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SUPPORTING INFORMATION

Poly(triazolyl methacrylate) glycopolymers as potential targeted unimolecular nanocarriers.

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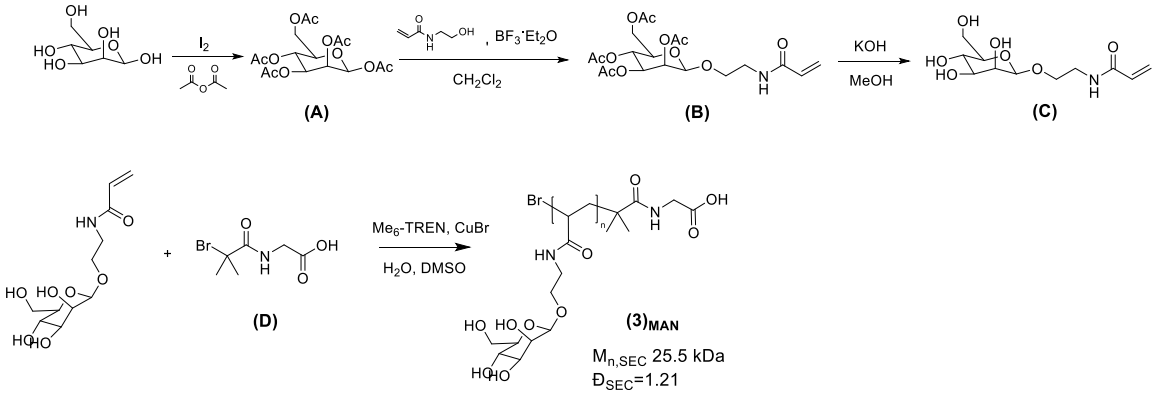
1 Materials and Methods. Synthesis and characterisation of sugar poly(triazolyl methacrylates) linear **(1)$_{MAN}$**, **(1)$_{GAL}$**, **(1)$_{LAC}$**, **(1)$_{TRE}$**, and 4-arm star **(2)$_{MAN}$**, **(2)$_{GAL}$**, **(2)$_{LAC}$**, **(2)$_{TRE}$**, are described in Madeira do O *et al*. *J. Mat. Chem. B* 2018, 6, 1044-1054.[1] Monofunctional **(D)**[2] and tetrafunctional **(E)**[1] initiators were synthesised according to literature methods. Agarose-bound Concanavalin A (Con A) and Peanut Agglutinin (PAN) (45-165 μm agarose beads) were purchased from Vector Laboratories, Peterborough, UK. All other reagents and solvents were obtained at the highest purity available from Aldrich Chemical Company or Fischer Scientific and used without further purification unless otherwise stated.

^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) analysis was carried out using a Bruker DPX400 UltraShield™ Spectrometer. The spectra were processed with MestReNova 6.0.2© 2009 Mestrelab Research S.L. All chemical shifts are reported in ppm (δ) relative to tetramethylsilane, referenced to the chemical shifts of residual solvents resonances. Peak multiplicities are defined by the following abbreviations: s = singlet, br = broad, d = doublet, t = triplet, m =multiplet. FT-IR spectra were recorded with an Attenuated Total Reflection spectrophotometer (Agilent Technologies Cary 630 FTIR) equipped with a diamond single reflection ATR unit. Spectra were acquired with a resolution of 4 cm^{-1}, in the range 4000-650 cm^{-1} by recording 32 interferograms. Mass Spectroscopy was carried out using a Micromass LCT KC453 spectrometer. Data were processed with OpenLynx software. Samples were prepared in suitable solvent with sodium trifluoroacetate added to them.

Aqueous SEC analysis of glycopolymers **(3)$_{MAN}$** and **(4)$_{MAN}$** was carried with a Wyatt dawn 8+ 1200 Infinity series, with RI detection, in a system calibrated with PEO narrow standards. Standard Dulbecco's Phosphate Buffered Saline (DPBS) was used as the mobile phase, at a flow rate of 1 ml min^{-1}.

Flow cytometry. Samples were analysed with a Beckman Coulter FC500 Series equipped with tetraCXP SYSTEM Software. At least $2x10^3$ cells were analysed for each sample. All experiments were performed in duplicates and repeated a minimum of two times.

2 Synthesis of glycopolymer $(3)_{MAN}$



Scheme S1. Synthesis of glycopolymer **$(3)_{MAN}$**.

6-(acetoxymethyl)tetrahydro-2H-pyran-2,3,4,5-tetrayl tetraacetate (A). D (+)-Mannose (50.0 g, 0.277 mol, 1 eq) was suspended in acetic anhydride (144 g, 133 mL, 1.41 mol, 5.1 eq.), in a 1 L beaker. Solid I_2 (360 mg, 1.42 mmol. 0.005 eq.) was slowly added under stirring over a period of 15 minutes. The suspension was then maintained under stirring until a dark brown solution was achieved. The solution was left to cool down at room temperature. Sodium thiosulfate (9 g in 300 mL of DI water) and a $NaHCO_3$ saturated aqueous solution (300 mL) were sequentially added to reduce the excess of iodine catalyst, and to neutralize the generated acetic acid, respectively.

The mixture was stirred at room temperature for 10 minutes and extracted with Et_2O (750 mL). The organic phase was washed 3 times with water. After addition of toluene, the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (SiO_2 column, 60 Å, 35−70 µm, Petroleum ether/EtOAc 7:3 then 6:4) and isolated as a viscous colourless oil (25 g, 0.064 mol, 23.1% yield).

^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 1.89 (s, 3H, CH_3), 1.94 (s, 3H, CH_3), 1.95 (s, 3H, CH_3), 1.99 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 4.16 (m, 2H, CH_2), 5.18 (m, 4H, CH), 5.30 (d, 1H, *J*= 3.3 Hz $CH_{anomeric}$).

^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 20.56 (1C, CH_3), 20.59 (1C, CH_3), 20.61 (1C, CH_3), 20.78 (1C, CH_3), 20.89(1C, CH_3), 62.54 (1C, CH_2), 66.17 (1C, CH), 68.14 (1C, CH), 68.84 (1C, CH), 70.21 (1C, CH), 91.96 (1C, $CH_{anomeric}$), 169.80 (C(O)O), 169.99 (C(O)O), 170.16 (C(O)O), 170.81 (C(O)O), 171.30 (C(O)O).

2-(acetoxymethyl)-6-(2-acrylamidoethoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (B). Product **(A)** (25 g, 0.064 mol) was dissolved in CH_2Cl_2 (100 mL) and *N*-Hydroxyethyl acrylamide (14.7 g, 13.2 mL, 0.128 mol) was added to the solution. BF_3Et_2O (18.2 g, 15.8 mL, 0.128 mol) was slowly added drop wise over a period of 30 minutes. The mixture was left to react for 16 hours at room temperature. The reaction was monitored by ^{13}C NMR, following the shifting of the anomeric carbon at 92 ppm to 105 ppm. The mixture was diluted with CH_2Cl_2 (100 mL), washed with $NaHCO_3$ saturated aqueous solution (3x200 mL) and finally two further washings with water (200 mL). The organic layer was collected, dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product was by flash chromatography (SiO_2 column, 60 Å, 35−70 μm, 100% Et_2O then Et_2O/EtOAc 9:1) to yield product **24** as a viscous oil (5.0 g, 11 mmol, 17.5% yield).

^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.91 (s, 3H, C*H*$_3$), 1.99 (s, 3H, C*H*$_3$), 2.00 (s, 3H, C*H*$_3$), 2. 11 (s, 3H, C*H*$_3$), 3.28 (m, 2H, NHC*H*$_2$), 3.66 (dtd, 2H, *J* = 16.5, 10.5, 5.8 Hz, OCH_2), 4.04 (m, 2H, CH), 4.19 (t, 1 H, *J* = 6.3 Hz, CH), 4.72 (d, 1H, *J* = 8.0 Hz, C*H*$_{anomeric}$); 4.94 (dd, 1H, *J* = 10.3, 8.0 Hz, C*H*), 5.15 (dd, 1H, *J* = 10.4, 3.5 Hz, C*H*), 5.25 (d, 1H, *J*= 3.1 Hz, C*H*), 5.57 (dd, 1H, *J*= 10.1, 2.3 Hz, CH=CH*H*$_2$), 6.07 (dd, 1H, *J*= 17.1, 2.2 Hz, CH=C*H*H), 6.22 (dd, 1H, *J*= 17.1, 10.1 Hz, C*H*=CH_2), 8.11 (t, 1H, *J*= 5.3 Hz, N*H*).

^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 20.30 (1C, *C*H_3), 20.37 (1C, *C*H_3), 20.45 (2C, *C*H_3), 20.51 (1C, *C*H_3), 38.67 (CH_2*C*H_2NH), 61.27 (1C, CH*C*H_2O(C)OCH_3), 67.34 (1C, *C*$H_2$$CH_2$NH), 67.66 (1C, *C*HO(C)$OCH_3$), 68.53 (1C, *C*HO(C)$OCH_3$), 69.90 (1C, *C*HO(C)$OCH_3$), 70.28 (1C, *C*HO(C)$OCH_3$), 100.00 (1C, $CH_{anomeric}$), 125.06 (1C, CH=*C*H_2), 131.66 (1C, CH=*C*H_2), 164.74 (*C*OCHCH_2), 169.12 (*C*OCH_3), 169.47 (*C*OCH_3), 169.87 (*C*OCH_3), 169.91 (*C*OCH_3).

***N*-(2-(3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)ethyl)acrylamide (C).**

Product **(B)** (5.00 g, 11.2 mmol) was dissolved in a solution of 63 mg of KOH in 50 mL of MeOH, and the resulting solution stirred for 18 hours at room temperature. The reaction mixture was passed through a short silica pad preconditioned with methanol, the product was eluted with the minimum amount of methanol, then the solvent was removed under reduced pressure and product **(C)** was isolated as a white powder (2.5 g, 9.0 mmol, 80% yield).

^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.30 (m, 2H, NHCH_2) 3.43 (m, 4H, OCH_2CH$_2$NH and CHCH_2O), 3.71 (m, 1H, CHCH$_2$OH), 3.77 (dd, 1H, *J* = 8.2, 3.0 Hz, CHOH), 3.86 (m, 1H, CHOH), 4.03 (m, 1H, CHOH), 4.41 (t, 1H, J= 5.6 Hz, CH$_2$OH), 4.61 (d, 1H, *J*= 5.6 Hz, CHOH), 4.79 (d, 2H, J= 3.8 Hz, C$H_{anomeric}$ and CHOH), 5.04 (d, 1H, *J*= 6.6, CHOH), 5.57 (dd, 1H, *J*= 10.1, 2.3 Hz, CH=CH_2), 6.07 (dd, 1H, *J*= 17.1, 2.3 Hz, CH=CH_2), 6.23 (dd, 1H, *J*= 17.1, 10.1 Hz, CH=CH$_2$), 8.11 (t, 1H, *J*= 5.4 Hz, NH).

^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 38.83 (1C, *C*H$_2$NH), 63.35 (1C, *C*H$_2$OH), 66.66 (1C, *C*H$_2$CH$_2$NH), 69.35 (1C, *C*H), 70.69 (1C, *C*H), 76.80 (1C, *C*H), 79.68 (1C, *C*H), 107.87 (1C, CH$_{anomeric}$), 125.09 (1C, *C*H=CH$_2$), 131.69 (1C, *C*H=CH$_2$), 164.67 (C(O)NH).

ESI-TOF Mass Spectrometry: expected *m/z* [M-Na]$^+$ 277.12, found 277.95.

FT-IR: v 3245, 2867, 1653, 1539, 1403, 1237, 1012, 712, 650 cm^{-1}

Synthesis of linear (3)$_{MAN}$. Non-triazolyl glycopolymer **(3)$_{MAN}$** used in this study were prepared by Cu-mediated living radical polymerization under the conditions described by Haddleton and co-workers,[3, 4] which relies on disproportionation of Cu(I) precursors to Cu(0) and Cu(II) species prior to the addition of the required polymerisation initiator. To a schlenk tube equipped with magnetic stir bar, DI H_2O (1.50 mL) and Me_6TREN (26 mg, 0.12 mmol, 0.8 eq) was added. The solution was deoxygenated by argon bubbling for 20 minutes, and left to stir for 10 minutes. Cu(I)Br (12 mg, 0.060 mmol, 0.4 eq) was added under inert conditions and the resulting Me_6TREN-Cu(I)Br complex allowed to disproportionate fully (2 minutes). A clean glass tube, was charged with initiator **(D)** (36 mg, 0.15 mmol, 1 eq), monomer **(C)** (2.91 g, 10.5 mmol, 70 eq), triethylamine (21 µL, 15 mg, 0.15 mmol, 1 eq), and DMSO:H_2O (4:1, 2 mL). The mixture was stirred until complete dissolution (~ 5 minutes) and further deoxygenated with argon bubbling for 10 minutes. This solution was then cannulated into the Schlenk tube, previously cooled to 0 ˚C and left to polymerise at this temperature for 2 hours, when 96% monomer conversion was achieved, as confirmed by ^{1}H NMR. The resulting reaction mixture was stirred overnight on Cuprisorb® resin to remove Cu(II) species in solution, and finally dialysed against water. Following freeze-drying the mannose glycopolymer **(3)$_{MAN}$** was obtained as a white light solid. DP_{Theo} 67, $M_{n,SEC}$ 23.9 kDa, Đ 1.22).

3 Fluorescence spectroscopy analysis of Nile Red-$(1)_{MAN}$ complexes.

In this work, two sets of fluorescence spectroscopy experiments were carried out:

3.1 an initial study aimed at investigating the formation of Nile Red:glycopolymer complexes, where the concentration of glycopolymer was kept constant, and that of Nile Red fluorophore was increased (Figure 1C in the manuscript), and

3.2 experiments where Nile Red was used as a fluorescent probe to assess the potential formation of self-assembled structures with a hydrophobic core able to enhance Nile Red incorporation (Figure 2C and 2D in the manuscript). Here the concentration of Nile Red was kept constant, and that of glycopolymers was increased.

Experimental procedures and additional results are reported below.

3.1 Formation of Nile Red:glycopolymer complexes (Figure 1C). Two stock solutions of linear **$(1)_{MAN}$** glycopolymer were prepared in water (or PBS, Figure S2) at 10 μg mL^{-1} and 100 μg mL^{-1} concentrations. A 1.3 mg mL^{-1} Nile Red stock solution was prepared in THF. Different dilutions in THF were made from this stock solution. For each dilution, 8 μL were taken and added to 200 μL of a 100 μg mL^{-1} polymer water solution, for final Nile Red:polymer molar ratios of 0.02:1, 0.05:1, 0.1:1, 0.5:1, 1:1, 5:1, 10:1, 15:1, 20:1 and 40:1 (0.02:1, 0.05:1, 0.075:1, 0.1:1, 0.2:1, 0.3:1, 0.5:1, 0.75:1, 1:1 and 2:1 for the experiments at narrower molar ratio range, Figure S1). For each ratio, the experiment was performed in triplicate.

The Nile Red stock solution was then diluted 10 times, for a final Nile Red concentration of 0.13 mg mL^{-1}. Different dilutions in THF were prepared from this stock solution. For each dilution, 8 μL were taken and added to 200 μL of a 10 μg mL^{-1} polymer water solution, for final Nile Red:polymer molar ratios of 0.02:1, 0.05:1, 0.1:1, 0.5:1, 1:1, 5:1, 10:1, 15:1, 20:1 and 40:1 (0.02:1, 0.05:1, 0.075:1, 0.1:1, 0.2:1, 0.3:1, 0.5:1, 0.75:1, 1:1 and 2:1 for the experiments at narrower molar ratio range, Figure S1). For each ratio, the experiment was performed in triplicate.

The samples were left for 5h protected from light to let the THF evaporate. 50 µL were then taken from each sample and transferred into a Corning black 384-well plate. Samples were analysed on a TECAN Spark 10M Multi-function Plate reader. Fluorescence was read from 580 to 700 nm, with λ_{ex}=550 nm.

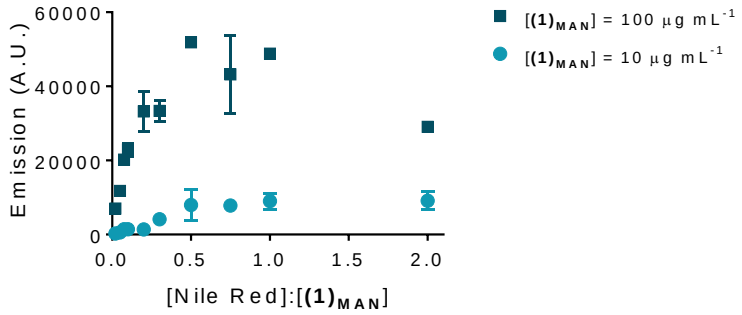


Figure S1. Fluorescence readings (λ_{ex} 550 nm, λ_{em} 630 nm) of Nile Red – **(1)$_{MAN}$** samples at variable [Nile Red]:[polymer chains] molar ratios, in DI H_2O. The experiment is analogous to that shown in Figure 1, but focuses on the 0-2.0 [Nile Red]:[**(1)$_{MAN}$**] range of molar ratios, with additional data points.

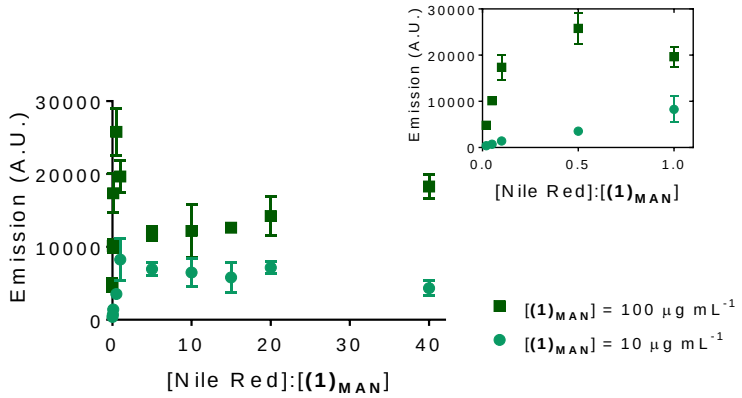


Figure S2. Fluorescence readings (λ_{ex} 550 nm, λ_{em} 630 nm) of Nile Red – **(1)$_{MAN}$** samples at variable [Nile Red]:[polymer chains] molar ratios, in PBS. Insets: magnification of the 0-1.0 [Nile Red]:[**(1)$_{MAN}$**] region.

3.2 Nile Red as fluorescent probe to assess the potential formation of glycopolymer self-assembled structures (Figure 2C and 2D). 2.0 mg mL^{-1} **(1)$_{MAN}$** and **(2)$_{MAN}$** stock solutions were prepared in water (80 µM for **(1)$_{MAN}$**, 68 µM for **(2)$_{MAN}$**), and these were used to prepare solutions of the glycopolymers in the 0.0010-2.0 mg mL^{-1} concentration range (Table S1). To each of these solution, 20

µL of a Nile Red stock solution in THF (3.2 mg mL^{-1}, 10 µM) were added, for a final Nile Red concentration of 2.0 µM. The solutions were left open under an aspirated fumehood protected from light for 5 hours, to allow the THF to evaporate. 50 µL from each solution were finally transferred into a Corning 384-black well plate and analysed using a Tecan Spark 10M Multi-function Plate reader. Fluorescence was read from 600 to 700 nm, with λ_{ex}=550 nm. Samples were analysed in triplicate (n=3). Data are shown in Figure 2C and 2D, and Figure S3 below.

Table S1. Polymer concentrations utilised in the fluorescence spectroscopy (ii) experiments, at constant Nile Red concentration (2.0 µM).

$(1)_{MAN}$		**$(2)_{MAN}$**	
Conc. (mg mL^{-1})	Conc. (µM)	Conc. (mg mL^{-1})	Conc. (µM)
0.0010	0.040	0.0010	0.034
0.0050	0.20	0.0050	0.17
0.010	0.40	0.010	0.34
0.020	0.80	0.020	0.68
0.040	1.6	0.040	1.4
0.060	2.4	0.060	2.0
0.080	3.2	0.080	2.7
0.10	4.0	0.10	3.4
0.20	8.0	0.20	6.8
0.40	16	0.40	14
0.60	24	0.60	21
0.80	32	0.80	27
1.0	40	1.0	34
1.25	50	1.25	42
1.5	60	1.5	51
1.75	70	1.75	59
2.0	80	2.0	68

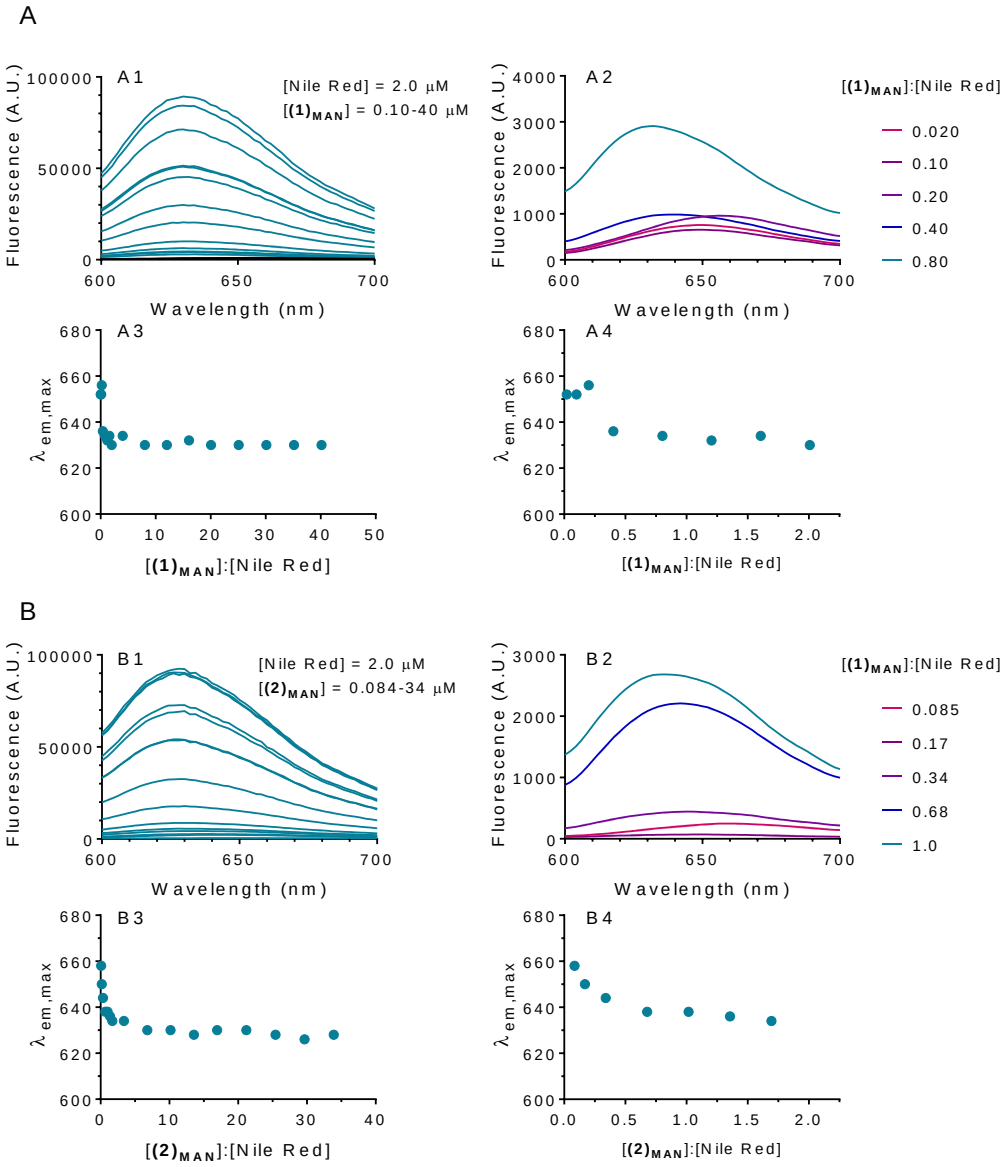
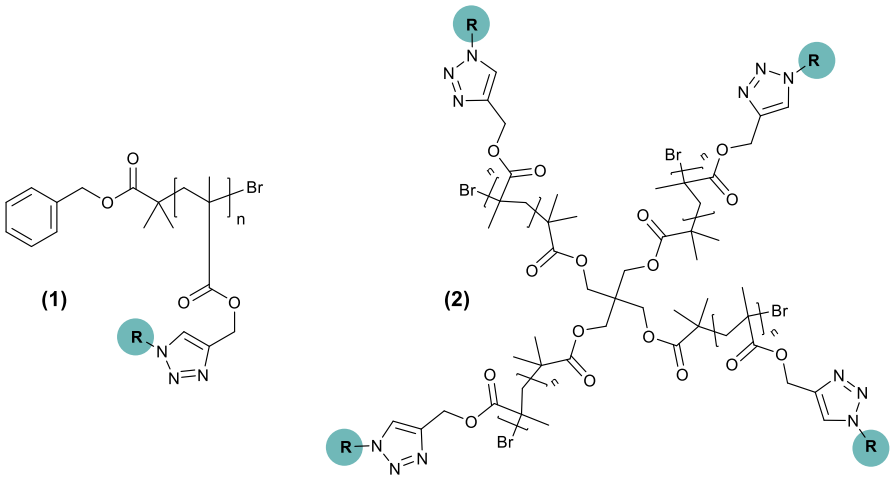


Figure S3. Fluorescence spectroscopy analysis of samples of Nile Red and **(1)**$_{MAN}$ (A), and **(2)**$_{MAN}$ (B) in deionised water. Nile Red was added from a stock solution in THF (targeting a theoretical [Nile Red]= 2.0 μM (0.64 μg mL^{-1}) in each sample) to solutions of **(1)**$_{MAN}$ and **(2)**$_{MAN}$ in deionised water, and samples were analysed following evaporation of THF. A1 and B1: emission spectra (λ_{ex} = 550 nm) for 0.0050-2.0 mg mL^{-1} solutions of **(1)**$_{MAN}$, and **(2)**$_{MAN}$. A2 and B2: emission spectra for polymer samples in the 0.0050-0.060 mg mL^{-1} range. A3 and B3: shift in $\lambda_{em,max}$ *vs.* (concentration of glycopolymer). A4 and B4: magnification of graphs A3 and B3 in the 0.0050-0.10 mg mL^{-1} concentration range.

4 Surface tension analysis. A DSA 100 Drop Shape Analyser with DSA 4 software (Kruss GmbH, Germany) was used. The pendant drop method at room temperature with Laplace–Young computational method was employed for surface tension analysis. Measurements were taken immediately after droplet formation. Samples were measured in triplicates, from two independent solutions.



(1)$_{MAN}$ n= 66, $M_{n,theo}$ 24.9 kDa, $M_{n,SEC}$ 23.9 kDa, Đ = 1.22
(1)$_{LAC}$ n= 66, $M_{n,theo}$ 35.6 kDa, $M_{n,SEC}$ 25.5 kDa, Đ = 1.25
(1)$_{TRE}$ n= 66, $M_{n,theo}$ 36.5 kDa, $M_{n,SEC}$ 37.6 kDa, Đ = 1.18

(2)$_{MAN}$ n= 77, $M_{n,theo}$ 29.5 kDa, $M_{n,SEC}$ 27.2 kDa, Đ = 1.25
(2)$_{LAC}$ n= 77, $M_{n,theo}$ 42.0 kDa, $M_{n,SEC}$ 42.6 kDa, Đ = 1.29
(2)$_{TRE}$ n= 77, $M_{n,theo}$ 43.0 kDa, $M_{n,SEC}$ 55.9 kDa, Đ = 1.17

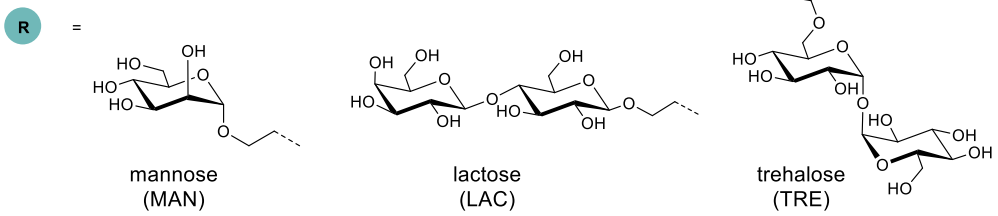


Chart S1. Glycopolymers utilised for the surface tension analysis measurements. Synthesis and characterisation of these materials are described in Madeira do O *et al. J. Mat. Chem. B* 2018, 6, 1044-1054.[1]

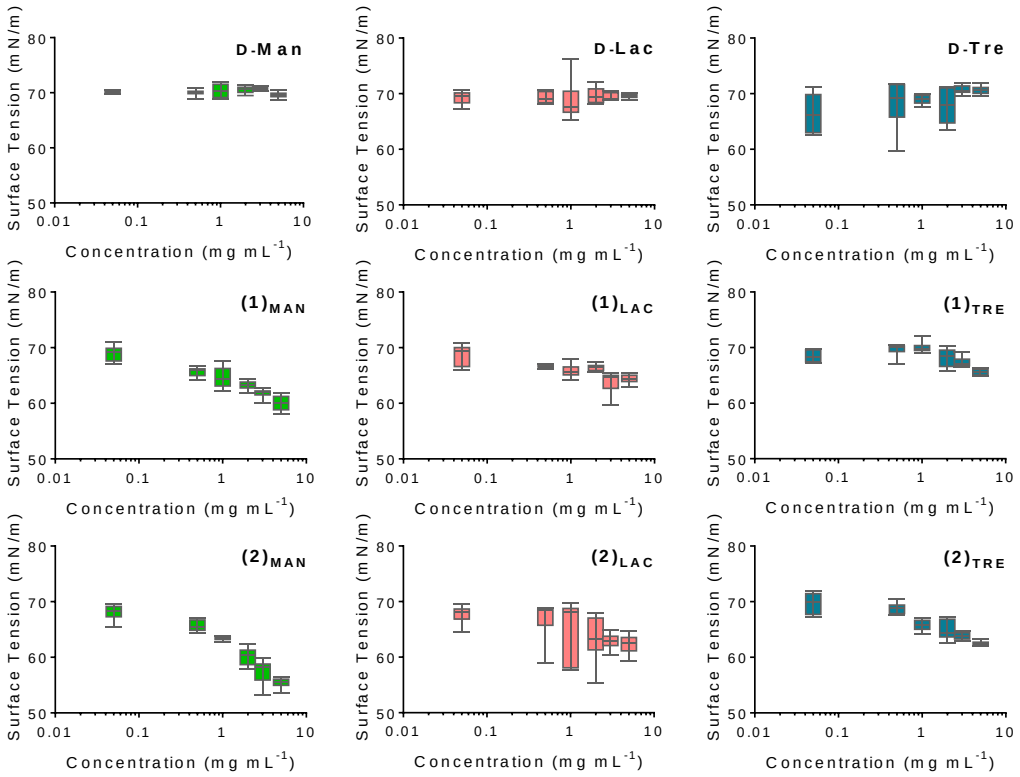


Figure S4. Surface tension analysis of solutions of mono- and disaccharides - mannose, and lactose and trehalose, respectively – (top line); and the corresponding linear (middle line), and star (bottom line) glycopolymers. Surface tension of sugar and polymer solutions (N=2, triplicates). Boxes represent median values, 25th and 75th percentile. Whiskers represent maximum and minimum values. All solutions were prepared in deionised water.

5 Dynamic Light Scattering (DLS) analysis. Samples were analysed on a Zetasizer Nano S spectrometer (Malvern Panalytical Ltd.) equipped with a 633 nm laser at a fixed angle of 173°. To ensure a correct conversion from intensity to volume plots, both refractive index and viscosity of a 2 mg mL^{-1} **(1)$_{MAN}$** and **(2)$_{MAN}$** solutions were measured, using an Anton Paar Abbematt 200 refractometer for refractive index and an Anton Paar Module Compact mcr 302 rheometer for viscosity, respectively. In both cases, values were found to be the same as those of pure DI water.

Particle size analyses were performed on a 10 mg mL^{-1} **(1)$_{MAN}$** water solution. To 1 mL of this solution, 12.9 µL of a Nile Red 1 mg mL^{-1} stock solution in THF were added for a final **(1)$_{MAN}$**: Nile Red 10:1 molar ratio. The mixture was left open to air for 5 hours to allow THF to evaporate. The particle size analysis

was then repeated. Finally, the sample was filtered using a 0.22 µm PVDF filter and the particle size analysis repeated one last time.

For CMC analyses, the polymers were dissolved in Milli-Q water for a final volume of 500 µL and concentrations in the 0.001-2.0 mg mL^{-1} range. The solutions were left gently mixing for 2 h, and then analysed. 3 repetitions were made for each sample, and the attenuator manually set in order to keep the scattering count rate below 1000. Intensity of scattering (Derived particle Count, kcps) was plotted against polymer concentration.

6 Taylor Dispersion analysis of Nile Red-$(1)_{MAN}$ complexes at variable dye:polymer ratios. A 2.0 mg mL^{-1} linear **$(1)_{MAN}$** glycopolymer solution was prepared in Milli-Q water. A 0.26 mg mL^{-1} Nile Red stock solution was prepared in THF. Different dilutions in THF were made from this stock solution. For each dilution, 12 µL were taken and added to 300 µL of the 2 mg mL^{-1} polymer water solution, for final Nile Red:**$(1)_{MAN}$** molar ratios of 1:10, 1:4, 1:2, 1:1 and 3:1.

The samples were left under an aspirated fumehood for 5 h protected from light to allow the traces of residual THF evaporate, and finally filtered using a 0.22 µm PVDF filter. 200 µL were then taken from each filtered sample and analysed on a Viscosizer (Malvern Panalytical, UK), to determine the hydrodynamic radius of the different **$(1)_{MAN}$**-Nile Red complexes. 40 nL were injected and analysed per sample. Absorbance was recorded at λ= 214 nm. Because of incomplete solubilisation of Nile Red, the concentrations of dye in the filtered samples used for these measurements, though still fluorescent, were lower than the nominal [**$(1)_{MAN}$**]:[Nile Red] 10:1, 4:1, 2:1, 1:1, 1:3 ratios. Under these conditions the hydrodynamic radii (R_h) of all these samples were all in the 2.0-2.2 nm range irrespective of the amount of added Nile Red.

7 Complexation of Nile Red and DCVJ by linear glycopolymers $(1)_{MAN}$ and $(3)_{MAN}$. 20 µL of 1.0 mg mL^{-1} stock solutions of Nile Red or DCVJ in THF were added to 1.00 mL aliquots of solutions of glycopolymers **$(1)_{MAN}$** and **$(3)_{MAN}$** at increasing glycopolymer concentrations (0, 100, 500 and 1000 µg

mL^{-1}) in deionised water. The resulting mixtures were centrifuged for 10 minutes at 14000 RPM to remove non-polymer entrapped dyes. The supernatants were collected, transferred into Eppendorf vials, and THF was allowed to evaporate over 5 hours. Fluorescence was measured *via* Plate Reader at λ_{ex} 550 nm, emission recorded in the λ_{em} = 520-700 nm region for Nile Red, and λ_{ex} = 430 nm, emission recorded in the λ_{em} = 400-600 nm region for DCVJ glycopolymer-dye complexes.

8 Filtration of $(1)_{MAN}$–Nile Red complexes through 0.22 and 0.1 µm membranes: ^{1}H NMR analysis. To confirm that glycopolymers were not lost following filtration of **$(1)_{MAN}$**–Nile Red complexes due to the hypothetical presence of large ≥100 nm glycopolymer particles, a 10 mg mL^{-1} **$(1)_{MAN}$** solution was prepared in D_2O, and Nile Red, previously dissolved in THF, added for a final 1:1 **$(1)_{MAN}$:**Nile Red molar ratio. To the resulting solution, *N*-hydroxyethylacrylamide (HEA), a hydrophilic molecule with ^{1}H NMR signals in the 5.5-6.5 ppm which do not overlap with those of glycopolymer **$(1)_{MAN}$**, was added as internal standard for NMR analysis under the assumption that a water-soluble probe would not interfere with the complexation process. After THF evaporation, the samples were filtered through a 0.22 µm, then a 0.10 µm PVDF filters. As the HEA standard can freely pass through the filter membrane, it can be used an internal standard to assess any decrease of concentration of polymer **$(1)_{MAN}$** following filtration through the 0.10 µm filter. Accordingly, the mixture was prepared by dissolving **$(1)_{MAN}$** (30 mg, 0.0012 mmol of polymer chains, 0.080 mmol of sugar repeating units) and HEA (~ 3 mg, the exact [sugar repeating units]:[HEA] molar ratio was determined by ^{1}H NMR, and found to be 1.8, below) in 3 mL of D_2O. The solution was split into three aliquots of 1 mL each. 50 µL of a 2.55 mg mL^{-1} stock solution of Nile Red ($4.0\ 10^{-4}$ mmol) in THF were added to each aliquot, for a final **$(1)_{MAN}$**:Nile Red 1:1 molar ratio. Residual traces of THF were found to evaporate overnight. One of the samples was analysed by ^{1}H NMR as is, another one was filtered through a 0.1 µm PVDF filter, and the third through a 0.1 µm PVDF filter. All filtered samples, still visually pink, were then analysed by ^{1}H NMR. The [sugar repeating units]:[HEA] molar ratio was calculated by comparing the integrals

of the HEA vinyl peaks in the 6.5-5.5 ppm range with the triazole proton of the sugar repeating units at 8 ppm. No detectable change of this ratio was observed after filtration, showing that all glycopolymer **(1)$_{MAN}$** was able to cross the 0.22 and 0.10 µm membranes.

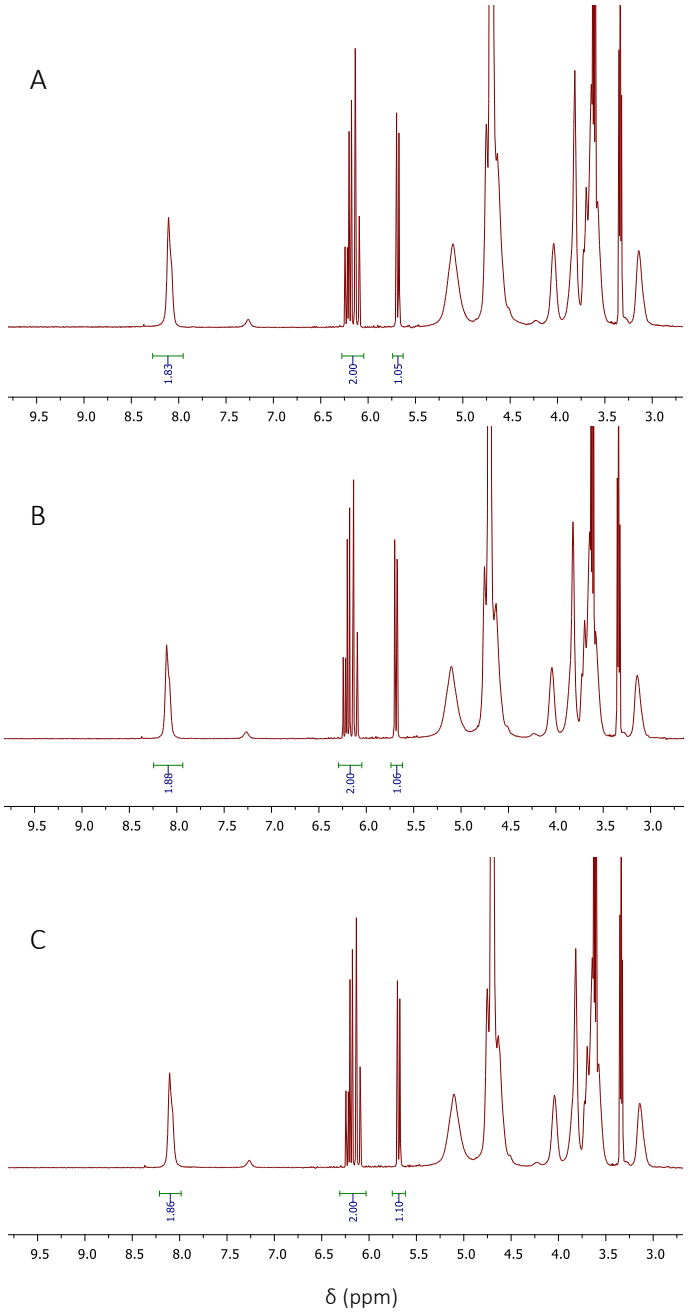


Figure S5. ^{1}H NMR analysis of solutions of **(1)$_{MAN}$** (4.0 10^{-4} mmol of polymer chains, 0.026 mmol of sugar repeating units), *N*-hydroxyethylacrylamide (HEA) internal standard (0.015 mmol), and Nile Red (4.0 10^{-4} mmol) in D_2O before (A), and after filtration through a 0.22 µm (B), and 0.10 µm (C) PVDF membranes. Glycopolymer triazole CH signal at 8.1 ppm was compared to the vinyl signals of HEA ^{1}H NMR standard.

9 Release of Nile Red from Nile Red-$(1)_{MAN}$ and Nile Red-$(2)_{MAN}$ complexes. Solutions of **$(1)_{MAN}$**- and **$(2)_{MAN}$**-Nile Red complexes were introduced into a Float-A-Lyzer® dialysis tubing (MWCO 5 kDa), and dialysed against PBS under gentle stirring. Released Nile Red was quantified by fluorescence spectroscopy analysis of the external dialysis medium, at regular intervals of time.

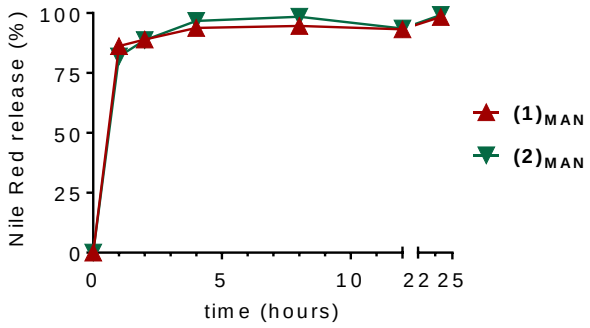


Figure S6. Release profile of Nile Red assessed by dialysis against PBS. Experiment was undertaken at room temperature and in sink conditions. Fluorescence readings of the sample were taken immediately before dialysis and values were taken as 100%, and were used for subsequent dye release quantification. Glycopolymers concentration: 1.0 mg mL^{-1}. Nile Red concentration of 20 µg mL^{-1}, 63 µM.

10 Reversible agglutination assay. A 2.0 mg mL^{-1} stock solution of Con A was prepared in 20 mM HEPES buffer (pH 7.4 with 50 mM NaCl, 5 mM $CaCl_2$ and 5 mM $MnCl_2$), and used to prepare 20, 500, 1000 and 2000 µg mL^{-1} Con A solutions in the same buffer. 100 µL of each of the latter solutions were placed into separate Eppendorf vials, followed by 100 µL of **$(1)_{MAN}$**- and **$(2)_{MAN}$**-Nile Red complexes (1:1 polymer:dye molar ratio, at a polymer concentration of 2.0 mg mL^{-1}) in the same buffer. Final concentration of Con A in these Eppendorf vials were 0, 10, 250, 500, 1000 µg mL^{-1}. The addition of the Con A immediately resulted in the formation of a cloudy suspension due to the formation of insoluble glycopolymer-Con A clusters.

The solutions were centrifuged for 2 min at 1000 rpm, and the supernatant was collected and analysed by fluorescence spectroscopy (λ_{ex} 550 nm, emission recorded in the 520-700 nm region) to obtain an estimation of residual **$(1)_{MAN}$**- and **$(2)_{MAN}$**-Nile Red complexes in solution. The precipitates from the

1000 μg mL^{-1} Con A samples were partially or completely redissolved by adding methyl-α-mannopyranoside at different final concentrations (200 μL; final methyl-α-mannopyranoside: 1, 5, 10, 15, 20 mg mL^{-1}) and, the fluorescence of the samples was recorded again.

11 Binding of $(1)_{MAN}$- and $(1)_{GAL}$-Nile Red complexes to immobilised Con A and PNA lectins. Agarose lectin beads (Con A and PNA beads) were washed with cold 20 mM HEPES buffer (pH 7.4 with 50 mM NaCl, 5 mM $CaCl_2$ and 5 mM $MnCl_2$) five times in order to remove sugar added to stabilize the lectins during storage. 100 μL aliquots of a 2 mg mL^{-1} lectin bead suspension was transferred into eppendorfs. To these, different amounts of linear **$(1)_{MAN}$**- and **$(1)_{GAL}$**-Nile Red complexes (1:1 polymer:dye molar ratio) were added to achieve final polymer concentrations of 0.10, 0.125, 0.25, 0.50, 1.0 mg mL^{-1}. The suspensions were gently mixed with a vortex. After 20 min, the suspensions were centrifuged for 1 min at 1200 rpm, followed by careful removal of the supernatants. The resulting pellets were resuspended in the same HEPES buffer again, and the centrifugation-washing cycle was repeated three times. The suspensions were then transferred into a 96-well plate for fluorescence imaging using an In Vivo Imaging System (IVIS) (excitation: 550 nm, detection 640 nm).

12 Affinity chromatography assay using Con A- and PNA stationary phases. Agarose lectin beads (Con A and PNA beads) were washed with cold 20 mM HEPES buffer (pH 7.4 with 50mM NaCl, 5 mM $CaCl_2$ and 5 mM $MnCl_2$) five times in order to remove sugar added to stabilize the lectins during storage. The lectin beads were resuspended in 1 mL of buffer solution, and transferred to Pasteur pipettes previously fitted with small piece of cottonwool to ensure that the resin is retained in the pipette. Following elution of the excess of suspending buffer from the column, 1.0 mL aliquots of of a 2.0 mg mL^{-1} linear **$(1)_{MAN}$**- and **$(1)_{GAL}$**-Nile Red complexes (1:1 polymer:dye molar ratio) in 20 mM HEPES buffer at pH 7.4 were added to the pipettes and again allowed to drain through the lectin beads under gravity. Next, the columns were eluted with 10-12 mL of HEPES buffer solution. Subsequently, 3-4 mL

of 10 mg mL^{-1} methyl-α-mannopyranoside and D-galactopyranoside solution in HEPES buffer was ran through Con A and PNA bead columns, respectively, followed by 5 mL of HEPES buffer solution.

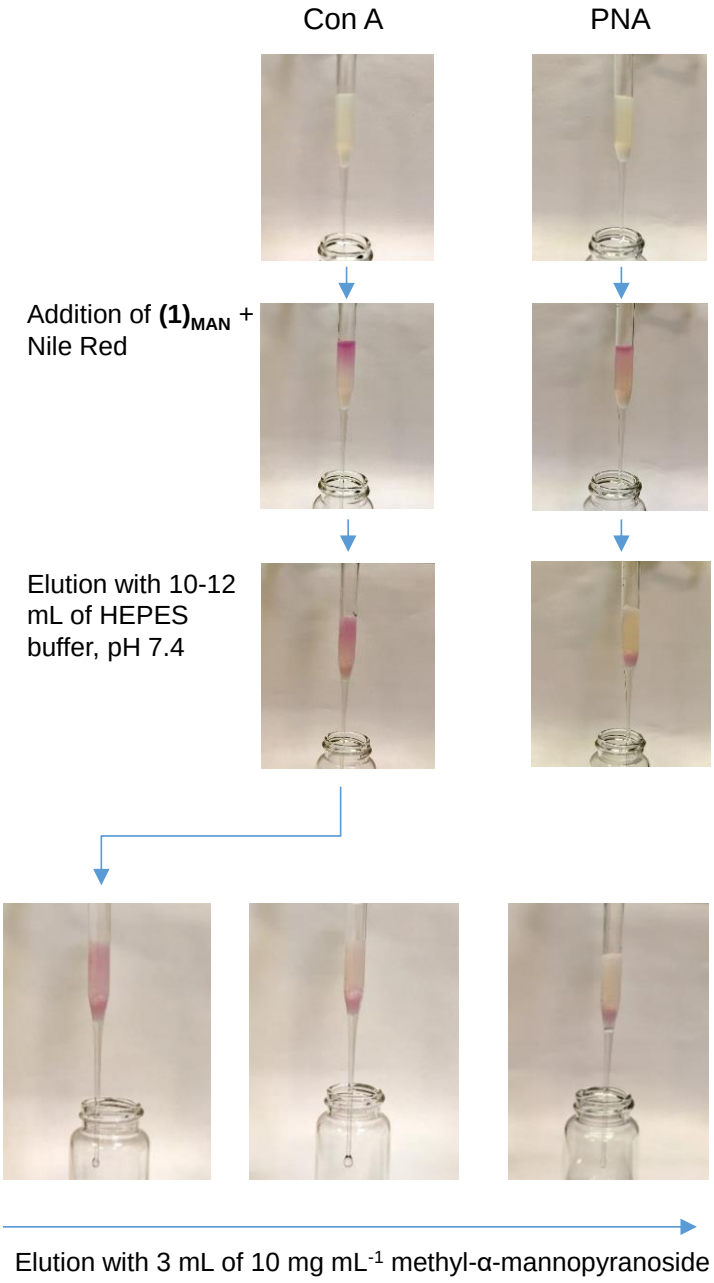


Figure S7. Affinity chromatography assay. Mannose-binding Con A- and galactose-binding PNA-immobilised beads were utilised as stationary phases for affinity chromatography for **(1)$_{MAN}$**-Nile Red complexes (1:1 polymer:dye molar ratio), eluting with HEPES buffer.

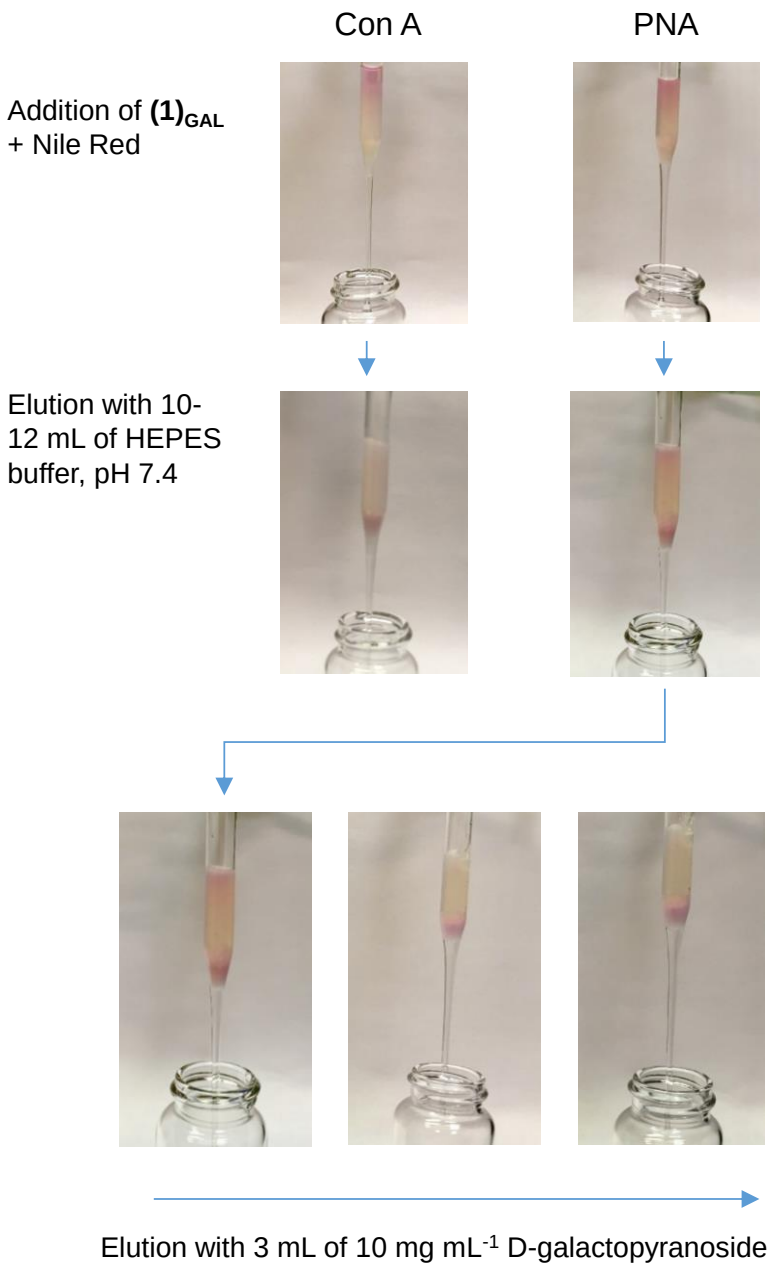


Figure S8. Affinity chromatography assay. Mannose-binding Con A- and galactose-binding PNA-immobilised beads were utilised as stationary phases for affinity chromatography for **(1)$_{GAL}$**-Nile Red complexes (1:1 polymer:dye molar ratio), eluting with HEPES buffer.

13 Quantification of entrapped Nile Red in polymer:dye complexes following 0.22 µm filtration. A 10 mg mL^{-1} (0.4 mM) of **(1)$_{MAN}$** solution was prepared in d.i. water and filtered through a 0.22 µM PVDF filter. To 1 mL of this filtered solution, 12.9 µL of a 1 mg mL^{-1} Nile Red solution in THF were added, for a final Nile Red concentration of 0.0129 mg mL^{-1} (0.04 mM) and a 10:1 Mannose Linear:Nile Red molar ratio. A control sample was prepared in the same way but without using glycopolymer **(1)$_{MAN}$**.Both solutions were left open under an aspirated fumehood for 5 h, to let THF evaporate. 200 µL ("unfiltered complexes") were subsequently withdrawn and diluted with 1800 µL of DMSO. The remaining 800 µL were filtered with a 0.22 PVDF filter ("filtered complexes"), then a 200 µL aliquot was withdrawn and diluted with 1800 µL of DMSO. From both DMSO-diluted solutions, three 50 µL aliquots of were transferred on a Corning-384 black wellplate and fluorescence detected with a TECAN Spark 10M Multi-function Platereader. Fluorescence was read from 580 to 700 nm, with λ_{ex}=550 nm. Results showed that following filtration through 0.22 µM PVDF filter 0.8% of Nile Red was retained within the glycopolymer complexes.

14 Cell uptake studies. Chinese Hamster Ovary expressing Mannose Receptor (MR^+CHO) cell lines were cultured in cell medium containing 10% Foetal Bovine Serum (FBS) (Sigma Aldrich, UK), 2 mM L-glutamine, 100 U/mL penicillin and 100 µg mL^{-1} streptomycin (Sigma Aldrich, UK), at 37°C, 5% CO_2 and 95% relative humidity. MR^+CHO stable transductants were selected by addition of 0.6 mg mL^{-1} geneticin (Sigma Aldrich, UK) in the culture medium.

Chinese Hamster Ovary (CHO) or Chinese Hamster Ovary expressing Mannose Receptor (MR^+CHO) were seeded in 24 wells plate (20 x 10^4 cells/well) and incubated overnight at 37°C, 5% CO_2 (DMEM/F12 medium). The medium was removed, cells were washed with 2 x 1 mL of PBS and incubate for 30 minutes with RPMI W/O phenol red, 5% CO_2. The media was then replaced with fresh media containing 1.00 mg mL^{-1} mannosylated linear **(1)$_{MAN}$** or star **(2)$_{MAN}$** glycopolymers -+ 10 mM Nile Red and cells were incubated at 37°C, 5% CO_2 for further 30 minutes. Subsequently, wells were rinsed with

PBS (3 x 1 mL), the cells harvested using trypsin/EDTA solution diluted 1:1 in PBS and fixed with 2% paraformaldehyde in PBS. Cells treated with media only were analysed as controls. The medium was then replaced with the glycopolymers-Nile Red solutions and cells were incubated for further 30 minutes at 37°C, 5% CO_2 in the dark. Wells were rinsed with 3 x 1 mL PBS, cells were harvested by treatment with trypsin/PBS 1:1, and fixed in 2% paraformaldehyde. Samples were subsequently analysed by flow cytometry (FACS).

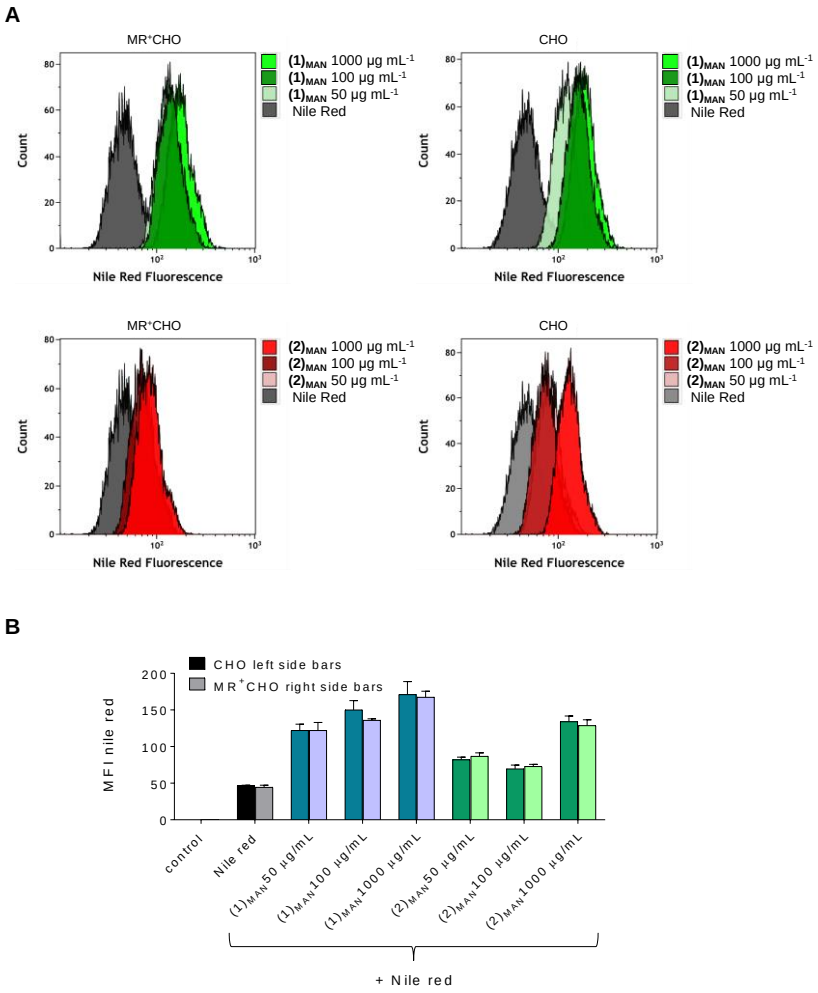


Figure S9. Uptake of nile red:glycopolymer complexes by CHO and MR+CHO cells as assessed by flow cytometry. A: histogram representing Nile red fluorescence intensity in cells treated with increasing concentration of nile red:polymer complexes. B: Bar diagram representing data shown in panel A.

15 Molecular dynamics experiments.

Simulation parameters. All MD simulations were run in GROMACS 5.1.0[5] using the High Performance Computing (HPC) cluster of the University of Nottingham or ARCHER, UK's National Supercomputer.[6] The GROMOS 54a7 force field was selected.[7] Following past work from our group[8], partial charges for the monomers and Nile Red, as well as their topology files were obtained from the Automated Topology Builder (ATB).[9] The polymers were built using *tleap* of AMBERtools and the number of monomers used matches exactly the DP as calculated via NMR (66 for both $\mathbf{(1)_{MAN}}$ and $\mathbf{(3)_{MAN}}$ glycopolymers).

The polymers were minimised (10000 steps of steepest descent energy minimisation, 100ps of Berendsen NVT and 100 ps NPT and 100 ns of MD simulation) in the presence of water. These aqueous equilibrations were done in triplicate. After that, the equilibrated single polymer chain was placed in a new cubic box and 5 molecules of Nile Red were placed randomly in the same box. All solvation was done via GROMACS [1] and the SPC water model was used. After solvation, the systems were again relaxed by 10000 steps of energy minimisation with the steepest descent algorithm followed by further equilibration via NVT ensembles at 298 K (V-rescale) for 100ps and NPT ensembles at 1 bar (Berendsen) for another 100ps. The cut-off distance for the van der Waals and the electrostatic interactions was set to 1 nm. At the end of each step of the previous processes the potential energies, temperature and pressure were checked retrospectively to ensure the proper equilibration of the system. All of the final MD simulations were run with V-rescale thermostat and the Parrinello-Rahman barostat, with a time step of 2 fs, under periodic boundary conditions. The end-to-end distances and radius of gyrations were calculated using the GROMACS tool *gmx polystat* while the distances between the centre of mass of a selected reference structure and the rest of the molecules was achieved with the tool *gmx pairdist*.

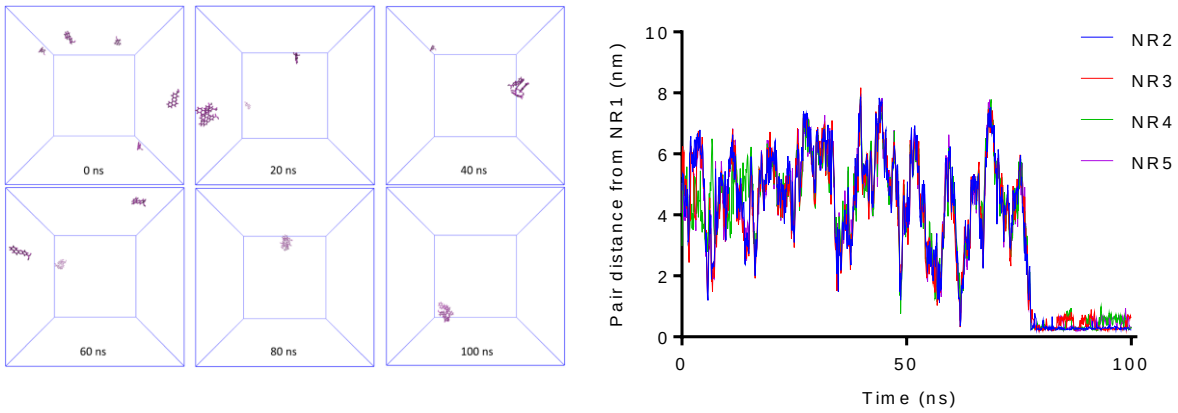


Figure S10. Metrics obtained from simulations with five molecules of Nile Red (NR) left to equilibrate in water. The evolution of the pair-distances between one Nile Red molecule (NR1) and the others is monitored over 100 ns (box size 10 x 10 x 10 nm^3).

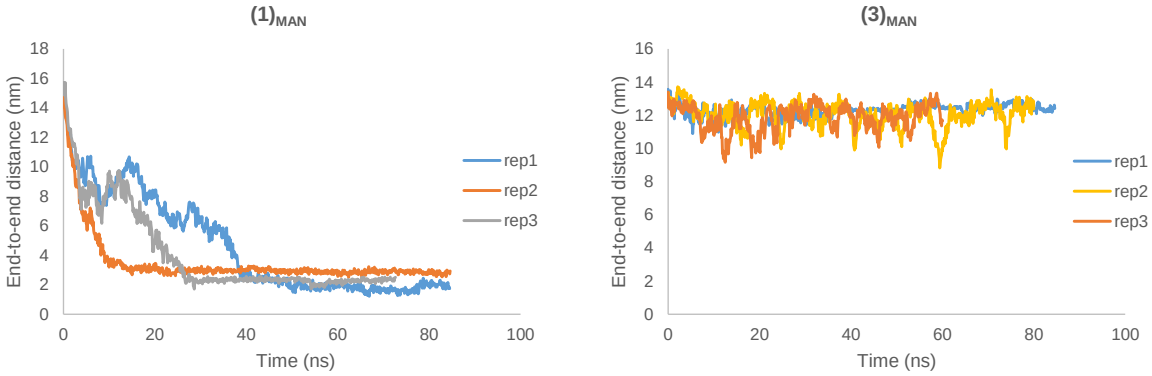


Figure S11. Metrics obtained from simulations with poly(mannose triazolyl methacrylates) **(1)$_{MAN}$** and poly(*N*-ethylacrylamidoyl-α-D-mannopyranoside) **(3)$_{MAN}$**. Evolution of end-to-end distance of the glycopolymers in water (3 replicas per polymer).

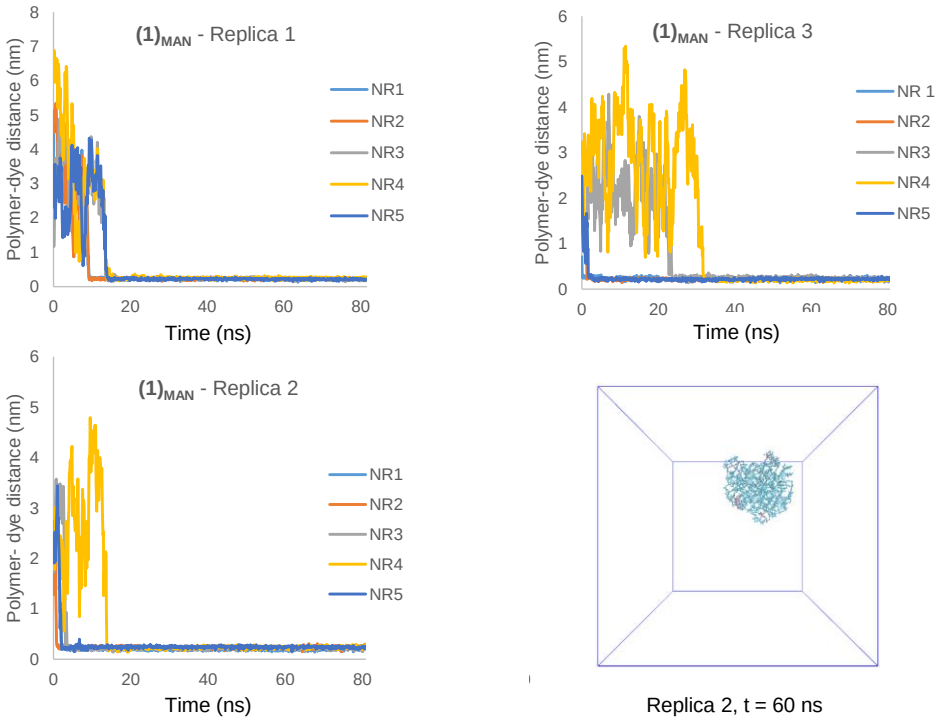


Figure S12. Metrics obtained from the 80 ns simulations with poly(mannose triazolyl methacrylates) **(1)$_{MAN}$**. Five Nile Red (NR) molecules were randomly placed in the space surrounding glycopolymers **(1)$_{MAN}$** and the evolution of the pair-distances between the centre of mass of the polymer chains and each Nile Red molecule was monitored. Data from all three replica experiments are shown. Bottom right: Snapshot of the system in the Replica 2, at t = 60 ns.

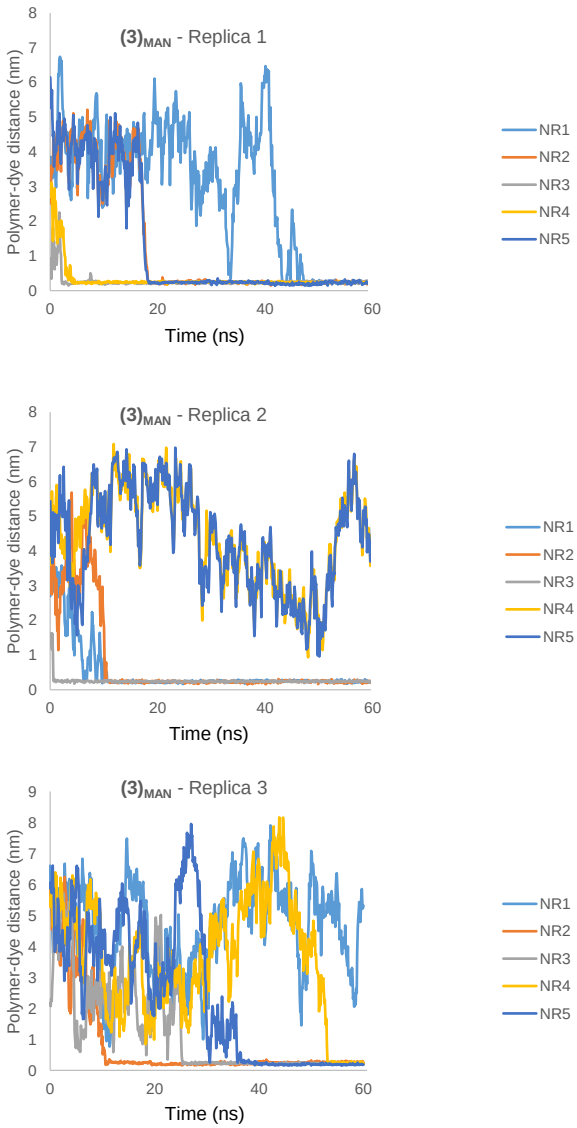


Figure S13. Metrics obtained simulations with poly(*N*-ethylacrylamidoyl-α-D-mannopyranoside) **(3)$_{MAN}$**. Five Nile Red (NR) molecules were randomly placed in the space surrounding glycopolymers **(3)$_{MAN}$** and the evolution of the pair-distances between the centre of mass of the polymer chains and each Nile Red molecule was monitored. Data from all three replica experiments are shown.

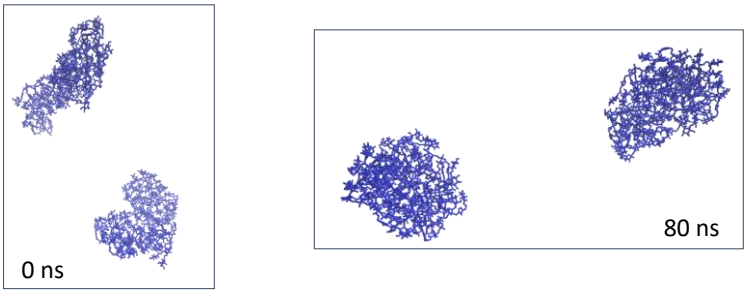


Figure S14. Snapshot of two water-equilibrated **(1)$_{MAN}$** polymer chains kept in a (15x15x15) nm^3 box for 80 ns. Within this timescale, no polymer-polymer aggregation was observed.

16 References

1. J. Madeira do O, F. Mastrotto, N. Francini, S. Allen, C. F. van der Walle, S. Stolnik and G. Mantovani, *J. Mater. Chem. B*, 2018, **6**, 1044-1054.
2. H. Murata, C. S. Cummings, R. R. Koepsel and A. J. Russell, *Biomacromolecules*, 2013, **14**, 1919-1926.
3. A. Simula, G. Nurumbetov, A. Anastasaki, P. Wilson and D. M. Haddleton, *Eur. Polym. J.*, 2015, **62**, 294-303.
4. Q. Zhang, P. Wilson, Z. Li, R. McHale, J. Godfrey, A. Anastasaki, C. Waldron and D. M. Haddleton, *J. Am. Chem. Soc.*, 2013, **135**, 7355-7363.
5. S. Pronk, S. Páll, R. Schulz, P. Larsson, P. Bjelkmar, R. Apostolov, M. R. Shirts, J. C. Smith, P. M. Kasson, D. van der Spoel, B. Hess and E. Lindahl, *Bioinformatics*, 2013, **29**, 845-854.
6. Service UNS: ARCHER. doi:http://www.archer.ac.uk/
7. N. Schmid, A. P. Eichenberger, A. Choutko, S. Riniker, M. Winger, A. E. Mark and W. F. van Gunsteren, *Eur. Biophys. J.*, 2011, **40**, 843-856.
8. R. Mackenzie, J. Booth, C. Alexander, M. C. Garnett and C. A. Laughton, *J. Chem. Theory Comput.*, 2015, **11**, 2705-2713.
9. A. K. Malde, L. Zuo, M. Breeze, M. Stroet, D. Poger, P. C. Nair, C. Oostenbrink and A. E. Mark, *J. Chem. Theory Comput.*, 2011, **7**, 4026-4037.