Electronic Supplementary Material (ESI) for Chemical Science.

Spatially-resolved soft materials for controlled release – hybrid hydrogels combining a robust photo-activated polymeric gel and an interactive supramolecular gel

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Contents

1. Materials and methods

General Experimental Methods. All compounds required in synthesis and analysis were purchased from standard commercial suppliers. The synthesis of DBS-$CONHNH_2$ and PEGDM were performed using literature methods.[1,2] Proton NMR spectra were recorded on a Jeol 400 spectrometer (^{1}H 400 MHz). Samples were recorded as solutions in deuterated NMR solvents as stated and chemical shifts (δ) are quoted in parts per million. UV-Vis spectroscopy was performed on a Shimadzu UV-2401 spectrometer. Rheological measurements were recorded using a Malvern Instruments Kinexus Pro+ rheometer fitted with a parallel plate geometry. T_{gel} values were recorded using a high precision thermoregulated oil bath using the tube inversion method. UV-vis absorbance was measured on a Shimadzu UV-2401 PC spectrophotometer. A high powered UV lamp (λ = 315 – 405 nm) was used for activation of the photoinitiator Irgacure 2959 (activation at λ = 365 nm).

2. Preparation of Gels

Preparation of DBS-$CONHNH_2$ hydrogels. A known quantity of DBS-$CONHNH_2$ was weighed into a 2.5 mL sample vial and 0.5 mL deionised water was added. The vial was then sonicated to disperse the solid and gels formed by a simple heat-cool cycle. Gels formed in a few minutes following the removal of the heat source. The same procedure was followed for gels containing DBS-hydrazide and naproxen.

Preparation of PEGDM hydrogels. Varying concentrations (% wt/vol) of PEGDM were dissolved in 0.5 mL of 2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (photoinititator, PI) solution (0.5% wt/vol) in 2.5 mL sample vials. The solutions were cured in uncapped vials under a long wavelength UV lamp (30 min) to form transparent hydrogels.

Preparation of DBS-hydrazide/PEGDM hybrid gels. DBS-hydrazide gels were prepared as above. A solution of PEGDM (various concentrations) dissolved in PI solution (0.5% wt/vol) was pipetted into the vial on top of the gel and allowed to stand in the sealed vial for 72 hours. The supernatant was then removed and the discs cured in the uncapped vials under a long wavelength UV lamp for between 10 and 30 min to yield opaque hybrid gel samples.

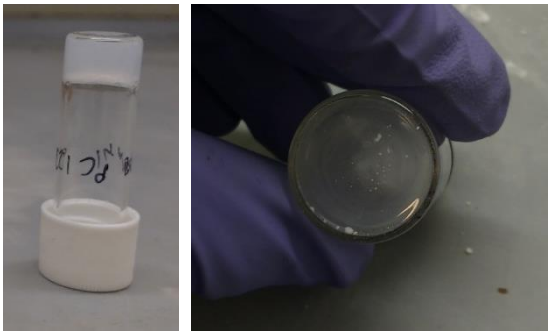

Figure S1: NPX-loaded hydrogels. (a) DBS-$CONHNH_2$ (6 mM), (b) PEGDM

Estimation of PEGDM concentration in hybrid gels. A NMR experiment allowed the PEGDM uptake in the LMWG to be quantified. PEGDM solutions of 5, 7 and 10% wt/vol (0.5 mL) were pipetted onto pre-formed DBS-hydrazide (6 mM, 0.5 mL) gels. The samples were allowed to stand for 72 h before removal of the supernatant. The gels were dried *in vacuo* and dissolved in DMSO-d_6. The resulting solution was analysed by ^{1}H NMR spectroscopy. Comparison of the integral of the methyl PEGDM peak (δ = 1.93) with that of the hydrazide (CON*H*NH_2, δ = 9.77) peak of DBS-$CONHNH_2$ allowed the concentration of PEGDM in the gel to be calculated – this was converted into a mass uptake and hence the % wt/vol was calculated (Table S1).

Table S1: Uptake of PEGDM into DBS-hydrazide gels after 3 days. All gel volumes were 0.5 ml.

PEGDM in solution % wt/vol	Peak ratio δ = 9.77 : 1.93	PEGDM uptake mg	PEGDM uptake %	PEGDM in gel % wt/vol
5	0.07	22.2	89	4.4
7	0.09	27.3	78	5.8
10	0.45	43.2	86	8.6

3. IR Spectra

Spectra

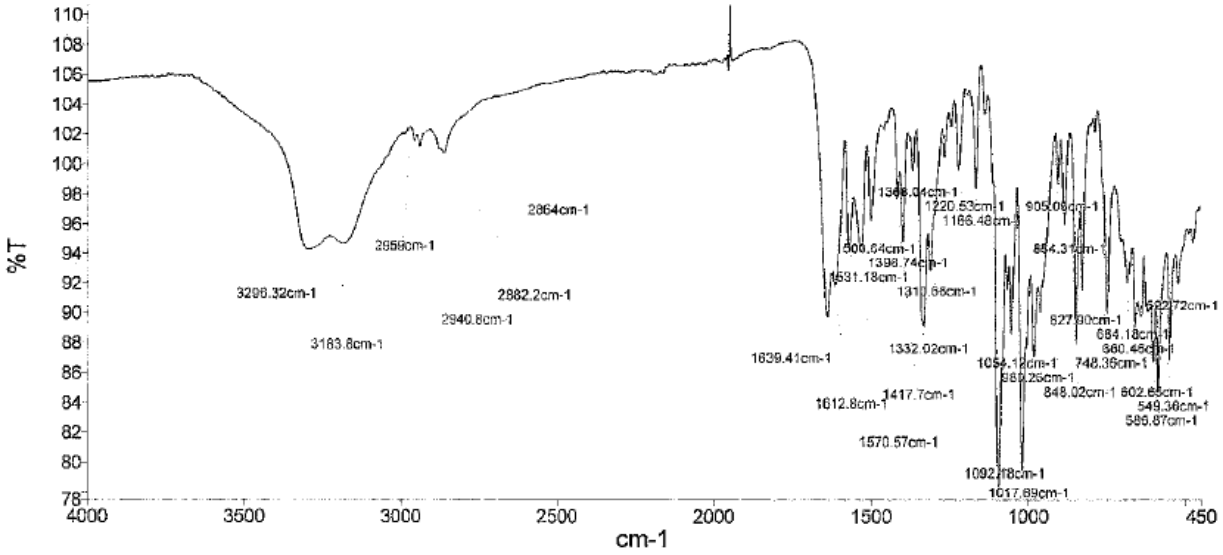

Figure S2. IR spectrum of xerogel formed from DBS-CONHNH$_2$ gel.

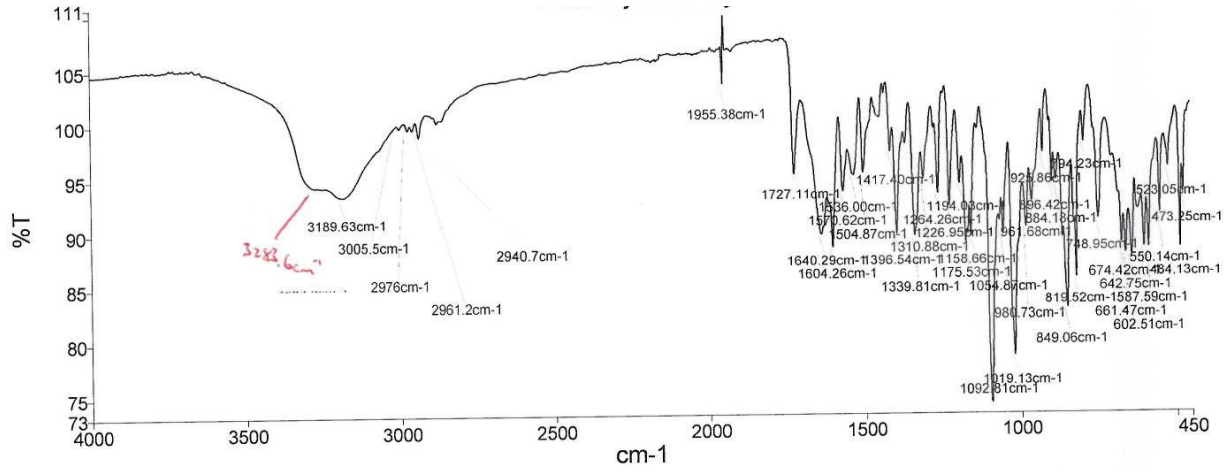

Figure S3. IR spectrum of xerogel formed from DBS-CONHNH$_2$ gel + NPX

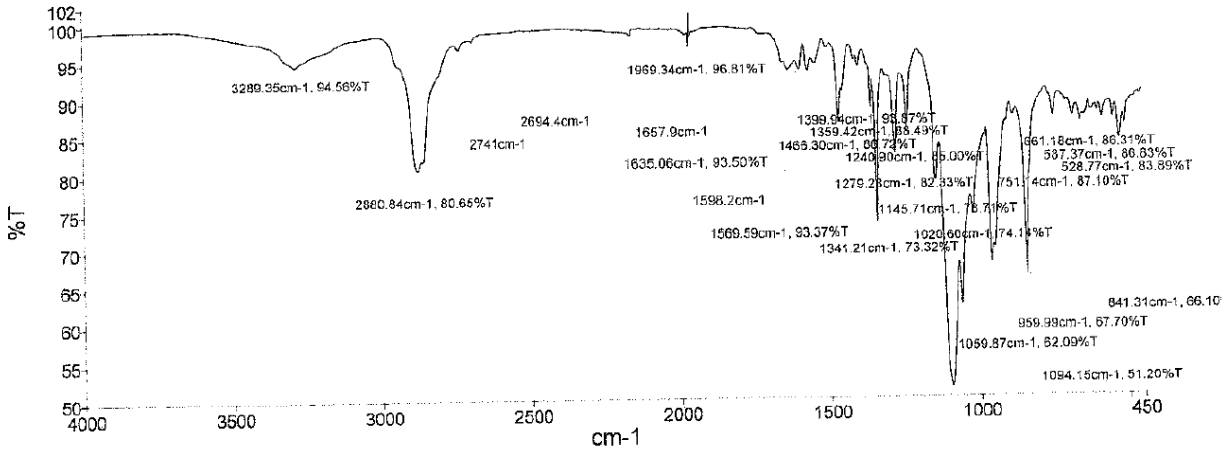

Figure S4. IR spectrum of xerogel formed from 10% hybrid

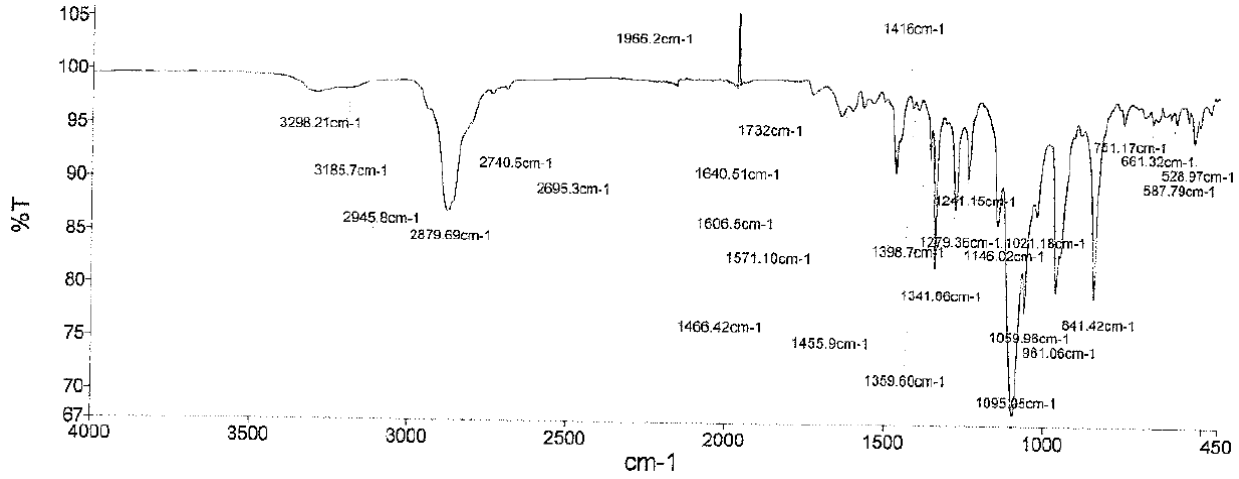

Figure S5. IR spectrum of xerogel formed from 10% hybrid gel + NPX

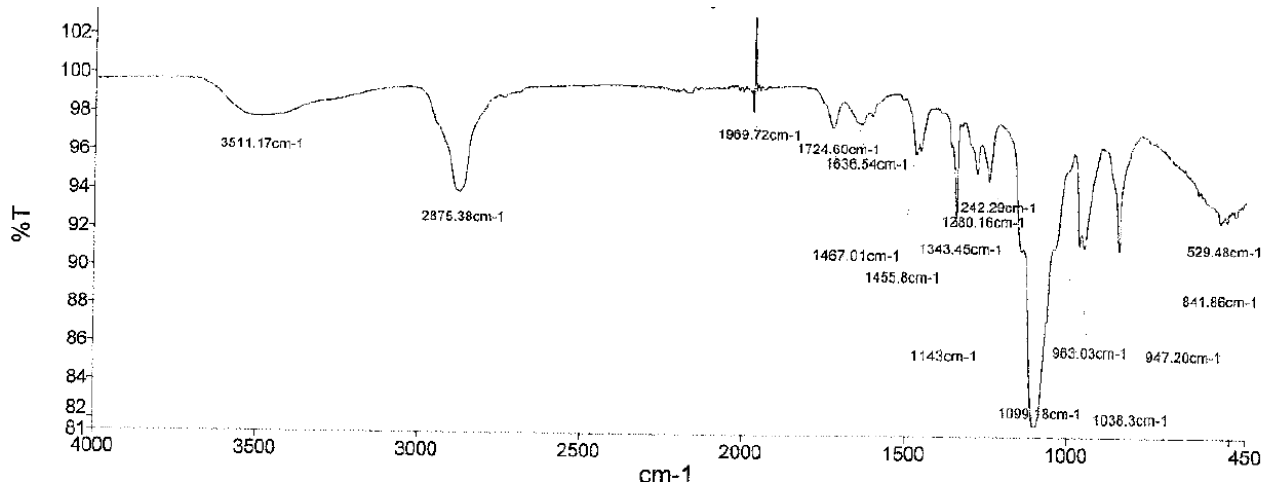

Figure S6. IR spectrum of xerogel formed from 10% PEGDM gel + NPX

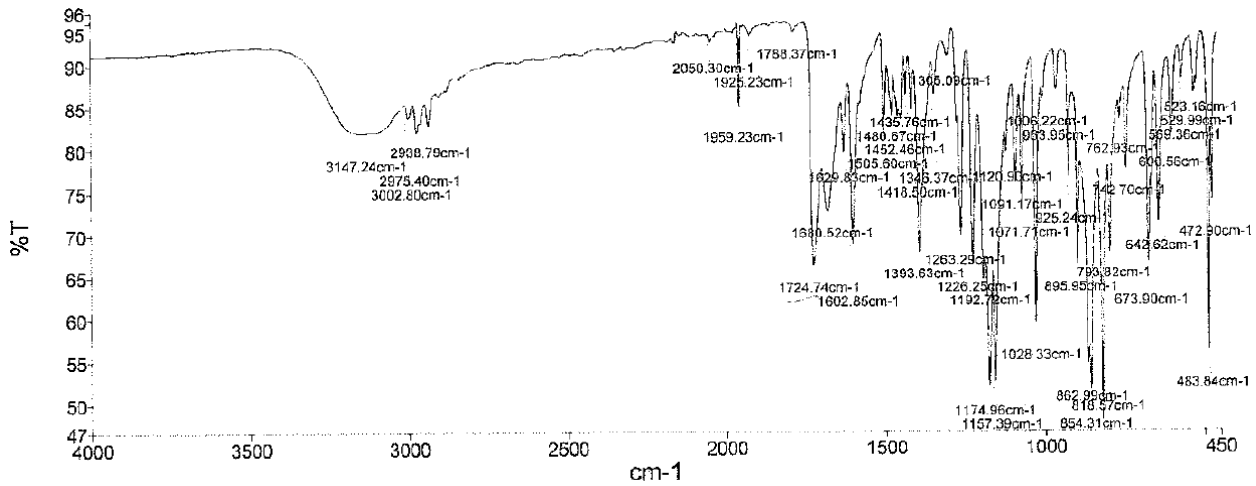

Figure S7. IR spectrum of solid NPX

4. SEM Images

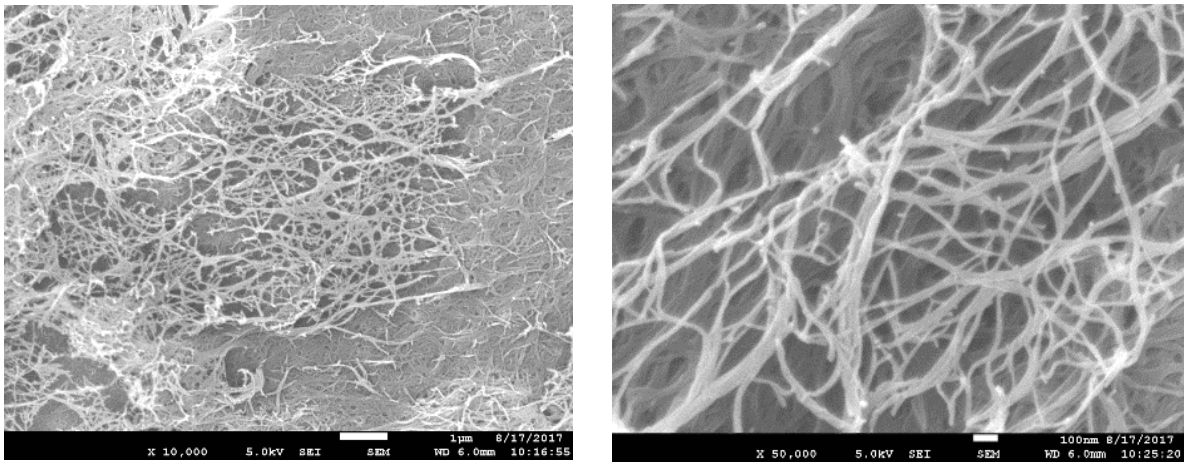

Figure S8. SEM images of cryo-dried DBS-$CONHNH_2$ xerogel. Scale bars: (left) 1 μm, (right) 100 nm.

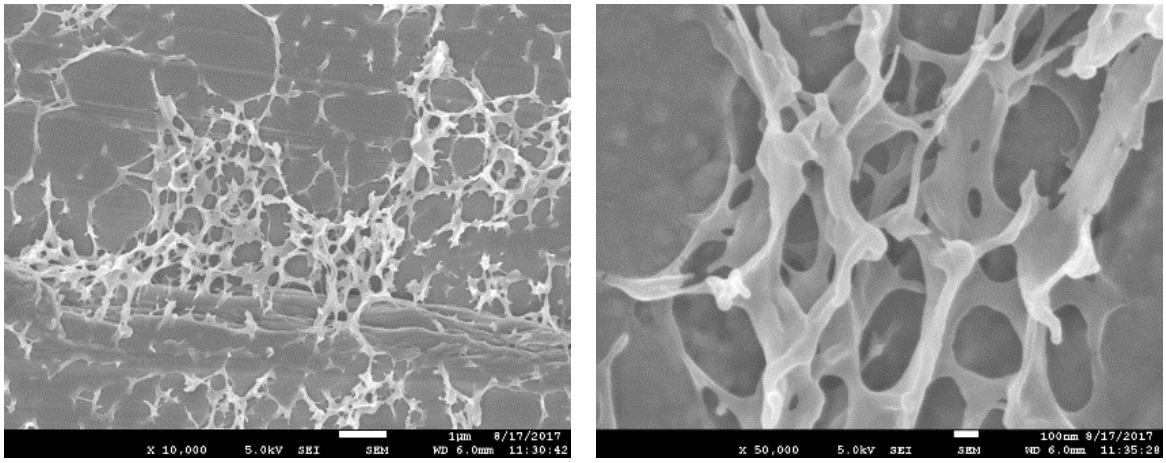

Figure S9. SEM images of cryo-dried PEGDM xerogel. Scale bars: (left) 1 μm, (right) 100 nm.

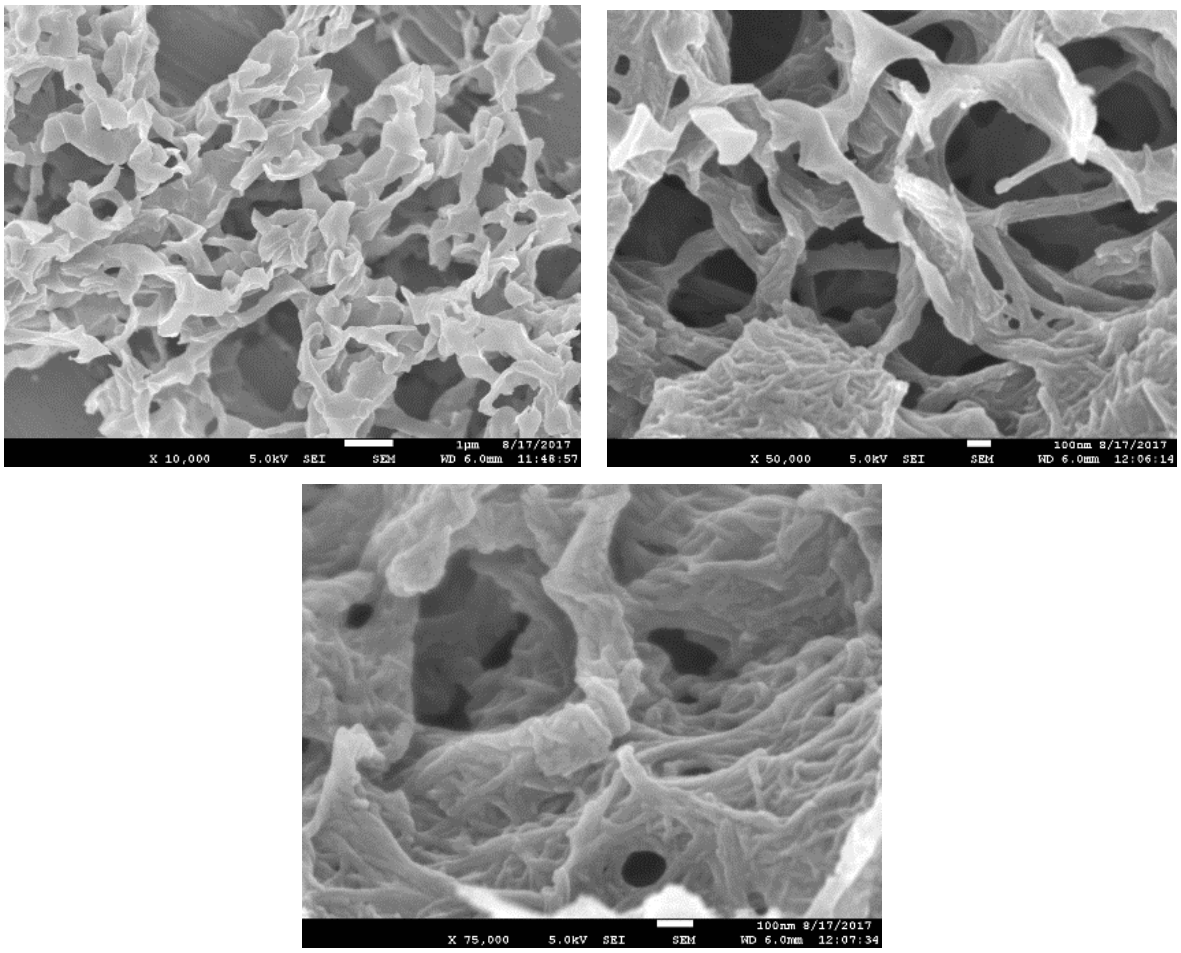

Figure S10. SEM images of cryo-dried 10% PEGDM/DBS-$CONHNH_2$ xerogel. Scale bars: (top left) 1 μm, (top right) 100 nm, (bottom) 100 nm.

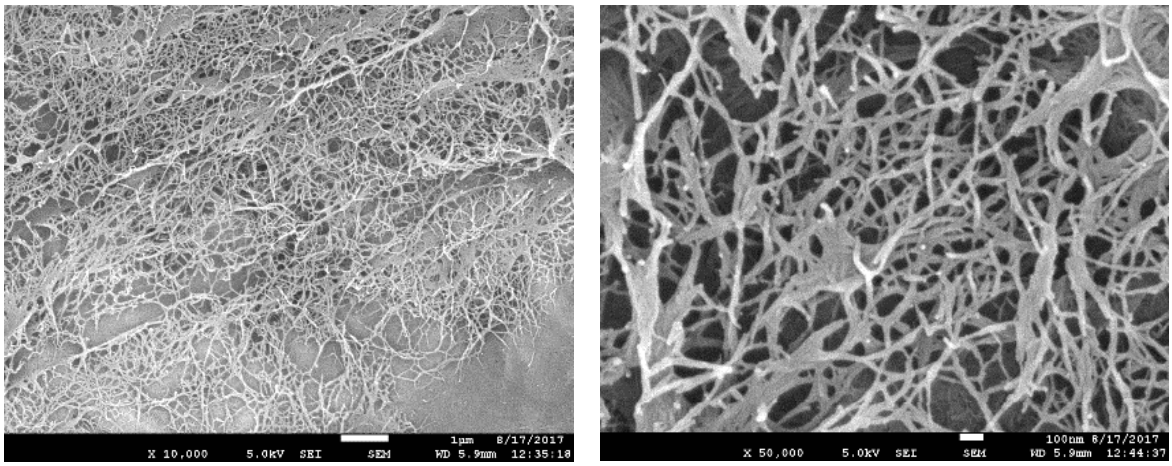

Figure S11. SEM images of cryo-dried DBS-$CONHNH_2$ xerogel loaded with NPX. Scale bars: (left) 1 μm, (right) 100 nm.

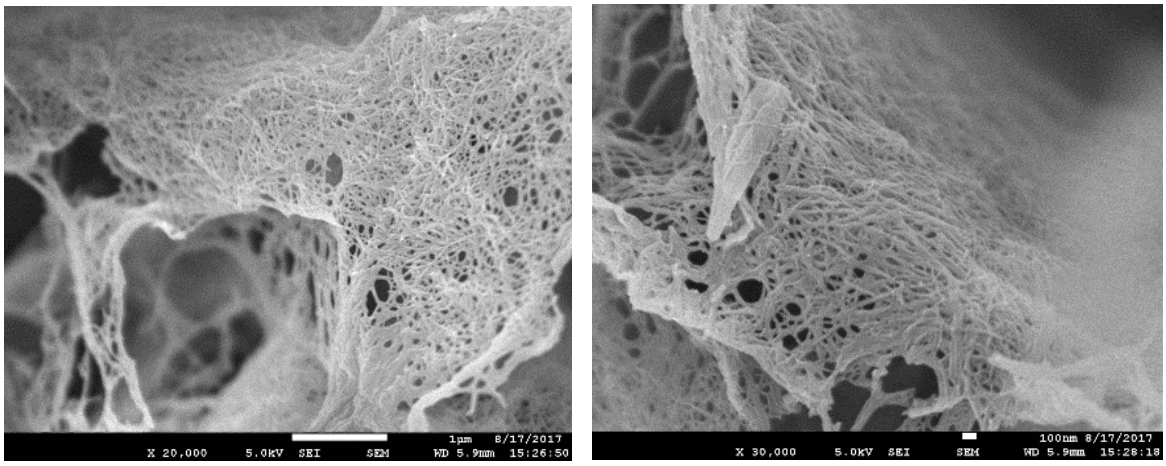

Figure S12. SEM images of cryo-dried DBS-$CONHNH_2$ xerogel loaded with NPX and soaked in pH 4 buffer. Scale bars: (left) 1 μm, (right) 100 nm.

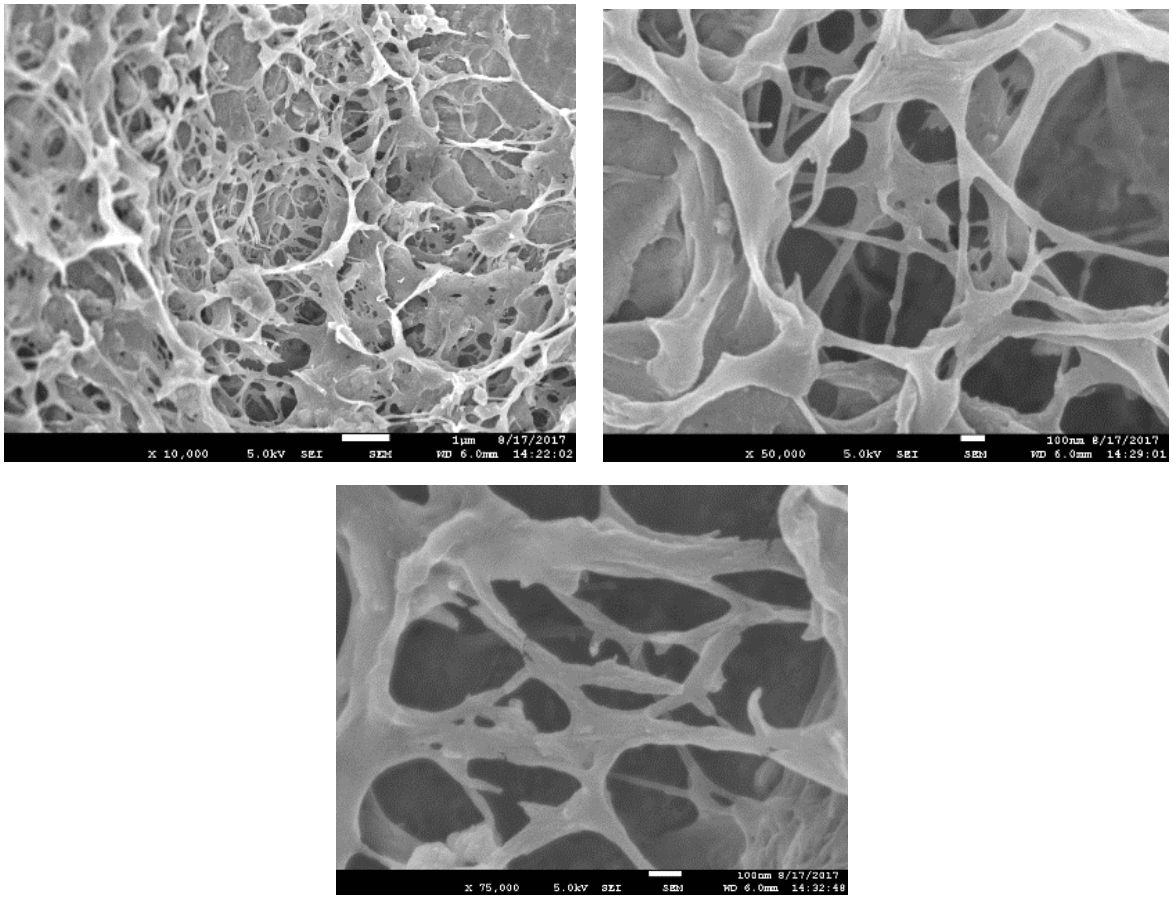

Figure S13. SEM images of cryo-dried 10% PEGDM/DBS-$CONHNH_2$ xerogel loaded with NPX. Scale bars: (left) 1 μm, (right) 100 nm, (bottom) 100 nm.

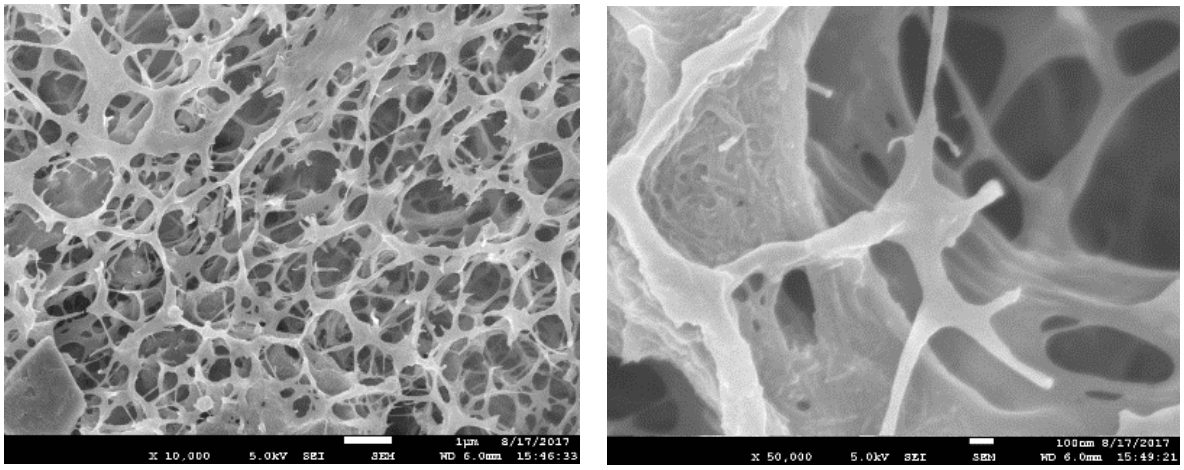

Figure S14. SEM images of cryo-dried 10% PEGDM/DBS-$CONHNH_2$ xerogel loaded with NPX and soaked in pH 4 buffer. Scale bars: (left) 1 μm, (right) 100 nm.

5. Rheological Characterisation

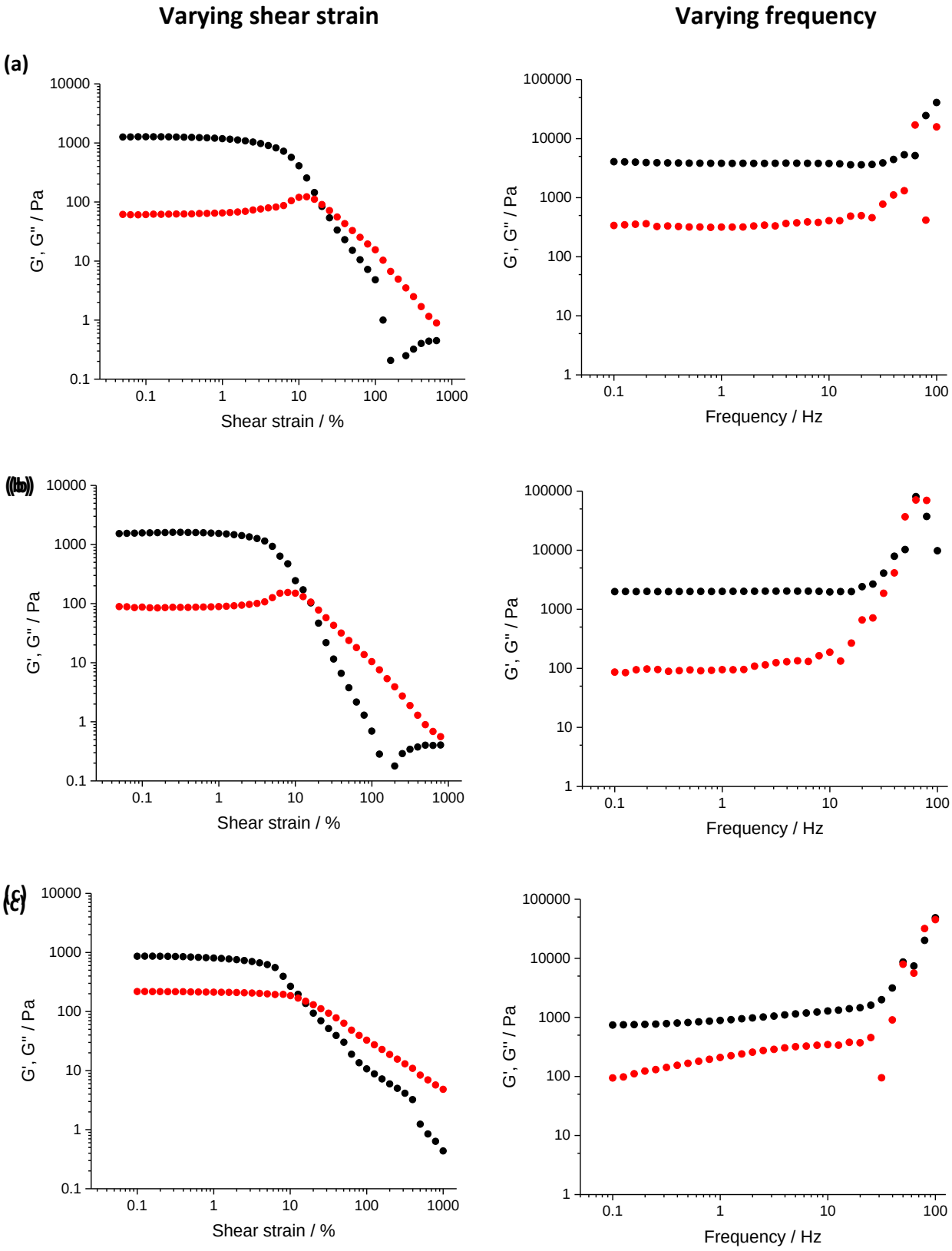

Figure S15: Elastic (G', black circles) and loss (G'', red circles) moduli of hydrogels with increasing shear strain (left) & frequency (right). (a) 6 mM DBS-$CONHNH_2$, (b) 8 mM DBS-$CONHNH_2$, (c) 5% hybrid.

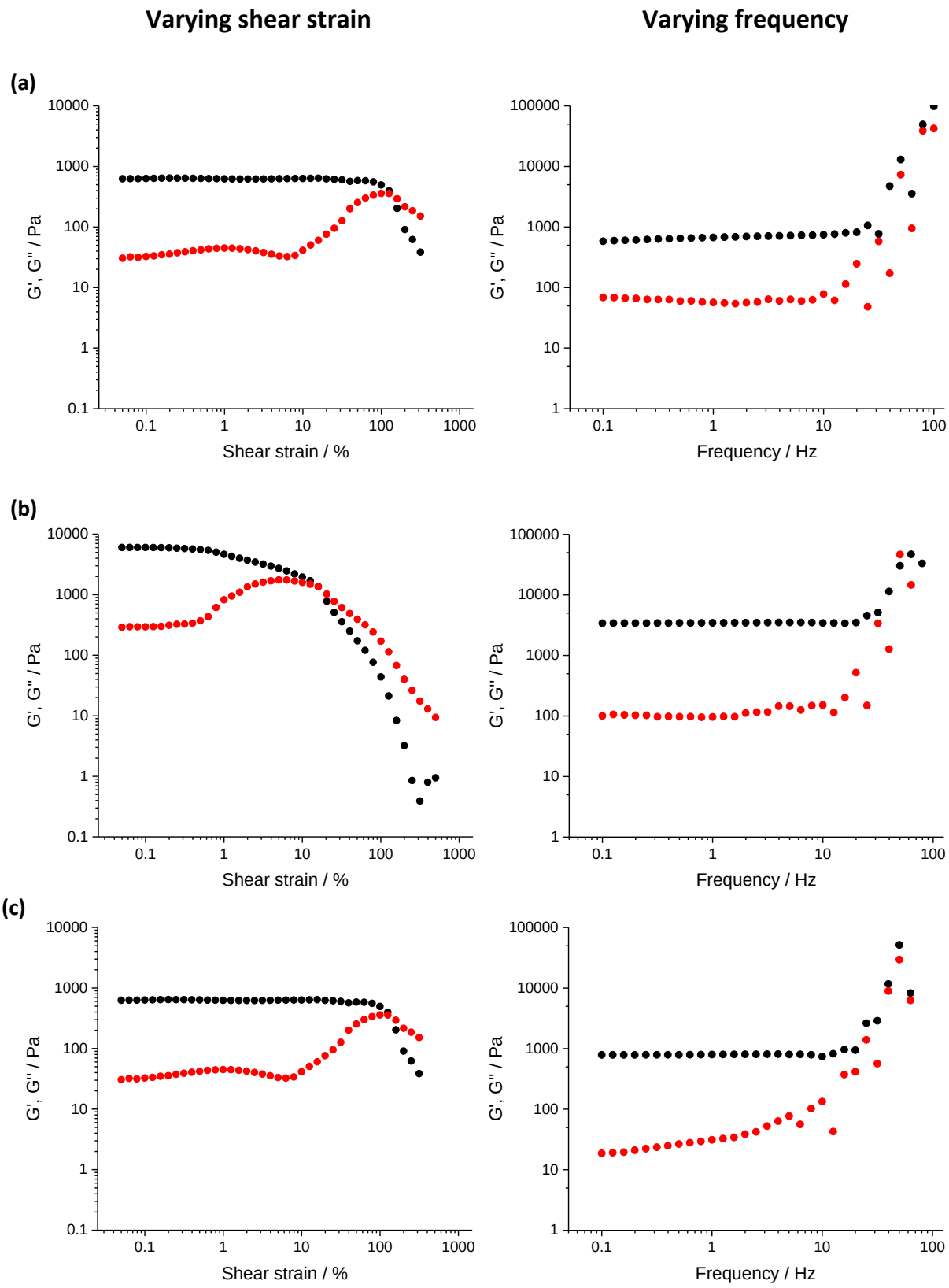

Figure S16: Elastic (G', black circles) and loss (G'', red circles) moduli of hydrogels with increasing shear strain (left) and frequency (right). (a) 7% hybrid, (b) 10% hybrid, (c) 5% PEGDM.

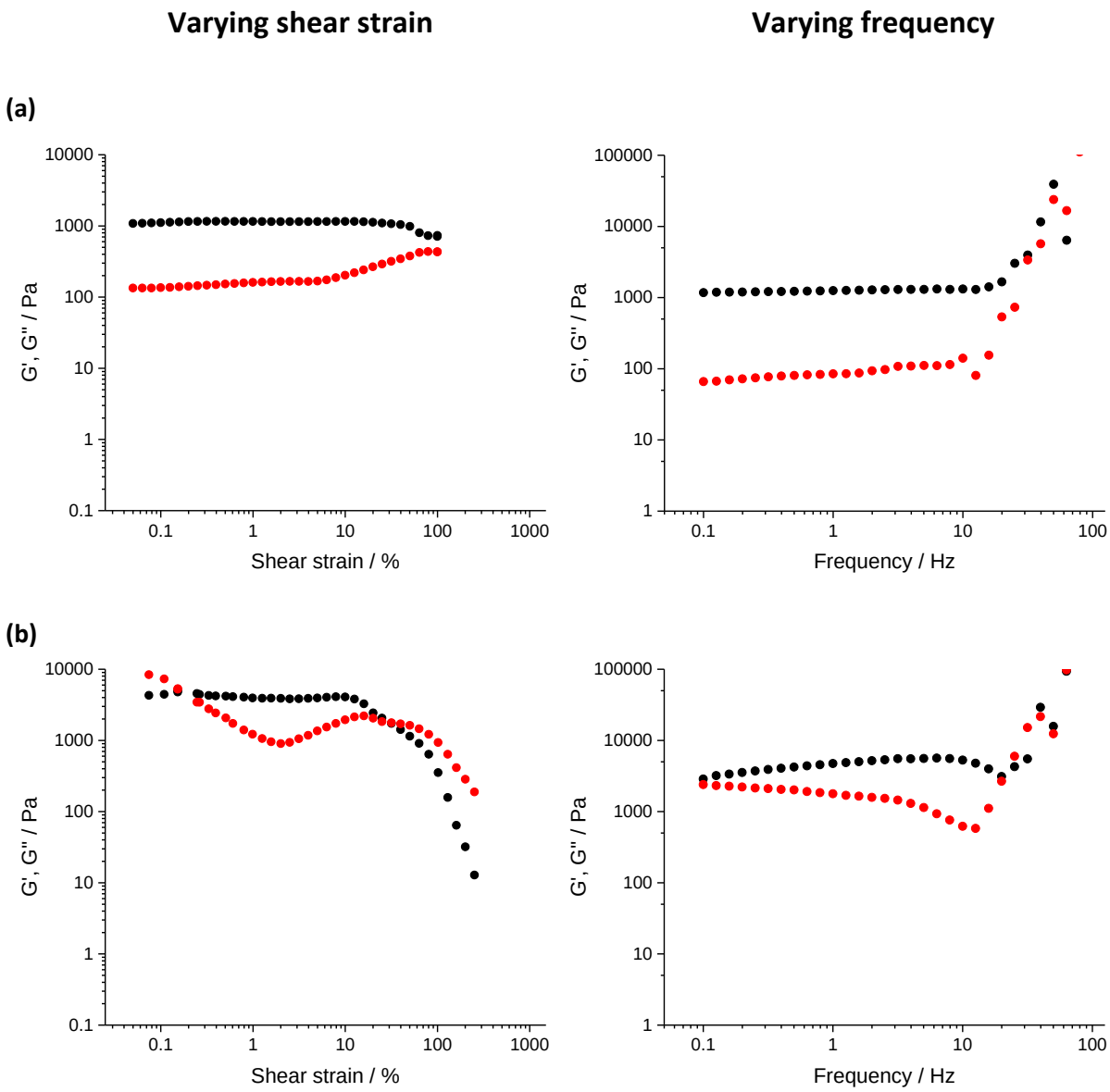

Figure S17: Elastic (G', black circles) and loss (G'', red circles) moduli of hydrogels with increasing shear strain (left) and frequency (right). (a) 7% PEGDM, (b) 10% PEGDM.

Gels with NPX

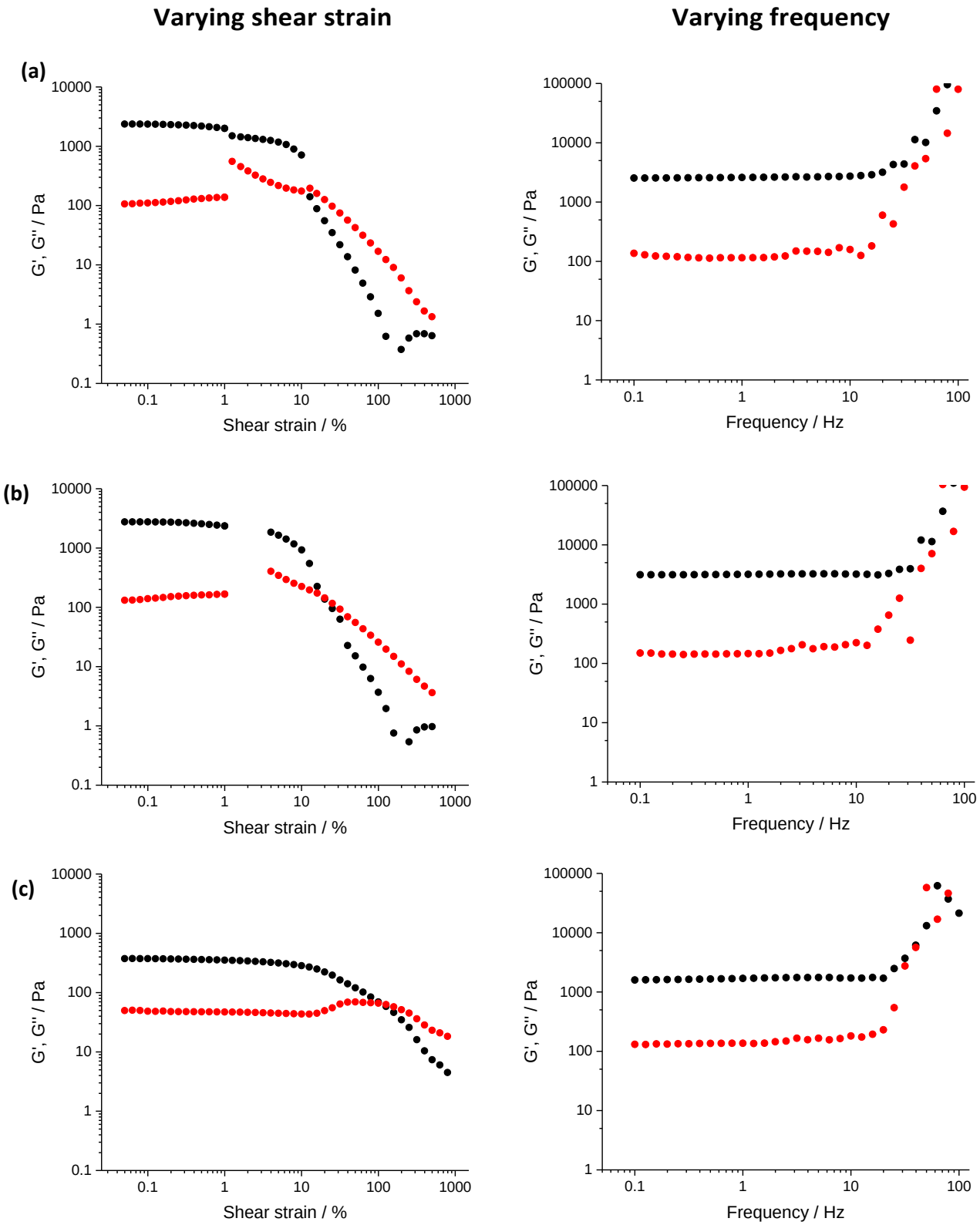

Figure S18: Elastic (G', black circles) and loss (G'', red circles) moduli of NPX-loaded hydrogels with increasing shear strain (left) and frequency (right). (a) 6 mM DBS-$CONHNH_2$, (b) 8 mM DBS-$CONHNH_2$, (c) 5% hybrid.

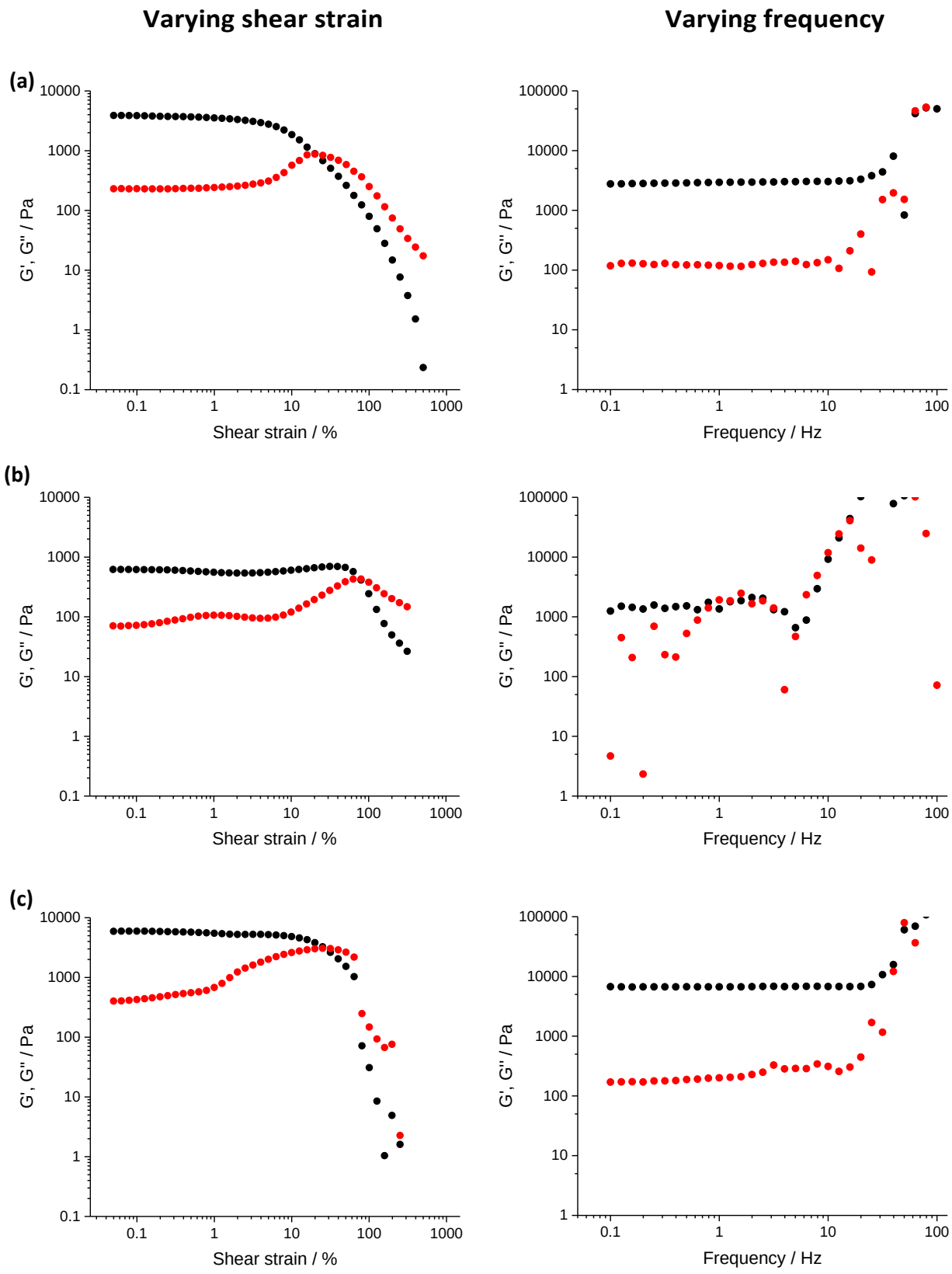

Figure S19: Elastic (G', black circles) and loss (G'', red circles) moduli of NPX-loaded hydrogels with increasing shear strain (left) and frequency (right). (a) 10% hybrid, (b) 5% PEGDM, (c) 10% PEGDM.

6. NPX Encapsulation and Release

NPX Encapsulation Experiment. DBS-hydrazide (1.99 mg, 6 mM) and NPX (0.97 mg, 6 mM) were suspended in D_2O (0.7 mL). DMSO (1.4 µL) was added and the mixture was sonicated for 15 mins to disperse the solids. The mixture was then heated to dissolution and transferred to an NMR tube. Upon cooling, a gel formed which was analysed by ^1H NMR spectroscopy (Fig. S3). NPX bound to the gel fibres is invisible by NMR, whilst unbound NPX can be observed, therefore the concentration of unbound NPX in the gel can be calculated. The integral of the peak corresponding to the NPX methyl group (δ = 1.27) was compared to that of DMSO (δ = 2.50), the concentration of which was calculated to be 28 mM (Table S2). From this it was calculated that for every mole of DMSO in the gel there are 0.017 moles unbound NPX. This corresponds to a concentration of 0.48 mM unbound NPX, which represents 8% of the NPX originally loaded in the sample.

Table S2: Quantification of NPX binding efficiency

Species	Integral	Relative quantity mol	Concentration mM	Unbound NPX %
DMSO	60.00	1	28	-
NPX	0.50	0.017	0.48	8

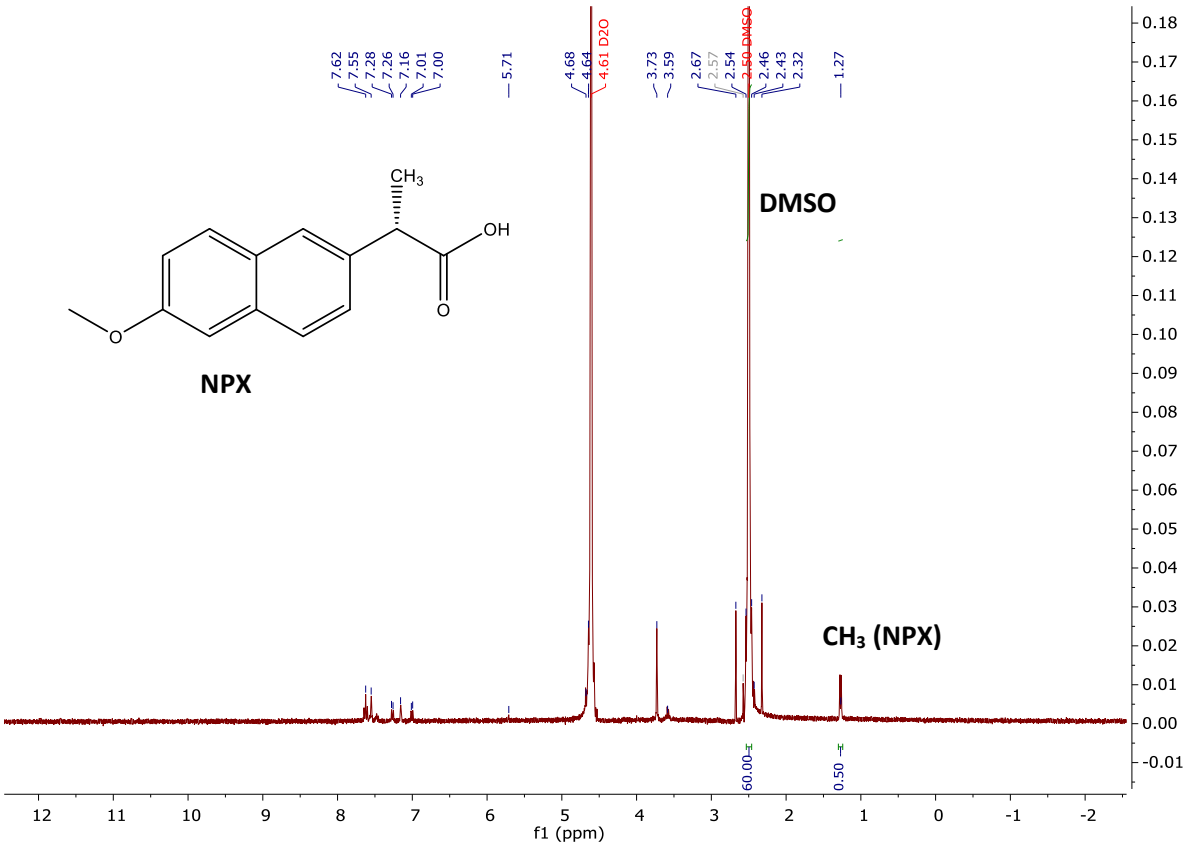

Figure S20: ^{1}H NMR spectrum of NPX (6 mM) loaded DBS-CONHNH$_2$ (6 mM) hydrogel made in D_2O solvent. Solution was spiked with DMSO (0.028 M) to quantify unbound NPX.

$$0.50/3 = 0.17$$

$$\left(\frac{0.17}{10}\right) \times 0.028\ M = 4.8\ \times 10^{-4} M$$

$$\frac{4.8\ \times 10^{-4} M}{6\ \times 10^{-3} M} \times 100\ \% = 7.93\ \%$$

Drug release studies. 1 mL volume gel samples were prepared by the methods outlined above. 6 mL of one of i) pH 2.8 citrate buffer, ii) pH 4.0 citrate buffer, iii) pH 5.5 phosphate-citrate buffer iv) pH 7 phosphate buffer, v) pH 8 phosphate buffer was pipetted onto the gel and the release of NPX monitored at 329 nm by UV-vis spectroscopy. The samples were incubated at 37°C for the duration of the study. NPX concentration in the supernatant was quantified using calibration curves. All experiments were carried out in duplicate and control experiments using gels containing no NPX were also carried out.

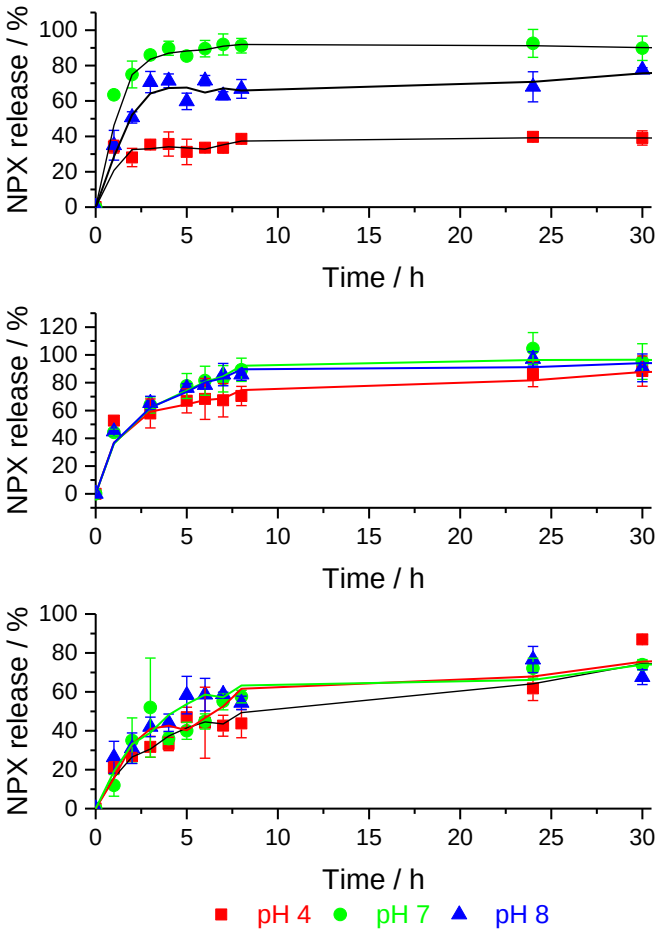

Figure S21: NPX release profile for (top) 8 mM DBS-CONHNH$_2$, (centre) 5% PEGDM, and (bottom) 10% PEGDM, into buffer solutions with different pH values in each case

Table S3: Initial NPX release rates into buffers of varying pH. All rates assume zero-order release kinetics

	Initial release rate × 10^{-9} / mol min^{-1}					
Buffer pH	DBS-CONHNH$_2$ 6 mM	DBS-CONHNH$_2$ 8 mM	5% hybrid	10% hybrid	5% PEGDM	10% PEGDM
2.8	5.7	-	-	-	-	-
4	6.9	5.3	7.2	13.2	57.9	13.1
5.5	22.6	-	5.1	-	-	-
7	33.5	33.7	27.9	39.9	44.2	17.8
8	26.4	24.9	24.3	37.0	44.8	15.3

7. Photopatterning

Multidomain Gel Photopatterning. DBS-$CONHNH_2$ (28.4 mg) was weighed into a sample vial and dissolved in 0.4 mL DMSO. This solution was added to boiling deionised H_2O (9.6 mL) and mixed before transferring to a square glass mould (25 mL capacity). On cooling, a gel was formed. A 10 mL solution of PI (0.05% wt/vol) and PEGDM (10% wt/vol) was then added on top of the gel and left for three days. The solution was removed and acetate photomasks placed over the top so only part of the gel was exposed. The mould was then sat in a tray of ice (to minimise heating) and placed under a long-wave UV light. The solution was cured for 30 min, after which the exposed region had formed a hybrid gel. This domain was very robust whereas the uncured (non-hybrid) domain was mechanically weak and could be removed by washing with water. To prepare naproxen-loaded multidomain gels the above procedure was followed using an equimolar mixture of DBS-hydrazide and naproxen.

8. Directional Release

Photopatterning. DBS-$CONHNH_2$ (28.4 mg) and NPX (13.8 mg) were weighed into a sample vial and dissolved in 0.4 mL DMSO. This solution was added to boiling deionised H_2O (9.6 mL) and mixed before transferring to a square glass mould (25 mL capacity). On cooling, a gel was formed. A 10 mL solution of PI (0.05% wt/vol) and PEGDM (10% wt/vol) was then added on top of the gel and left for three days. The solution was removed and acetate photomasks placed over the top so only part of the gel was exposed. The mould was then sat in a tray of ice (to minimise heating) and placed under a long-wave UV light. The solution was cured for 15 min, after which the exposed region had formed a hybrid gel.

Release study. The weak LMWG was removed by washing with water, leaving a band of robust hybrid gel. Buffer solutions (1.5 mL) of pH 2.8 and pH 7 were pipetted onto either side of the band and stirred using magnetic fleas. The mould was covered for the duration of the experiment. NPX release was monitored by UV-vis spectroscopy for 3 h. At each time point 100 µL of each of the stirred solutions was taken and diluted to 2 mL. The solution removed was replaced with fresh buffer. This experiment was repeated using hybrid gels containing no NPX.

9. References

1. B. O. Okesola and D. K. Smith, *Chem. Commun.*, 2013, **49**, 11164-11166.

2. S. Lin-Gibson, S. Bencherif, J. A. Cooper, S. J. Wetzel, J. M. Antonucci, B. M. Vogel, F. Horkay and N. R. Washburn, *Biomacromolecules*, 2004, **5**, 1280-1287.