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Two-Component Supramolecular Hydrogels for Controlled Drug Release

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Materials and Methods

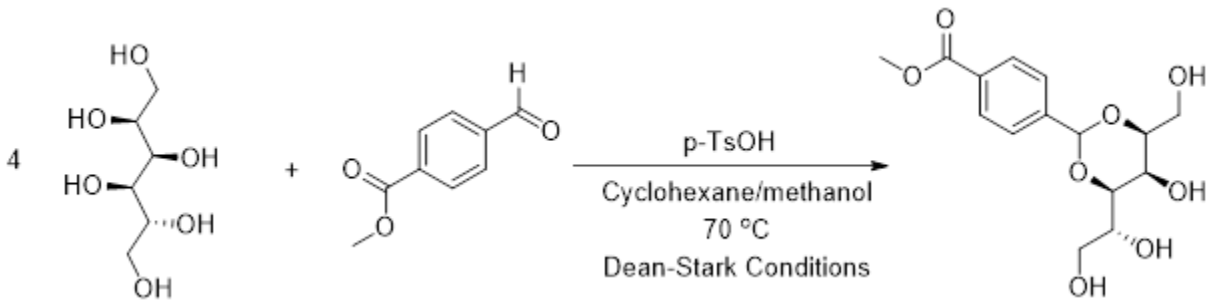
General Experimental Methods: All compounds required for synthesis and analysis were purchased from standard chemical suppliers and used without further purification. ^{1}H and ^{13}C NMR were recorded on a Jeol 400 spectrometer (^{1}H 400 MHz, ^{13}C 100 MHz), with the exception of the variable temperature NMR, which were recorded on a Bruker 500 (^{1}H 500 MHz). Coupling constants (*J*) are recorded in Hz. Mass spectrometry was performed by the University of York Mass Spectrometry Service. IR were recorded on a ThermoNicolet Avatar 370 FT-IR spectrometer. Melting points were recorded using a Stuart SMP3 apparatus. All rheological measurements were carried out using a Malvern Instruments Kinexus Pro+ rheometer. T_{gel} values were recorded using a high precision thermoregulated oil bath. Circular dichroism (CD) measurements were carried out using a Jasco J810 CD Spectrophotometer. UV-vis absorbance was measured on a Shimadzu UV-2401 PC spectrophotometer.

Synthesis and Characterisation of Gelator Molecules

Synthesis and Characterisation of DBS-CONHNH$_2$

DBS-CONHNH$_2$ was synthesised using the previously reported method,[1] and all characterisation data were in agreement with the previously reported data.[2]

Synthesis and Characterisation of MBS-CO$_2$Me



Scheme S1. Synthesis of MBS-CO$_2$Me

D-Sorbitol (15.9 g, 87 mmol) was added to a three-necked round bottomed flask fitted with Dean-Stark apparatus. Methanol (20 ml) and cyclohexane (40 ml) were added, and the mixture was stirred, under nitrogen, at 50°C for 20 minutes. 4-methylcarboxybenzaldehyde (3.5 g, 21.3 mmol) and *p*-TsOH (0.55 g, 2.89 mmol) were dissolved in methanol (20 ml) and stirred for 20 minutes at room temperature. This solution was then added dropwise to the D-sorbitol mixture. This was then heated to 70°C. After two to three hours, most of the solvent was removed, giving a white paste. The paste was washed with methanol, and cold water. The white solid was dissolved in boiling water, and filtered while hot. The filtrate was left to cool, to give a white solid. If necessary, this was then washed with further cold water. Yield: 2.11 g (30%), M.p: 198-201 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.97 (m, 2H, ArH_1), 7.63 (m, 2H, ArH_2), 5.64 (s, 1H, Ar-C*H*), 4.74-4.70 (m, 2H, C-O*H*), 4.48-4.44 (m, 2H, C-O*H*), 3.85-3.82 (m, 4H, OCH_3, H_5), 3.71-3.69 (m, 3H, H_6, H_7, H_8), 3.60-3.55 (m, 3H, H_3, H_4, H_9/H_{10}), 3.43-3.42 (m, 1H, H_9/H_{10}); ^{13}C NMR (100 MHz, DMSO-d_6): δ 166.11 (CH_3COO), 143.53 (ArC_1), 129.75 (ArC_2), 128.94 (ArC_3), 126.89 (ArC_4), 99.27 (Ar-*C*H), 81.09 (*C*H_3OO), 79.46 (*C*), 69.11 (*C*), 62.72 (C_{10}), 61.62 (*C*), 61.01 (C_5), 52.27 (C_6); ESI-MS (*m/z*) calc. for $C_{15}H_{20}O_8Na^+$ 351.1056; found 351.1049 (100 % $[M+Na]^+$); ν_{max} (cm^{-1}) (solid): 3293*s*, 2949*w*,

2877*w*, 1722*s*, 1616*w*, 1402*m*, 1340*w*, 1278*s*, 1218*w*, 1198*w*, 1151*m*, 1091*s*, 1077*m*, 1061*m*, 1031*s*, 1017*s*, 977*m*, 881*m*, 847*m*, 835*m*, 794*m*, 761*s*, 712*s*, 657*m*, 593*m*, 507*m*, 456*w*.

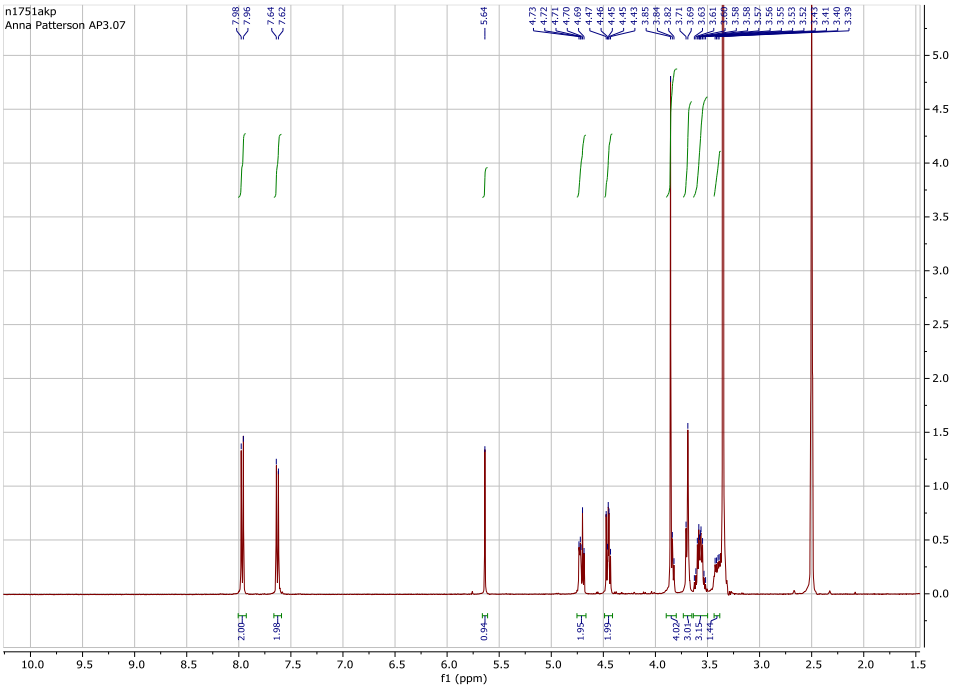


Figure S1. ^{1}H NMR spectrum of MBS-CO_2Me

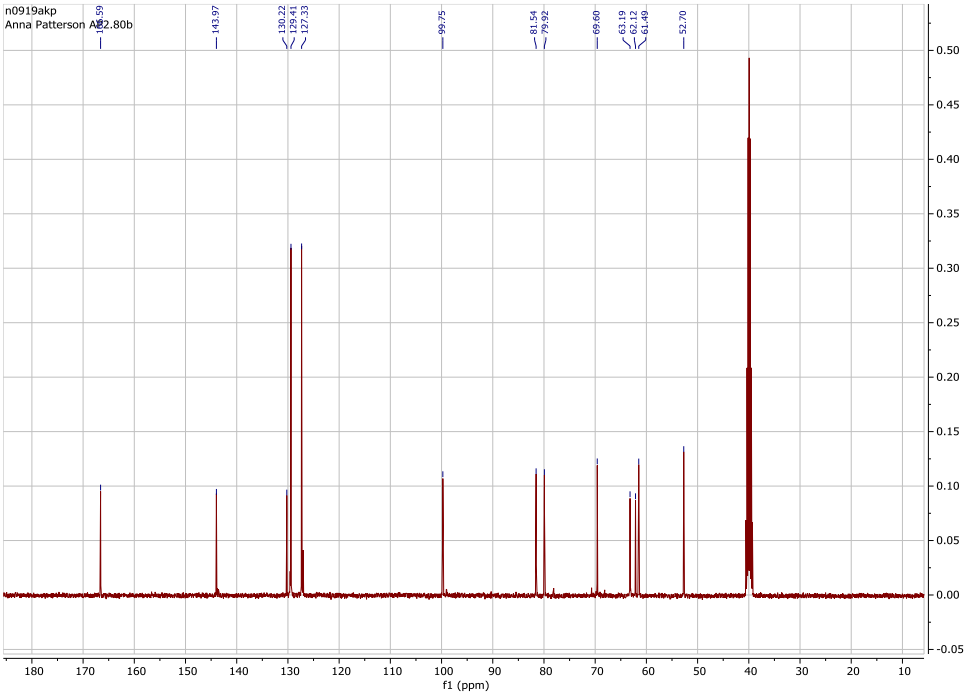


Figure S2. ^{13}C NMR spectrum of MBS-CO_2Me

Preparation of Gels

Preparation of MBS-CO_2Me hydrogels. A known mass of MBS-CO_2Me was weighed into a sample vial, and 0.5 ml of deionised water added. This was sonicated for 5 minutes, followed by a heat-cool cycle. On cooling, transparent gels were formed rapidly. The same procedure was followed for the preparation of MBS-CO_2Me gels containing NPX, with NPX (0.5 mg) added to the sample vial with the gelator. The pH of the gel was ca. 6.7.

Preparation of MBS-CO_2Me and DBS-$CONHNH_2$ Hybrid Hydrogels. A known mass of both MBS-CO_2Me and DBS-$CONHNH_2$ were weighed into a sample vial, and 0.5 ml of deionised water added. This was sonicated for 10 minutes, followed by a heat-cool cycle. On cooling, translucent gels were formed rapidly. The same procedure was followed for hybrid hydrogels containing NPX, with NPX (0.5 mg) added to the sample vial with the gelators. The pH of the gel was ca. 6.7.

(a) (b) (c) (d) (e) (f)

Figure S3: Images of hydrogels, (a) DBS-$CONHNH_2$ hydrogel (0.28% wt/vol), (b) DBS-$CONHNH_2$ hydrogel (0.28% wt/vol) with NPX, (c) MBS-CO_2Me (0.80% wt/vol) hydrogel, (d) MBS-CO_2Me (0.80% wt/vol) with NPX, (e) hybrid hydrogel (DBS-$CONHNH_2$ 0.20% wt/vol, MBS-CO_2Me 0.70% wt/vol), (f) hybrid hydrogel with NPX (DBS-$CONHNH_2$ 0.20% wt/vol, MBS-CO_2Me 0.70% wt/vol).

Preparation of MBS-CO_2Me and DBS-$CONHNH_2$ Hybrid Hydrogels for NMR Studies. MBS-CO_2Me (5.56 mg, 0.017 mmol) and DBS-$CONHNH_2$ (1.60 mg, 0.0034 mmol) were weighed into a sample vial and a 50:50 mix of D_2O and H_2O added (0.7 ml total), along with a DMSO standard (2 µl). This was sonicated for 10 minutes, followed by a heat-cool cycle. Once all the solid was dissolved, the solution was transferred rapidly to a warm NMR tube. On cooling, translucent gels were rapidly formed.

Preparation of MBS-CO_2Me Hydrogels for Rheology. MBS-CO_2Me (8.50 mg) was added to a sample vial, and 1 ml of deionised water added. This was then sonicated for 5 minutes, followed by heating. While hot, the solution was transferred to a bottomless vial attached to a solid surface. On cooling, transparent gels were formed. The bottomless vial could then be removed, and the gel disc transferred to the rheometer. The same procedure was followed for MBS-CO_2Me hydrogels with NPX, with NPX (1 mg) added to the samples vial along with the gelator.

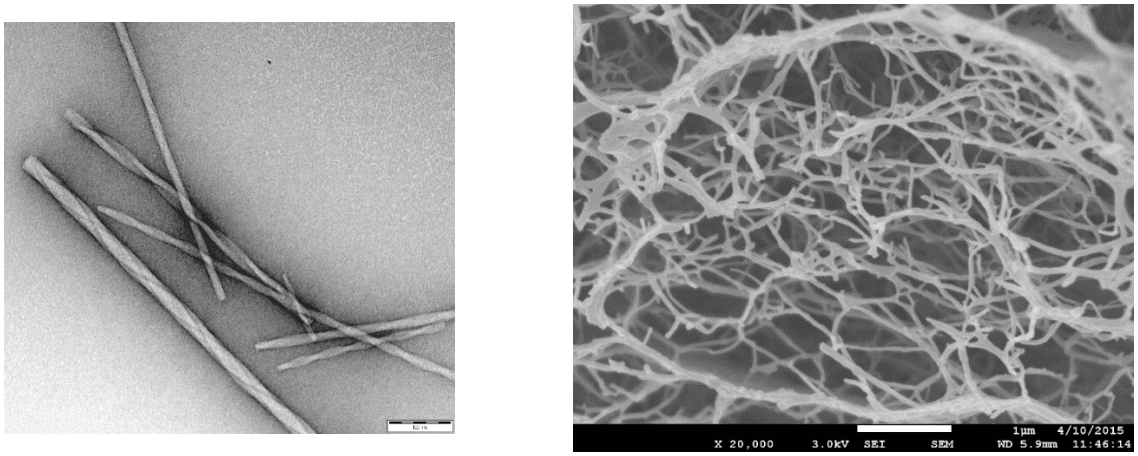
Preparation of MBS-CO_2Me and DBS-$CONHNH_2$ Hybrid Hydrogels for Rheology. MBS-CO_2Me (1.00 mg or 8.00 mg) and DBS-$CONHNH_2$ (2.40 mg or 2.80 mg) were added to a sample vial, and 1 ml of deionised water added. This was sonicated for 10 minutes, followed by heating. While hot, the solution was transferred to a bottomless vial fixed to a solid surface. On cooling, translucent gels formed. The bottomless vial could then be removed, and the gel disc transferred to the rheometer. The same procedure was followed for hybrid hydrogels containing NPX, with NPX (1 mg) added to the sample vial with the gelators.

Preparation of Samples for CD Studies. MBS-CO_2Me (5.74 mg) was added to a sample vial, and 1 ml deionised water added. This was sonicated for 5 minutes, followed by a heating. While hot, a portion of the solution (~0.7 ml) was transferred to a warm CD cuvette (path length 1 mm), then allowed to cool.

Imaging

Electron microscopy imaging was carried out by Meg Stark, at the Biology Technology Facility, Department of Biology, University of York. TEM imaging was carried out using the following method: A small portion of gel was transferred, by drop-casting, to a heat-treated copper support. Excess material was removed using a filter paper, and the samples air-dried for 20 minutes. TEM images were taken on a FEI Technai 12 G2. SEM images were obtained using the following method: A small portion of gel was transferred to a copper support, then freeze-dried by plunging into liquid nitrogen. The samples were then lyophilised for 12 hours, and any excess material removed. The dried sample was then sputter coated with a thin layer of gold/palladium, to prevent sample charging, and imaging carried out. SEM images were taken on either a JEOL JSM-7600f field emission SEM (DBS-$CONHNH_2$ hydrogels), or a JEOL JSM-6490LV (hybrid hydrogels).

(a)



(b)

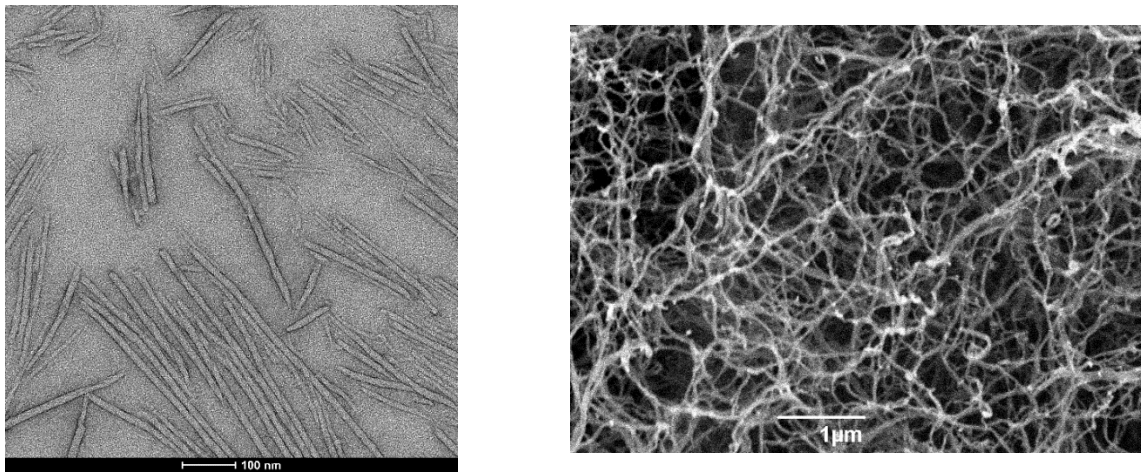


Figure S4. TEM (left) and SEM (right) images of (a) DBS-$CONHNH_2$ hydrogels (0.40% wt/vol);[3] (b) DBS-$CONHNH_2$ and MBS-CO_2Me hybrid hydrogels (0.20% wt/vol and 0.10% wt/vol respectively). Scale bars for TEM: 100 nm, for SEM 1 µm.

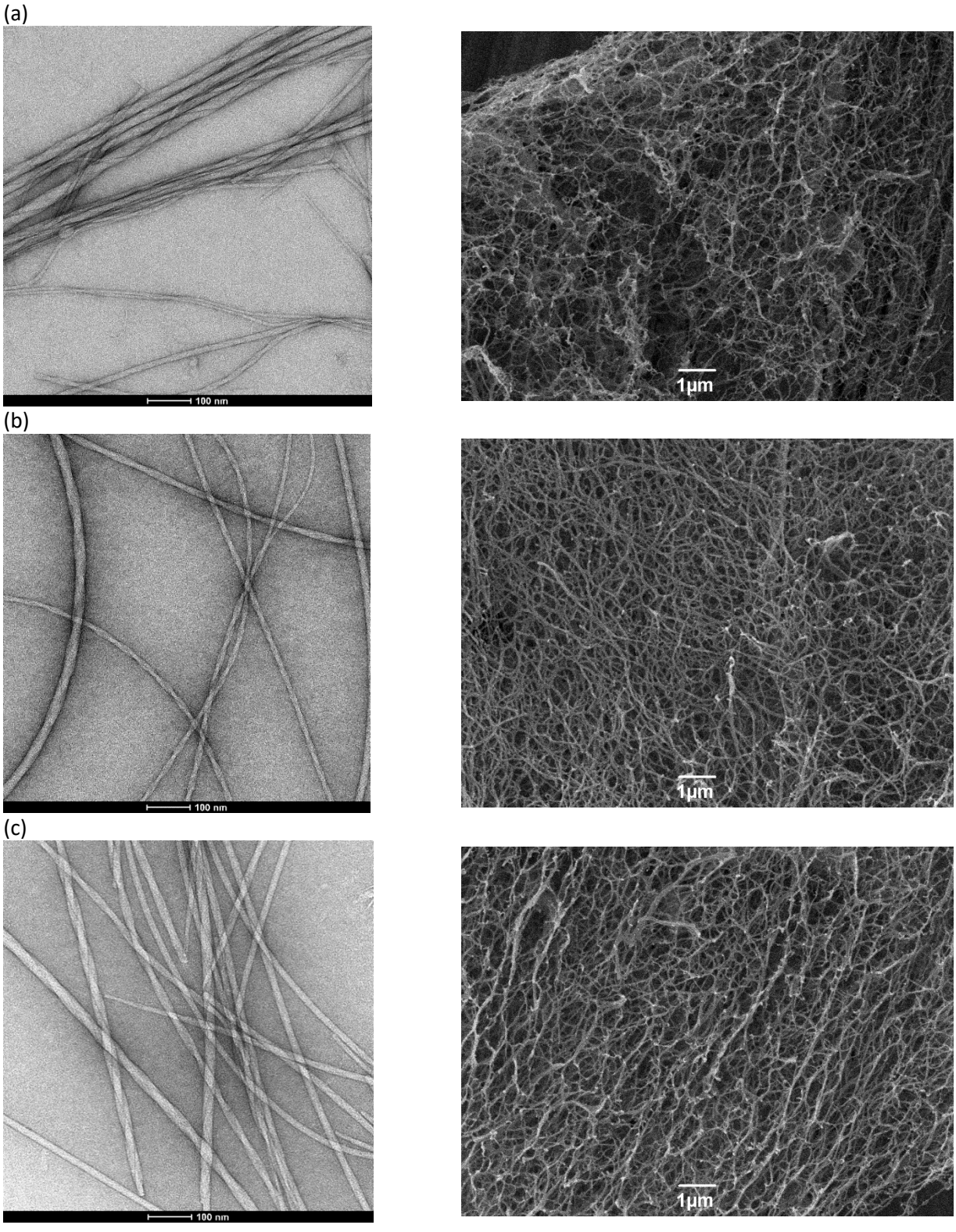


Figure S5. TEM (left) and SEM (right) images of (a) DBS-CONHNH$_2$ and MBS-CO$_2$Me hybrid hydrogels (0.20% wt/vol and 0.80% wt/vol respectively) (b) DBS-CONHNH$_2$ and MBS-CO$_2$Me hybrid hydrogels (0.28% wt/vol and 0.10% wt/vol respectively) (c) DBS-CONHNH$_2$ and MBS-CO$_2$Me hybrid hydrogels (0.28% wt/vol and 0.80% wt/vol respectively) Scale bar for TEM 100 nm, for SEM 1 µm.

Rheology

Without NPX

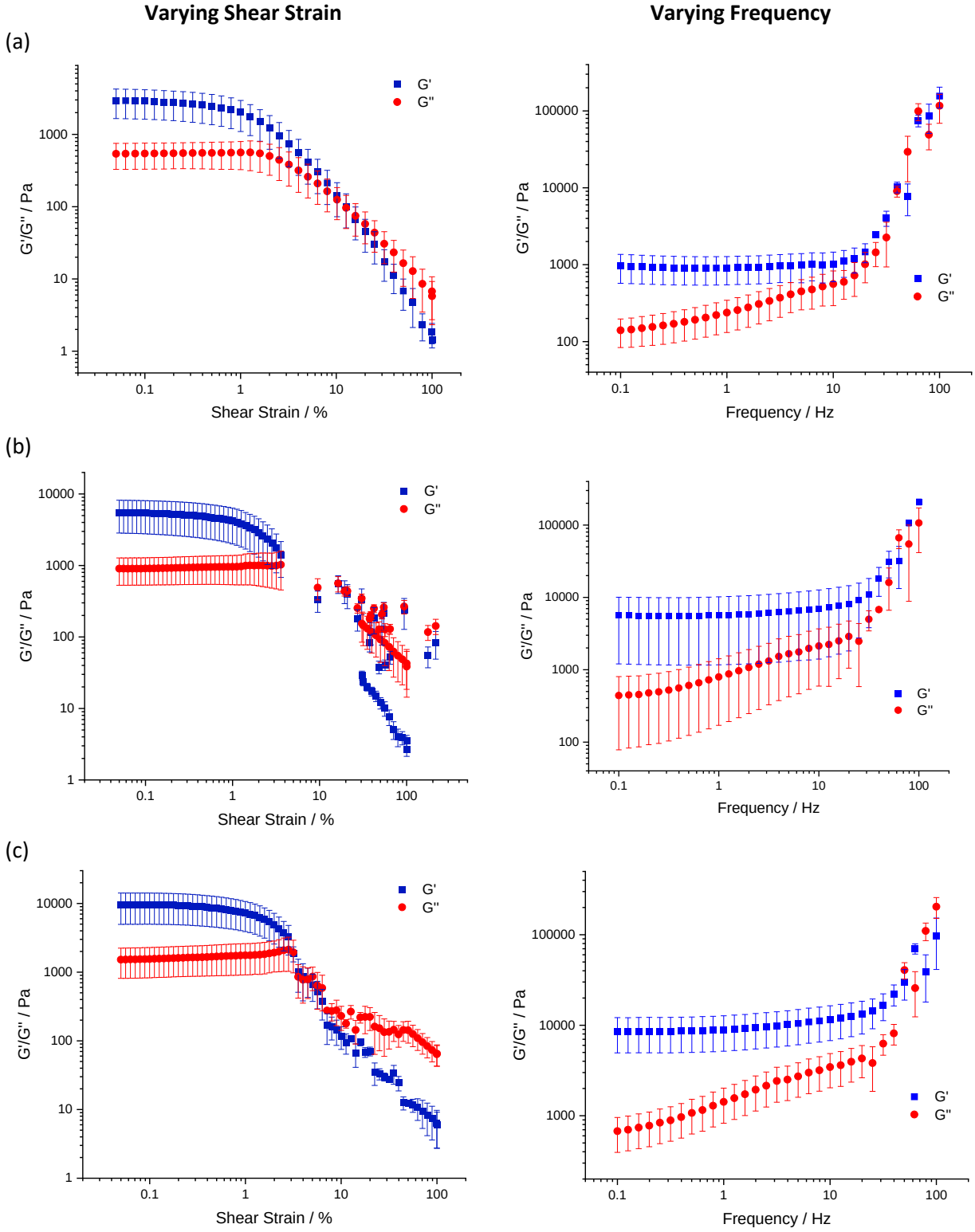


Figure S6. Elastic (G', blue squares) and loss (G'', red circles) moduli with increasing shear strain (left) and frequency (right) for (a) MBS-CO_2Me hydrogels (0.85% wt/vol) (b) DBS-$CONHNH_2$ and MBS-CO_2Me hybrid hydrogels (0.28% wt/vol and 0.80% wt/vol respectively) (c) DBS-$CONHNH_2$ and MBS-CO_2Me hybrid hydrogels (0.24 % wt/vol and 0.80% wt/vol respectively).

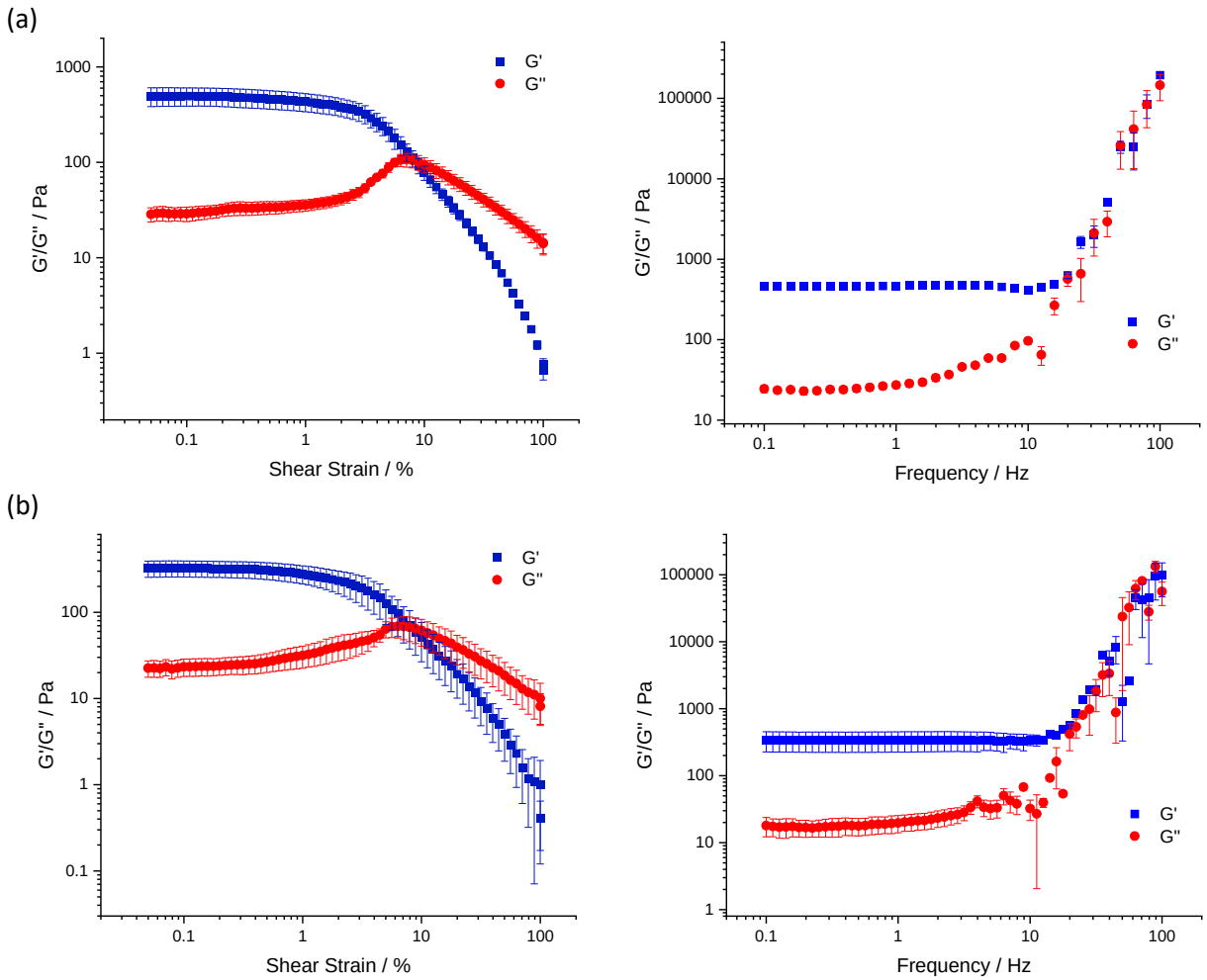


Figure S7. Elastic (G', blue squares) and loss (G'', red circles) moduli with increasing shear strain (left) and frequency (right) for (a) DBS-CONHNH$_2$ and MBS-CO$_2$Me hybrid hydrogels (0.28% wt/vol and 0.10% wt/vol respectively) (b) DBS-CONHNH$_2$ and MBS-CO$_2$Me hybrid hydrogels (0.24% wt/vol and 0.10% wt/vol respectively).

With NPX

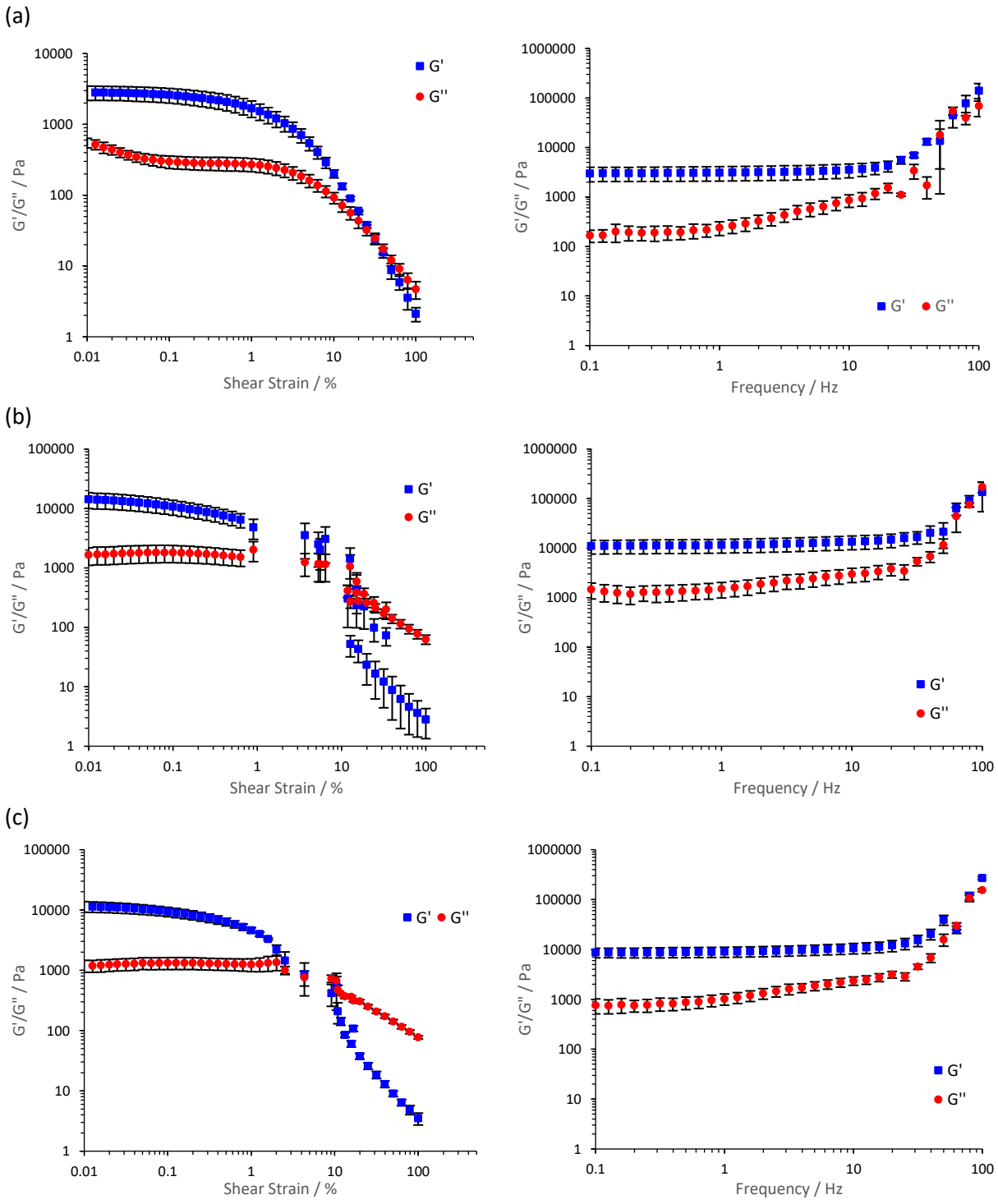


Figure S8. Elastic (G', blue squares) and loss (G'', red circles) moduli with increasing shear strain (left) and frequency (right) for (a) MBS-CO_2Me hydrogels with NPX, (b) DBS-$CONHNH_2$ and MBS-CO_2Me hybrid hydrogels (0.28% wt/vol and 0.80% wt/vol respectively) with NPX, and (b) DBS-$CONHNH_2$ and MBS-CO_2Me hybrid hydrogels (0.24% wt/vol and 0.80% wt/vol respectively) with NPX

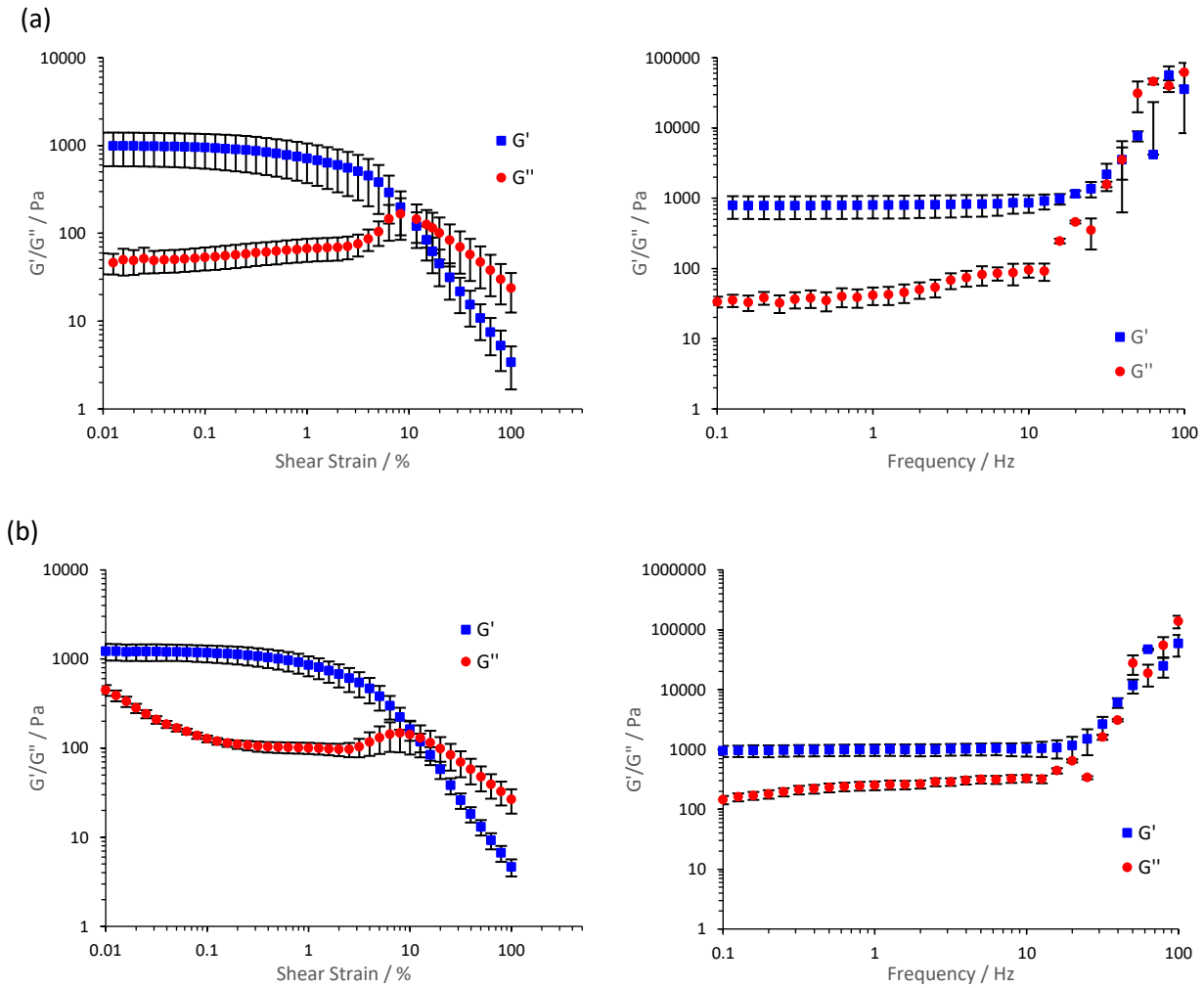


Figure S9. Elastic (G', blue squares) and loss (G'', red circles) moduli with increasing shear strain (left) and frequency (right) for (a) DBS-CONHNH$_2$ and MBS-CO$_2$Me hybrid hydrogels (0.28% wt/vol and 0.10% wt/vol respectively) with NPX, and (b) DBS-CONHNH$_2$ and MBS-CO$_2$Me hybrid hydrogels (0.24% wt/vol and 0.10% wt/vol respectively) with NPX.

T_{gel} Values

All hydrogels were prepared as described above. The method for determining the T_{gel} value was the reproducible tube inversion method. The temperature was recorded when the gels were no longer self-supporting under gravity.

Table S1. T_{gel} values for MBS-CO$_2$Me hydrogels at varying concentrations of gelator.

MBS-CO$_2$Me / % wt/vol	T_{gel} / °C
0.75	53
0.80	54
0.85	56
0.90	57
0.95	57
1.00	59

Table S2. T_{gel} values for MBS-CO_2Me hydrogels with NPX at varying concentrations of gelator.

MBS-CO_2Me / % wt/vol	T_{gel} / °C
0.75	34
0.80	51
0.85	53
0.90	56
0.95	56
1.00	61

Table S3. Selected T_{gel} values for the hybrid hydrogels, with varying proportions of MBS-CO_2Me. Total gelator concentration for all gels is 0.48 % wt/vol.

% MBS-CO_2Me	T_{gel} / °C
26	100
29	100
51	69
56	64
61	51
70	33

Table S4. T_{gel} values for MBS-CO_2Me hydrogels, DBS-$CONHNH_2$ hydrogels, and hybrid hydrogels.

DBS-$CONHNH_2$ / % wt/vol	MBS-CO_2Me / % wt/vol	T_{gel} / °C
0.28	-	80
-	0.90	57
0.36	0.12	100
0.34	0.14	100
0.24	0.24	69
0.21	0.27	64
0.19	0.29	51
0.14	0.34	33
0.20	0.11	27
0.24	0.11	53
0.16	0.20	49
0.28	0.11	62
0.20	0.22	58
0.32	0.10	66
0.16	0.30	50
0.28	0.20	63
0.36	0.13	100
0.20	0.29	74
0.40	0.10	100
0.32	0.19	99
0.23	0.29	79
0.36	0.19	100
0.39	0.19	100
0.28	0.31	100
0.32	0.31	100
0.25	0.39	51
0.36	0.30	100
0.40	0.25	100

Table S5. T_{gel} values for the hybrid hydrogels with NPX, with varying proportions of MBS-CO_2Me. Total gelator concentration for samples is 0.48% wt/vol.

% MBS-CO_2Me	T_{gel} / °C
30	100+
39	100+
43	100+
50	84
56	100+
62	63
71	79

Circular Dichroism Spectroscopy

All CD experiments were carried out using the following settings: Data Pitch = 0.5 nm, Scanning Mode = continuous, Scan Speed = 100 nm min^{-1}, Response = 1 s, Bandwidth = 2 nm, Accumulation = 5. Quartz cuvettes (pathlength 1 mm) were used. The loading of MBS-CO_2Me was 0.57% wt/vol.

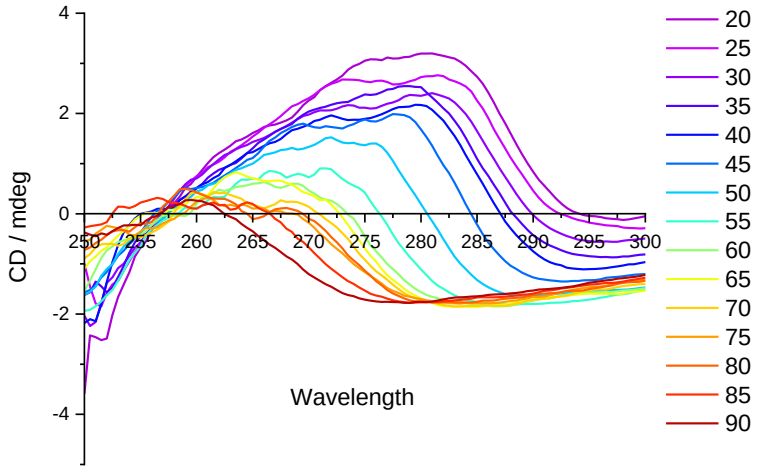


Figure S10. Variable temperature CD spectrum for MBS-CO_2Me.

Release studies

Gel samples with NPX, at a volume of 1 ml, were prepared following the methods outlined above. Samples contained DBS-$CONHNH_2$ (2 mg) and MBS-CO_2Me (7 mg), along with NPX (1 mg). 6 ml of one i) pH 5.5 phosphate-citrate buffer, ii) pH 7 phosphate buffer,was pipetted carefully onto the gel, and release of NPX monitored by UV-vis absorption at 329 nm. The samples were incubated at 37 °C for the duration of the release study. The concentration of NPX in the supernatant was quantified using calibration curves. For each pH, the experiment was carried out in triplicate, and control samples containing no NPX were also monitored.

Additional studies, with varying concentrations of gelator, were also carried out (see below).

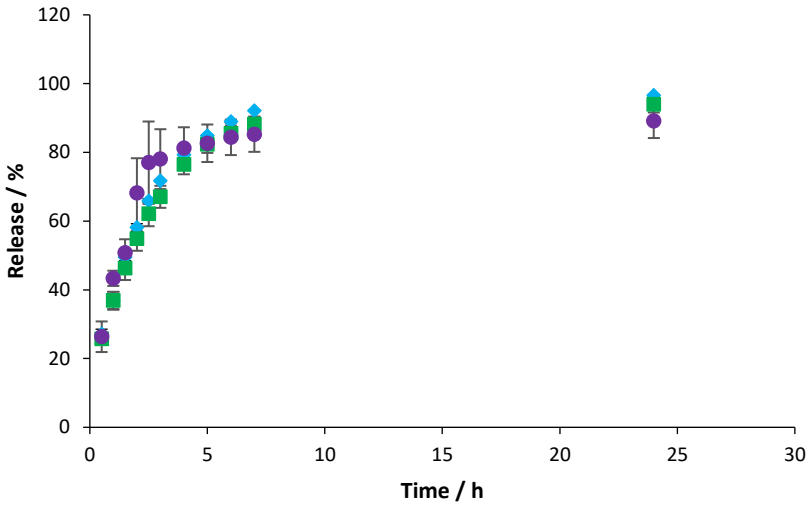


Figure S11. Release of NPX, at pH 7, with varying concentrations of gelator. All have 1 mg NPX. Green squares: DBS-CONHNH$_2$ 0.20% wt/vol, MBS-CO$_2$Me 0.70% wt/vol. Blue diamonds: DBS-CONHNH$_2$ 0.20% wt/vol, MBS-CO$_2$Me 0.60% wt/vol. Purple circles: DBS-CONHNH$_2$ 0.20% wt/vol, MBS-CO$_2$Me 0.10% wt/vol.

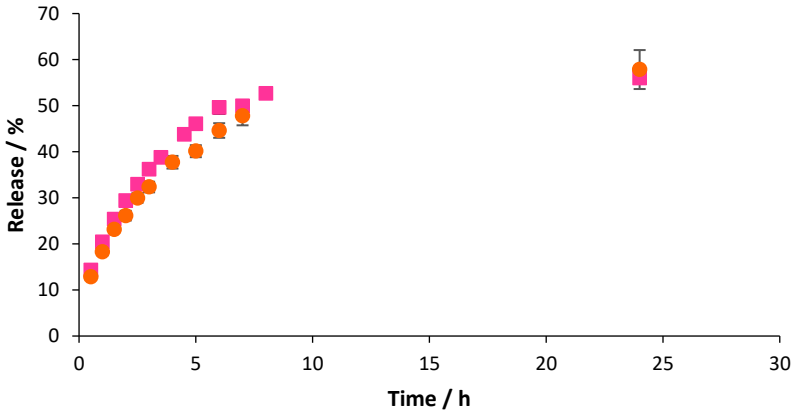


Figure S12. Release of NPX, at pH 5.5, with varying concentrations of gelator. All have 1 mg NPX. Orange circles: DBS-CONHNH$_2$ 0.20% wt/vol, MBS-CO$_2$Me 0.70% wt/vol. Pink Squares: DBS-CONHNH$_2$ 0.28% wt/vol, MBS-CO$_2$Me 0.70% wt/vol.

References

1. B. O. Okesola and D. K. Smith, *Chem. Commun.*, 2013, **49**, 11164-11166
2. P. R. A. Chivers, J. A. Kelly, M. J. S. Hill and D. K. Smith, *React. Chem. Eng.* 2020, **5**, 1112-1117.
3. V. M. P. Vieira, A. C. Lima, M. de Jong and D. K. Smith, *Chem-Eur. J.*, 2018, **24**, 15112-15118