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Macroscopic volume phase transitions in supramolecular gels directed by covalent crosslinking

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Supporting Information

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Experimental details

Materials: Compound **1** was synthesized as described previously.[1] Compound **2** and EDC hydrochloride were purchased from Fluorochem. Glutaraldehyde (50% aqueous solution) was obtained from Alfa Aesar. All other chemicals and solvents were purchased from commercial suppliers and used as received. Deionized water was used throughout all experiments.

Preparation of solutions: The stock solution of **1** was prepared in DMSO at a concentration of 100 mg/mL by warming gently at ~70 ^{o}C for 30 seconds using a heat gun. A stock solution of **2** was prepared at a concentration of 73.5 mg/mL by swirling in DMSO at room temperature. The stock solutions of EDC and glutaraldehyde were prepared in water at a concentration of 1 M and 0.5 M, respectively. The stock solutions of NaOH and HCl were prepared at a concentration of 1 M in H_2O. Solutions of **2**, EDC and glutaraldehyde were prepared freshly before each experiment.

Preparation of gels: All gels were prepared using the solvent switch method. Typically, water was added either to the DMSO solution of **1** or to the mixture of **1** and **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 H_2O was added in one aliquot. Hence the concentration of **1** in the gel was 10 mg/mL.

To prepare the multicomponent gels of (**1**+**2**) under different conditions, initially, 0.2 mL of DMSO solution of **1** was mixed with 0.2 mL of DMSO solution of **2**. To prepare the gels at pH 3.3, 1.60 mL of water was added to this mixture. Similarly, instead of water, either a mixture of 0.046 mL of NaOH and 1.554 mL of water or a mixture of 0.092 mL of NaOH and 1.508 mL of water was used to prepare the multicomponent gels at pH 9.3 and 10.8 respectively. Therefore, in all cases, the concentration of **1** was 10 mg/mL, and 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 9.3 and 10.8 were 1 and 2 molar equivalents respectively.

Cross-linking Using EDC and glutaraldehyde: To achieve covalent crosslinking between **1** and **2** in the gels, a post gelation functionalization technique was followed. In all cases, gels were initially prepared as mentioned earlier in 2 mL volume in 7 mL Sterilin vials. Then either a mixture of 0.046 mL of EDC and 0.5 mL of water (total volume 0.546 mL) or a mixture of 0.023 mL of glutaraldehyde and 0.5 mL of water (total volume 0.523 mL) was added carefully on the top of the gels and left undisturbed for ~24 hours. After that, the water was decanted, and the gels were used for further experiments. Therefore, the amount of EDC and glutaraldehyde used for crosslinking was 1 molar equivalent and 0.5 molar equivalents with respect to **1**, respectively.
The crosslinked gels obtained at pH 9.3 and 10.8 exhibit shrinking/swelling behaviours. To compare the final volume of the gels, the volume of the decanted water was measured and subtracted from the initial volume (0.546 mL or 0.523 mL) of the water.

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 HCl (1 M) to the solutions of **1** (concentration is 10 mg/mL) and **2** (1 equiv. with respect to **1**) containing 1 molar equivalents of NaOH in 20% DMSO in H_2O. During the titration, the solution was stirred continuously. The experimental temperature was 25 °C.

Rheological 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 0.2 % strain. All gels were left for ~16 hours before being measured. Time sweeps were performed at an angular frequency of 10 rad/s and with a strain of 0.2%. 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 (4 µL/mL of a 0.1 wt % solution in water). All gel 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 coverslip 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.

Optical Microscopy: Optical microscope images were collected under non-polarised light using a Nikon Eclipse LV100 microscope with a Nikon S Plan Fluor ELWD 20x/0.45 lens attached to an Infinity2-1C camera. For all experiments, gels 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 before imaging.

Circular dichroism: Circular dichroism data was acquired on a Chirascan VX spectrometer (Applied Photophysics) using a 0.01 mm path length quartz cuvette. All spectra were collected in the range 180-400 nm with a scanning step size of 1.0 nm and scanning rate of 0.25 s at 25 °C. All samples were prepared in Sterilin vials keeping the same volumes of the components as mentioned earlier. Then, small amounts of the gels were transferred to the cuvette for measurement.

Powder X-ray Diffraction: Powder X-ray diffraction (PXRD) patterns were recorded on a Rigaku MiniFlex 6G equipped with a D/teX Ultra detector, a 6-position sample changer (ASC-6) and Cu sealed tube. The $K\alpha_1$ and $K\alpha_2$ wavelengths were 1.5406 and 1.5444 Å respectively. All the patterns were collected as $\theta/2\theta$ scans over the range 3.0° to 40.0° and a scan step of 0.01°. Data collection and analysis were performed using the Rigaku SmartLab Studio II software (Rigaku Corporation, 2014). For all experiments, gels were prepared in 7 mL Sterilin vials keeping the same volumes of the components as mentioned earlier. Then required amounts of the gels were transferred to the sample holder for measurement.

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 270 nm. The excitation and emission slit widths were 10 nm and 5 nm respectively.

FTIR spectroscopy: Data were recorded using an Agilent Cary 630 FTIR spectrometer (with ATR attachment). All gels were prepared following the same methodology as described earlier and then the solvent was removed by freeze-drying. For the solid samples, the background of the empty ATR crystal was taken. Then small amounts of the freeze-dried samples were deposited on the ATR crystal before recording the spectra.

NMR spectroscopy and HRMS experiments: ^{1}H 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. Then either 1.25 equiv. or 2.25 equiv. of HCl (for pH 9.3 and 10.8 respectively) was added on the top of the gels and allowed to diffuse into the gels for ~16h. After that, the solvent was removed by freeze-drying. To record the NMR, the freeze-dried samples were dissolved in 3 mL of d_6-DMSO. 0.05 mL of these 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 either EDC or glutaraldehyde to the mixture of (**1**+**2**) in the absence and presence of different concentrations of NaOH. For these directly prepared gels, instead of H_2O a mixture of crosslinking agent and water was used keeping the compositions of the rest of the components identical. After ~24 hours, the samples were acidified using HCl (as described above) and then subjected to freeze-drying followed by dissolving in 3 mL of d_6-DMSO for NMR.

Supplementary Figures

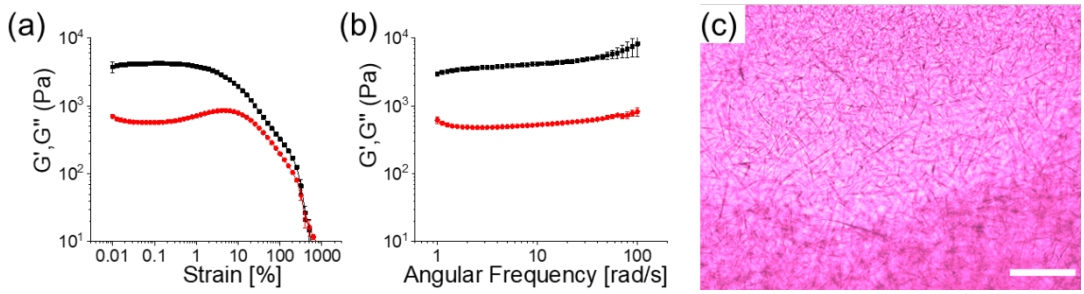


Figure S1. (a) Strain sweep, (b) frequency sweep and (c) optical microscopy (scale bar is 100 μm) image of the hydrogel of **1** in DMSO/H_2O (20/80, v/v). For (a, b), the black symbols represent G′, the red symbols G″. Measurements were performed in triplicate, and error bars were calculated from the standard deviation. In all cases, concentration of **1** is 10 mg/mL.

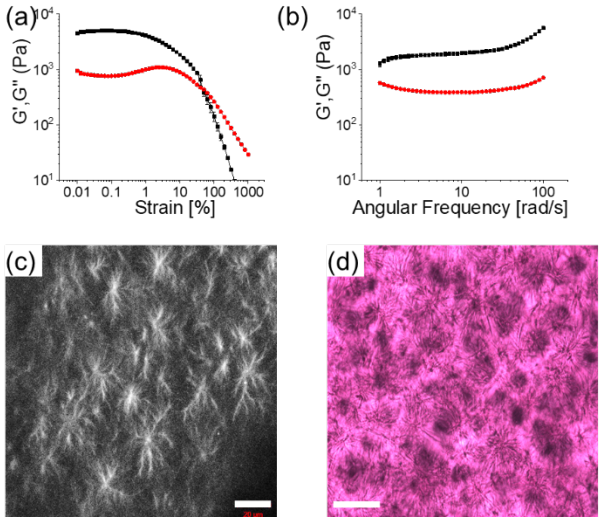


Figure S2. (a) Strain sweep, (b) frequency sweep, (c) confocal (scale bar is 20 μm) and (d) optical microscopy (scale bar is 100 μm) image of the hydrogel of **2** in presence of NaOH. For (a, b), the black symbols represent G′, the red symbols G″. Measurements were performed in triplicate, and error bars were calculated from the standard deviation. In all cases, concentration of **2** and NaOH is 1 equimolar with respect to the concentration of **1** (10 mg/mL) used this study. Solvent is DMSO/H_2O (20/80, v/v).

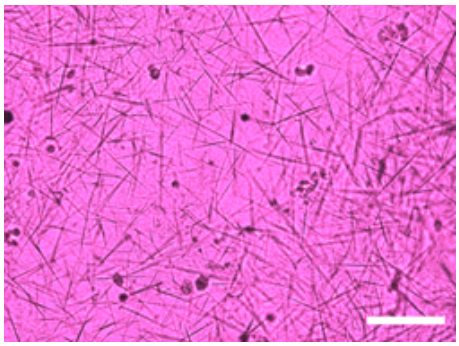


Figure S3. Optical microscopy (scale bar is 100 μm) image of the multicomponent gel of (**1**+**2**) at pH 3.3. Concentration of **1** is 10 mg/mL and the molar ratio of **1** and **2** is 1:1. Solvent is DMSO/H_2O (20/80, v/v).

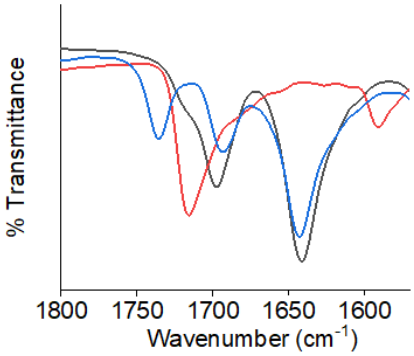


Figure S4. Partial FTIR spectra of **1** (black), **2** (red) and the multicomponent gel of (**1**+**2**) at pH 3.3 (blue). Concentration of **1** is 10 mg/mL and the molar ratio of **1** and **2** is 1:1.

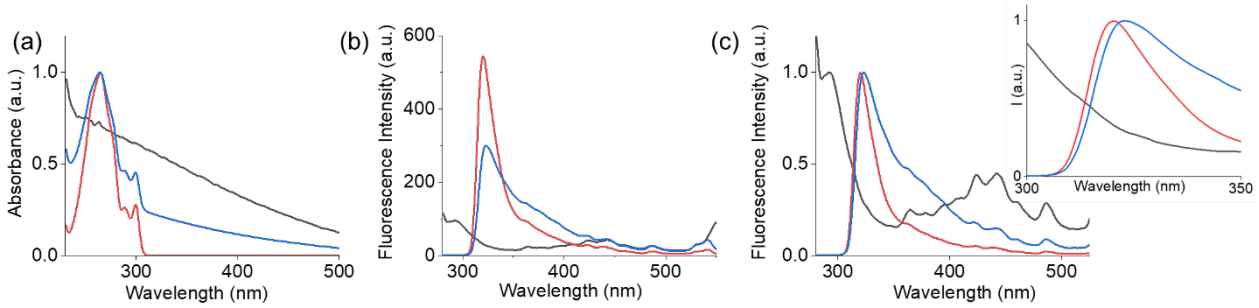


Figure S5. (a) UV-vis and (b) emission spectra of the hydrogel of **1** (black), solution of **2** (red), and the multicomponent gels of (**1**+**2**) at pH 3.3 (blue). Figure (c) represents normalized graph of figure (b). Inset represents expanded section of figure (c). In all cases, concentration of **1** is 10 mg/mL and the molar ratio of **1** and **2** is 1:1. Solvent is DMSO/H_2O (20/80, v/v).

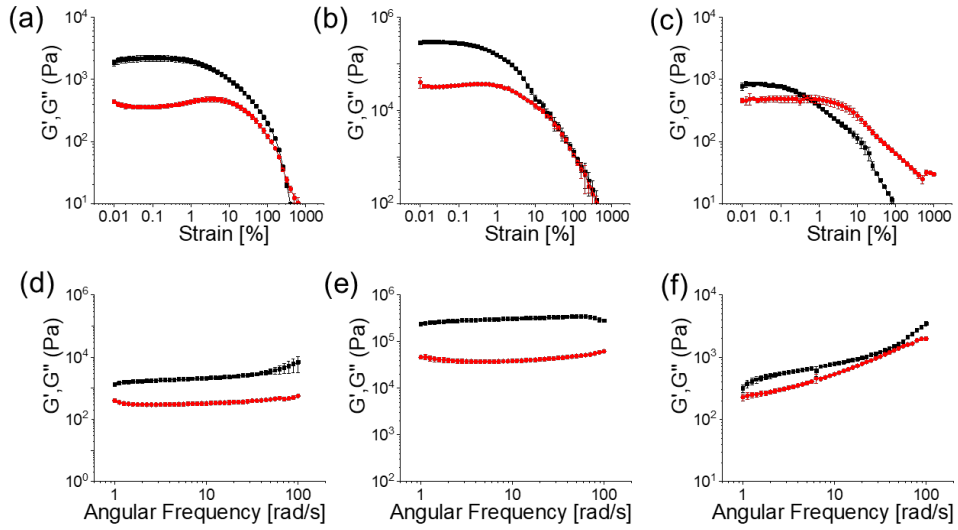


Figure S6. (a-c) Strain sweeps and (d-f) frequency sweeps for the hydrogels of (**1**+**2**) at pH 3.3 (a, d), 9.3 (b, e) and 10.8 (c, f). The black symbols represent G′, the red symbols G″. Measurements were performed in triplicate, and error bars were calculated from the standard deviation. In all cases, concentration of **1** is 10 mg/mL and the molar ratio of **1** and **2** is 1:1. Solvent is DMSO/H_2O (20/80, v/v).

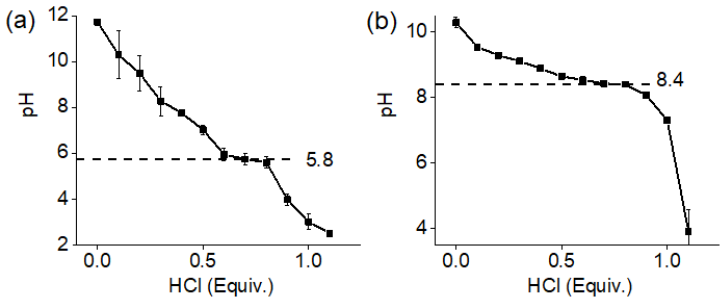


Figure S7. Determination of apparent pK_a of (a) **1** (10 mg/mL) and (b) **2** (1 eq.) in DMSO/H_2O (20/80, v/v). The plateau is taken to represent the apparent pK_a value, shown by the horizontal line. Measurements were performed in triplicate, and error bars were calculated from the standard deviation.

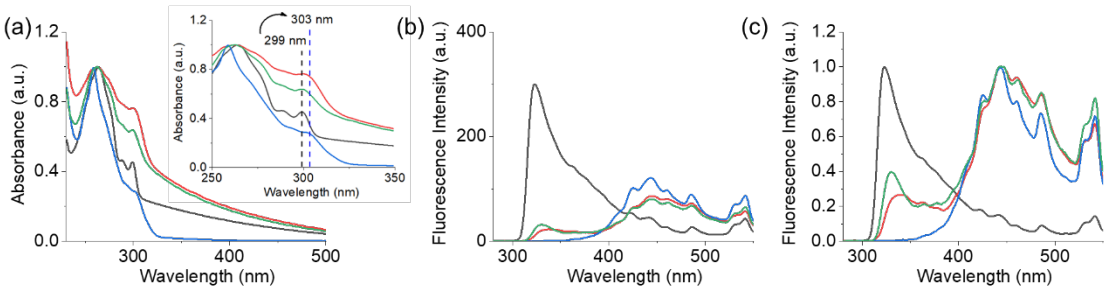


Figure S8. (a) Normalized UV-vis and (b) emission spectra of the multicomponent gels of (**1**+**2**) at pH 3.3 (black), 9.3 (red) and 10.8 (blue). The green data represent the hydrogel of **2** at high pH. Inset of (a) represents expanded section of figure (a). Figure (c) represents normalized graph of figure (b). In all cases, concentration of **1** is 10 mg/mL and the molar ratio of **1** and **2** is 1:1. Solvent is DMSO/H_2O (20/80, v/v).

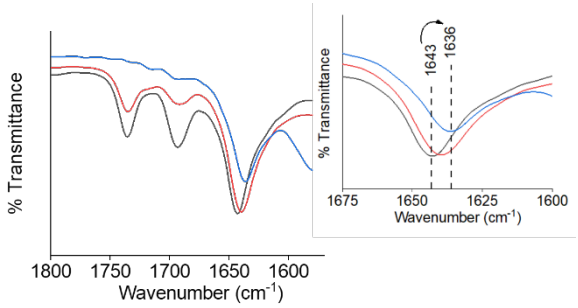


Figure S9. Partial FTIR spectra of the multicomponent gel of (**1**+**2**) at pH 3.3 (black), 9.3 (red) and 10.8 (blue). Inset represents expanded section of the graph. In all cases, concentration of **1** is 10 mg/mL and the molar ratio of **1** and **2** is 1:1.

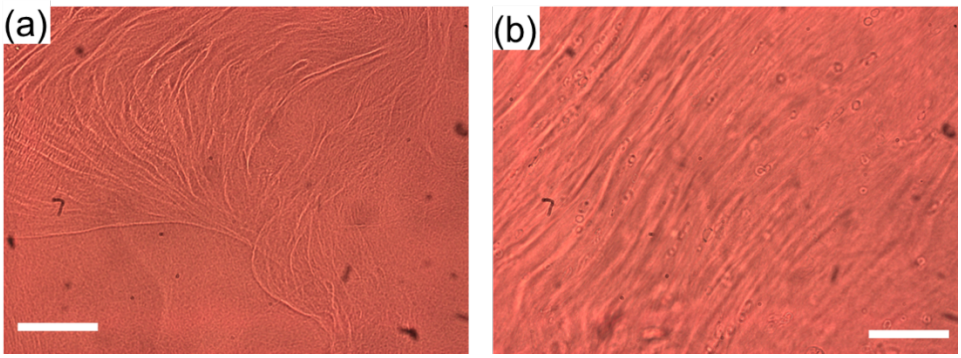


Figure S10. Optical microscopy (scale bars are 100 μm) image of the multicomponent gel of (**1**+**2**) at pH 9.3 (a) and 10.8 (b). In both cases, concentration of **1** is 10 mg/mL and the molar ratio of **1** and **2** is 1:1. Solvent is DMSO/H_2O (20/80, v/v).

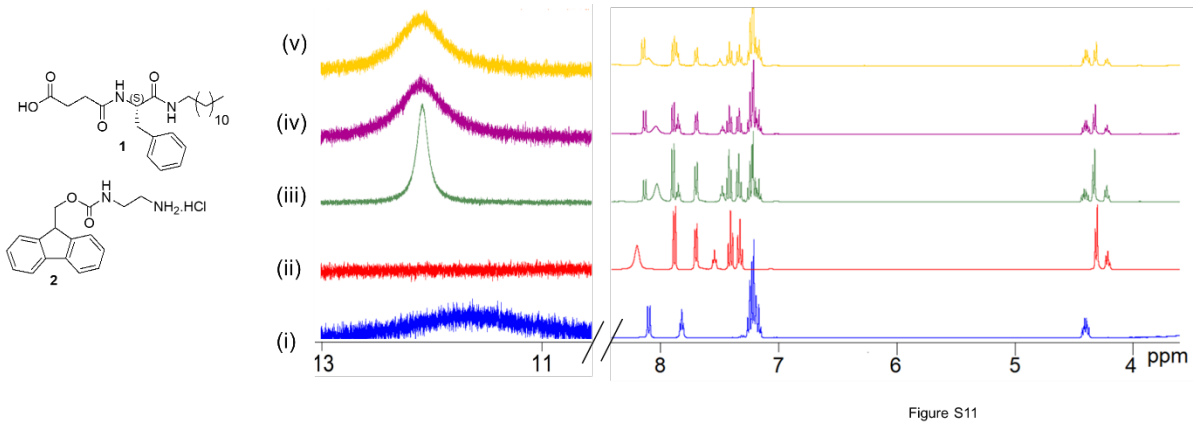
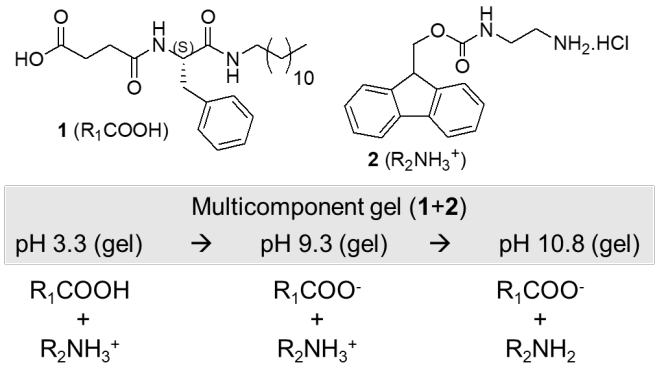


Figure S11

Figure S11. Partial ^{1}H NMR spectra (in DMSO-d_6) of **1** (i), **2** (ii), multicomponent gel of (**1**+**2**) at pH 3.3 (iii), 9.3 (iv) and 10.8 (v). For (iv-v), gels were acidified using HCl and then freeze-dried. In all cases, concentration of **1** is 10 mg/mL and the molar ratio of **1** and **2** is 1:1. Solvent is DMSO/H_2O (20/80, v/v).



Scheme S2. Chemical structures of **1** (R_1COOH) and **2** ($R_2NH_3^+$) responsible for gelation for the multicomponent system (**1**+**2**) at different pH.

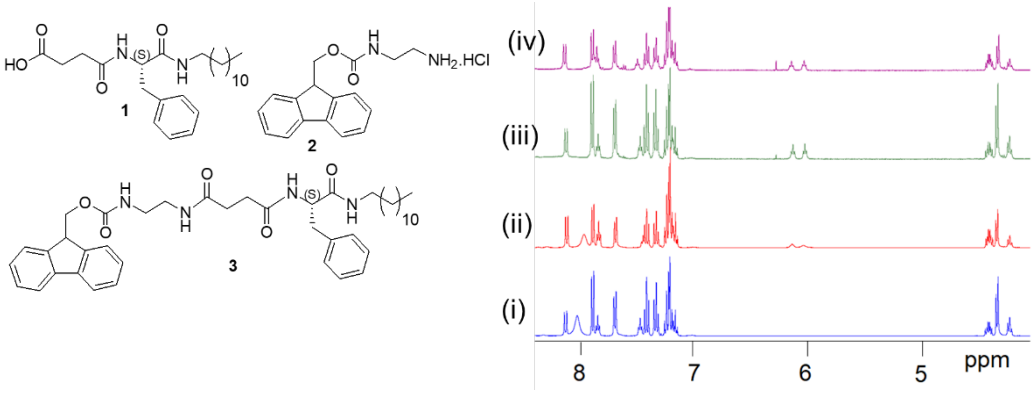


Figure S12. Partial ^{1}H NMR spectra (in DMSO-d_6) of the multicomponent gel of (**1**+**2**) at pH 3.3 (i), and the EDC treated gels of (**1**+**2**) at pH 3.3 (ii), 9.3 (iii) and 10.8 (iv). For (iii-iv), samples were acidified using HCl after ~24 h of addition of EDC and then freeze-dried. In all cases, concentration of **1** is 10 mg/mL and the molar ratio of **1**, **2** and EDC is 1:1:1. Solvent is DMSO/H_2O (20/80, v/v).

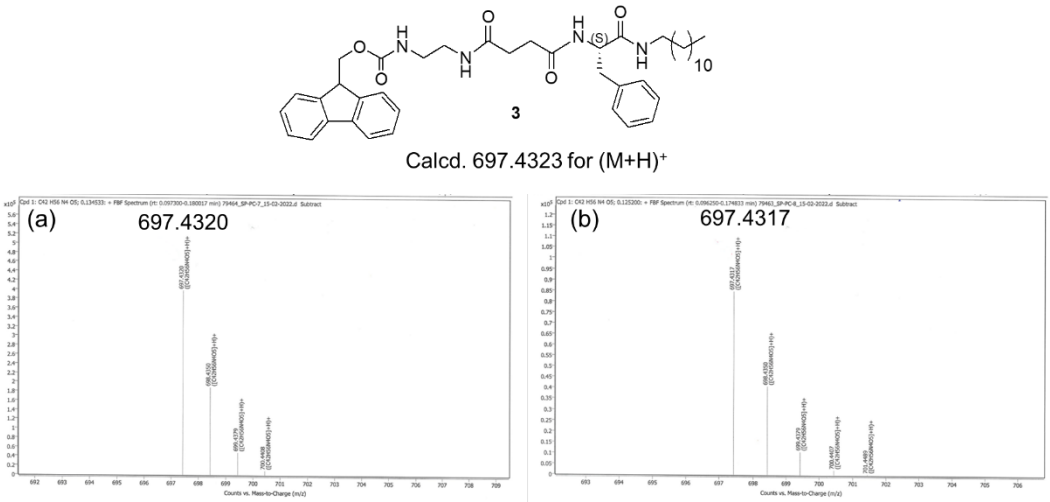


Figure S13. HRMS spectra of the EDC treated gels of (**1**+**2**) prepared at pH 9.3 (a) and 10.8 (b) involving post-assembly functionalization.

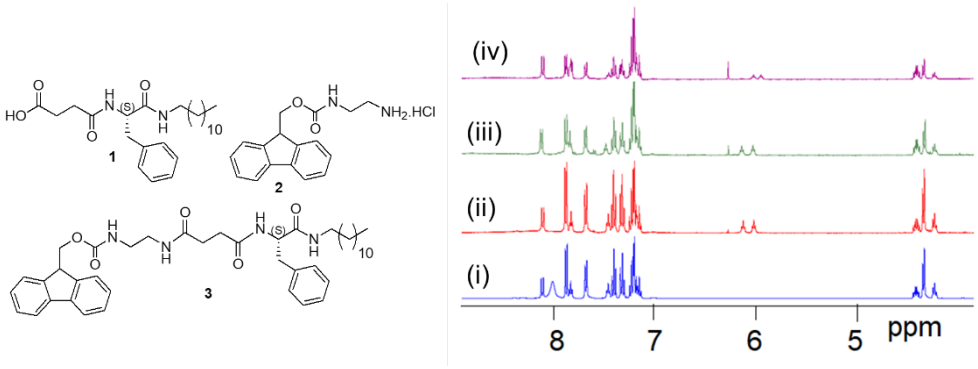


Figure S14. Partial ^{1}H NMR spectra (in DMSO-d_6) of the multicomponent gel of (**1**+**2**) at pH 3.3 (i), and the EDC-treated gels of (**1**+**2**) at pH 9.3 (ii) and 10.8 (iii) obtained by post-assembly functionalization technique. The spectrum (iv) represents the sample prepared by direct addition of EDC to the mixture of **1** and **2** in presence of base. For (ii-iv), samples were acidified using HCl after ~24 h of addition of EDC and then freeze-dried. In all cases, concentration of **1** is 10 mg/mL and the molar ratio of **1**, **2** and EDC is 1:1:1. Solvent is DMSO/H_2O (20/80, v/v).

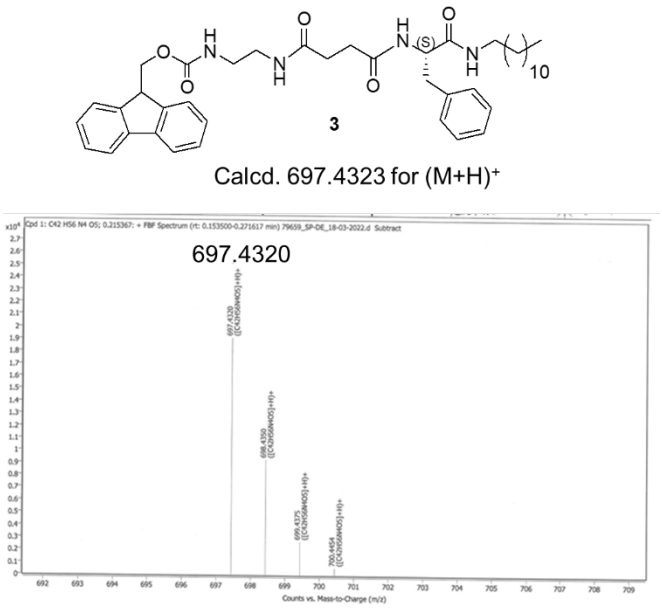


Figure S15. HRMS spectra of the sample prepared by direct addition of EDC to the mixture of **1** and **2** in presence of base.

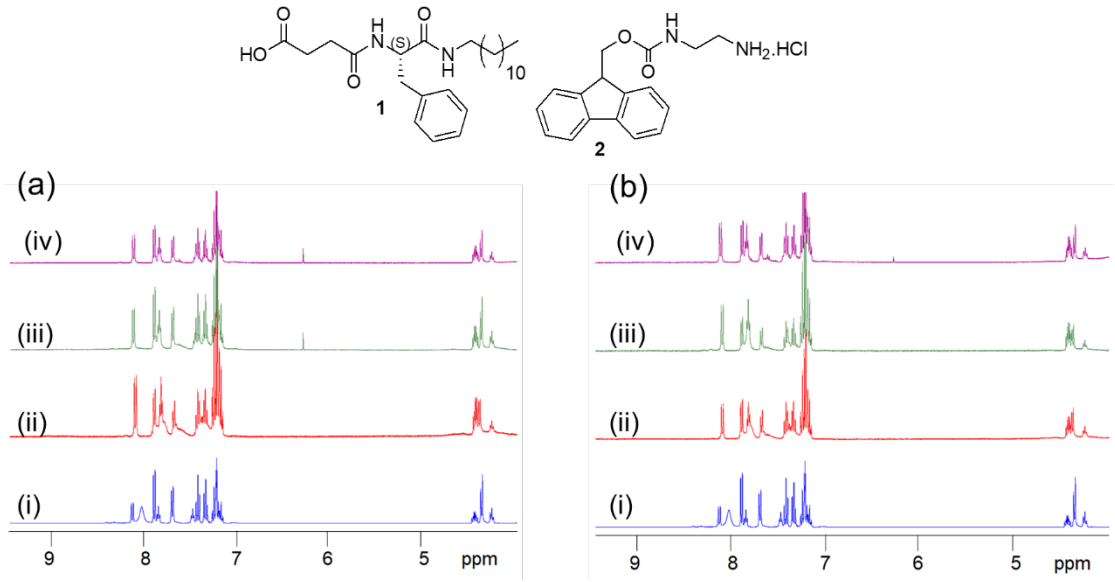


Figure S16. (a, b) Partial ^{1}H NMR spectra (in DMSO-d_6) of the multicomponent gel of (**1**+**2**) at pH 3.3 (i), and the glutaraldehyde treated gels of (**1**+**2**) at pH 3.3 (ii), 9.3 (iii) and 10.8 (iv). For (a), samples were prepared by post-assembly functionalization of respective gels. For (b), samples were prepared by direct addition of glutaraldehyde to the mixture of (**1**+**2**) under different conditions. For (a, b), samples (iii) and (iv) were acidified using HCl after ~24 h of addition of glutaraldehyde and then freeze-dried. In all cases, concentration of **1** is 10 mg/mL and the molar ratio of **1**, **2** and glutaraldehyde is 1:1:0.5. Solvent is DMSO/H_2O (20/80, v/v).

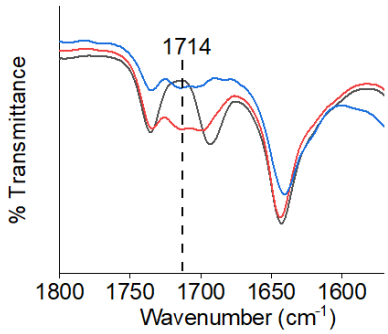


Figure S17. Changes in FTIR spectra of the gel of (**1**+**2**) at pH 3.3 (black) after treatment with glutaraldehyde (red and blue). For the red data, glutaraldehyde was added to the preformed gel (post assembly functionalization) of (**1**+**2**). For the blue data, glutaraldehyde was directly added to the mixture of (**1**+**2**) at different pH. In all cases, concentration of **1** is 10 mg/mL and the molar ratio of **1**, **2** and glutaraldehyde is 1:1:0.5. Solvent is DMSO/H_2O (20/80, v/v).

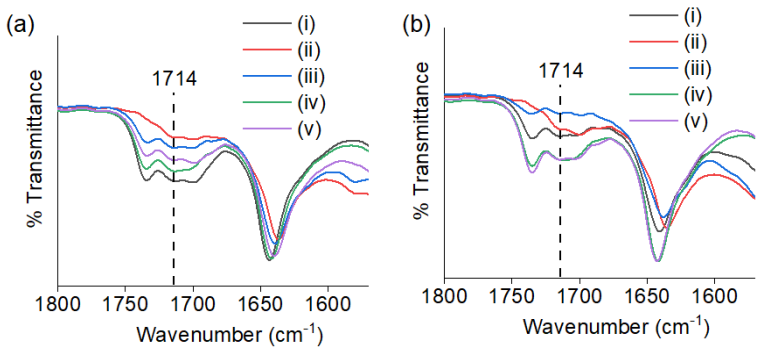


Figure S18. (a, b) Partial FTIR spectra of the glutaraldehyde treated samples of (**1**+**2**) at pH 3.3 (i), 9.3 (ii) and 10.8 (iii). (iv) and (v) represent the acidified samples of (ii) and (iii) respectively. For (a), samples are prepared by post assembly functionalization technique. For (b), samples are prepared by direct addition of glutaraldehyde to the mixture of (**1**+**2**) at different pH. In all cases, concentration of **1** is 10 mg/mL and the molar ratio of **1**, **2** and glutaraldehyde is 1:1:0.5. Solvent is DMSO/H_2O (20/80, v/v).

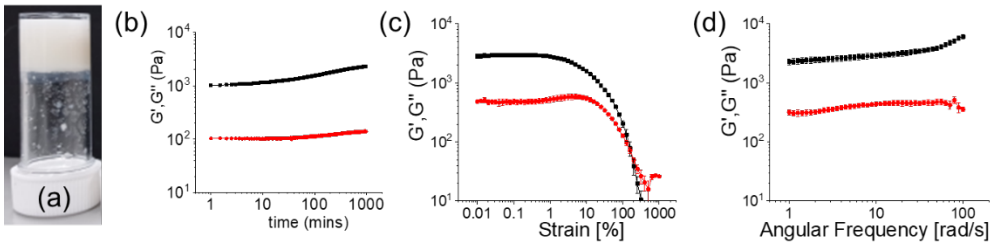


Figure S19. (a) Photograph of the hydrogel of (**1**+**2**) obtained after treatment with glutaraldehyde at pH 3.3. (b) Variation of G′ (black) and G″ (red) with time for the hydrogels of (**1**+**2**) at pH 3.3 upon addition of glutaraldehyde. (c) Strain and (d) frequency sweeps for the hydrogel of (**1**+**2**) obtained after treatment with glutaraldehyde at pH 3.3. The black symbols represent G′, the red symbols G″. Measurements were performed in triplicate, and error bars were calculated from the standard deviation. In all cases, concentration of **1** is 10 mg/mL and the molar ratio of **1**, **2** and glutaraldehyde is 1:1:0.5. Solvent is DMSO/H_2O (20/80, v/v).

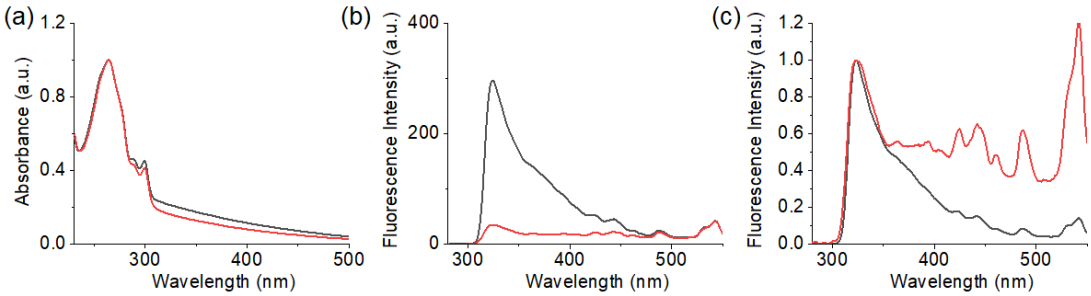


Figure S20. (a) Normalized UV-vis and (b) emission spectra of the hydrogel of (**1**+**2**) before (black) and after treatment with glutaraldehyde at pH 3.3. Figure (c) is the normalized graph of Figure (b). In all cases, concentration of **1** is 10 mg/mL and the molar ratio of **1**, **2** and glutaraldehyde is 1:1:0.5. Solvent is DMSO/H_2O (20/80, v/v).

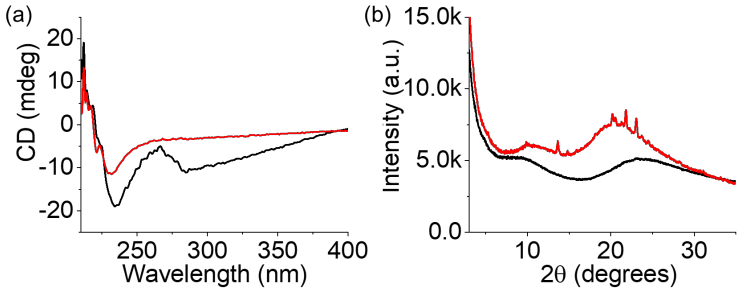


Figure S21. (a) CD spectra and (b) PXRD data of the hydrogel of (**1**+**2**) before (black) and after treatment with glutaraldehyde at pH 3.3. In all cases, concentration of **1** is 10 mg/mL and the molar ratio of **1**, **2** and glutaraldehyde is 1:1:0.5. Solvent is DMSO/H_2O (20/80, v/v).

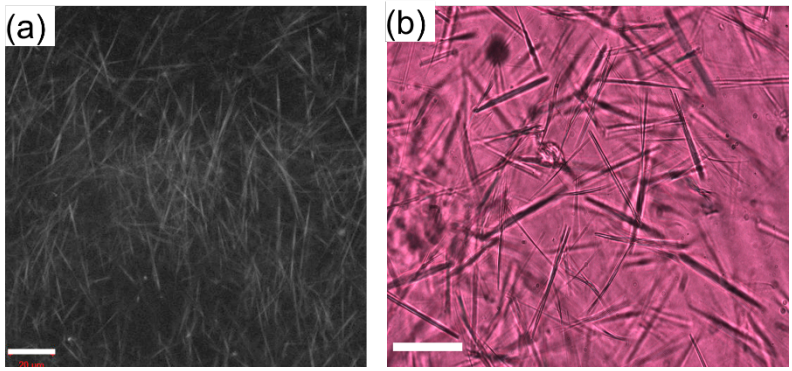


Figure S22. (a) Confocal (scale bar is 20 μm) and (b) optical (scale bar is 100 μm) microscopy images of the hydrogel of (**1**+**2**) obtained after treatment with glutaraldehyde at pH 3.3. In all cases, concentration of **1** is 10 mg/mL and the molar ratio of **1**, **2** and glutaraldehyde is 1:1:0.5. Solvent is DMSO/H_2O (20/80, v/v).

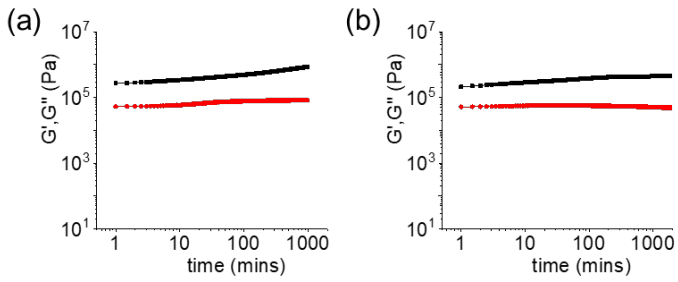


Figure S23. Variation of G′ (black) and G″ (red) with time for the hydrogels of (**1**+**2**) at pH 9.3 upon addition of (a) EDC and (b) glutaraldehyde. (In all cases, concentration of **1** is 10 mg/mL and the molar ratio of **1**, **2**, EDC and glutaraldehyde is 1:1:1:0.5. Solvent is DMSO/H_2O (20/80, v/v).

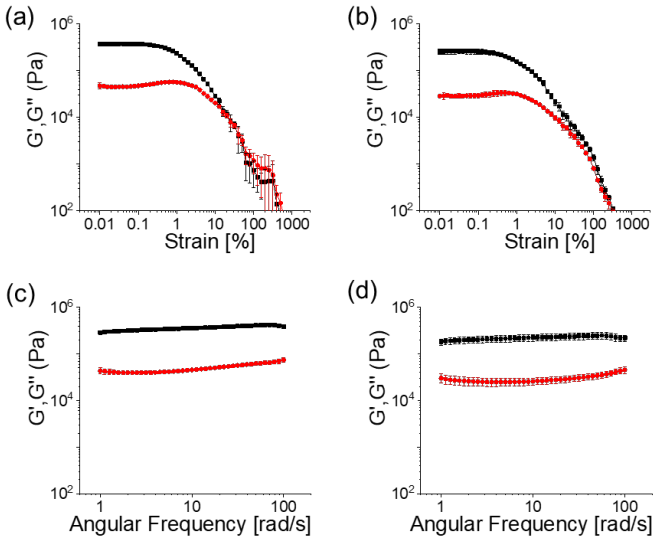


Figure S24. (a, b) Strain and (c, d) frequency sweeps for the hydrogel of (**1**+**2**) obtained after treatment with EDC (a, c) and glutaraldehyde (b, d) at pH 9.3. The black symbols represent G′, the red symbols G″. In all cases, concentration of **1** is 10 mg/mL and the molar ratio of **1**, **2**, EDC and glutaraldehyde is 1:1:1:0.5. Solvent is DMSO/H_2O (20/80, v/v).

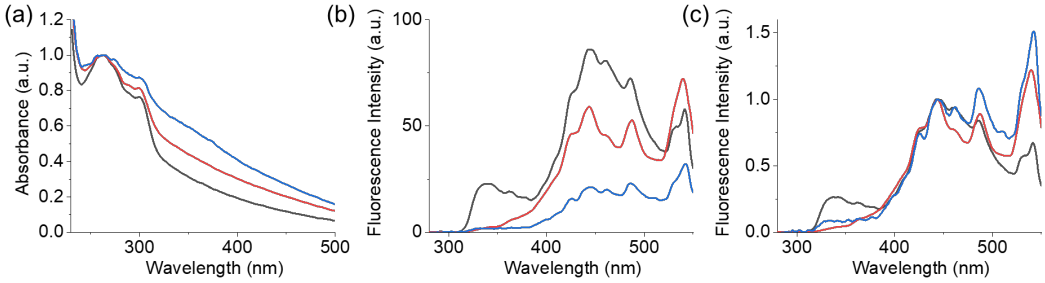


Figure S25. Change in (a) Normalized UV-vis and (b) emission spectra of the hydrogel of (**1**+**2**) (black) after treatment with EDC (red) and glutaraldehyde (blue) at pH 9.3. Figure (c) is the normalized graph of Figure (b). In all cases, concentration of **1** is 10 mg/mL and the molar ratio of **1**, **2**, EDC and glutaraldehyde is 1:1:1:0.5. Solvent is DMSO/H_2O (20/80, v/v).

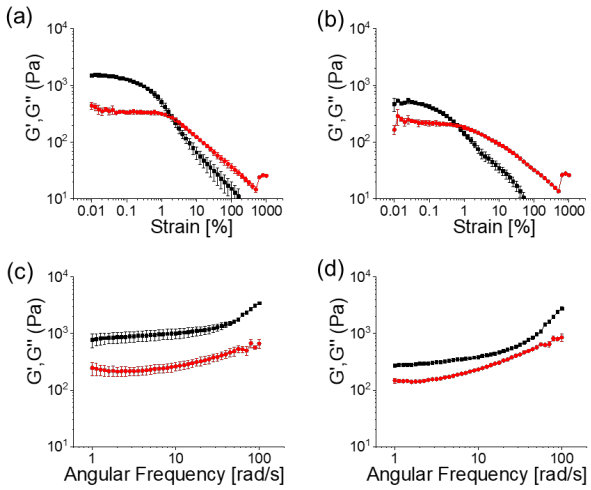


Figure S26. (a, b) Strain and (c, d) frequency sweeps for the hydrogel of (**1**+**2**) obtained after treatment with EDC (a, c) and glutaraldehyde (b, d) at pH 10.8. The black symbols represent G′, the red symbols G″. Measurements were performed in triplicate, and error bars were calculated from the standard deviation. In all cases, concentration of **1** is 10 mg/mL and the molar ratio of **1**, **2**, EDC and glutaraldehyde is 1:1:1:0.5. Solvent is DMSO/H_2O (20/80, v/v).

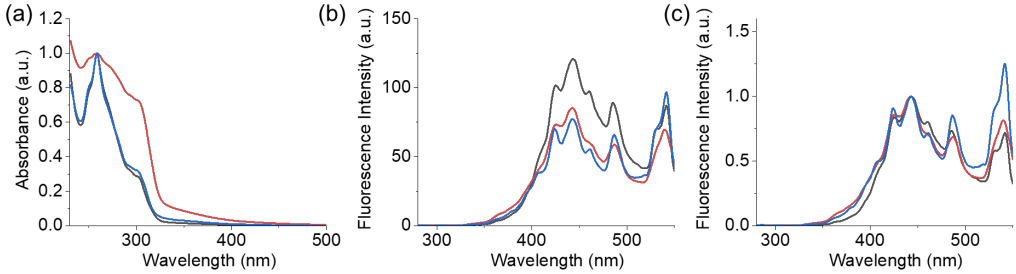


Figure S27. Change in (a) normalized UV-vis and (b) emission spectra of the hydrogel of (**1**+**2**) (black) after treatment with EDC (red) and glutaraldehyde (blue) at pH 10.8. Figure (c) is the normalized graph of Figure (b). In all cases, concentration of **1** is 10 mg/mL and the molar ratio of **1**, **2**, EDC and glutaraldehyde is 1:1:1:0.5. Solvent is DMSO/H_2O (20/80, v/v).

References

1. S. Panja, B. Dietrich and D. J. Adams, *ChemSystemsChem*, 2020, **2**, e1900038.