Electronic Supplementary Material (ESI) for ChemComm.

In-situ aldehyde-modification of self-assembled acyl hydrazide hydrogels and dynamic component selection from complex aldehyde mixtures

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Contents

1. Materials and Methods

NMR spectra were recorded on a Jeol ECX 400 (^{1}H 400 MHz) spectrometer using D_2O or d_6-DMSO as solvent. The chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane as an external standard for ^{1}H NMR spectra and calibrated against the solvent residual peak. To specify the signal multiplicity the following abbreviations are used: s (singlet), d (doublet), t (triplet), m (multiplet) and br (broad singlet). Coupling constants are in given in Hertz (Hz). ESI mass spectra were recorded on a Bruker Daltonics MicroTOF mass spectrometer, using a mixture of DMF and THF (1:1) as injection solvent. UV/Vis absorbances were measured on a Shimadzu UV-2401 PC spectrophotometer and infrared spectra were recorded on a Shimadzu IR Prestige-21 FTIR spectrometer. Rheology was performed using a Malvern Kinexus pro Rheometer. SEM was achieved with a Jeol JSM-7600F (Department of Biology, University of York). Commercially available compounds were used without further purification – all aldehydes were provided in 99%+ purity. Gelator DBS-$CONHNH_2$ was synthesised using standard methods, which have been reported elsewhere.[1]

2. Methodology for Gel Analysis

A DBS-$CONHNH_2$ hydrogel (0.5 mL, 0.8% wt/vol) was prepared via a heat cool cycle either in water or citrate buffer (pH 3.87). A solution (0.5 mL) in the same solvent containing two or four equivalents of aldehyde was pipetted carefully on top of the gel. The supernatant aldehyde solution was allowed to diffuse into the gel for 48 h. The supernatant was then removed and the gel vacuum dried at 50°C to remove solvent and excess aldehyde. The residual powder was then analysed – for example by dissolving in d_6-DMSO for ^{1}H NMR studies.

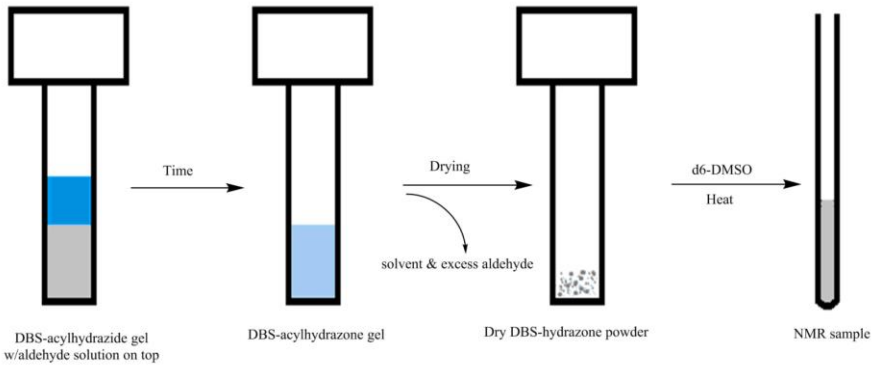
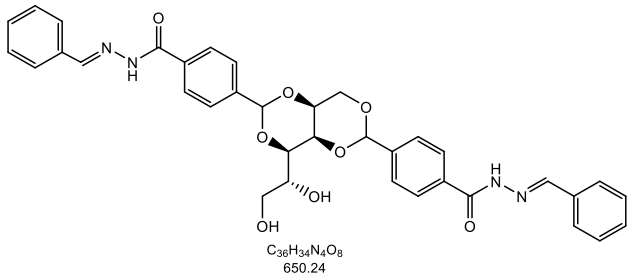


Fig. S1. Analytical workflow for aldehyde-loaded gels.

3. Characterisation of DBS-CONHNH$_2$ + Aldehydes

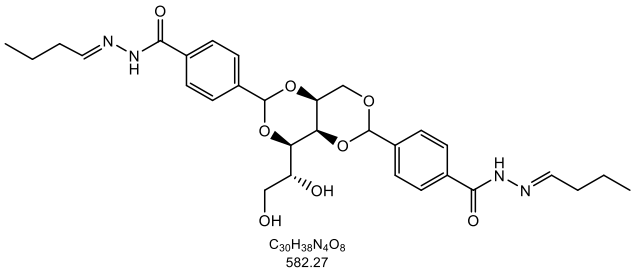
All of the samples contained the gelator DBS-CONHNH$_2$ as well as the mono- and disubstituted hydrazone derivatives. The analytical data relates only to the disubstituted reaction product.

DBS-CONHNH$_2$ + Benzaldehyde



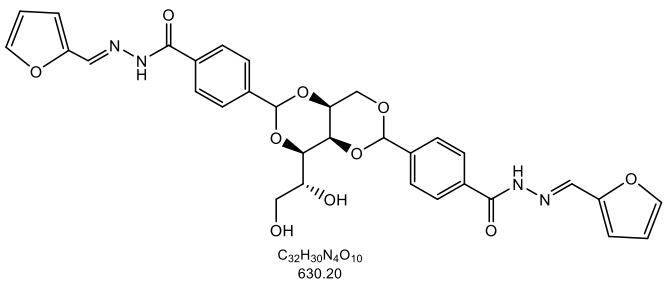
^{1}H-NMR (400 MHz, DMSO-d$_6$): δ 11.89 (s, 2H, CON*H*N), 8.47 (s, 2 H, N=C*H*), 7.96 -7.46 (m, 18H, Ar*H*), 5.71 (app s, 2H, Ar-C*H*), 4.92 (d, J = 5.6 Hz, CHO*H*, 1H), 4.46-4.43 (t, J = 12 Hz, CH$_2$O*H*, 1H), 4.21-4.18 (m, 3H, sugar), 3.98 (app s, 1H, sugar), 3.88-3.86 (m, 1H, sugar), 3.79-3.74 (m, 1H, sugar), 3.63-3.58 (m, 1H, sugar), 3.49-3.42 (m, 1H, sugar).
ESI-MS (*m/z*) calc. for $C_{36}H_{34}N_4O_8Na$ 673.2274; Found 673.2254 ([M+Na]$^+$).

DBS-CONHNH$_2$ + Butanal



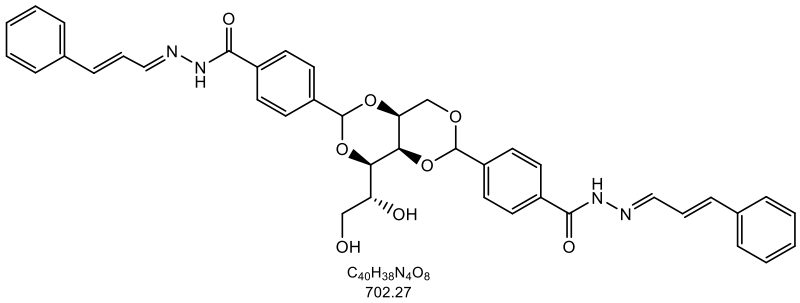
^{1}H-NMR (400 MHz, DMSO-d$_6$): δ 11.45 (s, 2H, CON*H*N), 7.84 -7.49 (m, 8H, Ar*H*), 7.74 (t, 2H, N=C*H*), 5.71 (app s, 2H, Ar-C*H*), 4.92 (d, J = 5.6 Hz, CHO*H*, 1H), 4.46-4.43 (t, J = 12 Hz, CH$_2$O*H*, 1H), 4.21-4.18 (m, 3H, sugar), 3.98 (app s, 1H, sugar), 3.88-3.86 (m, 1H, sugar), 3.79-3.74 (m, 1H, sugar), 3.63-3.58 (m, 1H, sugar), 3.49-3.42 (m, 1H, sugar), 2.24 (q, 4H, CH_2), 1.57 (m, 4 H, CH_2-CH$_3$), 0.93 (t, 6H, CH_3).
ESI-MS (*m/z*) calc. for $C_{30}H_{38}N_4O_8Na$ 605.2587; Found 605.2568 ([M+Na]$^+$).

DBS-CONHNH$_2$ + Furfural



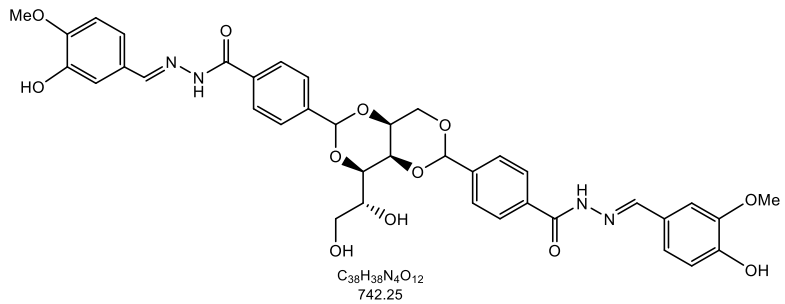
^{1}H-NMR (400 MHz, DMSO-d$_6$): δ 11.82 (s, 2H, CON*H*N), 8.39 (s, 2H, N=C*H*), 7.92-6.64 (m, 14H, Ar*H*), 5.71 (s, 2H, Ar-C*H*), 4.92 (d, J = 5.6 Hz, CHO*H*, 1H), 4.46-4.43 (t, J = 12 Hz, CH$_2$O*H*, 1H), 4.21-4.18 (m, 3H, sugar), 3.98 (app s, 1H, sugar), 3.88-3.86 (m, 1H, sugar), 3.79-3.74 (m, 1H, sugar), 3.63-3.58 (m, 1H, sugar), 3.49-3.42 (m, 1H, sugar).
ESI-MS (*m/z*) calc. for $C_{32}H_{30}N_4O_{10}Na$ 653.1860; Found 653.1834 ([M+Na]$^+$).

DBS-CONHNH$_2$ + Cinnamaldehyde



^{1}H-NMR (400 MHz, DMSO-d$_6$): δ 11.78 (s, 2H, CON*H*N), 8.26 (d, 2H, N=C*H*), 7.93-7.00 (m, 18H, Ar*H* and 4H, C=C*H*), 5.71 (s, 2H, C*H*OO), 4.92 (d, J = 5.6 Hz, CHO*H*, 1H), 4.46-4.43 (t, J = 12 Hz, CH$_2$O*H*, 1H), 4.21-4.18 (m, 3H, sugar), 3.98 (s, 1H, sugar), 3.88-3.86 (m, 1H, sugar), 3.79-3.74 (m, 1H, sugar), 3.63-3.58 (m, 1H, sugar), 3.49-3.42 (m, 1H, sugar).
ESI-MS (*m/z*) calc. for $C_{40}H_{38}N_4O_8$ 725.2587; Found 725.2559 ([M+Na]$^+$).

DBS-CONHNH$_2$ + Vanillin



^{1}H-NMR (400 MHz, DMSO-d$_6$): δ 11.78 (s, 2H, CON*H*N), 9.53 (s, 2H, Ar-O*H*), 8.35 (s, 2H, N=C*H*), 7.94-6.83 (m, 14 H, Ar*H*), 5.71 (app s, 2H, Ar-C*H*), 4.92 (d, J = 5.6 Hz, CHO*H*, 1H), 4.46-4.43 (t, J = 12 Hz, CH$_2$O*H*, 1H), 4.21-4.18 (m, 3H, sugar), 3.98 (s, 1H, sugar), 3.88-3.86 (m, 1H, sugar), 3.83 (s, 6H, O-C*H*$_3$), 3.79-3.74 (m, 1H, sugar), 3.63-3.58 (m, 1H, sugar), 3.49-3.42 (m, 1H, sugar).

ESI-MS (*m/z*) calc. for $C_{38}H_{38}N_4O_{12}Na$ 765.2384; Found 765.2352 ($[M+Na]^+$).

4. NMR Uptake Assay

DBS-CONHNH$_2$ hydrogels (8.42 mM) were prepared in citrate buffer (0.7 mL D$_2$O). A hot solution of the gelator was added to an NMR tube, and the gel was prepared *in situ*. The aldehyde (2 eq., 16.84 mM), also dissolved in citrate buffer (0.7 ml, D$_2$O) and added to the top of the gel. The system was then left to diffuse for 14 days (a long time period of diffusion was used because of the low interfacial area between the gel and the supernatant solution). Assuming an even distribution of aldehyde, the total aldehyde concentration in the citrate buffer (1.4 mL) would, on average, be 8.42 mM. The supernatant was removed and analysed by ^{1}H NMR, with citrate buffer being used as an internal standard to quantify the amount of aldehyde. The gel sample was then also analysed by standard ^{1}H NMR. In this way, the free/mobile aldehyde in the gel could be quantified. Any aldehyde that could no longer be observed via NMR spectroscopy, was assumed to have reacted with the gel nanofibres. We were thus able to quantify the percentage of the aldehyde within the gel that was free, and that which had become attached to the gel nanofibres. This assay avoids any drying effects, and hence helps confirm the reaction of free aldehydes with the acyl hydrazide functionalised nanofibres, leading to acylhydrazone derivatives that are retained within the gel nanofibres, and are hence immobile on the NMR timescale.

Table S1. Percentage of each aldehyde (total concentration 16.84 mM) within the gel that is immobile, and hence reacted with, and attached to, the gel nanofibres after 14 days diffusion.

Aldehyde	% Aldehyde in Gel Attached to Gel Nanofibres
Vanillin **1**	86%
Benzaldehyde **2**	74%
Cinnamaldehyde **3**	95%
Furfural **4**	89%
Hexanal **5**	17%

5. Rheology of DBS-CONHNH$_2$ and Aldehyde-Modified Gels

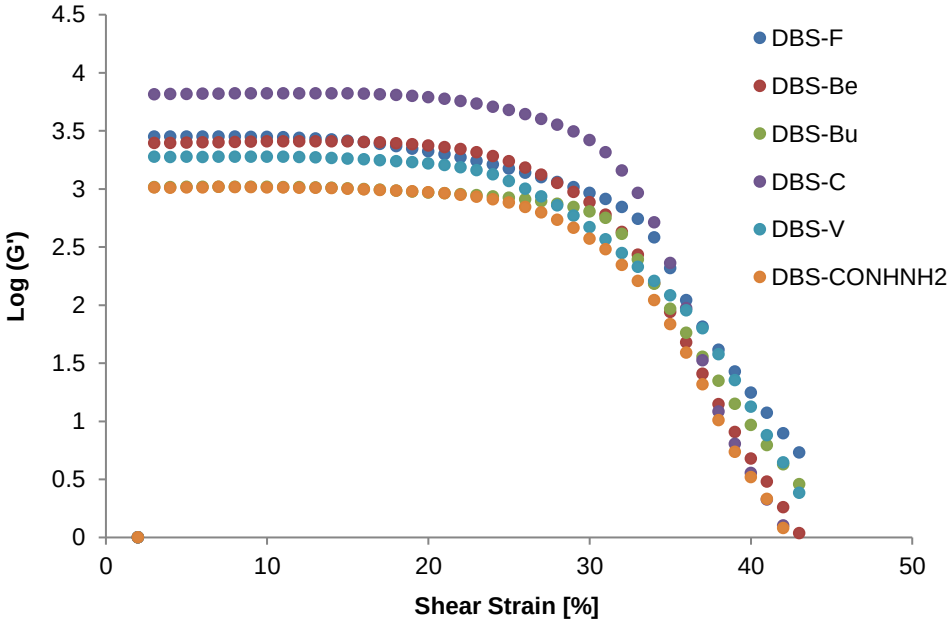


Fig. S2. Rheology of DBS-CONHNH$_2$ in citric acid buffer (pH 3.87) at a concentration of 8.42 mM and DBS-CONHNH$_2$ at the same concentration modified by diffusion of 2 eq. of aldehyde into the gel: DBS-F (+ furfural), DBS-Be (+ benzaldehyde), DBS-Bu (+ butanal), DBS-C (+cinnamaldehyde), and DBS-V (+ vanillin).

6. Scanning Electron Microscopy

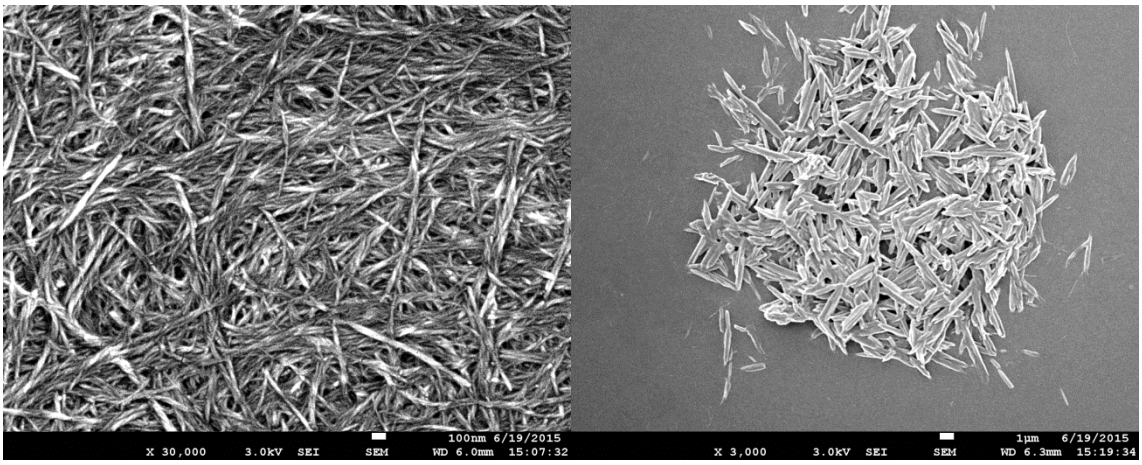


Figure S3. (Left) DBS-$CONHNH_2$ hydrogel (8.42 mM); scale bar = 100 nm. (Right) DBS-$CONHNH_2$ hydrogel (8.42 mM) after functionalization with vanillin (16.84 mM, 2 eq.); scale bar = 1 μm. Both samples have been dried *in vacuo* prior to imaging.

7. Aldehyde Competition Assay Data

Table S2. Competition between pairs of aldehydes (total concentration 16.84 mM) for interaction with DBS-CONHNH2 (8.42 mM) in water (pH 7.0).

		Competitor Aldehyde				
		1	**2**	**3**	**4**	**6**
Measured Aldehyde	Vanillin (**1**)	-	59	56	60	88
	Benzaldehyde (**2**)	41	-	33	56	80
	Cinnamaldehyde (**3**)	44	67	-	73	87
	Furfural (**4**)	40	44	27	-	75
	Butanal (**6**)	12	20	13	25	

Table S3. Competition experiments between three aldehydes for interaction with DBS-$CONHNH_2$ in citrate buffer (pH 3.87) showing the % uptake onto the gel nanofibres. In cases where butanal and hexanal could not be distinguished, the total amount of both is provided in square brackets.

			Competitor Aldehydes									
			2+3	**2+4**	**2+5**	**2+6**	**3+4**	**3+5**	**3+6**	**4+5**	**4+6**	**5+6**
Aldehyde Being Measured		Vanillin **1**	59	49	57	54	62	56	50	55	68	70
			1+3	**1+4**	**1+5**	**1+6**	**3+4**	**3+5**	**3+6**	**4+5**	**4+6**	**5+6**
		Benzaldehyde **2**	23	23	26	33	38	36	46	35	44	53
			1+2	**1+4**	**1+5**	**1+6**	**2+4**	**2+5**	**2+6**	**4+5**	**4+6**	**5+6**
		Cinnamaldehyde **3**	18	18	28	35	31	48	36	40	36	59
			1+2	**1+3**	**1+5**	**1+6**	**2+3**	**2+5**	**2+6**	**3+5**	**3+6**	**5+6**
		Furfural **4**	29	20	29	20	31	39	36	31	37	63
			1+2	**1+3**	**1+4**	**1+6**	**2+3**	**2+4**	**2+6**	**3+4**	**3+6**	**4+6**
		Hexanal **5**	17	16	16	[30]	16	26	[47]	29	[41]	[37]
			1+2	**1+3**	**1+4**	**1+5**	**2+3**	**2+4**	**2+5**	**3+4**	**3+5**	**4+5**
		Butanal **6**	15	15	12	[30]	18	20	[47]	27	[41]	[37]

Table S4. Percentage of aldehydes attached to DBS-$CONHNH_2$ (8.42 mM) in citrate buffer (pH 3.87) when functionalized first with 2 eq. of vanillin and then subsequently with 2 eq. of a second aldehyde.

	Aldehyde 1 [%]	Aldehyde 2 [%]
1. Vanillin; 2. Benzaldehyde	84	16
1. Vanillin; 2 Cinnamaldehyde	81	19
1. Vanillin; 2. Furfural	80	20
1. Vanillin; 2. Hexanal	85	15
1. Vanillin; 2 Butanal	90	10

8. References

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