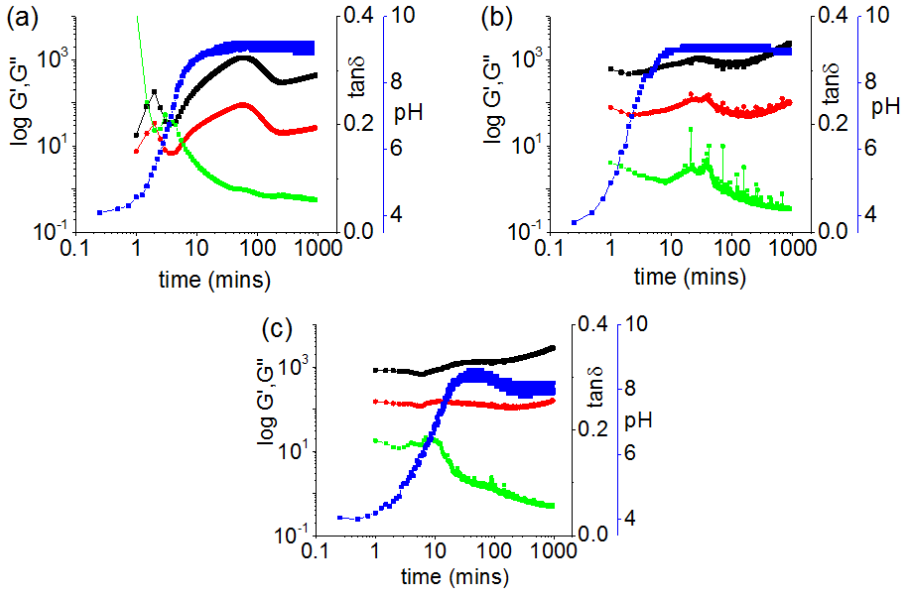

Figure S14. Variation of G′ (black), G″ (red), tanδ (green) and pH (blue) with time for **1** in presence of urea-urease reaction involving initial conditions: (a) and (b) [urease] = 0.2 mg/mL, [urea] = 0.02 M; (c) [urease] = 0.1 mg/mL, [urea] = 0.01 M. Concentration of Ca^{2+} is 0.5 molar equivalents with respect to **1** for (a) and 2 molar equivalents with respect to **1** for (b) and(c). In all cases conc. of **1** is 2 mg/mL, solvent is 20/80 DMSO/water (v/v).

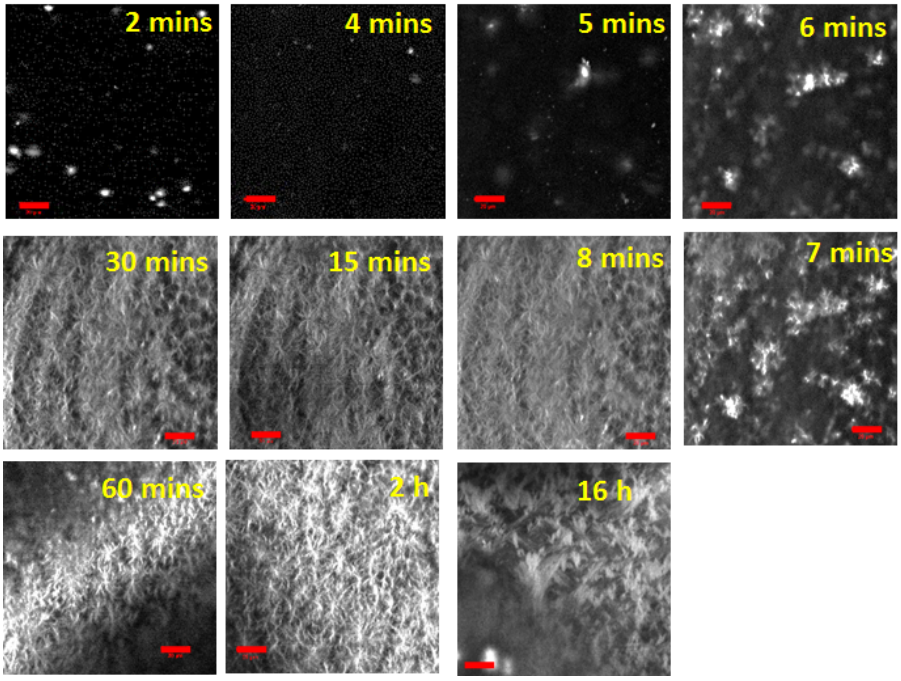

Figure S15. Time dependent confocal microscopy images of **1** in presence of urea-urease reaction involving initial conditions [urease] = 0.2 mg/mL, [urea] = 0.02 M, [Ca^{2+}] = 0.5 equivalents with respect to **1**, [**1**] is 2 mg/mL, solvent is 20/80 DMSO/water (v/v). Scale bar is 20 μm in all cases.

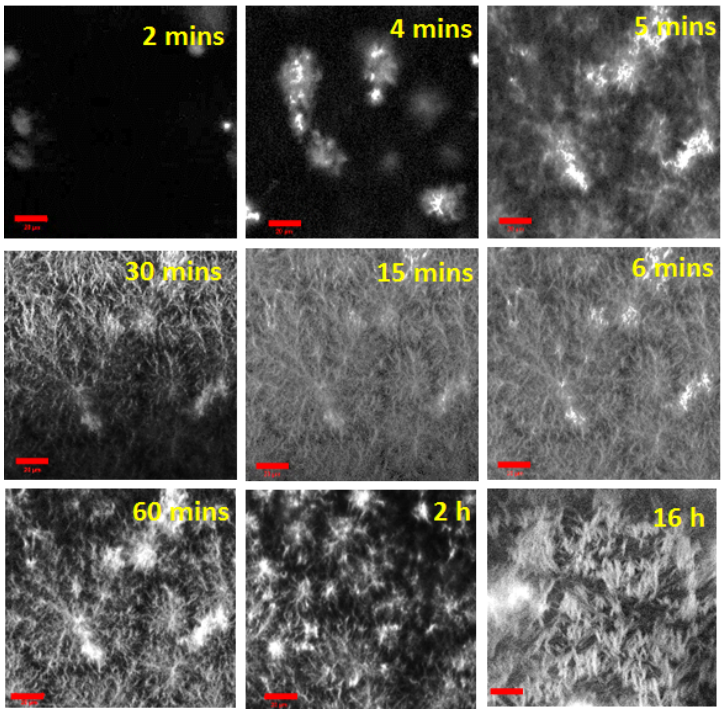

Figure S16. Time dependent confocal microscopy images of **1** in presence of urea-urease reaction involving initial conditions [urease] = 0.2 mg/mL, [urea] = 0.02 M, [Ca^{2+}] = 2 equivalents with respect to **1**, [**1**] is 2 mg/mL, solvent is 20/80 DMSO/water (v/v). Scale bar is 20 μm in all cases.

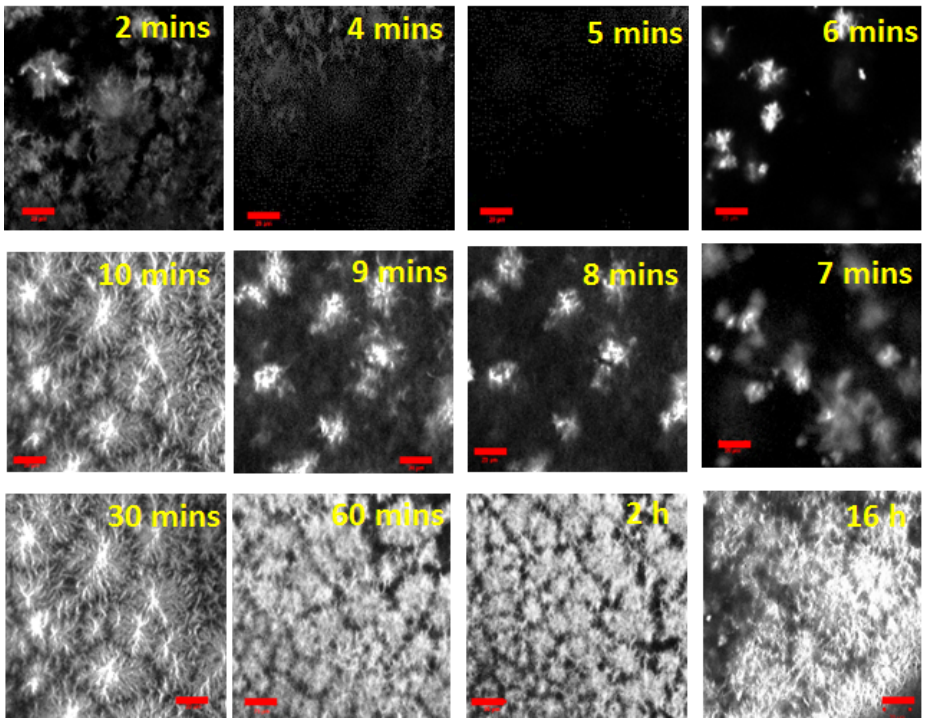

Figure S17. Time dependent confocal microscopy images of **1** in presence of urea-urease reaction involving initial conditions [urease] = 0.1 mg/mL, [urea] = 0.01 M, [Ca^{2+}] = 2 equivalents with respect to **1**, [**1**] is 2 mg/mL, solvent is 20/80 DMSO/water (v/v). Scale bar is 20 μm in all cases.

UV-vis studies showed that the DMSO-water gel of **1** exhibited three strong absorption bands at 278 nm, 318 nm and 332 nm (Figure S18). In comparison to this, in the Ca^{2+}-triggered gels obtained by dissipative process, the intensity of these bands decreases while new blue shifted bands appeared at 272 nm, 310 nm and 325 nm. Additionally, at a fixed Ca^{2+}-ion concentration, gels obtained from slow pH change showed a weak shoulder at 332 nm. Moreover, at a fixed enzyme and urea concentration, an increase in the concentration of Ca^{2+} ions resulted in considerable increase in intensity at 310 nm and 325 nm. On the other hand, Ca^{2+}-triggered gelation induced very small changes in emission spectra where only a 3 nm blue shift in the monomer emission was noticed compared to the DMSO-water gel. Such differences in the absorption and emission spectra of the gels emphasizes different molecular packing and hence different mechanical behaviour of the gels.

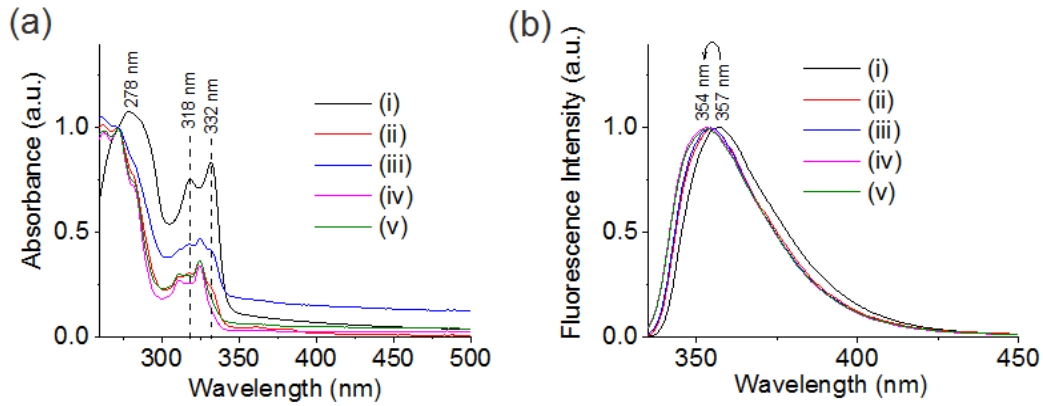

Figure S18. Comparison of normalized UV-vis (a) and emission (b) spectra of **1** in gel states obtained from (i) DMSO-H_2O and (ii)-(v) enzymatic reaction in presence of Ca^{2+}-ions. In all cases, [**1**] = 2 mg/mL and solvent is 20/80 DMSO/water (v/v). The initial conditions were: (ii) [urease] = 0.1 mg/mL, [urea] = 0.01 M, [Ca^{2+}] = 0.5 equiv.; (iii) [urease] = 0.1 mg/mL, [urea] = 0.01 M, [Ca^{2+}] = 2 equiv.; (iv) [urease] = 0.2 mg/mL, [urea] = 0.02 M, [Ca^{2+}] = 0.5 equiv.; (v) [urease] = 0.2 mg/mL, [urea] = 0.02 M, [Ca^{2+}] = 2 equiv.

Time variable absorption and emission spectra were recorded to realize the effect of the disassembly and reassembly kinetics on the molecular packing (Figure S19 and S20). In time dependent emission studies, all the dissipative gels initially showed strong emission at 357 nm. With time the intensity of the bands at 357 nm initially increases but after some time slowly decreases and blue shifted to 354 nm. In UV-vis, as soon as water was added to the DMSO solution of **1**, strong band at 332 nm was appeared. With time as the pH of the medium increased, the intensity of the band decreases suggesting the pH-induced disassembly of aggregates. With further time, a new peak at 325 nm generated and intensified indicating the salt induced reassembly of aggregates. Such changes in the absorption and emission spectra were more prominent when the rate of pH change was slow.

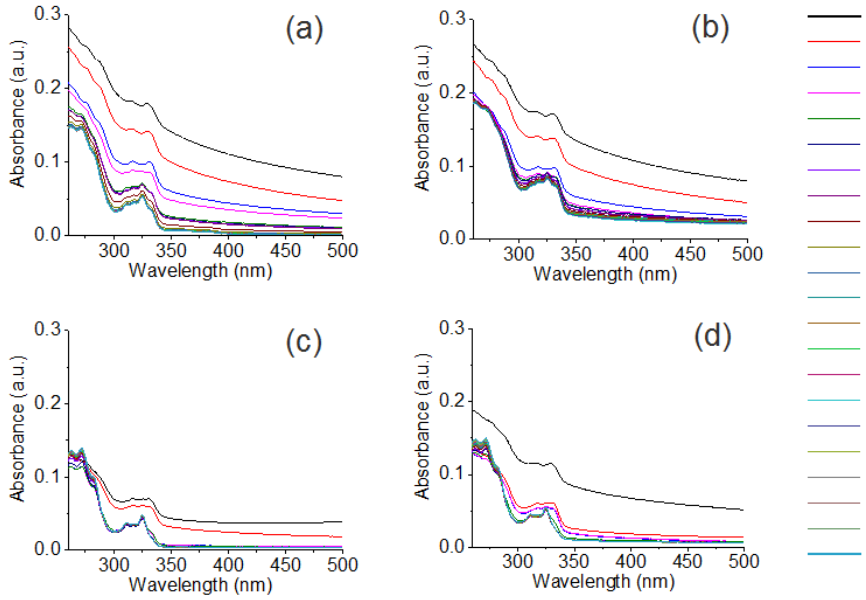

Figure S19. Change in UV-vis spectra for **1** with time from the urease–urea reaction in presence of Ca^{2+}. Initial conditions were (a) [urease] = 0.1 mg/mL, [urea] = 0.01 M, [Ca^{2+}] = 0.5 equiv.; (b) [urease] = 0.1 mg/mL, [urea] = 0.01 M, [Ca^{2+}] = 2 equiv.; (c) [urease] = 0.2 mg/mL, [urea] = 0.02 M, [Ca^{2+}] = 0.5 equiv.; (d) [urease] = 0.2 mg/mL, [urea] = 0.02 M, [Ca^{2+}] = 2 equiv. In all cases, [**1**] = 2 mg/mL and solvent is 20/80 DMSO/water (v/v). Data recorded at different time intervals (from top to bottom of the colour bar): after 1 min, 2 mins, 5 mins, 10 mins, 30 mins, 1 h, 1.5 h, 2 h, 3 h, 4 h, 5h, 6 h, 7 h, 8 h, 9 h, 10 h, 11 h, 12 h, 13 h, 14 h, 15 h, 16 h.

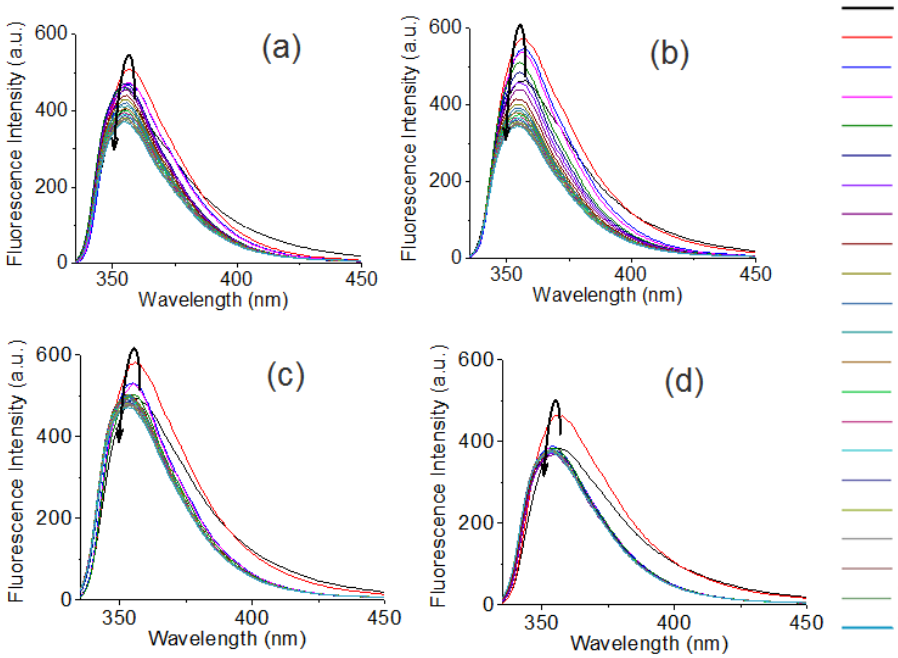

Figure S20. Change in emission for **1** with time from the urease–urea reaction in presence of Ca^{2+}. Initial conditions were (a) [urease] = 0.1 mg/mL, [urea] = 0.01 M, [Ca^{2+}] = 0.5 equiv.; (b) [urease] = 0.1 mg/mL, [urea] = 0.01 M, [Ca^{2+}] = 2 equiv.; (c) [urease] = 0.2 mg/mL, [urea] = 0.02 M, [Ca^{2+}] = 0.5 equiv.; (d) [urease] = 0.2 mg/mL, [urea] = 0.02 M, [Ca^{2+}] = 2 equiv. In all cases, [**1**] = 2 mg/mL and solvent is 20/80 DMSO/water (v/v). Data recorded at different time intervals (from top to bottom of the colour bar): immediate after addition, 1 min, 5 mins, 10 mins, 30 mins, 1 h, 1.5 h, 2 h, 3 h, 4 h, 5h, 6 h, 7 h, 8 h, 9 h, 10 h, 11 h, 12 h, 13 h, 14 h, 15 h, 16 h.

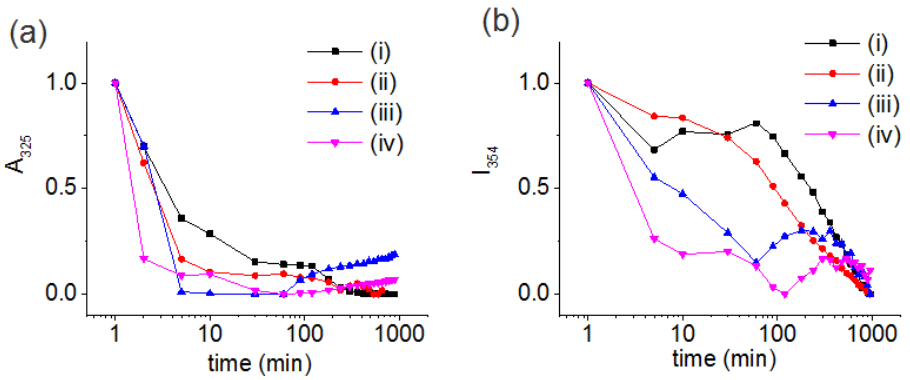

Figure. S21. Change in normalised (a) absorbance at 325 nm and (b) emission at 354 nm with time for **1** from the urease–urea reaction in presence of Ca^{2+}. Initial conditions were (i) [urease] = 0.1 mg/mL, [urea] = 0.01 M, [Ca^{2+}] = 0.5 equiv.; (ii) [urease] = 0.1 mg/mL, [urea] = 0.01 M, [Ca^{2+}] = 2 equiv.; (iii) [urease] = 0.2 mg/mL, [urea] = 0.02 M, [Ca^{2+}] = 0.5 equiv.; (iv) [urease] = 0.2 mg/mL, [urea] = 0.02 M, [Ca^{2+}] = 2 equiv. In all cases, [**1**] = 2 mg/mL and solvent is 20/80 DMSO/water (v/v).

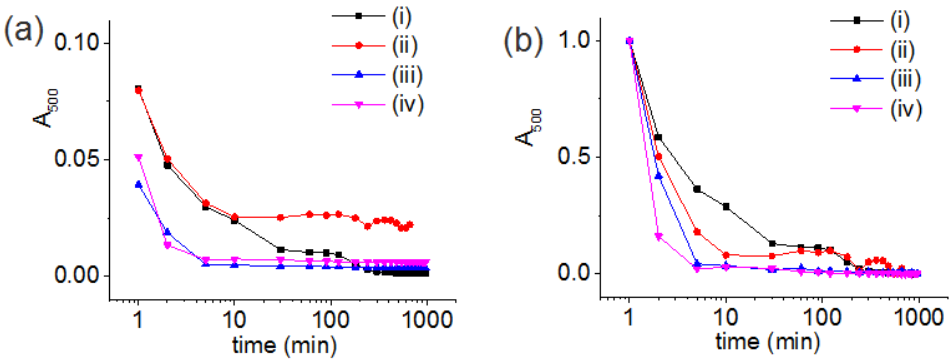

Figure S22. Changes in turbidity with time. (a) Change in absorbance at 500 nm with time for **1** from the urease–urea reaction in presence of Ca^{2+}. Initial conditions were (a) [urease] = 0.1 mg/mL, [urea] = 0.01 M, [Ca^{2+}] = 0.5 equiv.; (b) [urease] = 0.1 mg/mL, [urea] = 0.01 M, [Ca^{2+}] = 2 equiv.; (c) [urease] = 0.2 mg/mL, [urea] = 0.02 M, [Ca^{2+}] = 0.5 equiv.; (d) [urease] = 0.2 mg/mL, [urea] = 0.02 M, [Ca^{2+}] = 2 equiv. In all cases, [**1**] = 2 mg/mL and solvent is 20/80 DMSO/water (v/v). Figure (b) represents the normalized spectra of the data shown in (a).

Method	[Ca^{2+}] (Molar equiv.)	[urease] (mg/mL)	[urea] (M)	G′ (Pa) [at 0.5% strain]	Critical strain (%)	% Strain at crossover point
Urea+urease+Ca^{2+}	0.5	0.2	0.02	220	50	540
	0.5	0.1	0.01	710	35	715
	2	0.2	0.02	495	40	460
	2	0.1	0.01	1570	20	No crossover

Remark: [**1**] = 2 mg/mL, solvent is 20/80 DMSO/water (v/v).

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, irrespective of initial reaction conditions, an increase in Ca^{2+} ion concentration resulted in significant increase in gel stiffness. At a fixed Ca^{2+} ion concentration, gels obtained from slow kinetics are found to be stiffer but less strong (i.e. less strain withstanding capability). Moreover, among the enzyme-triggered gels the yield/crossover point increases when the gelation rate is slow.

Table S1: Comparison of strain sweep data of different Ca^{2+}-triggered gels of **1** obtained from the enzymatic reactions.

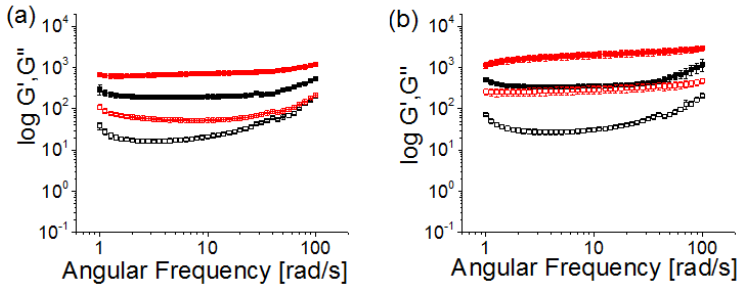

Figure S23. Frequency sweep experiments of the Ca^{2+}-triggered ((a) 0.5 equivalents and (b) 2 equivalents with respect to **1**) hydrogels of **1** (2 mg/mL) obtained from the enzymatic reactions involving initial conditions: [urease] = 0.2 mg/mL, [urea] = 0.02 M (black data); [urease] = 0.1 mg/mL, [urea] = 0.01 M (red data). In all cases, the closed symbols represent G′, the open symbols G″. Solvent is 20/80 DMSO/water (v/v).

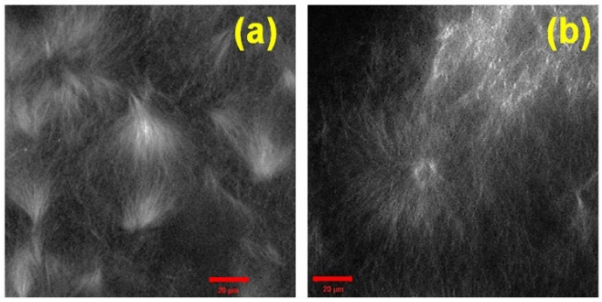

Figure S24. Confocal fluorescence microscopy images of the Ca^{2+}- gels of **1** (concentration = 2 mg/mL) prepared by external addition of 2 molar equivalents of Ca^{2+} ions to the solutions of **1** obtained from (a) NaOH and (b) enzymatic reaction involving initial conditions: [urease] = 0.1 mg/mL, [urea] = 0.01 M. In both cases, solvent is 20/80 DMSO/water (v/v). Scale bar is 20 μm.

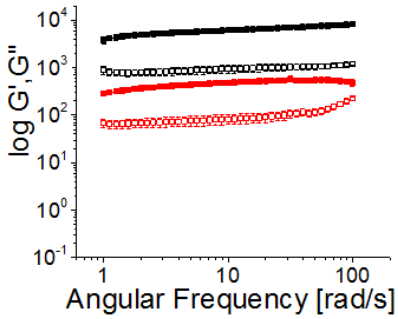

Figure S25. Frequency sweep experiments of the Ca^{2+}- gels of **1** (concentration = 2 mg/mL) prepared by external addition of 2 molar equivalents of Ca^{2+} ions to the solutions of **1** obtained from NaOH (black data) and enzymatic reaction (red data) involving initial conditions: [urease] = 0.1 mg/mL, [urea] = 0.01 M. In both cases, the closed symbols represent G′, the open symbols G″. Solvent is 20/80 DMSO/water (v/v).

Method	[urease] (mg/mL)	[urea] (M)	G′ (Pa) [at 0.5% strain]	Critical strain (%)	% Strain at crossover point
Urea+urease+Ca^{2+}	0.2	0.02	495	40	460
	0.1	0.01	1570	20	No crossover
NaOH followed by addition of Ca^{2+}	-	-	4965	6	25
Enzymatic reaction followed by addition of Ca^{2+}	0.1	0.01	250	10	95
Remark: [**1**] = 2 mg/mL, [Ca^{2+}] = 2 molar equivalents; solvent is 20/80 DMSO/water (v/v). The significance of G′, critical strain and cross over point are discussed in Table S1. In our case, all the homogeneous Ca^{2+}-triggered gels obtained from dissipative pH change showed significantly higher critical strain as well as crossover point compare to gels obtained from separate addition of Ca^{2+}.					

Table S2: Comparison of strain sweep data of Ca^{2+}-triggered gels of **1** prepared by different methods.