

Chemical crosslinking in ‘reactive’ multicomponent gels

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Supporting Information

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Experimental details

Materials: Compound **1** was synthesized as described previously.¹ EDC hydrochloride and 1-pyrenemethylamine hydrochloride (**2**) were purchased from Fluorochem and Sigma-Aldrich respectively and used as received. All other chemicals and solvents were purchased from commercially available suppliers and used without further purification. Deionized water was used throughout all experiments.

Preparation of solutions: Stock solutions of **1** and **2** were prepared in DMSO at a concentration of 20 mg/mL and 31 mg/mL, respectively. A stock solution of EDC was prepared in H₂O at a concentration of 1 M. The stock solutions of NaOH and HCl were prepared at a concentration of 0.1 M in H₂O.

Preparation of gels: To prepare the hydrogels, a solvent switch method was followed. In general, water was added to the DMSO solution of **1** (in absence and presence of **2**) in one aliquot to prepare the gels at a total volume of 2 mL in which the ratio of DMSO and water was 20:80.

To prepare the hydrogel of **1**, 0.2 mL solution of **1** was diluted by 0.2 mL of DMSO. To this solution, 1.60 mL of H₂O was added in one aliquot. Hence the concentration of **1** in the gel was 2 mg/mL.

The multicomponent hydrogels were prepared by mixing compound **1** with **2** in DMSO/H₂O (20/80, v/v) under different conditions. To prepare the gels at pH 3.1, 1.60 mL of water was added to a mixture of 0.2 mL solution of **1**, 0.069 mL of solution of **2**, and 0.131 mL of DMSO. Similarly, the addition of 1.520 mL of water to the mixture of 0.08 mL of NaOH, 0.2 mL solution of **1**, 0.069 mL of solution of **2**, and 0.131 mL of DMSO resulted in gels at pH 7.8. To prepare the gels at pH 10.4, 1.440 mL of water was added to the mixture of 0.16 mL of NaOH, 0.2 mL solution of **1**, 0.069 mL of solution of **2**, and 0.131 mL of DMSO. Therefore, in all cases, the concentration of **1** was 2 mg/mL, the molar ratio of **1** and **2** was 1:1. Note that the amounts of NaOH required to deprotonate **1** and **2** sequentially in the multicomponent gels were calculated from the molar concentration of **1**. Hence, the concentrations of NaOH in the gels at pH 7.8 and 10.4 were 1 and 2 molar equivalents respectively.

To achieve crosslinking between **1** and **2** in the gels, a post gelation functionalization (PAF) method was followed. In all cases, gels were initially prepared as mentioned earlier in 2 mL volume in 7 mL Sterilin vials. Then a mixture of 0.008 mL of EDC and 0.5 mL of water was added carefully on the top of the gels and left undisturbed for ~20 hours. After that, the water was separated by decantation and the gels were used for further experiments. Therefore, the concentration of EDC used for crosslinking was 1 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 .

pK_a determination was carried out by recording the pH values after each addition of NaOH (0.1 M) to the solution of **2** (concentration is 1.07 mg/mL) in 20% DMSO in H₂O. During the titration, the solution was continuously. The experimental temperature was 25 °C.

Rheological measurements: All rheological measurements were undertaken on an Anton Paar Physica MCR 301 and 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 overnight 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 7 mL Sterilin vials.

Confocal microscopy: A Zeiss LSM710 confocal microscope (Zeiss, Göttingen, Germany) with an LD EC Epiplan NEUFLUAR 50X, 0.55 DIC (Carl Zeiss, White Plains, NY, USA) objective was used for imaging. All gel samples were prepared in presence of Nile blue (2 $\mu\text{L}/\text{mL}$ of a 0.1 wt % solution in water). For the hydrogel of **1** and the multicomponent gels of **(1+2)** at different pH, samples were prepared as mentioned earlier in CELLview culture dishes (35 mm diameter). For the EDC-triggered crosslinked gels, samples were prepared in 7 mL Sterilin vials keeping the same volumes of the components as mentioned earlier. Then small amounts of the gels were deposited onto glass microscope slides. A cover slip was placed carefully on the gel before imaging. All the samples were excited at 633 nm using a He-Ne laser. Images were captured using Carl Zeiss ZEN 2011 v7.0.3.286 software.

UV-Vis spectroscopy: Absorption spectra of **1** and **2** under different conditions (i.e gels and sols) were recorded on an Agilent Technologies Cary 60 UV-Vis spectrophotometer using a 0.01 mm path length quartz cuvette. All gel samples were prepared in Sterilin vials using the same methodology as described earlier and were left overnight. Then, small amounts of the gels were transferred to the cuvette for measurement.

Fluorescence spectroscopy: Emission data were collected on an Agilent Technologies Cary Eclipse fluorescence spectrometer. Samples were prepared in PMMA cuvettes with a path length of 1 cm by following the same procedure as mentioned earlier. All gels were left overnight before measurements were carried out. In all cases, the excitation wavelength was 280 nm. Both the excitation and emission slit widths were 5 nm.

FTIR spectroscopy: Data were recorded using an Agilent Cary 630 FTIR spectrometer (with ATR attachment). For all samples, 20% DMSO- d_6 in D_2O was used for the background correction. All gels were prepared using DMSO- d_6 , D_2O and NaOD following the same methodology as described above. Then, small amounts of the gels were deposited on the ATR crystal before recording the spectra.

NMR spectroscopy and HRMS experiments: ^1H 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.

All the gels were initially prepared following the same methodology as described earlier. The solvent was then removed by freeze-drying. To record NMR, the freeze-dried samples were dissolved in 0.6 mL of d_6 -DMSO. 0.05 mL of the same solutions were further diluted with 0.5 mL of CH_3CN to record the mass spectra.

To confirm the crosslinking, gels were also prepared by direct addition of EDC to the mixture of **(1+2)** in absence and presence of different concentrations of NaOH. For these directly prepared samples, instead of H_2O a mixture of EDC (0.008 mL) and water was used keeping the compositions of the rest of the components identical. Again, the samples were freeze-dried after almost 20 h of addition of EDC followed by dissolving in 0.6 mL of d_6 -DMSO for NMR. 0.05 mL of the same solutions were diluted with 0.5 mL of CH_3CN to record the mass spectra.

We also recorded proton NMR of the crosslinked gels under acidic conditions. For these experiments, gels were initially prepared by following the same methodology as described earlier (for both PAF and directly prepared gels). Then either 1.25 equiv. or 2.25 equiv. of HCl (for pH 7.8 and 10.4 respectively) was added on the top of the gels and allowed diffusion of HCl into the gels for 8 h. After that, the samples were freeze-dried.

Supplementary Figures

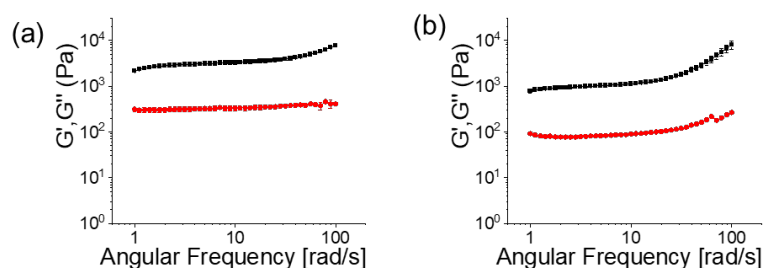


Figure S1. Frequency sweeps of (a) the hydrogel of **1** and (b) the multicomponent gels of **(1+2)** at pH 3.1. In all cases, concentration of **1** is 2 mg/mL and molar ratio of **1** and **2** is 1:1. Solvent is DMSO/H₂O (20/80, v/v). The black symbols represent G' , the red symbols G'' . Measurements were performed in triplicate, and error bars were calculated from the standard deviation.

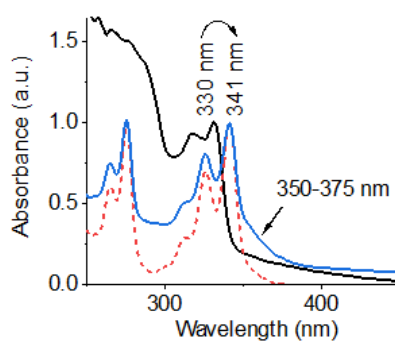


Figure S2. UV-vis spectra of hydrogel of **1** (black), sol of **2** (red) and the multicomponent gel of **(1+2)** at pH 3.1 (blue). In all cases, concentration of **1** is 2 mg/mL and molar ratio of **1** and **2** is 1:1. Solvent is DMSO/H₂O (20/80, v/v).

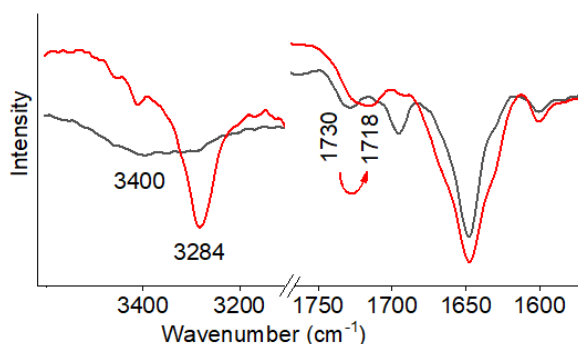


Figure S3. Partial FTIR spectra of the hydrogel of **1** (black) and the multicomponent gels of **(1+2)** at pH 3.1 (red). In all cases, concentration of **1** is 2 mg/mL and molar ratio of **1** and **2** is 1:1. Solvent is DMSO/H₂O (20/80, v/v).

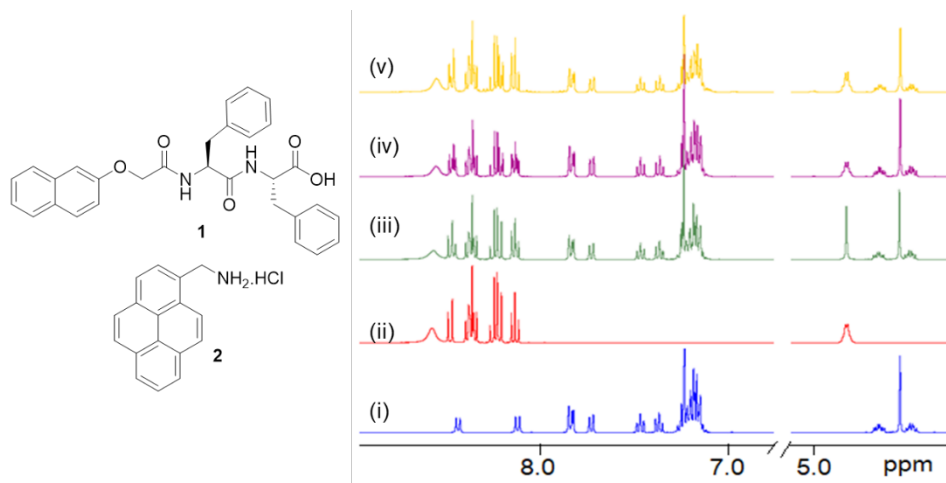


Figure S4. Partial ^1H NMR spectra (in DMSO-d_6) spectra of (i) **1**, (ii) **2**, (iii) hydrogel of (**1+2**) at pH 3.1. (iv) and (v) represent proton NMR of the multicomponent gel of (**1+2**) after treatment with EDC. For (iv), EDC was added to the preformed gel (post assembly functionalization) of (**1+2**). For (v), EDC was directly added to the mixture of (**1+2**). In all cases, concentration of **1** is 2 mg/mL and molar ratio of **1** and **2** is 1:1, concentration of EDC is 1 equiv. Solvent is $\text{DMSO/H}_2\text{O}$ (20/80, v/v).

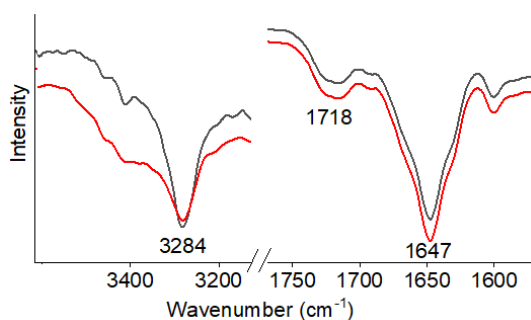


Figure S5. Partial FTIR spectra of the hydrogel of (**1+2**) before (black) and after (red) addition of EDC at pH 3.1. In all cases, concentration of **1** is 2 mg/mL and molar ratio of **1** and **2** is 1:1, concentration of EDC is 1 equiv. Solvent is $\text{DMSO/H}_2\text{O}$ (20/80, v/v).

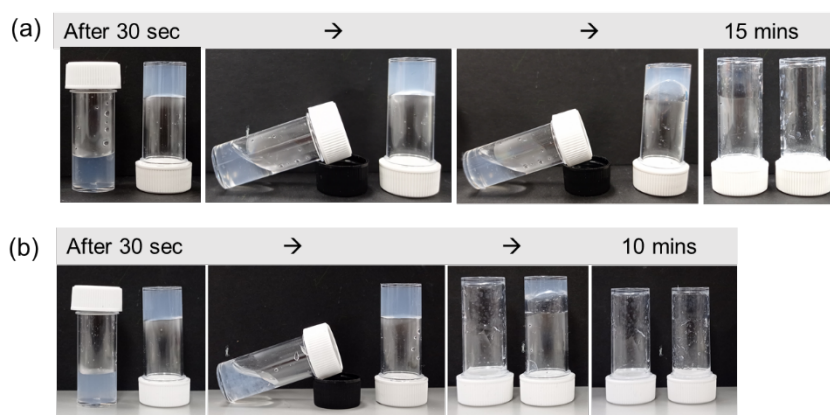


Figure S6. (a, b) Photographs showing the phase changes of **1** in absence (left vial) and presence of EDC (right vial). For (a) and (b) gelation tests are performed in absence and presence of 1 equiv. of HCl respectively. In all cases, concentration of **1** is 0.75 mg/mL and concentration of EDC is 1 equiv. Solvent is $\text{DMSO/H}_2\text{O}$ (20/80, v/v).

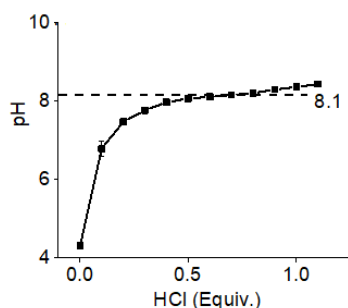


Figure S7. Determination of apparent pK_a of **2** in DMSO/H₂O (20/80, v/v). The plateau is taken to represent the apparent pK_a value, shown by the horizontal line.

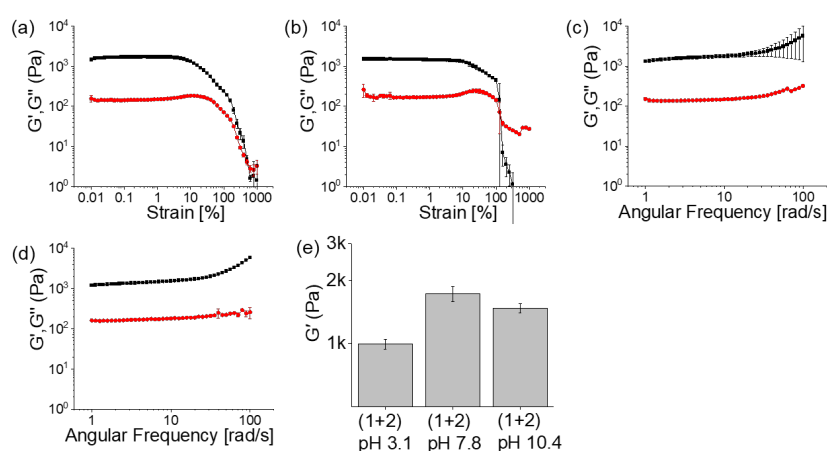


Figure S8. (a, b) Strain sweeps and (c, d) frequency sweeps for the hydrogels of (1+2) at pH 7.8 (a, c) and 10.4 (b, d). The black symbols represent G' , the red symbols G'' . (e) Bar graph representing the stiffness (G' , calculated at 0.5% strain from strain sweeps) of the hydrogels of (1+2) prepared at different pH. In all cases, concentration of **1** is 2 mg/mL and molar ratio of **1** and **2** is 1:1, concentration of EDC is 1 equiv. Solvent is DMSO/H₂O (20/80, v/v). Measurements were performed in triplicate, and error bars were calculated from the standard deviation.

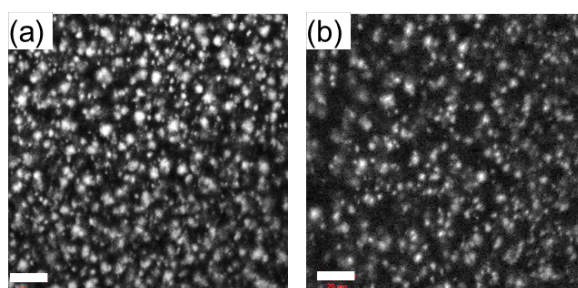


Figure S9. Confocal fluorescence microscopy images (scale bars represent 20 μm) of the hydrogels of (1+2) at pH 7.8 (a) and 10.4 (b). In both cases, concentration of **1** is 2 mg/mL and molar ratio of **1** and **2** is 1:1. Solvent is DMSO/H₂O (20/80, v/v).

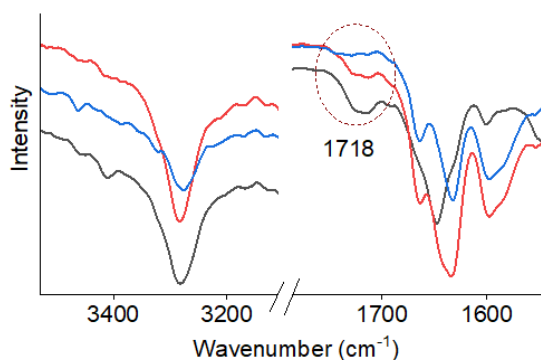


Figure S10. Partial FTIR spectra of the hydrogel of (1+2) at pH 3.1 (black), 7.8 (blue) and 10.4 (red). In all cases, concentration of **1** is 2 mg/mL and molar ratio of **1** and **2** is 1:1. Solvent is DMSO/H₂O (20/80, v/v).

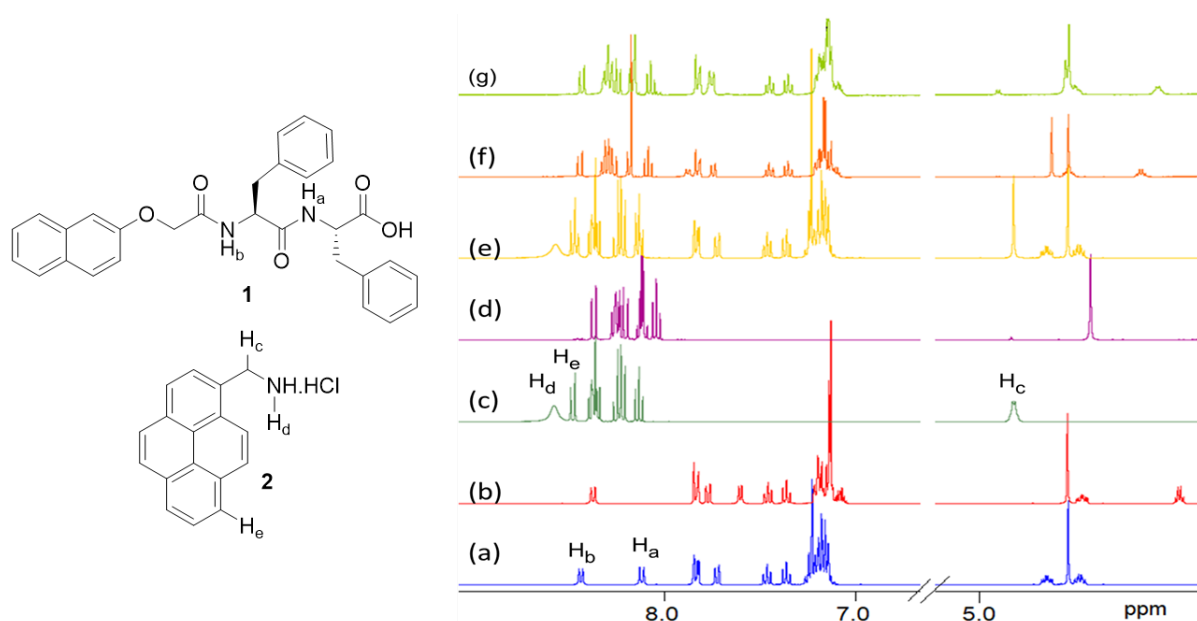


Figure S11. Partial ¹H NMR spectra (in DMSO-d₆) spectra of (a) **1**, (b) **1** + 1 equiv. NaOH, (c) **2**, (d) **2** + 1 equiv. NaOH, and hydrogels of (1+2) at pH (e) 3.1, (f) 7.8 and (g) 10.4. In all cases, concentration of **1** is 2 mg/mL and molar ratio of **1** and **2** is 1:1. Solvent is DMSO/H₂O (20/80, v/v).

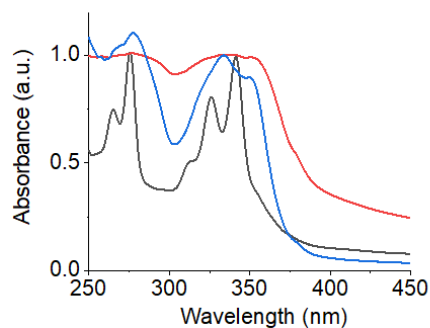


Figure S12. UV-Vis spectra of the multicomponent gels of (1+2) at pH 3.1 (black), 7.8 (red) and 10.4 (blue). In all cases, concentration of **1** is 2 mg/mL and molar ratio of **1** and **2** is 1:1. Solvent is DMSO/H₂O (20/80, v/v).

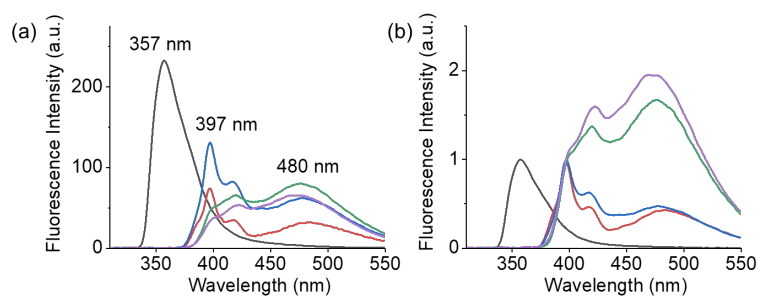


Figure S13. (a) Emission spectra of the hydrogel of **1** (black), solution of **2** (red), and the multicomponent gels of **(1+2)** at pH 3.1 (blue), 7.8 (green) and 10.4 (purple). Figure (b) represent normalized graph of figure (a). In all cases, concentration of **1** is 2 mg/mL and molar ratio of **1** and **2** is 1:1. Solvent is DMSO/H₂O (20/80, v/v).

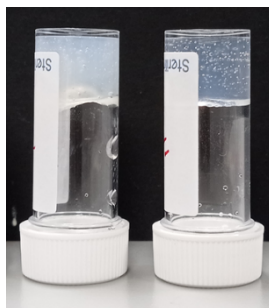


Figure S14. Photograph of the crosslinked gels of **(1+2)** obtained after treatment with EDC at pH 7.8 (left) and 10.4 (right). In all cases, initial concentration of **1** is 2 mg/mL and molar ratio of **1** and **2** is 1:1, concentration of EDC is 1 equivalent. Solvent is DMSO/H₂O (20/80, v/v).

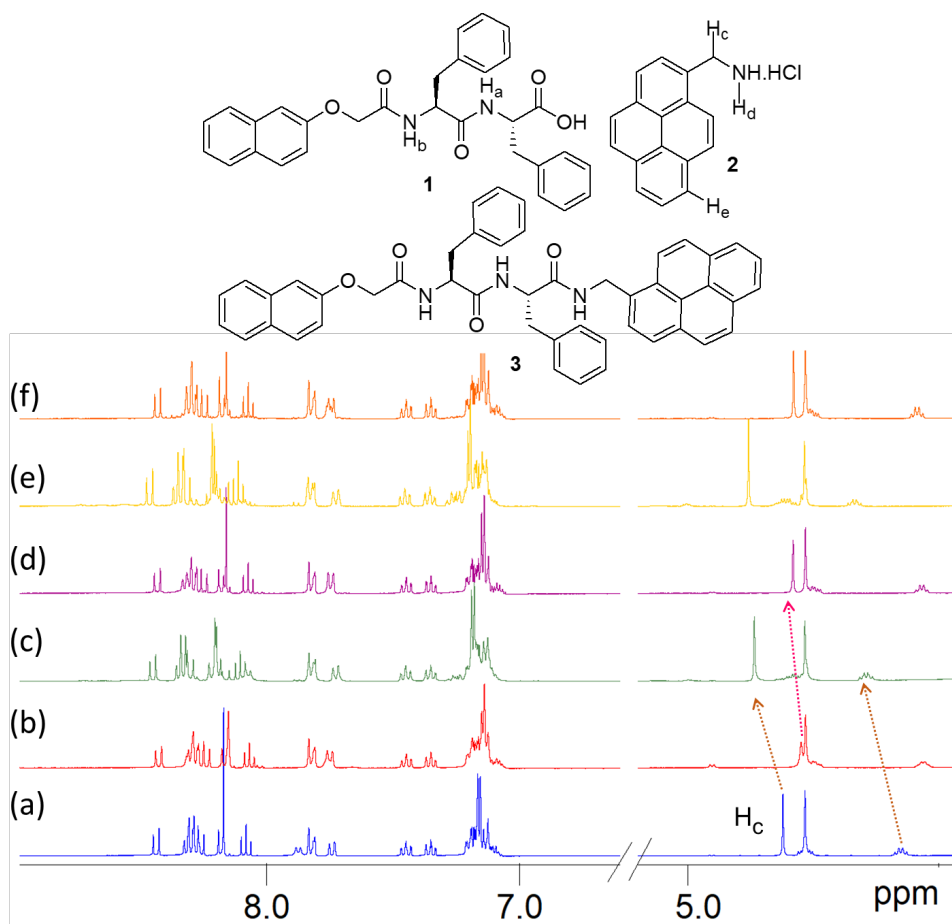
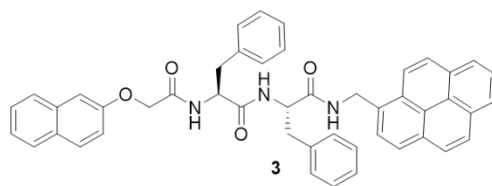


Figure S15. Partial ^1H NMR spectra (in DMSO-d_6) spectra of the hydrogel of (1+2) at pH 7.8 (a) and 10.4 (b). (c-d) represent partial proton NMR spectra of the EDC-treated gels prepared at pH 7.8 (c) and 10.4 (d) involving post assembly functionalization. For (e) and (f), EDC was directly added to the mixture of (1+2) in presence of 1 equiv. and 2 equiv. amounts of NaOH respectively. In all cases, initial concentration of 1 is 2 mg/mL and molar ratio of 1 and 2 is 1:1, concentration of EDC is 1 equivalent. Solvent is $\text{DMSO}/\text{H}_2\text{O}$ (20/80, v/v).



Calcd. 710.3013 for (M+H)⁺
Calcd. 728.3119 for (M+H₂O+H)⁺

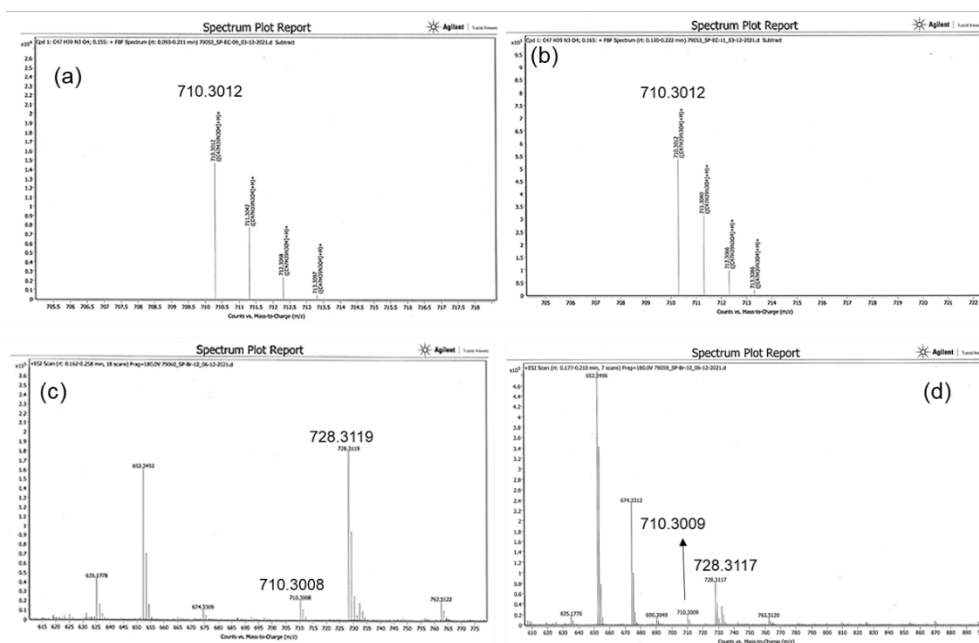


Figure S16. HRMS spectra of the EDC treated gels of (1+2) prepared at pH 7.8 (a, c) and 10.4 (b, d) involving post assembly functionalization (a, b) and direct addition of EDC (c, d).

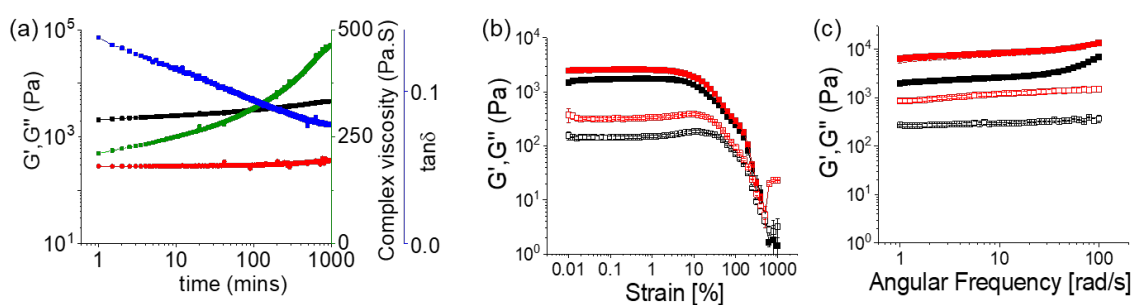


Figure S17. (a) Variation of G' (black), G'' (red), complex viscosity (green) and $\tan\delta$ (blue) with time for the hydrogels of (1+2) at pH 7.8 upon addition of EDC. (b) Strain sweeps of hydrogels of (1+2) obtained before (black) and after (red) addition of EDC at pH 7.8. (c) frequency sweeps of the EDC treated gels prepared by post assembly functionalization of the multicomponent gels of (1+2) at pH 7.8 (black) and 10.4 (red). For (b, c), the closed symbols represent G' , the open symbols G'' . In all cases, initial concentration of **1** is 2 mg/mL and molar ratio of **1** and **2** is 1:1, concentration of EDC is 1 equivalent. Solvent is DMSO/H₂O (20/80, v/v). Measurements were performed in triplicate, and error bars were calculated from the standard deviation.

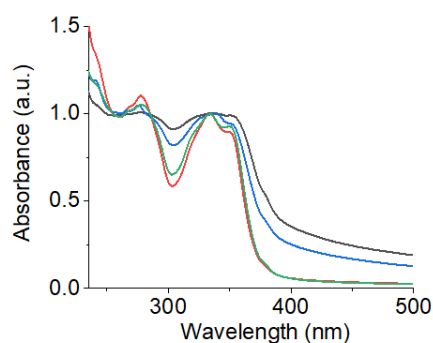


Figure S18. Changes in UV-Vis spectra of the multicomponent gels of (1+2) at pH 7.8 (black) and 10.4 (red) upon treatment with EDC (blue and green respectively). In all cases, initial concentration of **1** is 2 mg/mL and molar ratio of **1** and **2** is 1:1, concentration of EDC is 1 equivalent. Solvent is DMSO/H₂O (20/80, v/v).

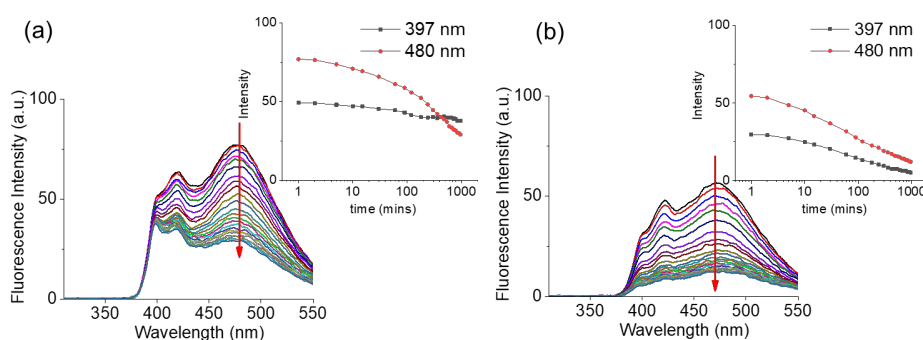


Figure S19. Time variable changes in emission spectra of the multicomponent gels of (1+2) at pH 7.8 (a) and 10.4 (b) upon addition of EDC. Insets of (a) and (b) show changes in emission intensity at 397 nm and 480 nm with time. In all cases, initial concentration of **1** is 2 mg/mL and molar ratio of **1** and **2** is 1:1, concentration of EDC is 1 equivalent. Solvent is DMSO/H₂O (20/80, v/v).

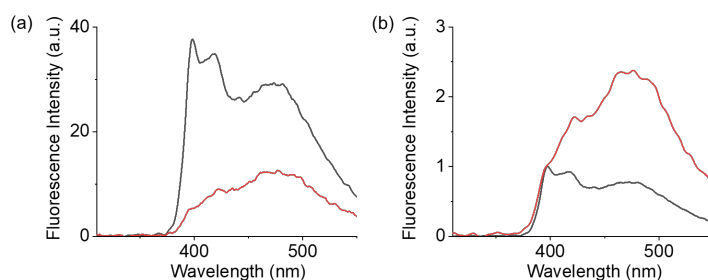


Figure S20. Emission spectra of the EDC treated gels of (1+2) obtained at pH 7.8 (black) and 10.4 (red). Figure (b) represents normalized graph (normalized at 397 nm) of figure (a). In all cases, concentration of **1** is 2 mg/mL and molar ratio of **1** and **2** is 1:1, initial concentration of EDC is 1 equivalent. Solvent is DMSO/H₂O (20/80, v/v).

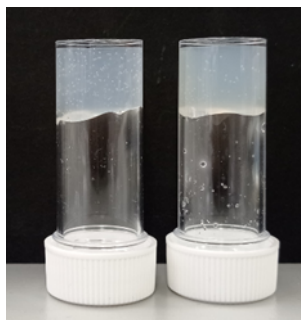


Figure S21. Photographs of the gels obtained from the freeze-dried samples of the EDC-treated gels at pH 7.8 (left) and 10.4 (right). For these experiments, the EDC-treated gels at pH 7.8 and 10.4 were freeze-dried first and then dissolved in DMSO, followed by the addition of water. In both cases, we observed instant gelation (within 30 seconds). In both cases, concentration of **1** is 2 mg/mL and molar ratio of **1** and **2** is 1:1. Solvent is DMSO/H₂O (20/80, v/v).

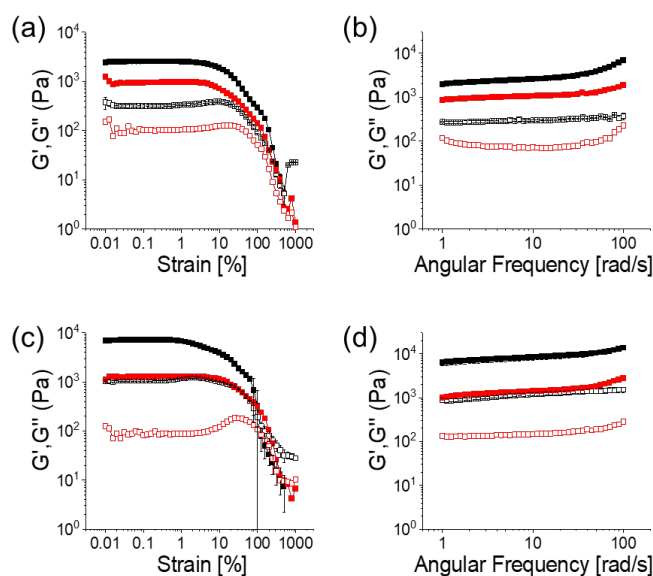


Figure S22. (a, c) Strain sweeps and (b, d) frequency sweeps of the EDC treated gels of (**1+2**) obtained at pH 7.8 (a, b) and 10.4 (c, d). In all cases, the black data represent the EDC-treated gels obtained by post assembly functionalization of the multicomponent gels of (**1+2**). The red data represent the gels prepared from the freeze-dried samples of the EDC-treated gels of (**1+2**), i.e., performing **3** and using this directly as a gelator. For (a-d), the closed symbols represent G' , the open symbols G'' . In all cases, initial concentration of **1** is 2 mg/mL and molar ratio of **1** and **2** is 1:1, concentration of EDC is 1 equivalent. Solvent is DMSO/H₂O (20/80, v/v).

References

1. L. Chen, S. Revel, K. Morris, L. C. Serpell and D. J. Adams, *Langmuir*, 2010, **26**, 13466-13471.