Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Programming properties of transient hydrogels by an enzymatic reaction

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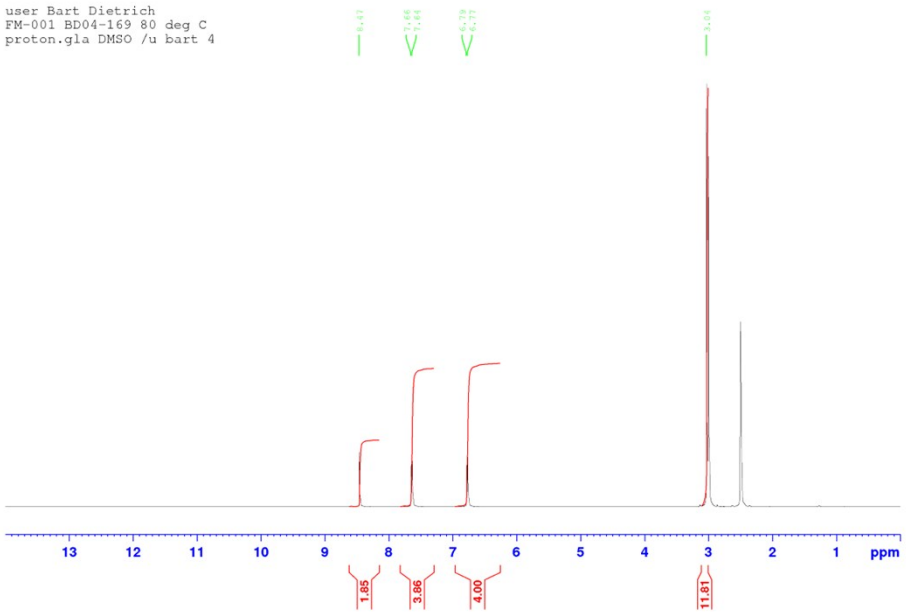
Materials and synthesis: 4-(Dimethylamino)benzaldehyde (**1**) and hydrazine monohydrochloride (**2**) were purchased from Sigma Aldrich and used as received. Urea (ultrapure 99%) and urease (J61455 urease, Jack Beans, minimum 45.0 units/mg solid) were purchased from Alfa Aesar. HCl and NaOH solutions (1 M) were obtained from Sigma Aldrich. DMSO-d_6 used in NMR experiments was also obtained from Sigma Aldrich. Deionised water was used throughout all experiments.

Synthesis of compound 3: Compound **3** was synthesized according to the following procedure and the characterization data were compared with previously reported values.[1, 2]

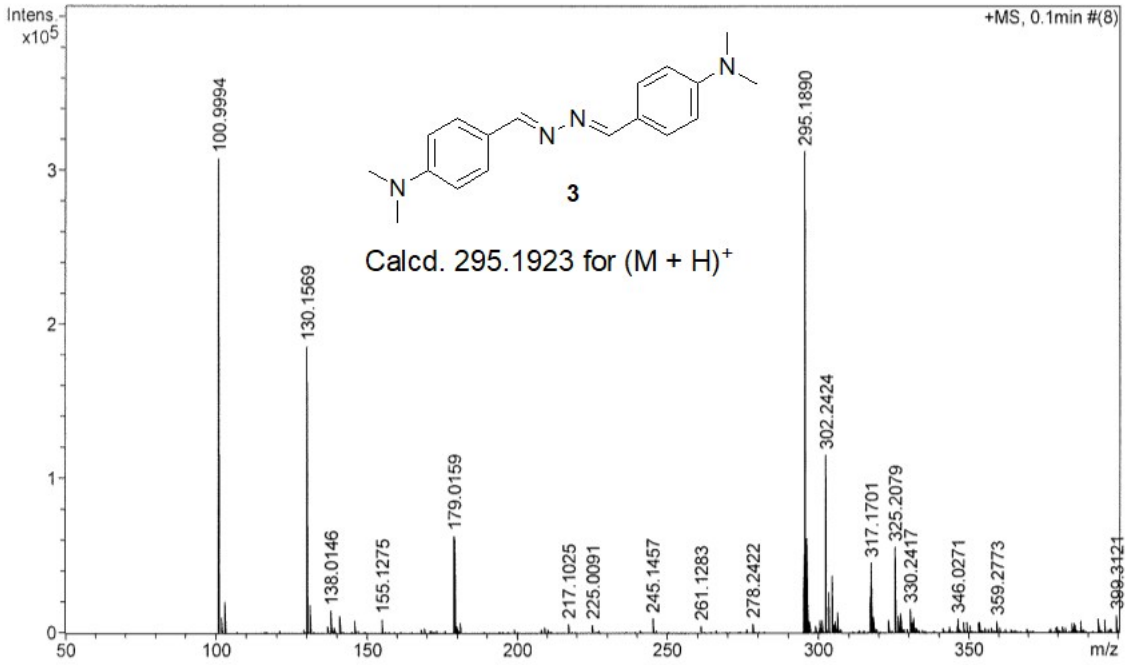
To a solution of 4-(*N*,*N*-dimethylamino)benzaldehyde (2 eq, 25.1 g, 0.168 mol) in DMSO (100 mL) at 80 °C was added a solution of hydrazine hydrate (1 eq, 4.21 g, 84.1 mmol) in DMSO (50 mL) and the mixture was stirred at 80 °C overnight. After this time a thick orange precipitate was found in the flask. This was difficult to filter as it tended to block the pores of the filter paper. The reaction mixture was instead centrifuged and the supernatant decanted. The remaining solid was washed with water, after which filtration became easier. The solid was washed with plenty of water in the filter and dried under vacuum. The title compound was obtained as an orange solid (19.3 g, 39%). Characterization data were in agreement with previously reported values.[1, 2]

^{1}H NMR (DMSO-d_6, 400 MHz, ppm, 80 °C): 8.47 (2H, s, N=C$\underline{H}$), 7.65 (4H, d, *J* 8.79, $\underline{H_{Ar}}$), 6.78 (4H, d, *J* 8.78, $\underline{H_{Ar}}$), 3.04 (12H, s, C$\underline{H_3}$). No carbon NMR was obtained due to the compound's very limited solubility. HRMS (ESI) m/z: $[M+H]^+$ calcd for $C_{18}H_{23}N_4$ 295.1923; found 295.1890.

^{1}H NMR of compound **3** in DMSO-d_6 at 80 °C.



HRMS spectrum of compound **3**.



Experimental details

Preparation of gels

Gels were prepared from the mixture of **1** and **2** in DMSO/H_2O (20/80, v/v) under different conditions in which the molar ratio of **1** and **2** was maintained as 2:1 at the begining of the reaction in all cases. Stock solution of **1** was prepared in DMSO at a concentration of 100 mg/mL by stirring. Stock solutions of **2**, urea and urease were prepared in H_2O at a concentration of 2 M, 2 M and 0.065 mg/mL respectively. Compound **2**, urea and urease were highly soluble in H_2O and therefore did not require stirring. The enzyme concentration in the stock solution was determined from the mass (in mg) dissolved in known volume of H_2O. Solutions of **1**, **2**, urease and urea were prepared freshly before each experiment.

To prepare the gels in absence and presence of NaOH, 0.40 mL of the solution of **1** was transfered in a 7 mL Sterilin vial. To this solution, either a mixture of 1.533 mL of H_2O and 0.067 mL of solution of **2** or a mixture of 1.399 mL of H_2O and 0.067 mL of solution of **2** and 0.134 mL of NaOH was added in one aliquot. Therefore, the ratio of DMSO and water was 20:80. Initial concentration of **1** was 20 mg/mL, initial concentrations of **2** and NaOH were 0.5 molar equivalent with respect to **1**.

Gelation experiments were also carried out in presence of urease. For these experiments, instead of aqueous solution of **2**, a mixture of 1.533 mL of urease and 0.067 mL of **2** was used. The pH of the mixture was pH 4.9 and it was further adjusted to pH 6.9, 7.2, 7.5, 7.6 and 7.75 (as required) by addition of NaOH (1 M). To prepare the gels, 1.60 mL of these solutions were added to 0.40 mL of **1**. The same solutions were used for the enzymatic reactions performed in presence of urea. For these experiments, 1.60 mL of the above mentioned solutions were transferred to the vial containing 0.40 mL of **1** and urea (either 20 µL or 40 µL) and left undisturbed. Therefore, the final ratio of DMSO and H_2O was 20:80 (v/v). Initial concentration of **1** was 20 mg/mL, initial concentration of urease was 0.05 mg/mL and the initial concentration of **2** was 0.5 molar equivalent with respect to **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. Samples 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.

Rheological measurements

All rheological measurements were carried out on an Anton Paar Physica MCR 101 and MCR 301 rheometers 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 0.2 % strain. Samples were prepared following the same procedure as described above and left ~18 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 in a 7 mL Sterilin vials keeping the same volumes of the components as mentioned earlier.

NMR spectroscopy and HRMS experiments

NMR spectra were recorded on a Bruker Avance III or Avance III HD 400 or 500 MHz instruments. HRMS spectra were recorded at the University of Glasgow on a Bruker micrOTOFQ instrument. Chemdraw prime (version 16) was used to calculate the mass values of the compounds.

For the gel (or sol), initially the sample was prepared using following the same methodology as described above. After ~18 hours, excess volume of DMSO-H_2O (20/80, v/v) was added to it and the mixture was

stirred for 10 mins. The resulting precipitate was filtered and washed well with hexane to obtain crude compound **3**. For, ^{1}H NMR, 4 mg of this crude sample was dissolved in 0.6 mL of DMSO-d_6. To record the the mass spectra, 0.05 mL of this solution was further diluted with 0.5 mL of CH_3CN.

Scanning Electron Microscopy (SEM)

To prepare samples for SEM experiments, gels (or sols) were deposited onto glass cover slips which were stuck onto aluminium SEM stubs using sticky carbon tabs and left to dry for overnight. Scanning electron microscopy images were obtained using an XL30 ESEM Phillips tungsten filament electron microscope with a secondary electron detector operating at 20 kV after gold coating (for 80 seconds) using a Polaron SC7640 sputter coater.

UV-Vis measurements

Absorption spectra of **1** and **2** under different conditions were recorded on an Agilent Technologies Cary 60 UV-Vis spectrophotometer using a 0.01 mm path length quartz cuvette. All the gel samples were prepared in Sterilin vials using the same methodology as described before and were immidiately transferred to the cuvette for measurement.

Fluorescence spectroscopy

Emission spectra of **1** and **2** under different conditions were recorded on an Agilent Technologies Cary Eclipse fluorescence spectrofluorophotometer. Samples were prepared in PMMA cuvettes with a path length of 1 cm by following the same procedure as mentioned before. In all cases, the excitation wavelength was 350 nm. Both the excitation and emission slit widths were 10 nm.

Supplementary Figures

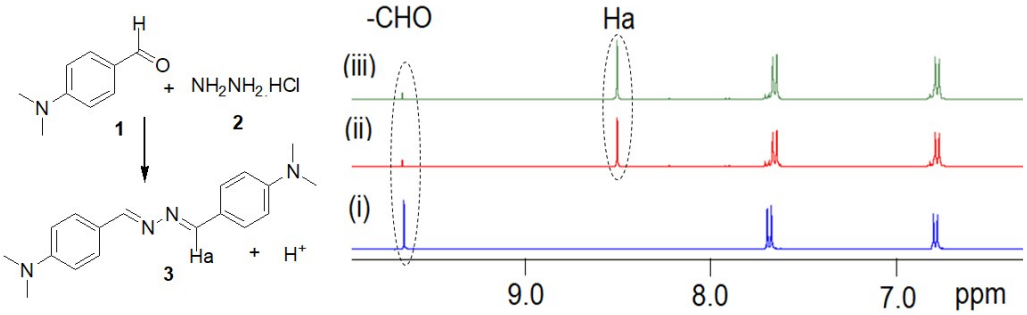


Figure S1. Partial ^{1}H NMR (in DMSO-d_6) spectra of (i) **1**, (ii) sol obtained from the mixture of **1** and **2** in absence of NaOH, (iii) gel obtained from the mixture of **1** and **2** in presence of NaOH. In all cases, initial concentration of **1** is 20 mg/mL, [**2**] = 0.5 equivalent. Molar ratio of **2** and NaOH is 1:1.

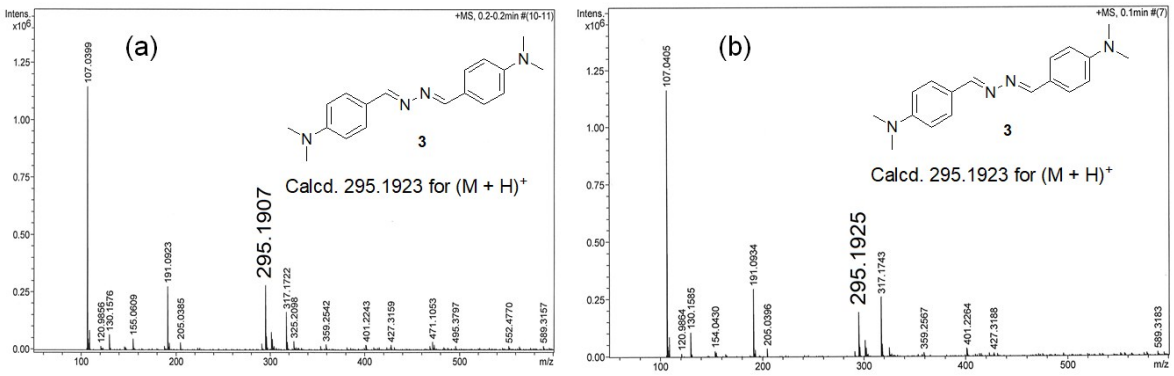


Figure S2. (a) and (b) represent the HRMS of the mixtures of **1** and **2** in absence (a) and presence (b) of NaOH. In all cases, initial concentration of **1** is 20 mg/mL, [**2**] = 0.5 equivalent. Molar ratio of **2** and NaOH is 1:1.

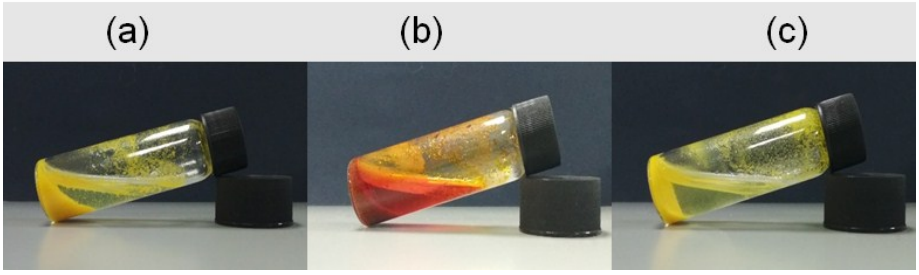


Figure S3. Photographs showing the phase change of **3** in DMSO/H_2O (20/80, v/v); (a) **3**, (b) **3** with equimolar amount of HCl and (c) **3** with equimolar amount of NaOH. In all cases [**3**] = 20mg/mL.

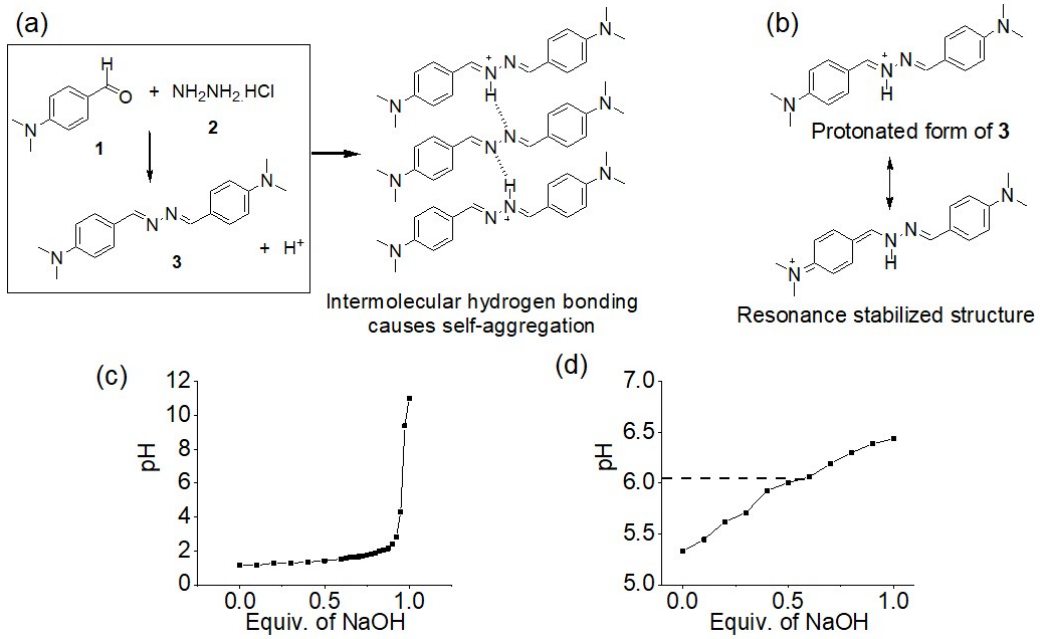


Figure S4. (a) Suggested mode of interaction of **3** during transient hydrogel formation at low pH involving the chemical reaction between **1** and **2**. (b) Resonance stabilized structure of the protonated form of **3** responsible for orange coloration of the gel under acidic condition. (c) and (d) represent the pH-metric titration of the mixture of compound **3** and equimolar amounts of HCl by NaOH in DMSO-H_2O (20/80, V/V). Concentration of **3** is 20 mg/mL (a) and 1 x 10^{-5} M (b).

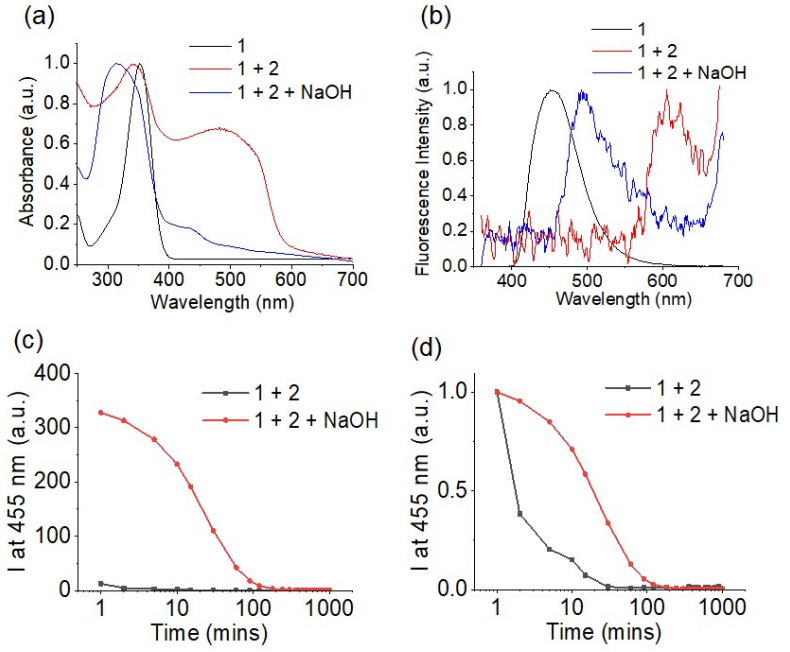


Figure S5. Normalised UV-vis (a) and emission (b) spectra of **1** and **2** under different conditions (after 18 hours). (c) Change in emission intensity at 455 nm with time for the mixture of **1** and **2** in absence and presence of NaOH. Figure (d) represents the normalized spectra of figure (c). In all cases, initial concentration of **1** is 20 mg/mL, [**2**] = 0.5 equivalent. Molar ratio of **2** and NaOH is 1:1. Solvent is DMSO/H_2O (20/80, v/v).

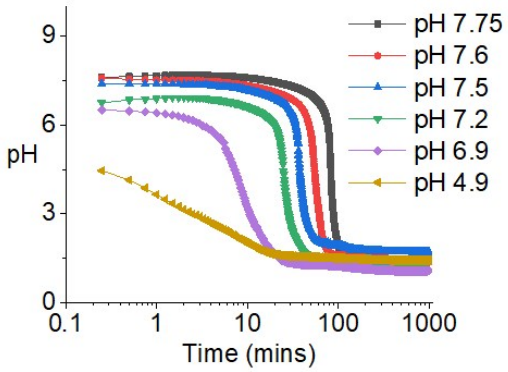


Figure S6. Change in pH with time for the mixture of **1** and **2** in the presence of urease starting at various initial pH. In all cases initial [**1**] = 20 mg/mL, [**2**] = 0.5 equivalent, [urease] = 0.05 mg/mL. Solvent is DMSO/H_2O (20/80, v/v).

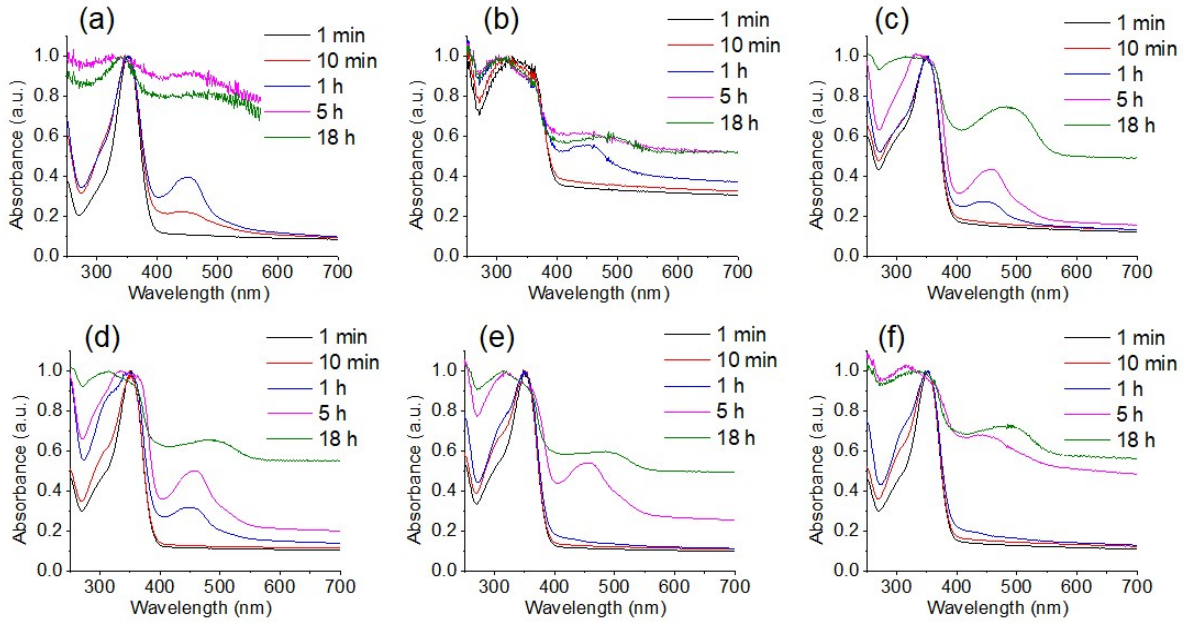


Figure S7. (a)-(f) Change in UV-vis spectra (normalized) of the mixture of **1** and **2** with time in the presence of urease involving initial pH 4.9, 6.9. 7.2. 7.5, 7.6 and 7.75 respectively. In all cases initial [**1**] = 20 mg/mL, [**2**] = 0.5 equivalent, [urease] = 0.05 mg/mL. Solvent is DMSO/H_2O (20/80, v/v).

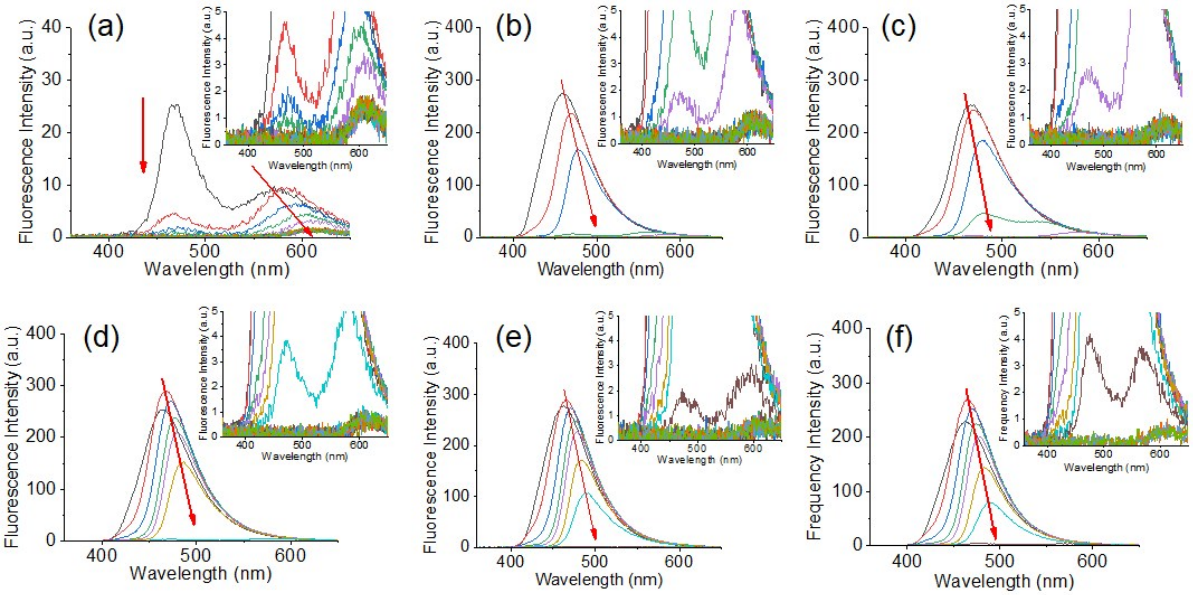


Figure S8. (a)-(f) Change in emission of the mixture of **1** and **2** with time in the presence of urease involving initial pH 4.9, 6.9. 7.2. 7.5, 7.6 and 7.75 respectively. Insets represent the expanded sections of the corresponding graph showing the final peak at 600 nm. In all cases initial [**1**] = 20 mg/mL, [**2**] = 0.5 equivalent, [urease] = 0.05 mg/mL. Solvent is DMSO/H_2O (20/80, v/v).

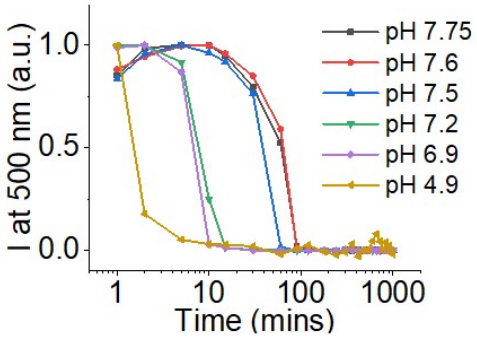


Figure S9. Change in emission intensity at 500 nm (normalized) with time for the mixture of **1** and **2** in presence of urease involving different initial pH. In all cases initial [**1**] = 20 mg/mL, [**2**] = 0.5 equivalent, [urease] = 0.05 mg/mL. Solvent is DMSO/H_2O (20/80, v/v).

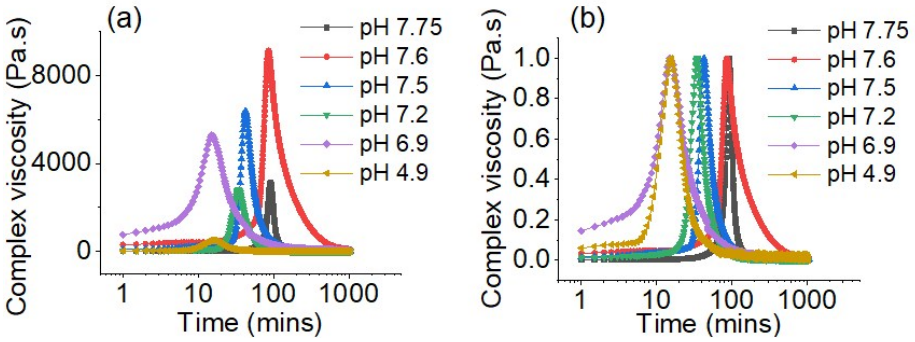


Figure S10. (a) variation of complex viscosity of the mixture of **1** and **2** with time in the presence of urease involving different initial pH. Figure (b) is the normalized graph of (a). In all cases initial [**1**] = 20 mg/mL, [**2**] = 0.5 equivalent, [urease] = 0.05 mg/mL. Solvent is DMSO/H_2O (20/80, v/v).

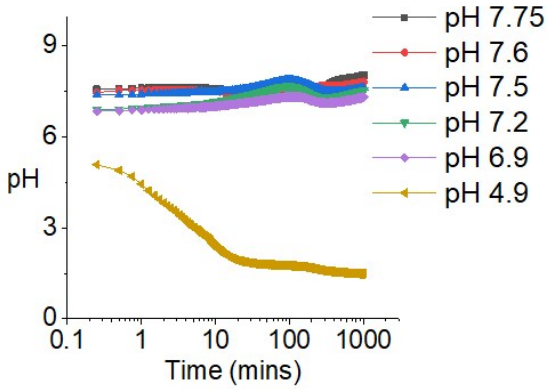


Figure S11. Change in pH with time for the mixture of **1** and **2** involving urea (40 µL of 2 M) and urease reaction starting at different initial pH. In all cases initial [**1**] = 20 mg/mL, [**2**] = 0.5 equivalent, [urease] = 0.05 mg/mL. Solvent is DMSO/H_2O (20/80, v/v).

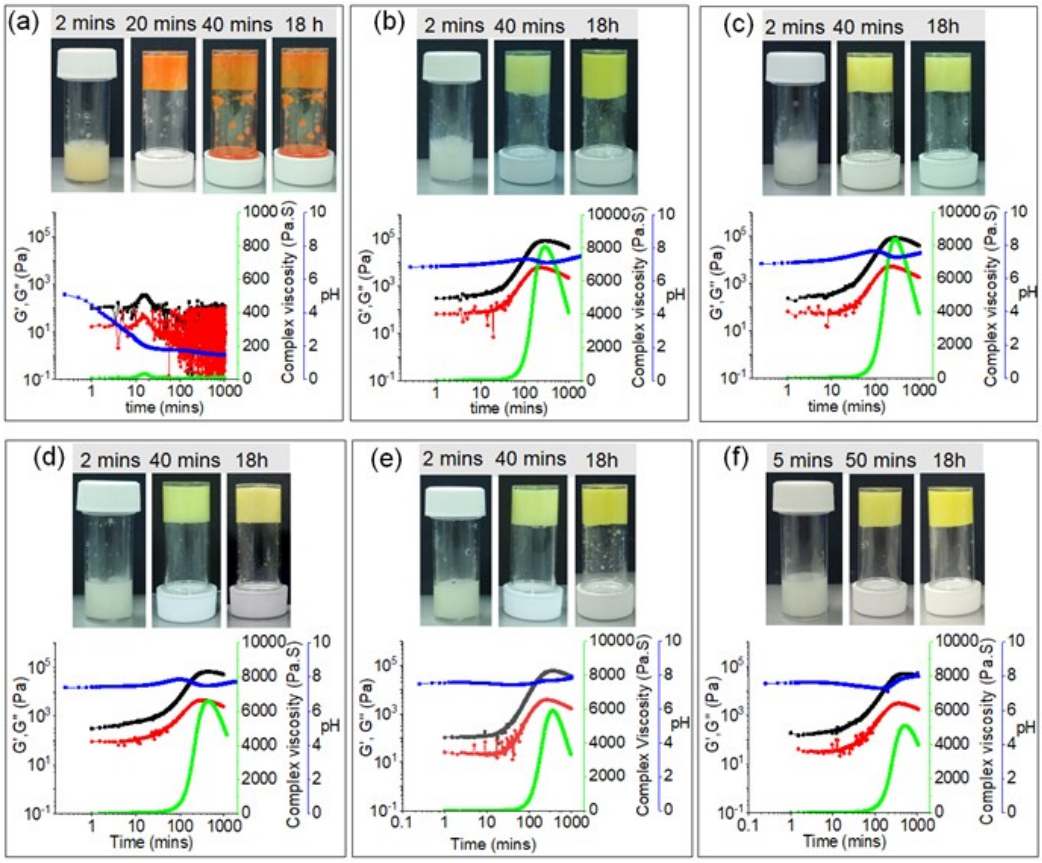


Figure S12. (a-f) Variation of G′ (black), G″ (red), complex viscosity (green) and pH (blue) with time for mixture of **1** and **2** involving urea (40 μL of 2 M) and urease reaction starting at different initial pH: 4.9, 6.9, 7.2, 7.5, 7.6 and 7.75 respectively. Photographs showing the phase changes of the mixture of **1** and **2** with time under respective conditions. In all cases, initial concentration of **1** is 20 mg/mL, [**2**] = 0.5 equivalent, [urease] = 0.05 mg/mL. Solvent is DMSO/H_2O (20/80, v/v).

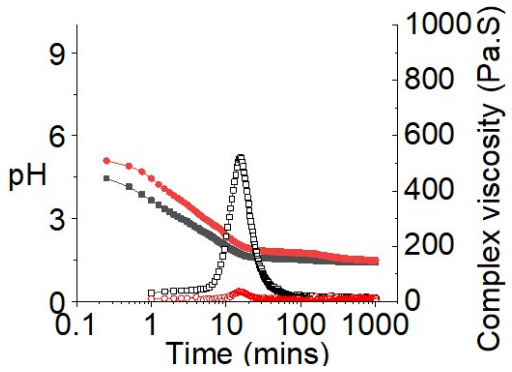


Figure S13. Variation of pH (closed symbols) and complex viscosity (open symbols) for the mixture of **1**, **2** and urease in absence (black data) and presence (red data) of urea (40 μL of 2 M). In all cases, initial pH of the mixture of **2** and urease is pH 4.9. Initial [**1**] = 20 mg/mL, [**2**] = 0.5 equivalent, [urease] = 0.05 mg/mL. Solvent is DMSO/H_2O (20/80, v/v).

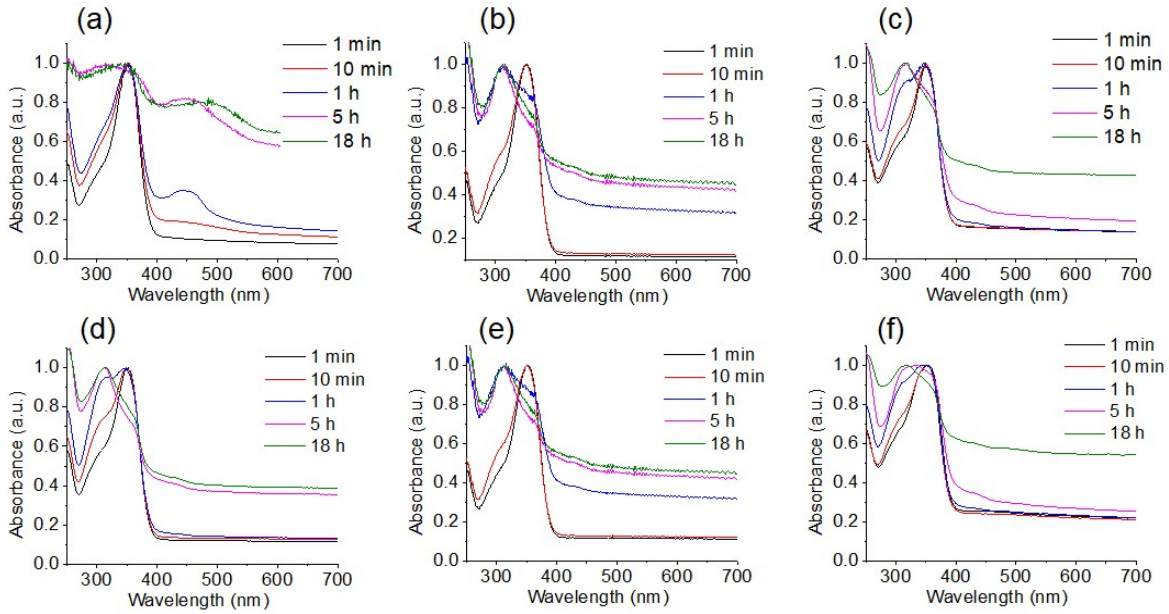


Figure S14. (a)-(f) Change of UV-vis spectra (normalised) of **1** and **2** with time involving urea (40 µL of 2 M) and urease reaction starting at initial pH 4.9, 6.9. 7.2. 7.5, 7.6 and 7.75 respectively. In all cases initial [**1**] = 20 mg/mL, [**2**] = 0.5 equivalent, [urease] = 0.05 mg/mL. Solvent is DMSO/H_2O (20/80, v/v).

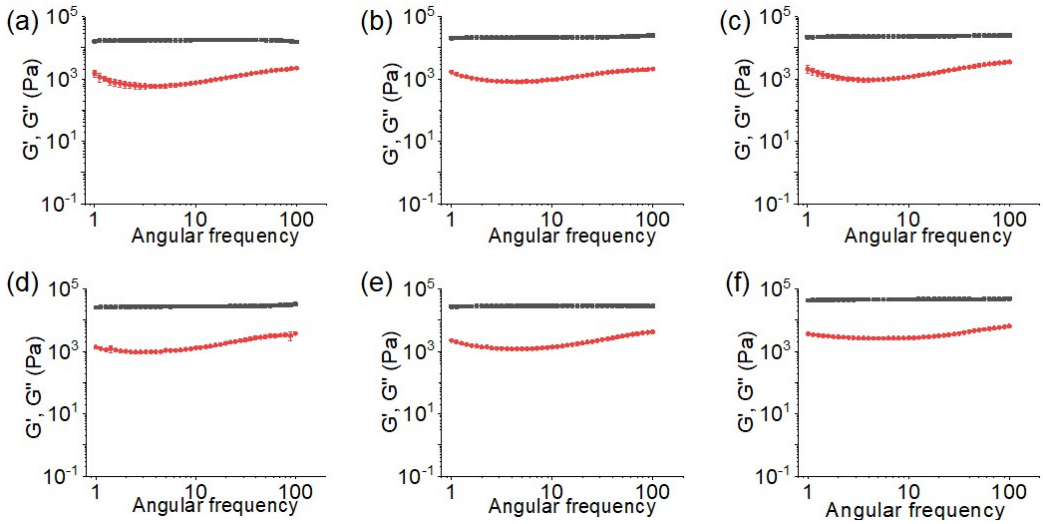


Figure S15. (a-e) Frequency sweep experiment for the hydrogels obtained from the mixture of **1** and **2** involving urea (40 µL of 2 M) and urease reaction starting at different initial pH: 6.9, 7.2, 7.5, 7.6 and 7.75 respectively. (f) is the frequency sweep experiment for the hydrogel obtained from the mixture of **1** and **2** in presence of NaOH. The black data represents G' and the red data G″. In all cases, initial concentration of **1** is 20 mg/mL, [**2**] = 0.5 equivalent, [urease] = 0.05 mg/mL. Molar ratio of **2** and NaOH is 1:1. Solvent is DMSO/H_2O (20/80, v/v).

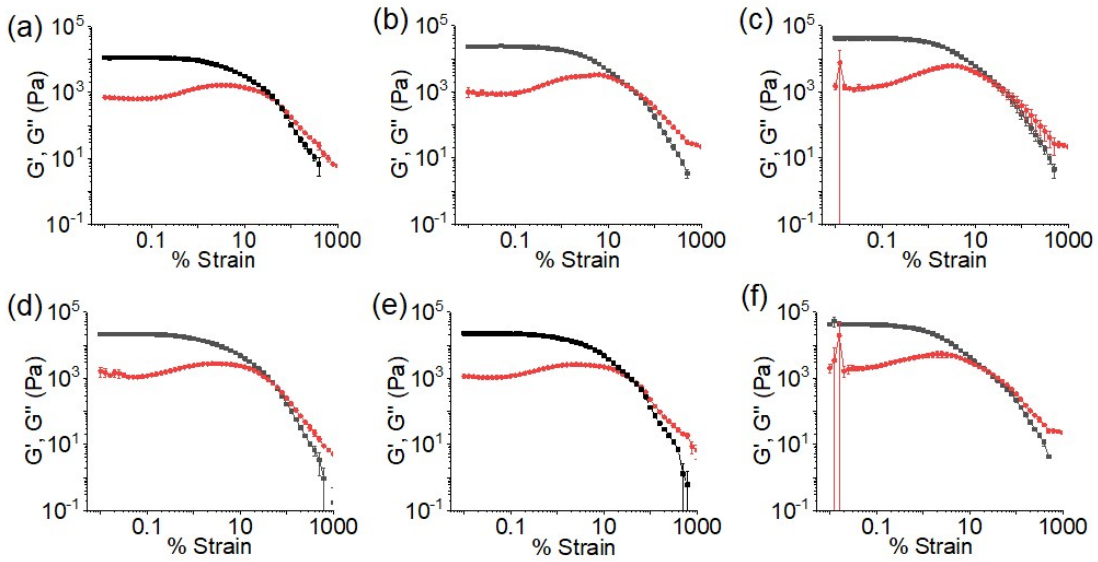


Figure S16. (a-e) Strain sweep experiment for the hydrogels obtained from the mixture of **1** and **2** involving urea (40 µL of 2 M) and urease reaction starting at different initial pH: 6.9, 7.2, 7.5, 7.6 and 7.75 respectively. (f) is the strain sweep experiment for the hydrogel obtained from the mixture of **1** and **2** in presence of NaOH. The black data represents G' and the red data G″. In all cases, initial concentration of **1** is 20 mg/mL, [**2**] = 0.5 equivalent, [urease] = 0.05 mg/mL. Molar ratio of **2** and NaOH is 1:1. Solvent is DMSO/H_2O (20/80, v/v).

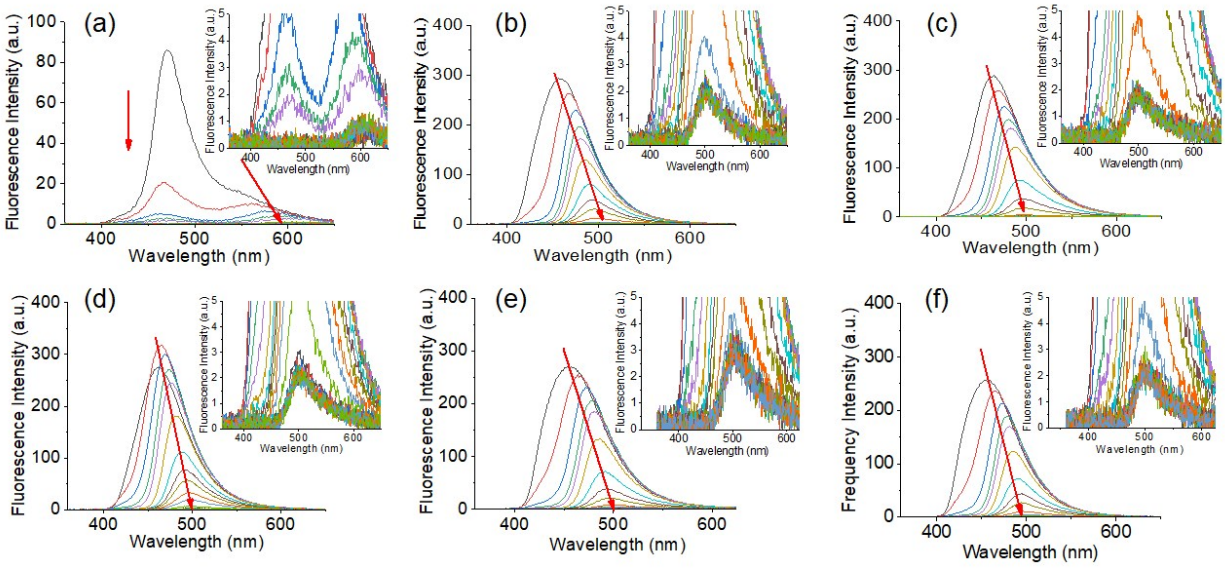


Figure S17. (a)-(f) Change in emission of **1** and **2** involving urea (40 µL of 2 M) and urease reaction starting at initial pH 4.9, 6.9. 7.2. 7.5, 7.6 and 7.75 respectively. Insets represent the expanded sections of the corresponding graph showing the final absorbance in each case. In all cases initial [**1**] = 20 mg/mL, [**2**] = 0.5 equivalent, [urease] = 0.05 mg/mL, [urea] = 2 M. Solvent is DMSO/H_2O (20/80, v/v).

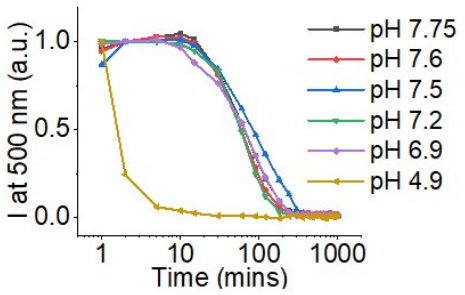


Figure S18. Change in emission intensity at 500 nm (normalized) for **1** in presence of 40 µL of urea and **2** in presence of urease involving different initial pH. In all cases initial [**1**] = 20 mg/mL, [**2**] = 0.5 equivalent, [urease] = 0.05 mg/mL, [urea] = 2 M. Solvent is DMSO/H_2O (20/80, v/v).

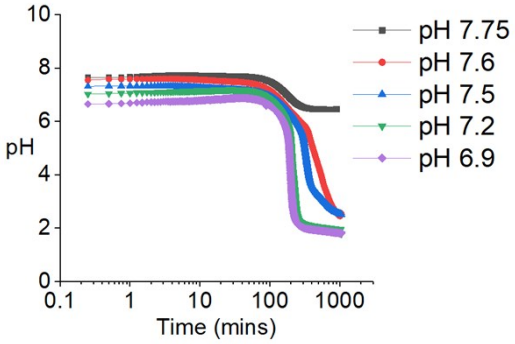


Figure S19. Change in pH with time for the mixture of **1** and **2** involving urea (20 µL of 2M) and urease reaction starting at different initial pH. In all cases initial [**1**] = 20 mg/mL, [**2**] = 0.5 equivalent, [urease] = 0.05 mg/mL. Solvent is DMSO/H_2O (20/80, v/v).

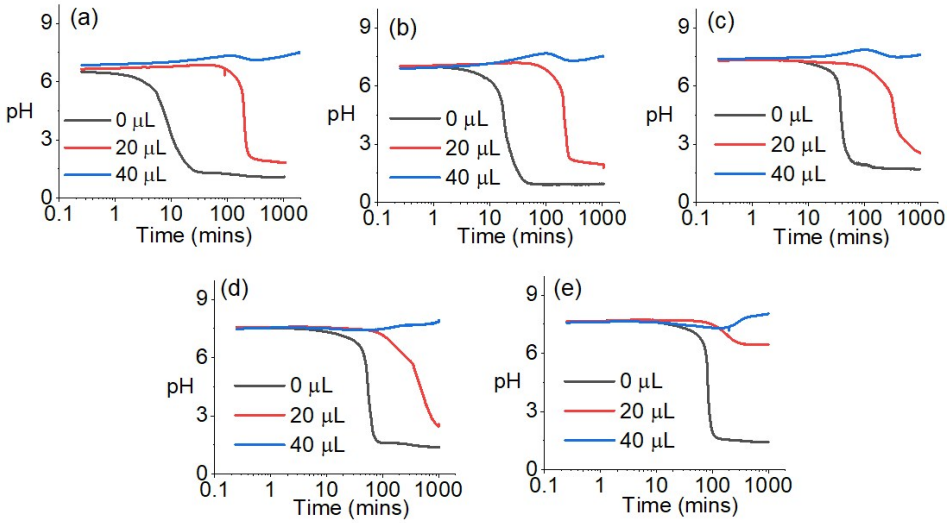


Figure S20. Change in pH with time for the mixture of **1** and **2** involving urea (20 µL of 2M) and urease reaction starting at different initial pH. In all cases initial [**1**] = 20 mg/mL, [**2**] = 0.5 equivalent, [urease] = 0.05 mg/mL. Solvent is DMSO/H_2O (20/80, v/v).

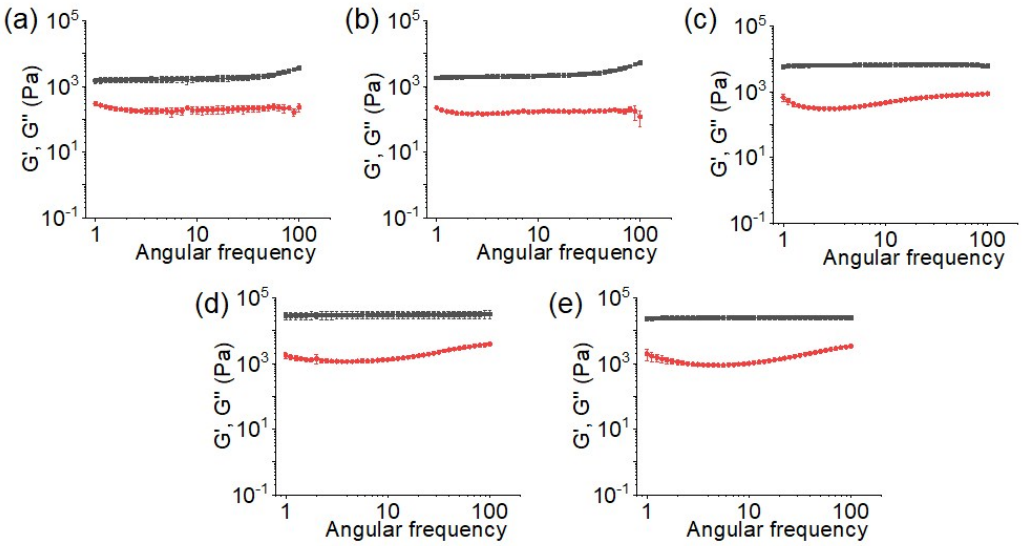


Figure S21. (a-e) Frequency sweep experiments for the hydrogels obtained from the mixture of **1** and **2** involving urea (20 µL of 2 M) and urease reaction starting at different initial pH: 6.9, 7.2, 7.5, 7.6 and 7.75 respectively. The black data represents G' and the red data G″. In all cases, initial concentration of **1** is 20 mg/mL, [**2**] = 0.5 equivalent, [urease] = 0.05 mg/mL. Solvent is DMSO/H_2O (20/80, v/v).

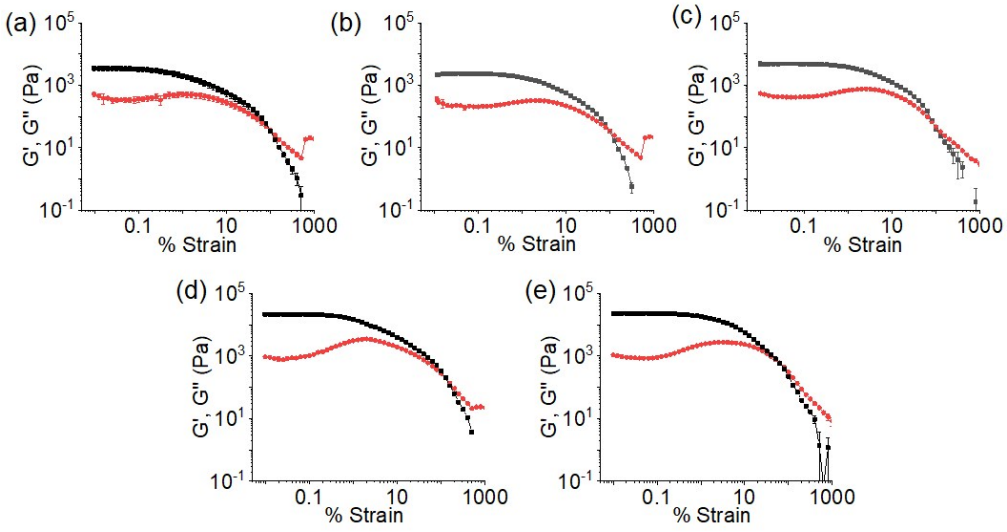


Figure S22. (a-e) Strain sweep experiments for the hydrogels obtained from the mixture of **1** and **2** involving urea (20 µL of 2 M) and urease reaction starting at different initial pH: 6.9, 7.2, 7.5, 7.6 and 7.75 respectively. The black data represents G' and the red data G″. In all cases, initial concentration of **1** is 20 mg/mL, [**2**] = 0.5 equivalent, [urease] = 0.05 mg/mL. Solvent is DMSO/H_2O (20/80, v/v).

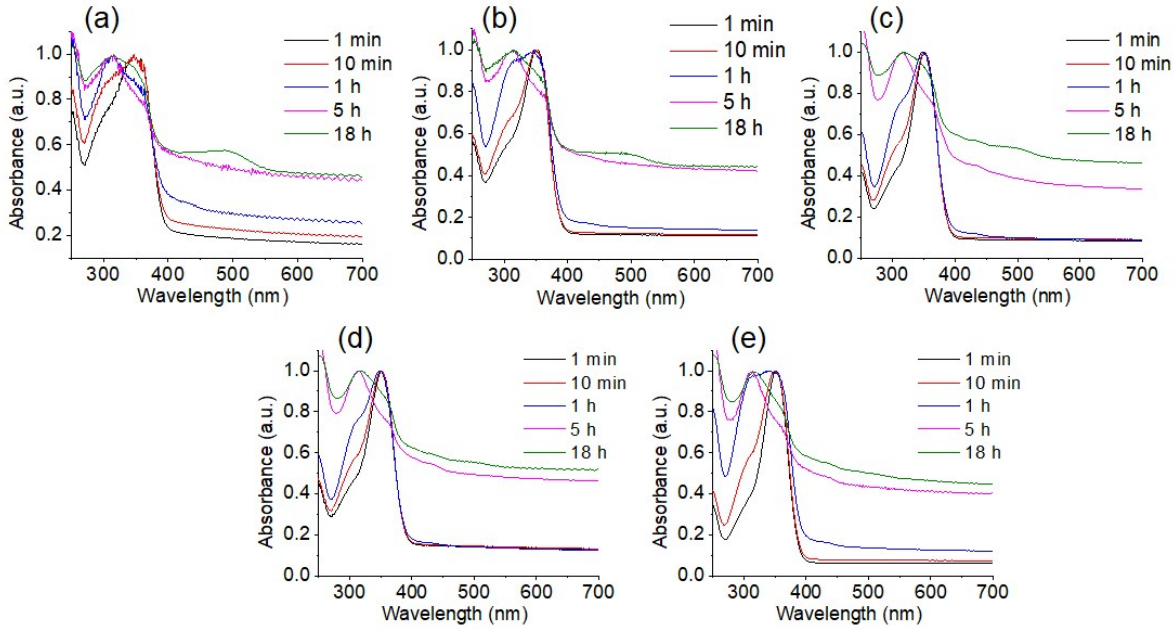


Figure S23. (a)-(e) Change of UV-vis spectra of the mixture of **1** and **2** with time involving urea (20 μL of 2 M) and urease reaction starting at initial pH 6.9. 7.2. 7.5, 7.6 and 7.75 respectively. In all cases initial [**1**] = 20 mg/mL, [**2**] = 0.5 equivalent, [urease] = 0.05 mg/mL. Solvent is DMSO/H_2O (20/80, v/v).

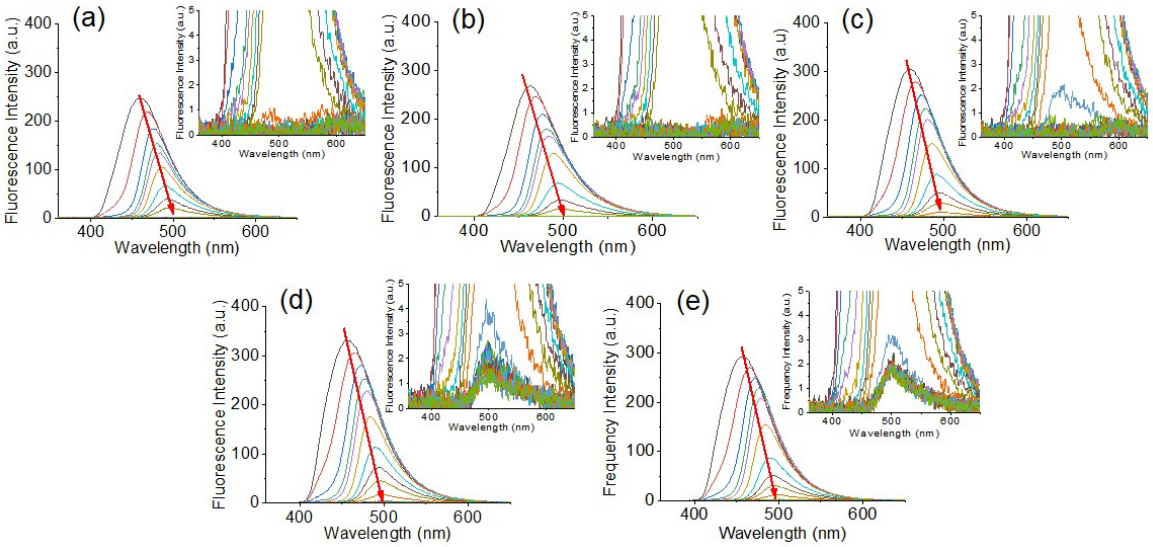


Figure S24. (a)-(e) Change in emission of the mixture of **1** and **2** involving urea (20 μL of 2 M) and urease reaction starting at initial pH 6.9. 7.2. 7.5, 7.6 and 7.75 respectively. Insets represent the expanded sections of the corresponding graphs. In all cases initial [**1**] = 20 mg/mL, [**2**] = 0.5 equivalent, [urease] = 0.05 mg/mL, [urea] = 2 M. Solvent is DMSO/H_2O (20/80, v/v).

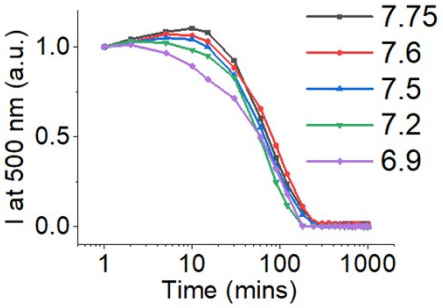


Figure S25. Change in emission intensity at 500 nm (normalized) for the mixture of **1** and **2** in presence of urea (20 µL of 2 M) – urease reaction involving different initial pH. In all cases initial [**1**] = 20 mg/mL, [**2**] = 0.5 equivalent, [urease] = 0.05 mg/mL, [urea] = 2 M. Solvent is DMSO/H_2O (20/80, v/v).

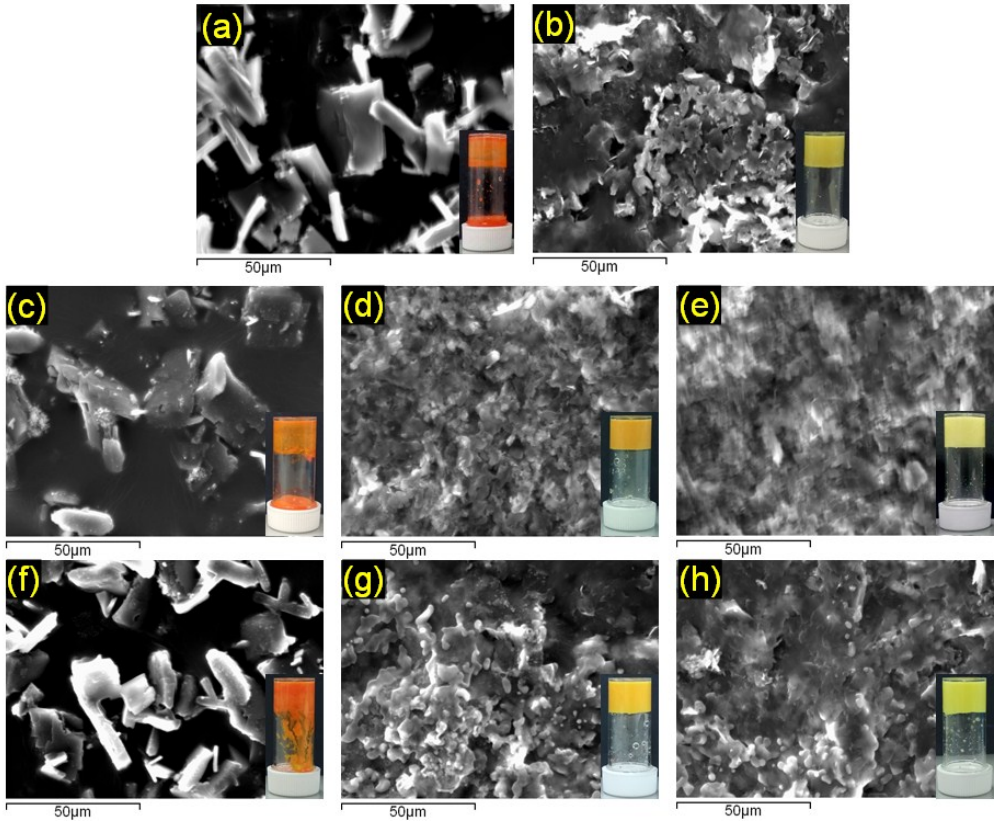


Figure S26. (a-b) SEM image of the of the mixture of **1** and **2** in absence (a) and presence (b) of NaOH. (c-e) SEM image of the of the mixture of **1** and **2** in presence of urease and urea (0 µL, 20 µL and 40 µL of 2 M respectively) starting at initial pH 7.5. (f-h) SEM image of the of the mixture of **1** and **2** in presence of urease and urea (0 µL, 20 µL and 40 µL of 2 M respectively) starting at initial pH 7.6. Photographs showing the corresponding mixture of **1** and **2** after 18h under respective conditions. In all cases initial [**1**] = 20 mg/mL, [**2**] = 0.5 equivalent, [urease] = 0.05 mg/mL, [urea] = 2 M. Solvent is DMSO/H_2O (20/80, v/v). The scale bar represents 50 µm.

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