# Supporting Information

Investigating Templating within Polymer-Scaffolded Dynamic Combinatorial Libraries

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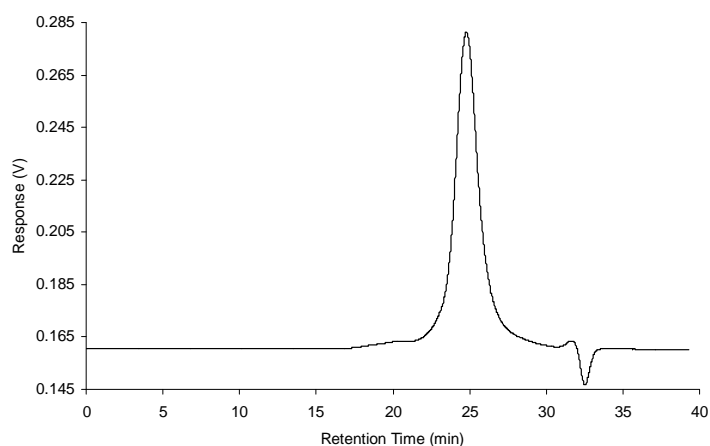
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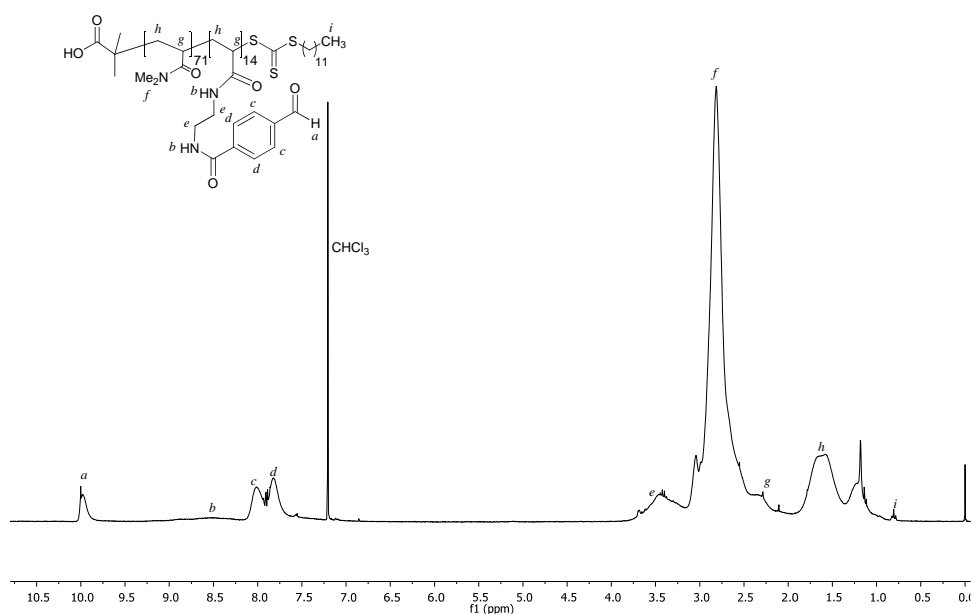
S1. Representative ^{1}H NMR spectrum of **P1** (300 MHz, CDCl$_3$).


S2. Representative gel permeation chromatography (GPC) refractive index (dRI) trace in DMF (0.6 mL/ min) of **P1**.

General procedure for preparation of PS-DCLs

PS-DCLs were prepared so as to contain 2.05×10^{-5} mol aldehyde functionalities, with the amount of polymer added adjusted accordingly.

Polymer (**P1-P9**) was combined with Girard's reagent T (**R1**) (4.2 mg, 2.5×10^{-5} mol) or sulfoacetylhydrazide (**R3**) (4.8 mg, 2.5×10^{-5} mol) in 0.1 M $NH_4OAc/AcOH$ deuterated buffer (pH 4.5, 0.5 mL) and sonicated until a clear solution was obtained. 2-(2-hydroxyethoxy)acetohydrazide (**R2**) (3.4 mg, 2.5×10^{-5} mol) was added to the reaction mixture, which was left overnight to equilibrate. Equilibration to a 1.0:1.0 ratio of **R1** and **R2**, or **R2** and **R3**, was confirmed by ^{1}H NMR spectroscopic analysis prior to template addition.

Representative ^{1}H NMR spectra of PS-DCLs before and after addition of each template are shown below. In all cases, no signal corresponding to aldehyde protons may be observed, indicating that acylhydrazone equilibria lie to the side of acyl hydrazone products.
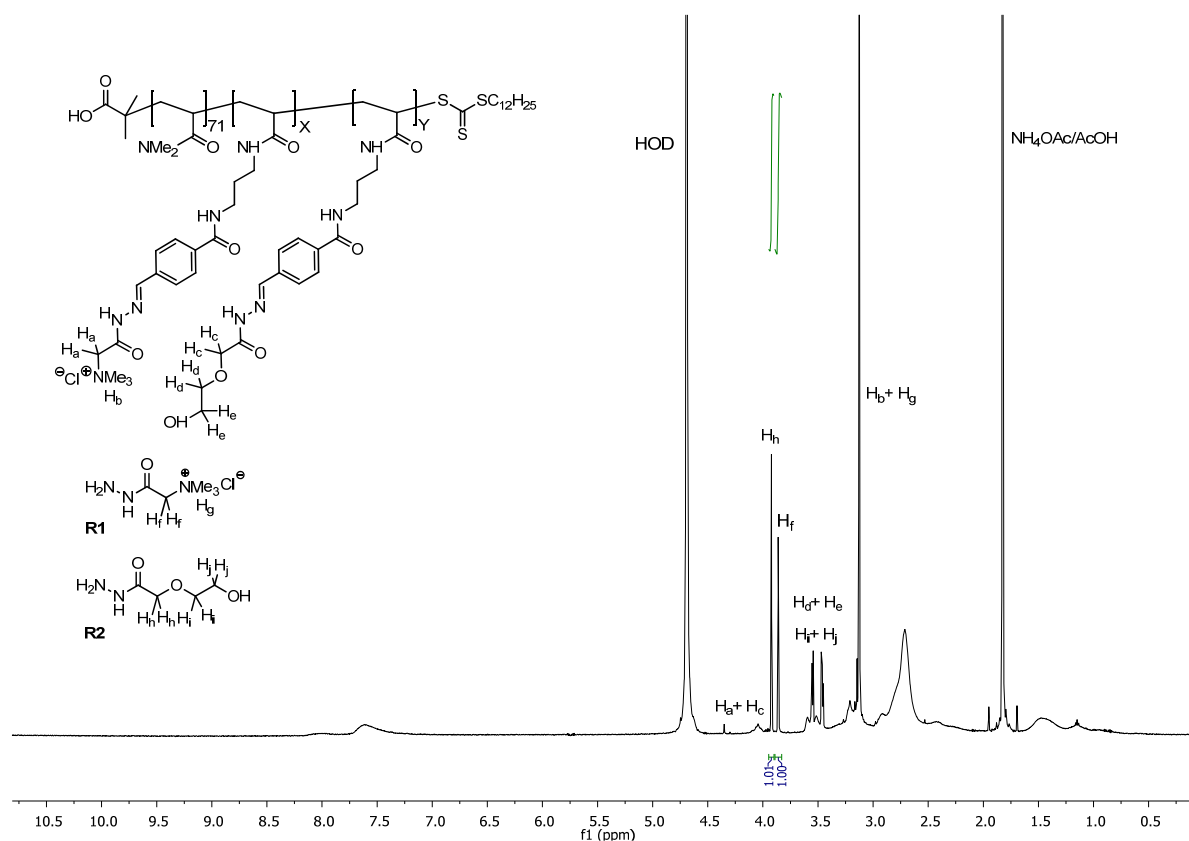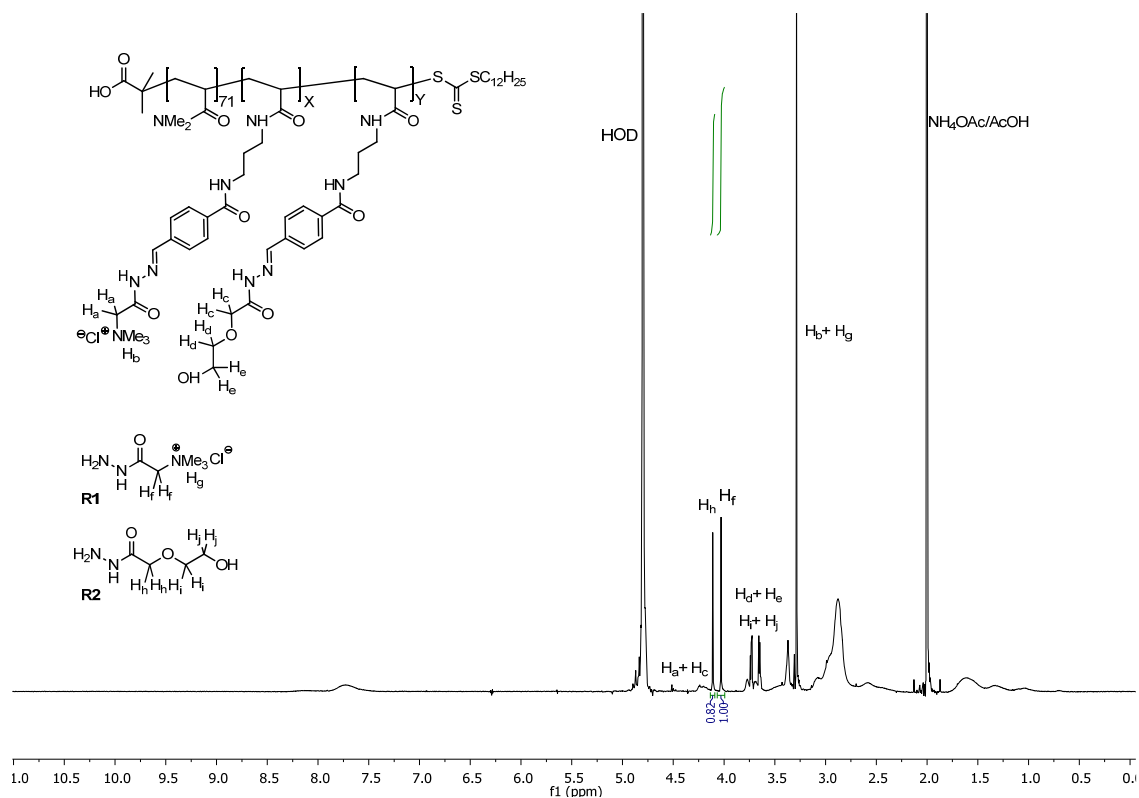

S3. ^{1}H NMR spectrum (500 MHz, D_2O, pH 4.5) of a PS-DCL constructed on **P1** using acylhydrazides **R1** and **R2**, prior to the addition of a template.
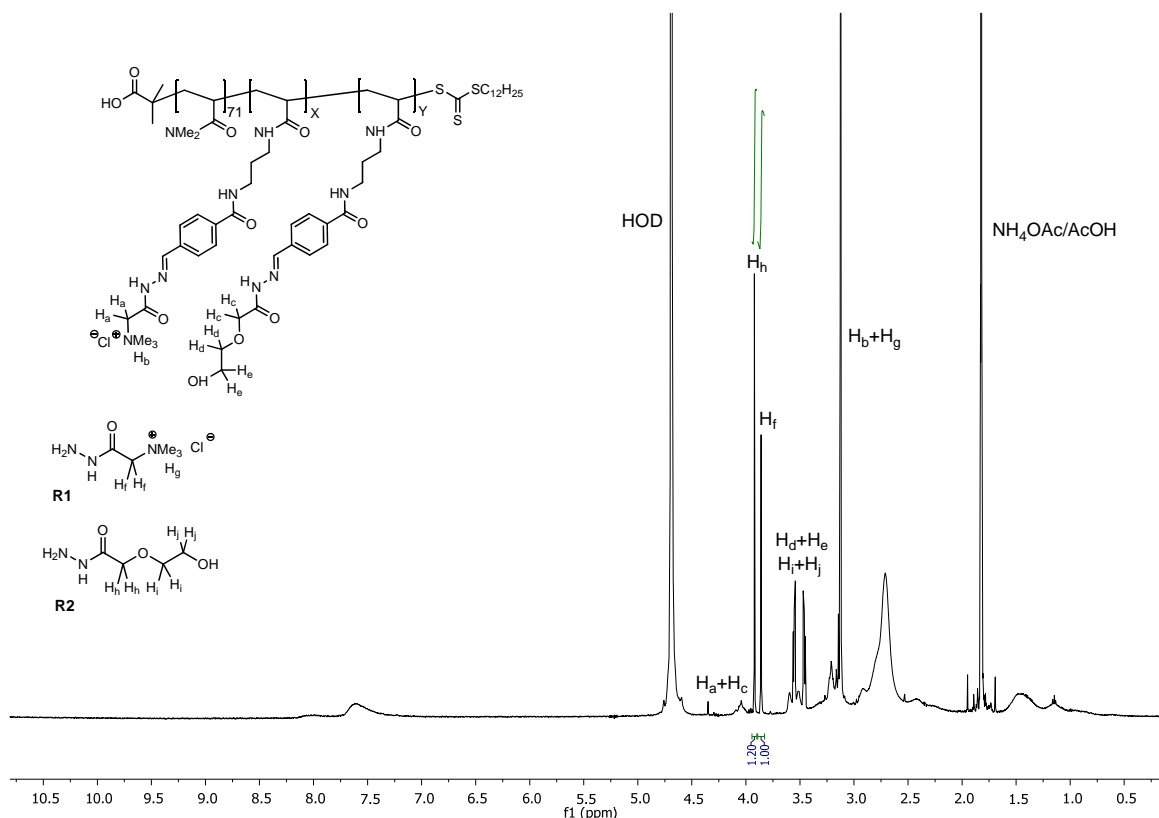
S4. ^{1}H NMR spectrum (500 MHz, D_2O, pH 4.5) of a PS-DCL constructed on **P1** using acylhydrazides **R1** and **R2**, 16 h after addition of poly(sodium-4-styrenesulfonate).
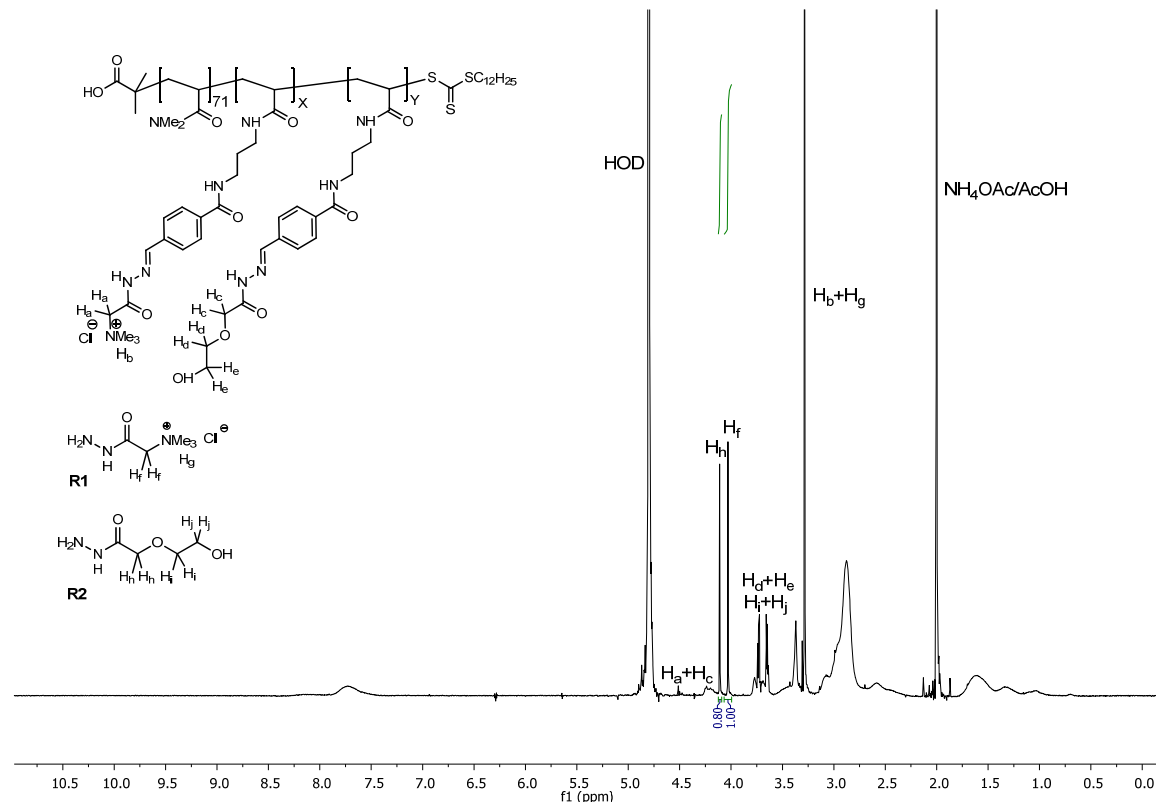
S5. ^{1}H NMR spectrum (500 MHz, D_2O, pH 4.5) of a PS-DCL constructed on **P1** using acylhydrazides **R1** and **R2**, 16 h after addition of BSA.

S6. ^{1}H NMR spectrum (500 MHz, D$_2$O, pH 4.5) of a PS-DCL constructed on **P1** using acylhydrazides **R1** and **R2**, 16 h after addition of bovine trypsin.

S7. ^{1}H NMR spectrum (500 MHz, D$_2$O, pH 4.5) of a PS-DCL constructed on **P1** using acylhydrazides **R2** and **R3**, prior to the addition of template.
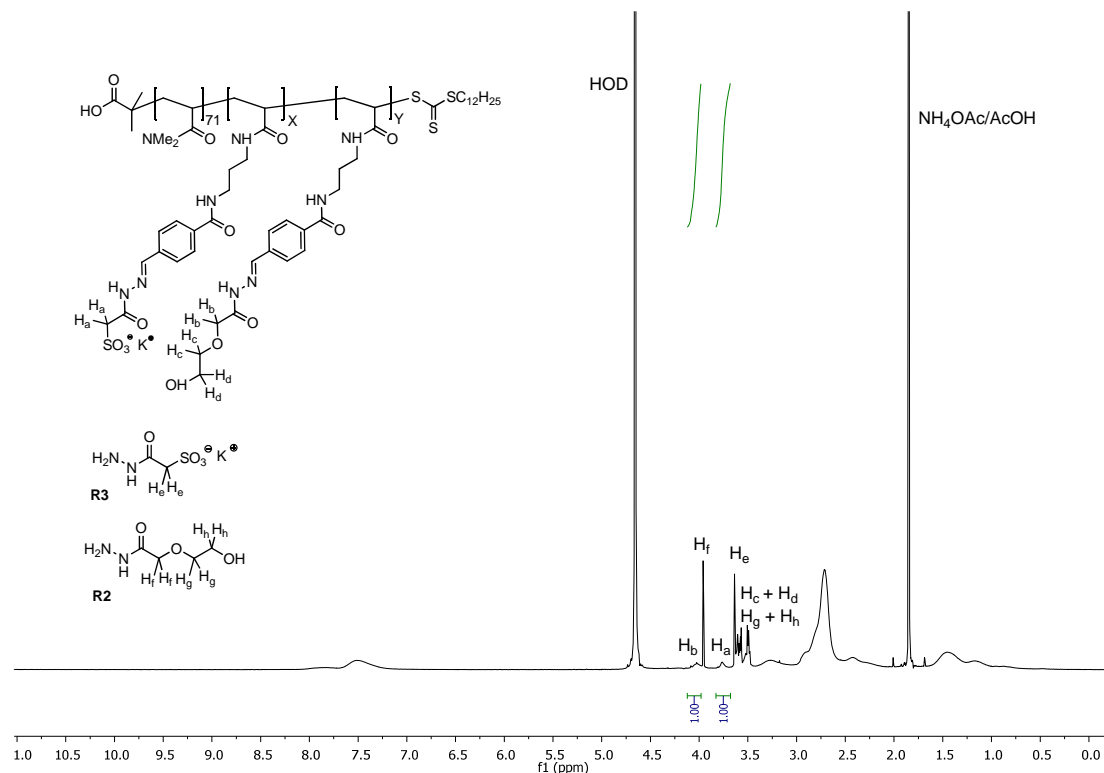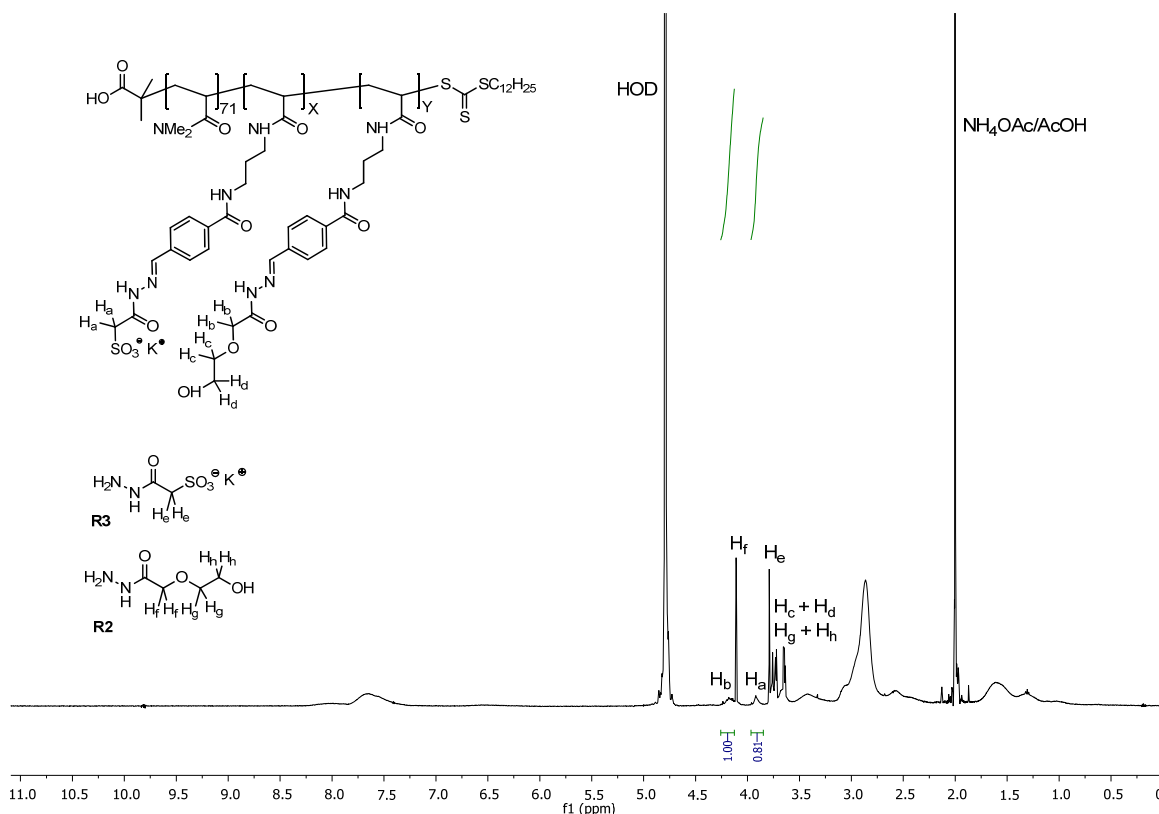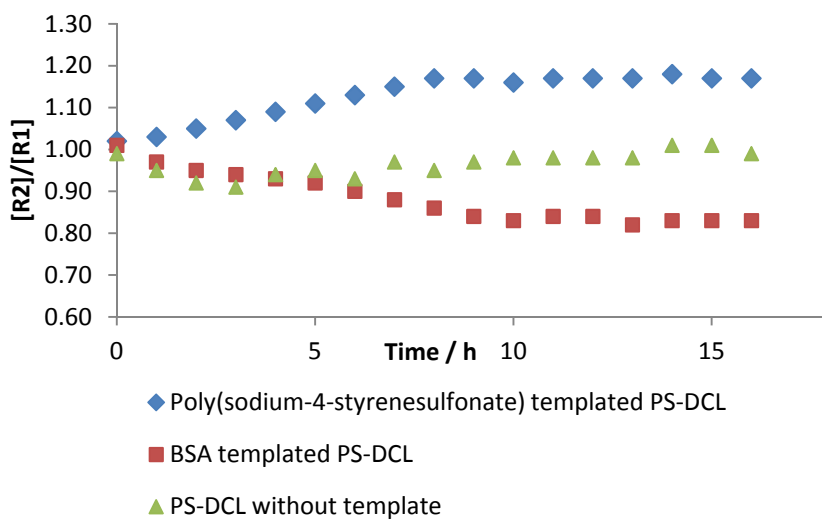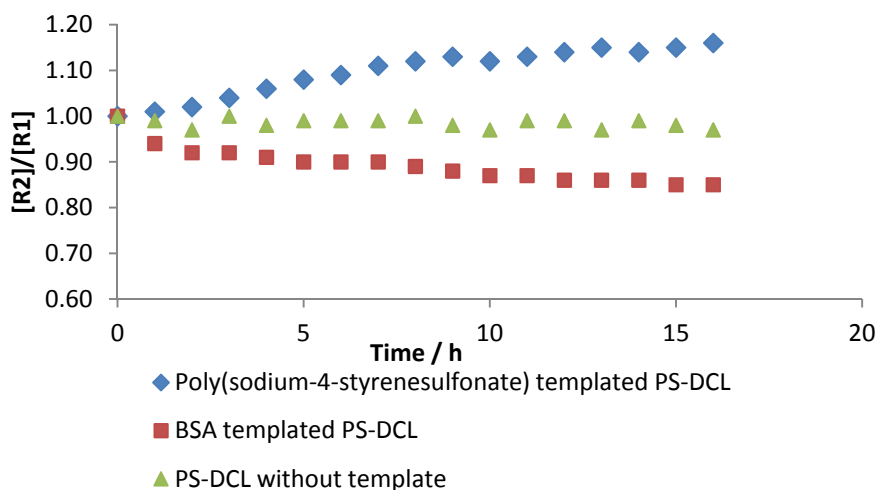
S8. ^{1}H NMR spectrum (500 MHz, D$_2$O, pH 4.5) of a PS-DCL constructed on **P1** using acylhydrazides **R2** and **R3**, 16 h after the addition of poly(sodium-4-styrenesulfonate).
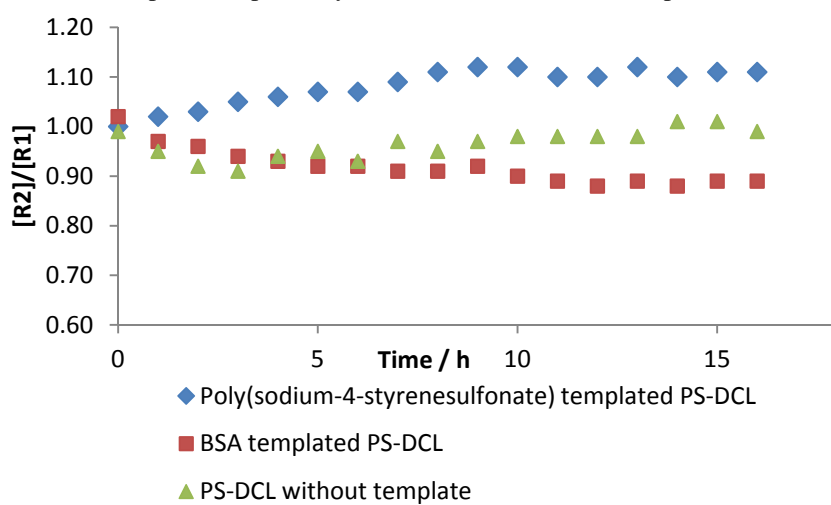
^{1}H NMR spectroscopic analysis

S9. ^{1}H NMR spectroscopic analysis of PS-DCLs constructed upon scaffold **P2**.

S10. ^{1}H NMR spectroscopic analysis of PS-DCLs constructed upon scaffold **P3**.

S11. ^{1}H NMR spectroscopic analysis of PS-DCLs constructed upon scaffold **P4**.

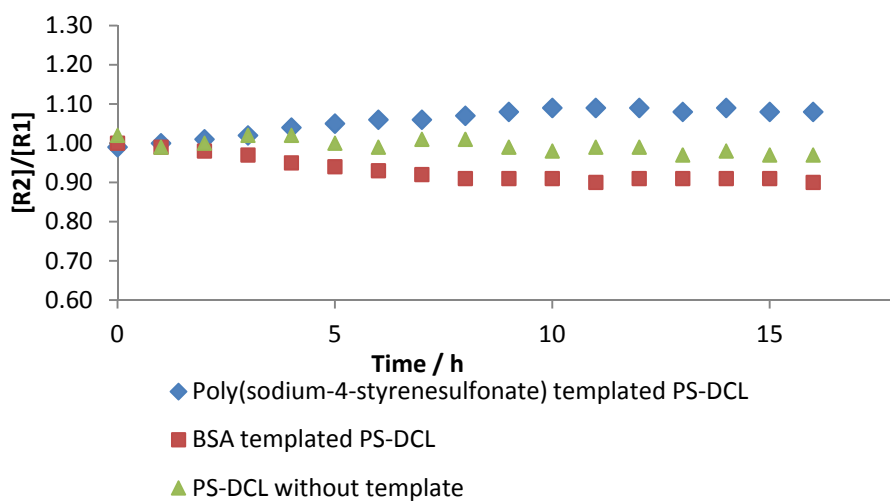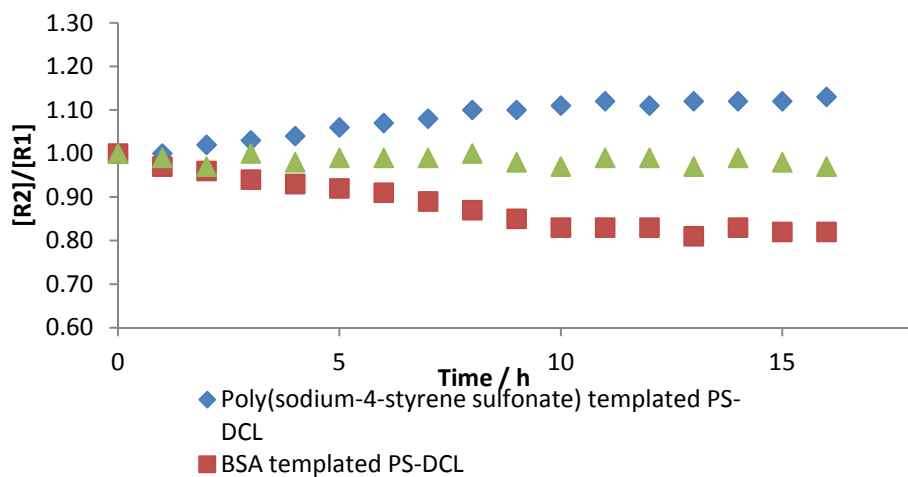S12. ^{1}H NMR spectroscopic analysis of PS-DCLs constructed upon scaffold **P5.**

S13. ^{1}H NMR spectroscopic analysis of PS-DCLs constructed upon scaffold **P6**.

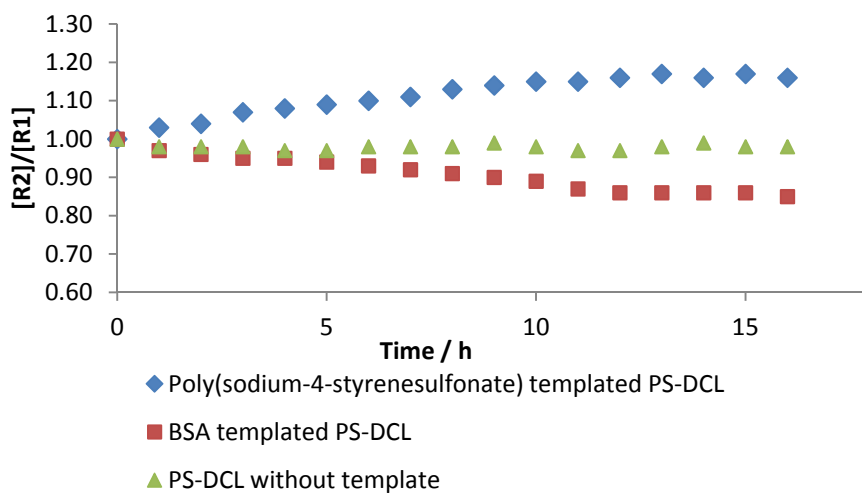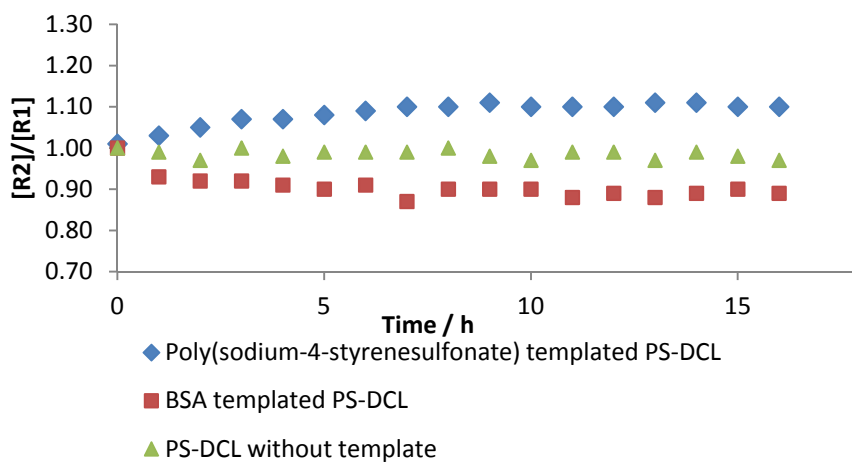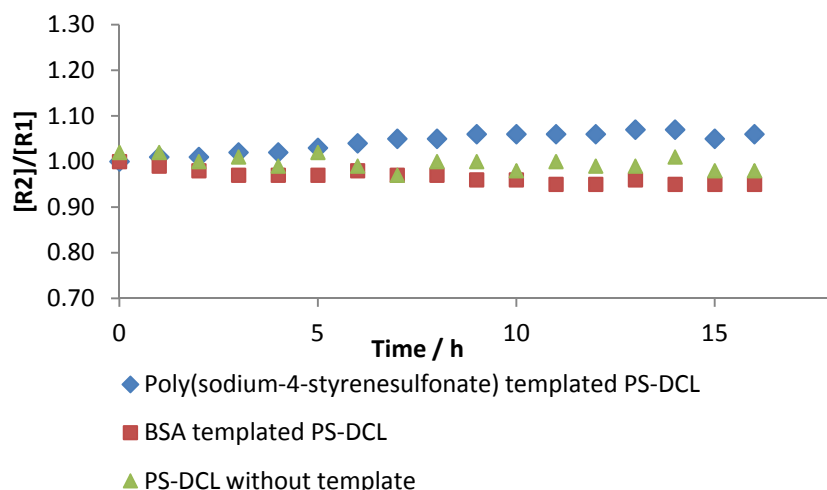S14. ^{1}H NMR spectroscopic analysis of PS-DCLs constructed upon scaffold **P7**.

S15. ^{1}H NMR spectroscopic analysis of PS-DCLs constructed upon scaffold **P8**.

- ◆ Poly(sodium-4-styrenesulfonate) templated PS-DCL
- ■ BSA templated PS-DCL
- ▲ PS-DCL without template

S16. ^{1}H NMR spectroscopic analysis of PS-DCLs constructed upon scaffold **P9**.

Response of PS-DCLs to template addition as the degree of polymerisation of the polymer scaffold is varied
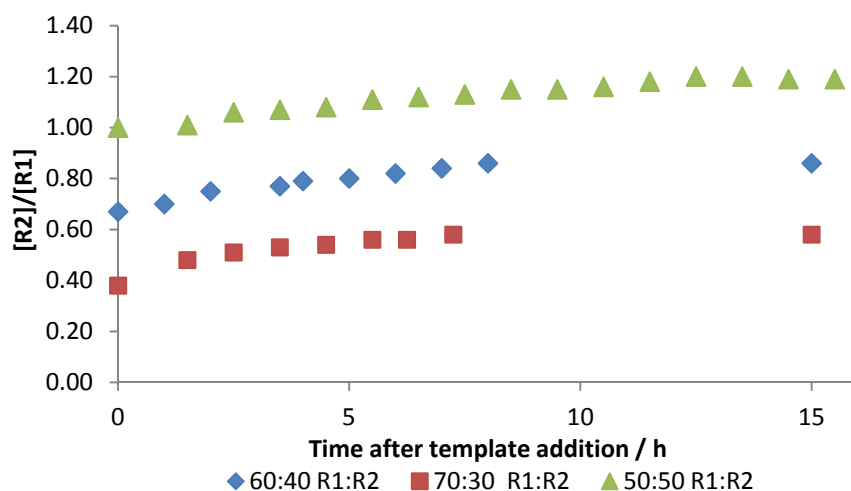

- ◆ Poly(sodium-4-styrene sulfonate) templated PS-DCLs
- ■ BSA templated PS-DCLs
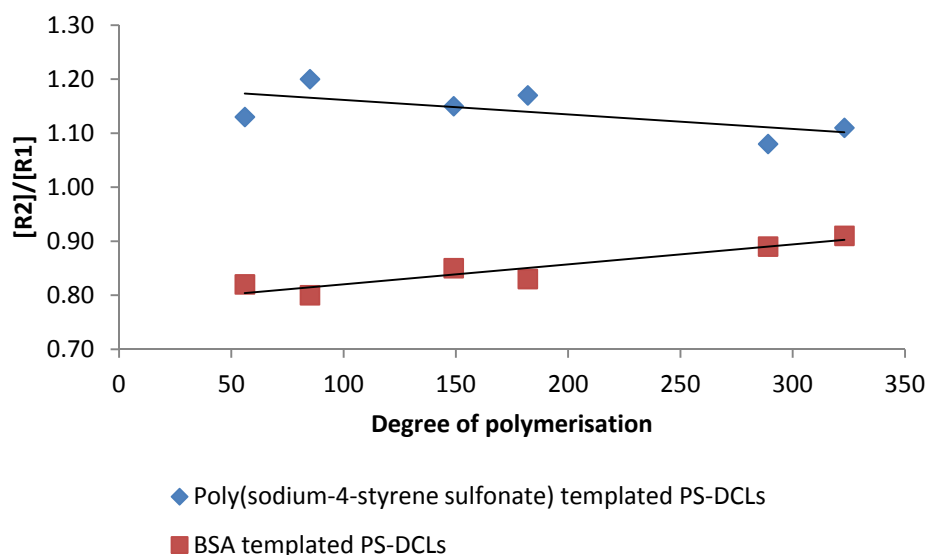
S17. The final library composition after template addition of a series of PS-DCLs constructed upon scaffolds **P1**-**P6**, as a function of the degree of polymerisation of the polymer scaffold.

The main article shows the correlation between the molecular weight of the polymer scaffold (as determined by ^{1}H NMR spectroscopy) and the composition of the PS-DCL after templation. This treatment produces a similar, though more linear correlation. We propose that this difference arises as a consequence of the difference in the molecular weights of the monomer units (*N,N*-dimethylacrylamide: 99.03 g mol^{-1}; *N*-ethylacrylamide-4-benzaldehyde: 262.24 g mol^{-1}).
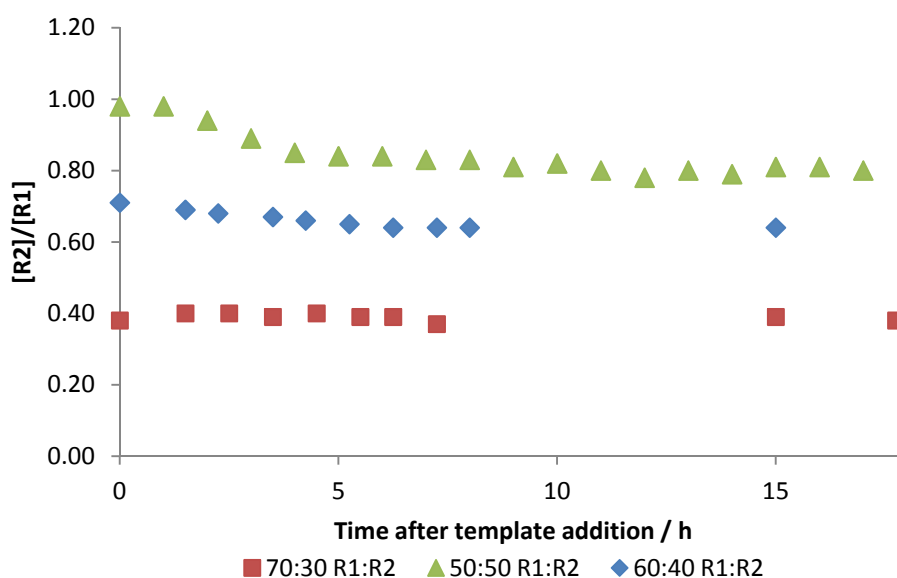
PS-DCLs with unequal initial concentrations of R1 and R2

PS-DCLs have been shown to respond to the addition of various templates by amplifying the concentration upon the polymer scaffold of those residues which are expected to interact most favourably with the template. In order to further investigate the response of these systems to template

addition, PS-DCLs were generated upon scaffold **P1**, using residues **R1** and **R2**, so as to generate PS-DCLs with unequal concentrations of the two residues. The total acylhydrazide concentration in each PS-DCL was maintained at 100 mM, whilst incorporating different concentrations of **R1** and **R2**. This treatment produces PS-DCLs in which polymer scaffolds are already preferentially functionalised with a particular residue. These PS-DCLs were exposed to poly(sodium-4-styrene sulfonate) and BSA templates, and ^{1}H NMR spectroscopy was used to determine the composition of PS-DCLs, as described previously.

S18. ^{1}H NMR spectroscopic analysis (400 MHz, D$_2$O, pH 4.5) of PS-DCLs constructed upon scaffold **P1,** containing an excess of **R1** relative to **R2**, exposed to poly(sodium-4-styrene sulfonate). Control data where initial concentrations of **R1** and **R2** are equal are also shown (green triangles).

S19. ^{1}H NMR spectroscopic analysis (400 MHz, D$_2$O, pH 4.5) of PS-DCLs constructed upon scaffold **P1,** containing an excess of **R1** relative to **R2**, exposed to BSA. Control data where initial concentrations of **R1** and **R2** are equal are also shown (green triangles).
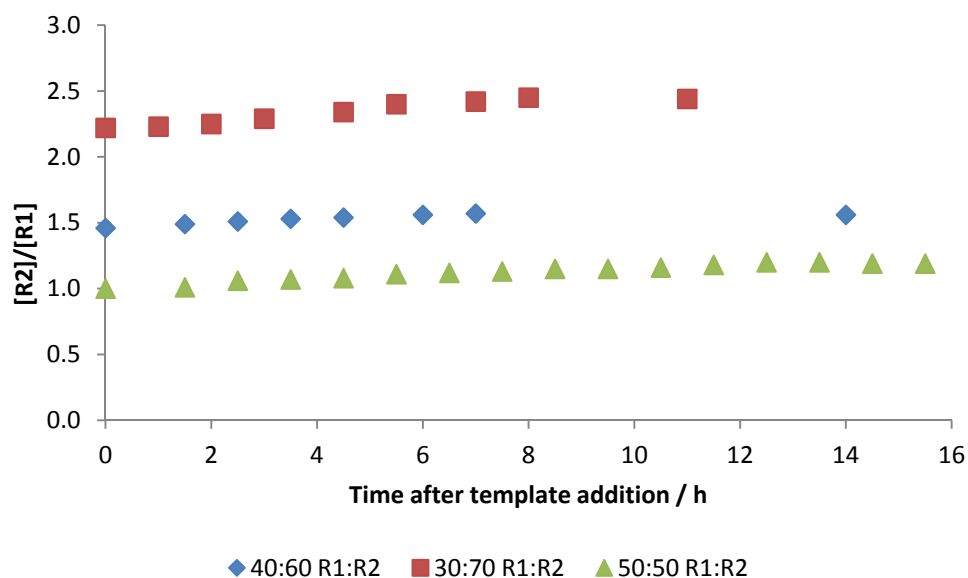
S20. ^{1}H NMR spectroscopic analysis (400 MHz, D$_2$O, pH 4.5) of PS-DCLs constructed upon scaffold **P1,** containing an excess of **R2** relative to **R1**, exposed to poly(sodium-4-styrene sulfonate). Control data where initial concentrations of **R1** and **R2** are equal are also shown (green triangles).
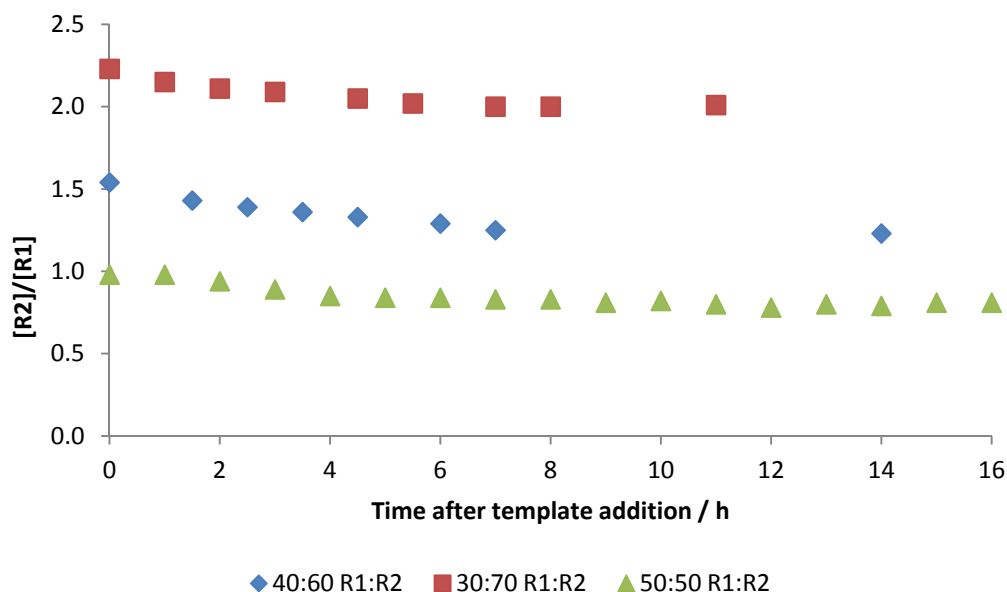

S21. ^{1}H NMR spectroscopic analysis (400 MHz, D$_2$O, pH 4.5) of PS-DCLs constructed upon scaffold **P1,** containing an excess of **R2** relative to **R1**, exposed to BSA. Control data where initial concentrations of **R1** and **R2** are equal are also shown (green triangles).

In all cases above, the system responds to the addition of the template in the expected manner, by amplifying the proportion of the residue expected to interact most favourably with the template. These PS-DCLs do not, however, reach compositions identical to those of systems which contain equal concentrations of **R1** and **R2**. In all of our PS-DCLs, library members are present in a large excess with respect to templates, therefore only a small proportion of library members may interact with the template at a given time. This composition was chosen to incorporate the idea that amplification of the "best-binding species" is observed when library

members must compete in order to interact with the template.[1] Our ^{1}H NMR spectroscopic analysis determines the overall composition of the system, and therefore may not reflect the composition of the "best-binding" fraction of the library. It may, therefore, be the case that the best-binding members of these PS-DCLs do have compositions that reflect the composition observed for PS-DCLs in which **R1** and **R2** are present initially in equal concentrations. It may also be the case that only a limited excess of the preferred residue on the polymer scaffold is necessary to facilitate strong binding of the template. For instance, in PS-DCLs templated with poly(sodium-4-styrene sulfonate), it may become unfavourable to incorporate too many units of the positively charged **R1,** as a consequence of electrostatic repulsion of these residues along the polymer scaffold. There may, in fact, exist an optimum concentration of **R1** on the polymer scaffold, so that enough positive charges are present to ensure good binding to the template, but with a smaller proportion of the neutral acylhydrazide **R2** present to minimise electrostatic repulsion.

References

1.   P. T. Corbett, S. Otto and J. K. M. Sanders, *Chem. Eur. J.*, 2004, **10**, 3139-3143.