Electronic Supplementary Material (ESI) for Soft Matter.

Construction of supramolecular hydrogels using photo-generated nitric oxide radicals

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Supporting information

Experimental

Radical induced supramolecular hydrogels were prepared by first dissolving *N*-fluorenylmethyloxycarbonyl tyrosine phosphate (FMOC tyrosine phosphate, FYP) in 250 µL of a pH 10.1 alkaline buffer (50 mM Tris-HCl, 50 mM Na_2CO_3, 1 mM $MgCl_2$) at concentrations of 100 mM. Vortexing and sonication were necessary to aid the dissolution of the FYP. Subsequently, an aqueous solution of sodium nitroprusside dihydrate (SNP) (250 µL) was added at varying molar ratios (SNP: FYP)10:1, 5:1, 2:1 and 1:1), and vortexed, giving a final FYP concentration of 50 mM. Samples were then irradiated with UV light (λ = 254 nm) for 30 minutes. To compare the properties of the above hydrogels, control hydrogel samples were prepared by using enzymatic pathway.[1] In this case, 50 mM buffered solution of FYP was mixed with calf intestine alkaline phosphatase (ALP) (10 µL, 1000 U mL^{-1}) and the mixture was incubated at 37^{o}C for 6 hours and then left to cool to room temperature. Differential scanning calorimetry (DSC) was carried out using a Mettler Toledo TGA/DSC1 Star System at a scan rate of 1 °C min^{-1} with a nitrogen flow of 25 mL min^{-1}. Rheometry was performed using a Malvern Kinexus fitted with a parallel plate geometry (gap width of 200 µm) at room temperature. Prior to rheology experiments, all hydrogel samples were aged for one day and were added to the rheometer using a spatula to minimise shear. The top plate is lowered, and the normal force is measured and allowed to reach equilibrium. Transmission electron microscopy (TEM) was performed in bright-field mode using a JEOL TEM 1400 electron microscope and JEOL TEM 2010 electron microscope operating at 120 keV. TEM samples were prepared by drop casting a dilute suspension of hydrogel (5 µL) onto carbon-coated copper TEM grids for three minutes and wicking excess fluid away using filter paper. All samples were left to dry overnight at room temperature. Cryo-TEM samples were imaged using an FEI Tecnai Twin Lens electron microscope fitted with an FEI Eagle 4k x4k CCD camera and is operated at 200 keV. AFM tapping mode images were obtained using a Bruker Multimode atomic force microscope with Nanoscope V controller and Picoforce Extender. Samples were prepared by diluting the sample either 10 or 100-fold in deionised water and drop cast on either freshly cleaved mica.

Fluorimetry measurements were recorded at room temperature using a Horiba FluoroMax 4 spectrometer. Fluorimetry was carried on supramolecular hydrogels with an excitation wavelength of 265 nm, with excitation bandwidth of 1 nm and emission bandwidth of 5 nm. Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) was performed using a PerkinElmer Spectrum 100 FTIR spectrometer fitted with a universal attenuated total reflection accessory. The hydrogels were lyophilized by freezing in liquid nitrogen followed by freeze-drying for at least 24 hours. CD spectra were recorded at room temperature using a JASCO J-810 spectrometer through the two quartz plates. Samples were prepared by spreading hydrogels (ca. 25 µL) between two quartz plates to produce a homogeneous film and to reduce scattering of light by the hydrogel sample. Experiments were conducted at a scan speed of 20 nm min^{-1} with ten accumulations. For NMR studies gelled samples were freeze-dried and lyophilised (in the dark) and resuspended in deuterated DMSO. ^{31}P NMR experiments were undertaken the next day and ^{13} C after this due to the time taken to prepare the samples. ^{13}C NMR and ^{31}P NMR were recorded respectively at 125.7 and 202.4 MHz. Carbon (^{13}C) and Phosphorous (^{31}P) NMR was performed on the FMOC-Tyr hydrogels to confirm dephosphorylation. Norell Select Series 500 MHz NMR Tubes were used. Spectra were reported using 0.01% triethyl phosphate as an internal standard and 10% D_2O. Due to solubility issues, for ^{13}C NMR experiments required dissolving the hydrogel in deuterated DMSO.[2,3] Chemical shifts were recorded in parts per million (ppm).

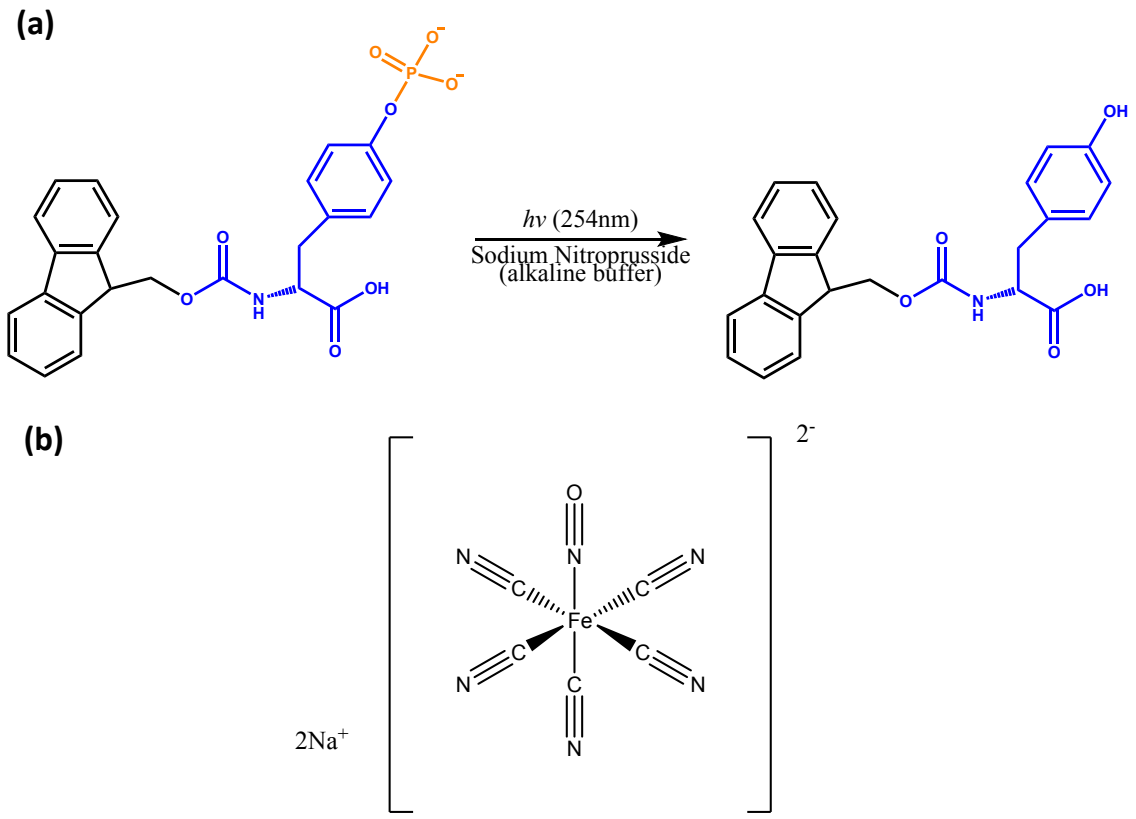
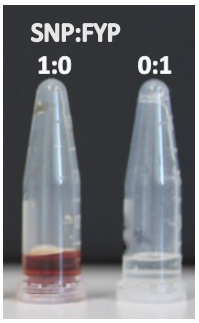


Figure S1. (a) Scheme for the formation of a supramolecular hydrogel through dephosphorylation of fluorenylmethyloxycarbonyl-tyrosine-phosphate (FYP) to form fluorenylmethyloxycarbonyl-tyrosine using sodium nitroprusside (SNP), and (b) Structure of sodium nitroprusside (SNP).

(a)



(b)

$$[Fe^{II}(CN)_6(NO)]^{2-} + H_2O \rightarrow [Fe^{III}(CN)_5(H_2O)]^{3-} + NO^{\bullet}$$

Figure S2 (a) Control samples with either FYP or SNP absent, respectively, demonstrating that both must be present for gelation to occur. (b) Scheme demonstrating the generation of nitric oxide radicals resulting from the irradiation of SNP.

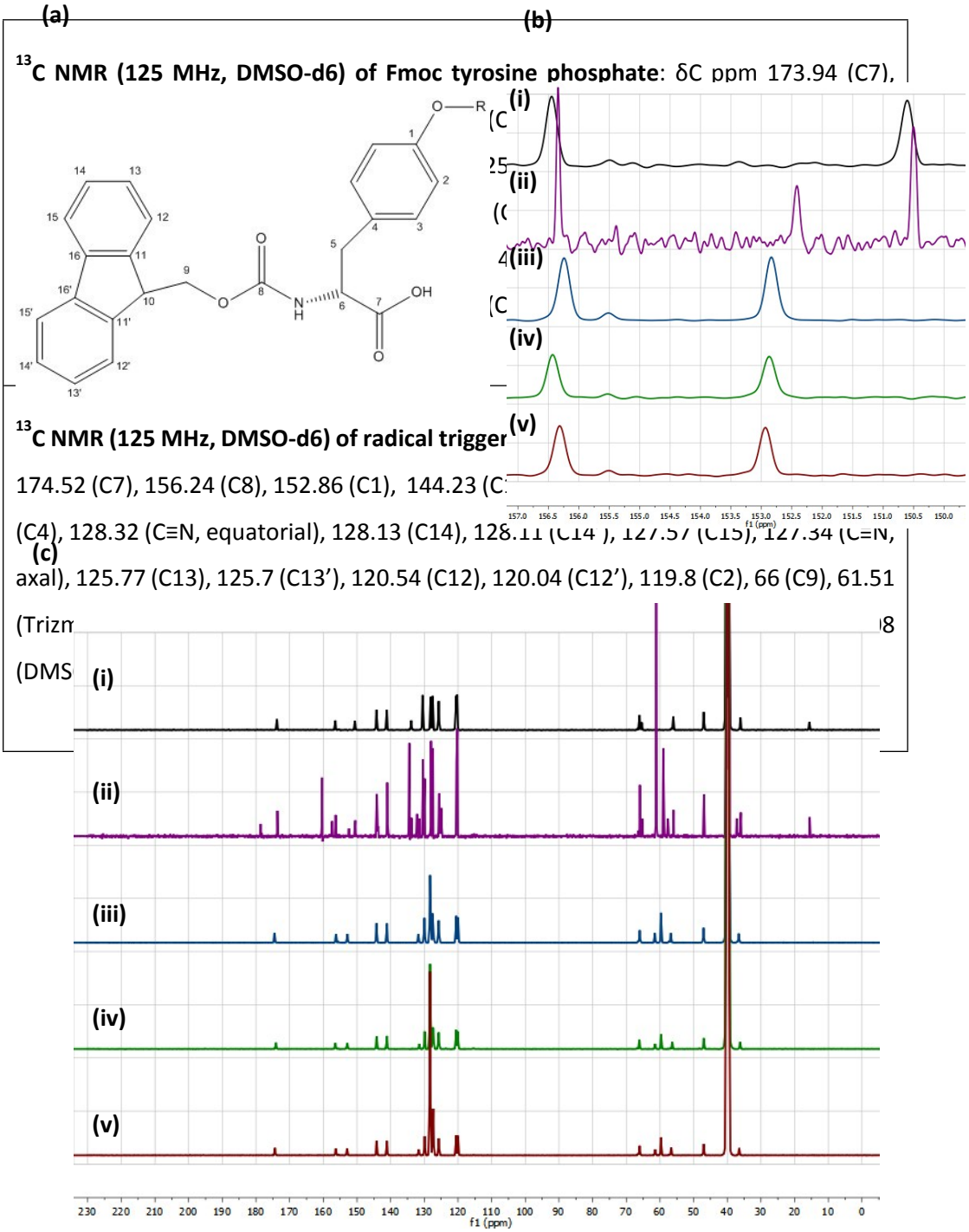
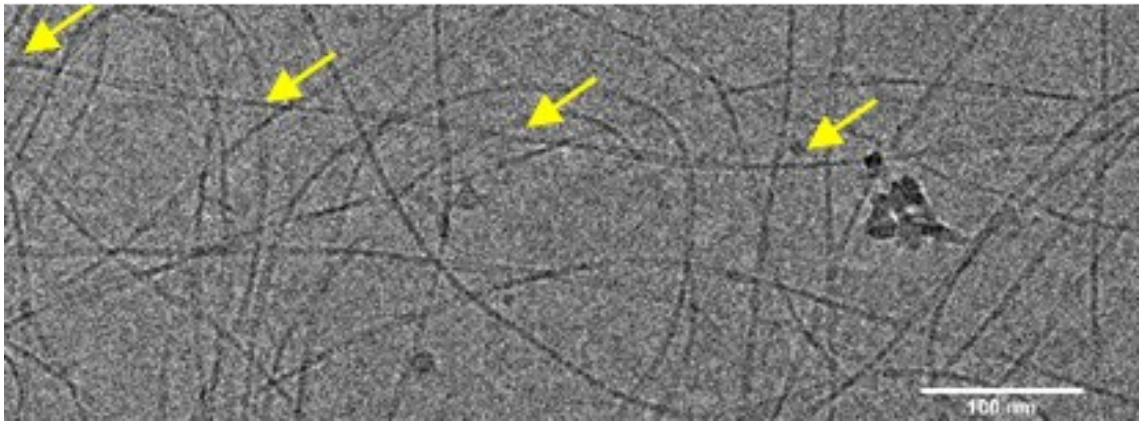


Figure S3 (a) Structure of FMOC-tyrosine-R, whereby R refers to either PO32- or H, (b) Selected area ^{13}C NMR (i) FMOC tyrosine phosphate (precursor), (ii) 1:1 gel, (iii) 2:1 gel, (iv) 5:1 gel, and (v) 10:1 gel. (c) Full spectra of 13C NMR on (i) FMOC tyrosine phosphate (precursor), (ii) 1:1 gel, (iii) 2:1 gel, (iv) 5:1 gel, and (v) 10:1 gel. (500 MHz, DMSO-d6).

Table 1

(a)



(b)

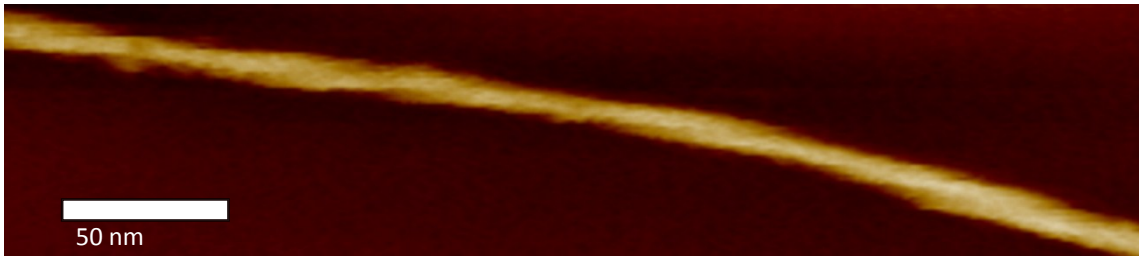


Figure S4 (a) Cryo-TEM image of SNP-FYP hydrogel showing narrow and twisted filaments (arrows indicate location of twisting). (b)AFM height scan showing the twisting of nanofilaments.

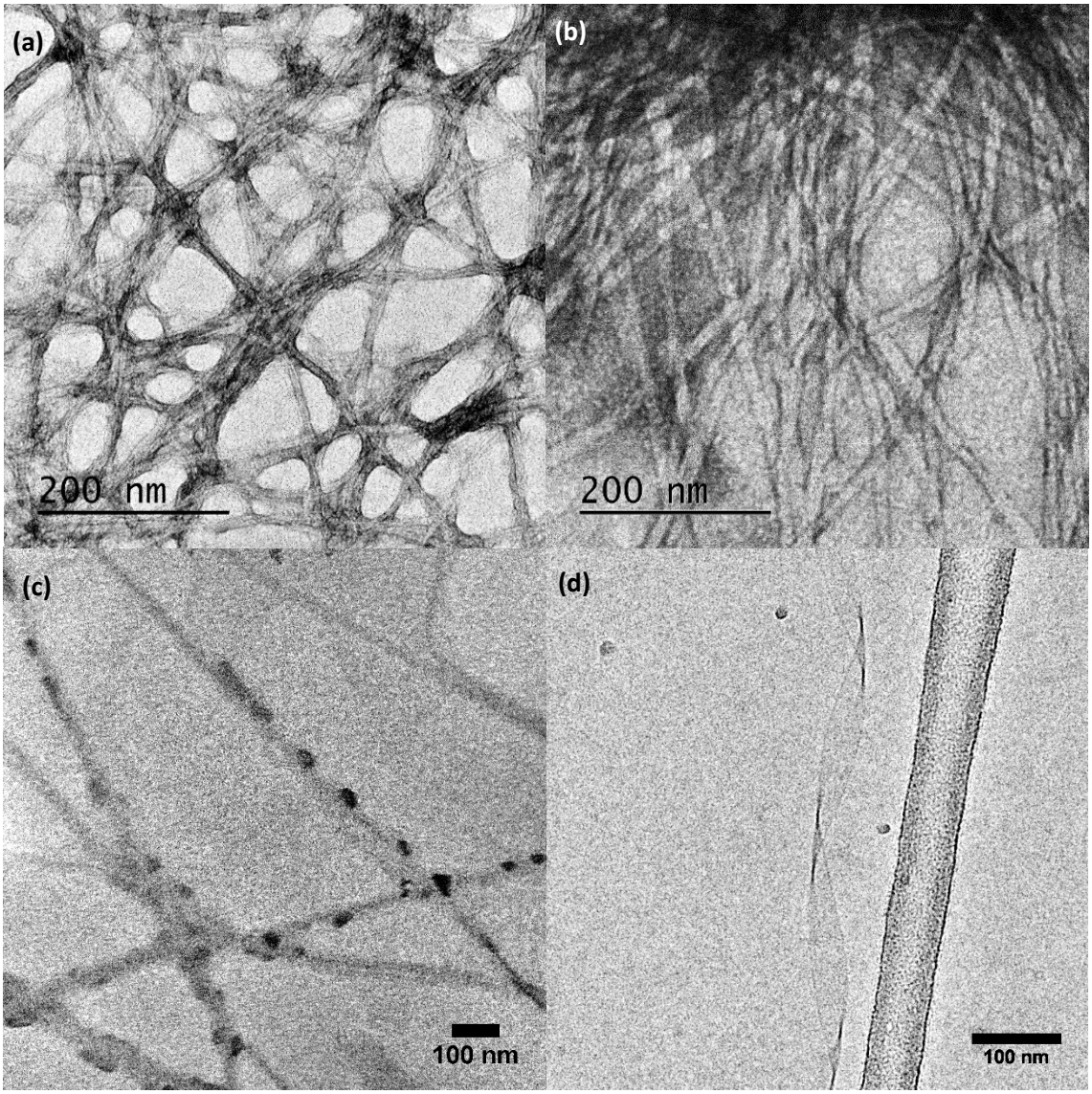


Figure S5 TEM image of a negatively stained hydrogels (a) SNP-FYP, (b) ALP-FYP, (c) unstained TEM image of SNP-FYP hydrogel nanofilaments, decorated with SNP salts and (d) cryo-TEM image of single twisted nanofilament formed through conventional, enzymatic route (ALP-FYP gel).

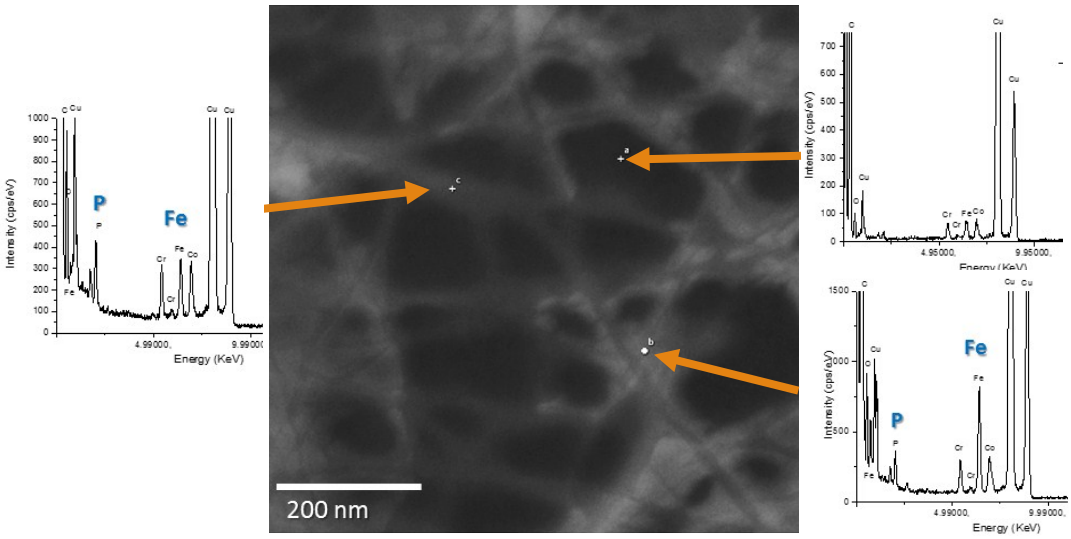
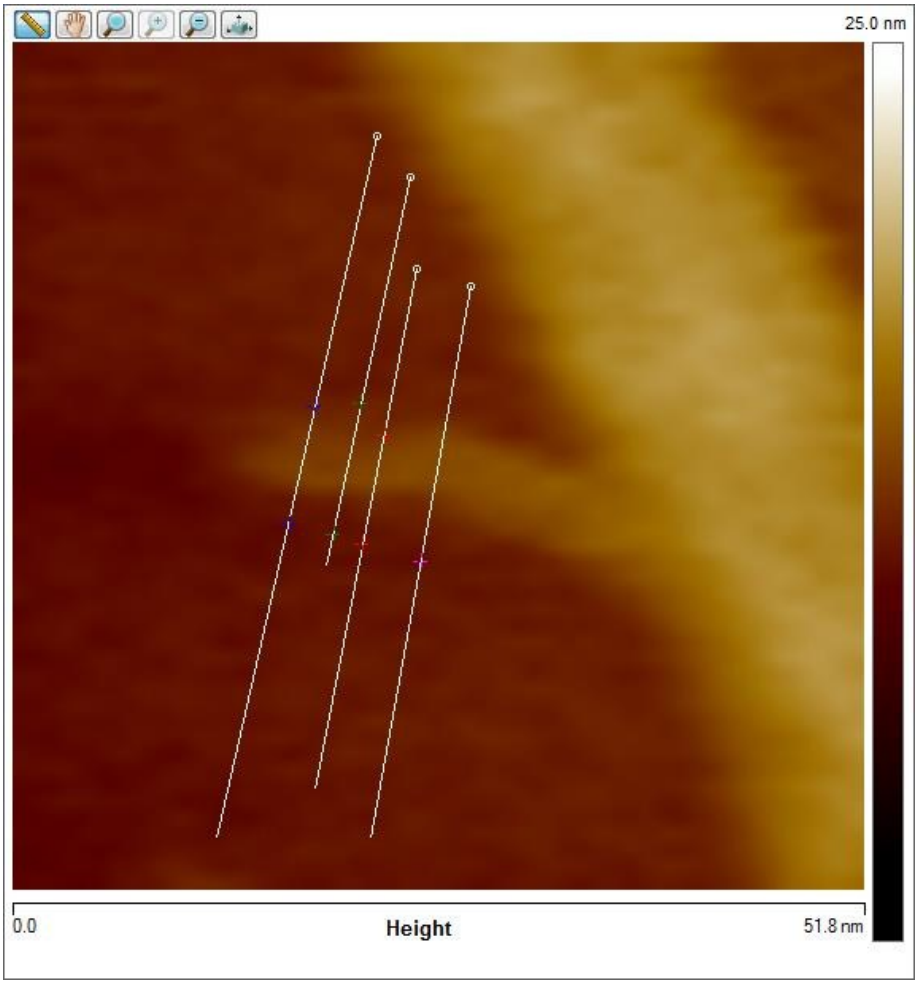


Figure S6 STEM image of SNP-FYP hydrogel; graphs show the energy-dispersive X-ray (EDX) spectra data. Locations: (a) carbon grid with small peak due to TEM grid holder, (b) and (c) iron, associated with SNP, and phosphorus peaks observed localised with the filaments.

(a)



(b)

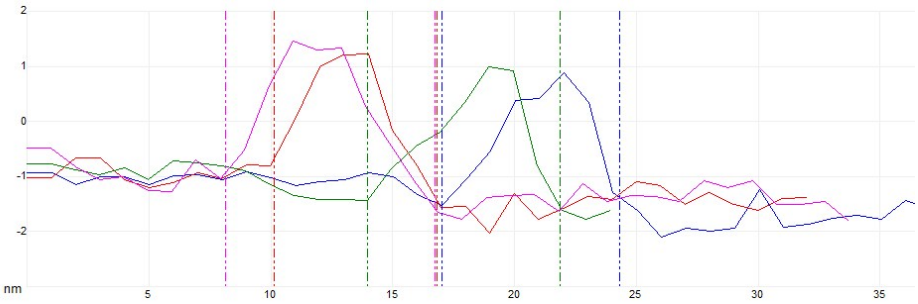


Figure S7 (a) Height scan (retrace), drop cast on bare freshly cleaved mica with line plots and (b) corresponding height profile across filament(s) with multiple maxima (see pink and blue lines in particular.)

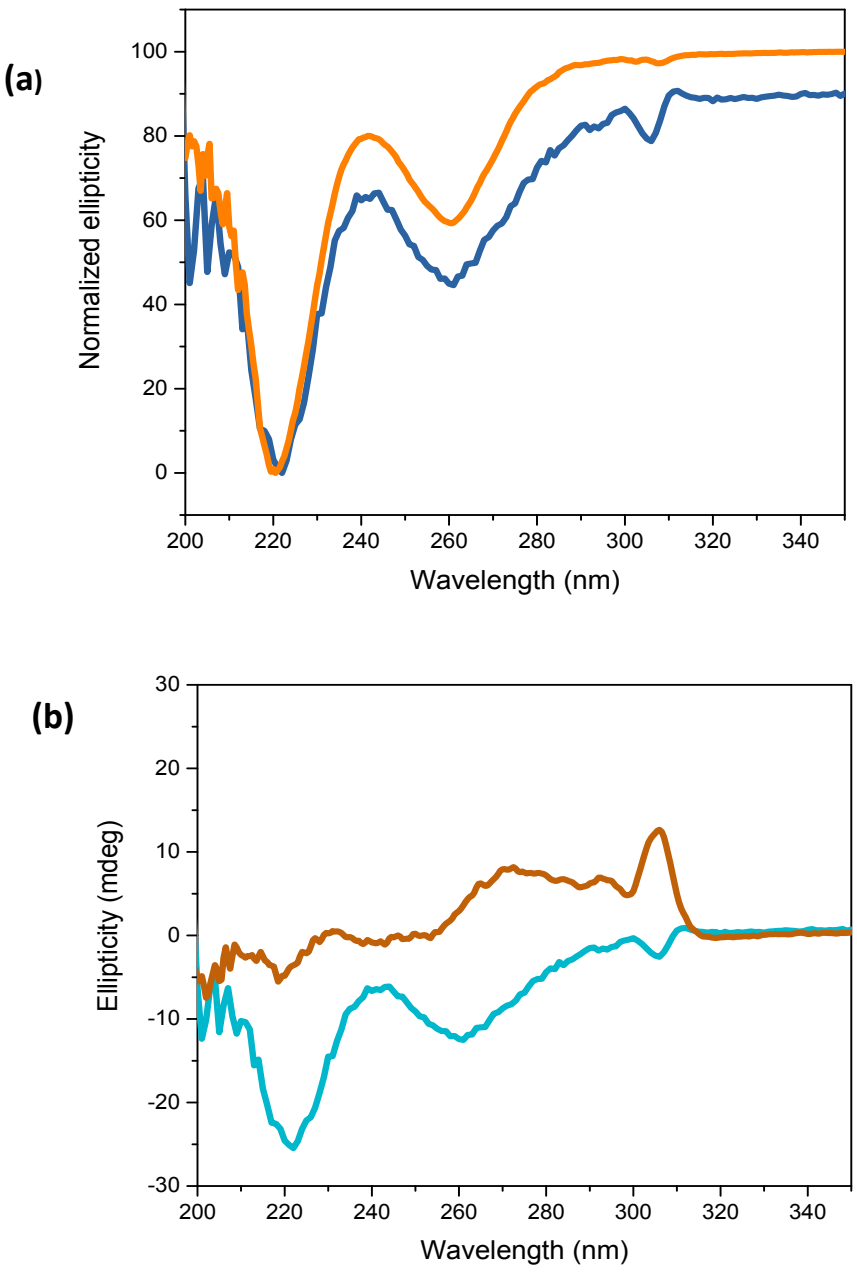


Figure S8 CD spectra of doped control gels (a) Fmoc tyrosine-phosphate (50mM) mixed with ALP (1kU/mL) at 37 °C (In tin foil) in the presence of SNP (250 mM) (orange) and as prepared 2-1 gel (blue). (b) CD spectra of melted hydrogels demonstrating that the chirality of the SNP-FYP can be reversed to the expected chirality after a heating and cooling cycle.

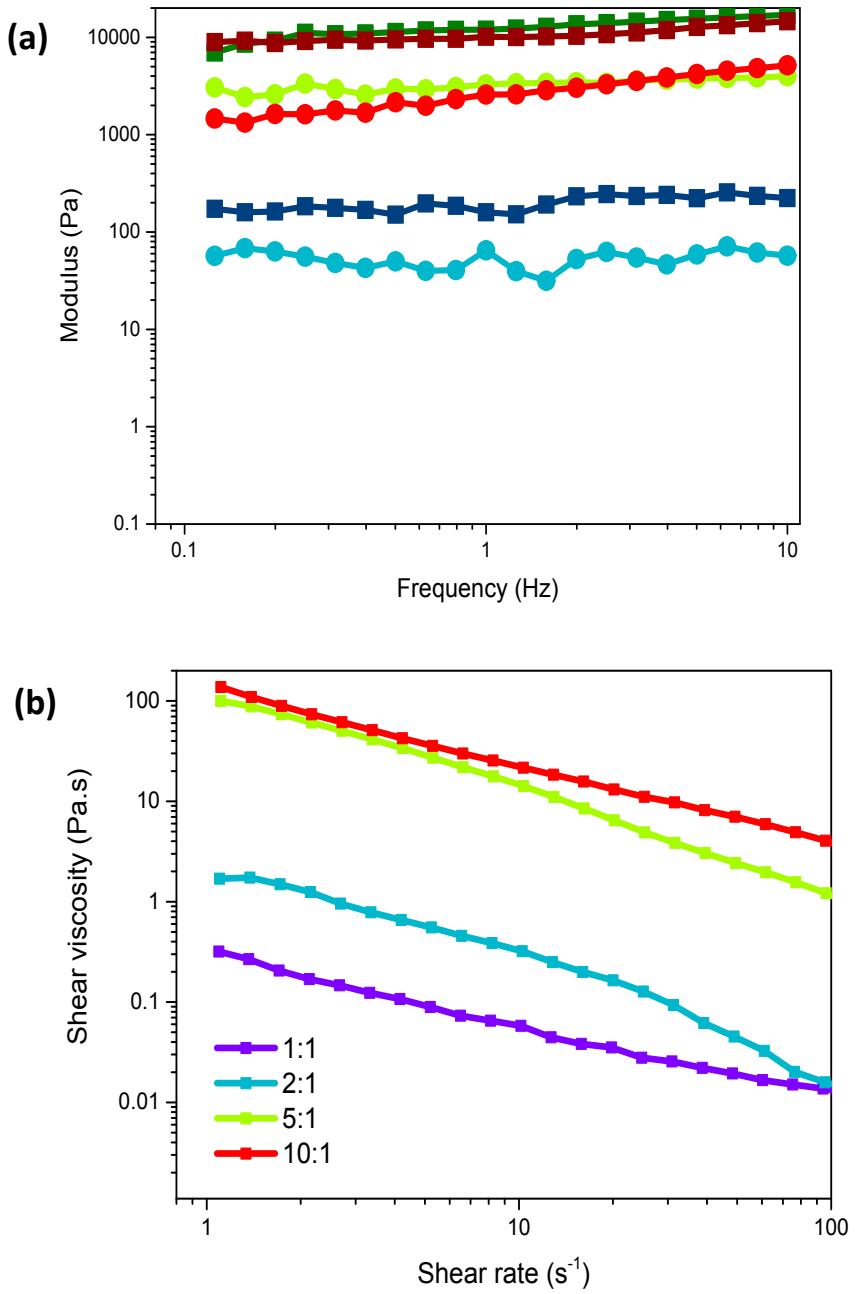


Figure S9: In all cases SNP: FYP ratios of 1:1 (purple), 2:1 (blue), 5:1 (green) and 10:1 (red). (a) Oscillatory frequency sweep, moduli demonstrating an indifference to frequency, typical of supramolecular hydrogels. ▪ refer to G′ and ● refer to G′′, the storage modulus and viscous modulus, respectively. G′ values were greater than G′′ values across this range of shear stress, indicative of solid-like viscoelastic behaviour of the gel samples, (b) Viscosity profile of hydrogels prepared at different SNP:FYP molar ratios, demonstrating shear-thinning behaviour. This corresponds to a lowering of shear viscosity with increasing shear rates as the applied shear can overcome the non-covalent interactions between gelators. As a result, the samples flow more easily, corresponding to a lower shear viscosity.

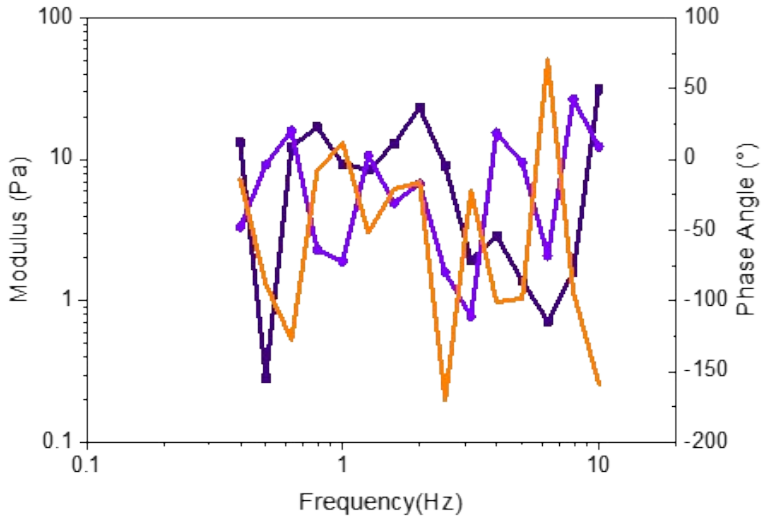


Figure S10: Oscillatory amplitude sweep at a constant frequency (1Hz) for SNP: FYP molar ratio of 1:1. Note that this sample failed to exhibit a linear viscoelastic regime. Indicating that at 1:1 ratio samples produced viscous solution. In all cases ▪ refer to G' (elastic moduli) and ● refer to G'' (viscous moduli) Phase angle also included (orange).

References

1 Z. Yang, H. Gu, D. Fu, P. Gao, J. K. Lam and B. Xu, *Adv. Mater.*, 2004, **16**, 1440–1444.

2 Z. Yang, G. Liang, L. Wang and B. Xu, *J. Am. Chem. Soc.*, 2006, **128**, 3038–3043.

3 K. J. Skilling, B. Kellam, M. Ashford, T. D. Bradshaw and M. Marlow, *Soft Matter*, 2016, **12**, 8950–8957.