Supporting Information for: **Functional Hyperbranched Polymers: Towards Targeted *in vivo* ^{19}F Magnetic Resonance Imaging using Designed Macromolecules**

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Materials and Methods

Materials. All chemicals and solvents were purchased from Sigma Aldrich and used as received unless otherwise stated. Monomers were passed through alumina prior to use to remove inhibitors. 2-dodecylsulfanyl thiocarbonyl sulfanyl-2-methyl propionic acid[1] and the alkyne analogue[2] were synthesized as previously published. AIBN was recrystallized from methanol three times before use. Mannose azide was synthesized as previously described.[3]

Characterisation. GPC-MALLS was carried out on a Waters GPC equipped with an RI detector and a Wyatt 8 angle DAWN Helios MALLS detector operating at 658 nm. The polymers were eluted at 1 mL/min in dimethylacetamide (DMAc). Dn/dc for each polymer was calculated by measuring the refractive index of a series of polymer dilutions in DMAc.

^{1}H NMR spectra were conducted using a Bruker Avance 300 spectrometer. $CDCl_3$ or d_6-DMSO were generally used as solvent for the hyperbranched polymers. High resolution ^{19}F spectra were recorded on a Bruker Avance 500 spectrometer (11.7 T) in D20. The chemical shift was referenced to CFCl3. All samples showed a single ^{19}F peak at -73 ppm which was assigned to the CF3 resonance. An example ^{19}F spectrum of P1 is shown in Figure S1.

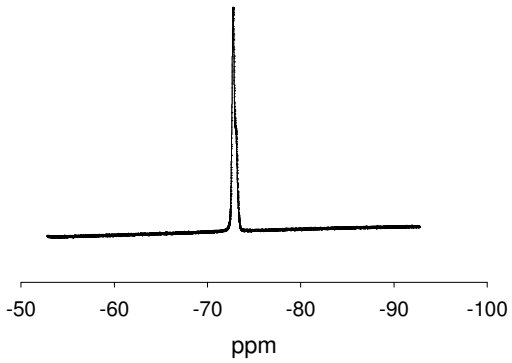

Figure S1. ^{19}F spectrum of P1 in D_20. Chemical shift is referenced to $CFCl_3$.

Hyperbranch particle sizes in pure water were measured using a Malvern Zetasizer. The diameter that is recorded (Scheme 1) is a volume average and is the average result of 5 experiments conducted at 25 ^oC. Samples were filtered through 450 micron filters prior to measurement.

^{19}F T_2 measurements were recorded on a Bruker 300 MHz wide-bore system (7 T). Typically, the polymer is dissolved in water to a concentration of 20 mg/mL. The samples are analysed using a standard spin echo pulse sequence with a repetition time of 3 seconds. Typically, two or three T_2 relaxation times are observed (Ref 4 in main text). The data presented refers only to the long relaxation time; this is the population that contributes to the ^{19}F images (as discussed in reference 4 in main text).

MRI images of live mice were taken on a Bruker 700 MHz wide-bore microimaging system (16.4 T). Proton images were taken using a standard Bruker MSME pulse sequence (SE3D). Only a single echo was used in the acquisition. The ^{19}F images were taken using a spin-echo 3D pulse sequence with a repetition time of 1 s and echo time of 6.4 ms. 32 scans were averaged to achieve sufficient signal to noise ratio and 8 slices were generally taken with a field of view of 3 cm (matrix size: 128x96x64). The total scan time was approximately 8 minutes. Ethics clearance was obtained from the University of Queensland for live mice testing (AIBN/076/08). The respiration rate of the mouse was monitored at all times during the imaging experiment.

Figure S2 shows the MRI image of P1 phantom solutions at 5, 10, 15, 20 mg/mL in pure water. A plot of S/N as a function of concentration is also shown in the figure.

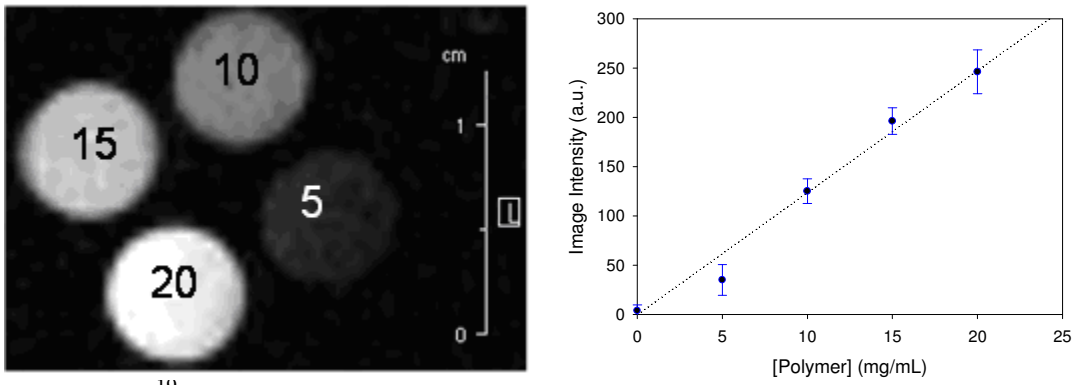

Figure S2. ^{19}F phantom images of P 1 at 5, 10, 15, 20 mg/mL in pure water. Plot of S/N as a function of concentration is shown on the right (10 mg/mL of polymer = 3.8 x 10^{-2} moles ^{19}F / L).

Synthesis of hyperbranched PDMAEA-stat-tFEA (P1). 1 g dimethylaminoethyl acrylate (DMAEA) (1.06 mL, 6.98 x 10^{-3} mol), 269 mg trifluoroethyl acrylate (tFEA) (221 uL, 1.74 x 10^{-3} mol), 157 mg 2-dodecylsulfanyl thiocarbonyl sulfanyl-2-methyl propionic acid (4.34 x 10^{-4} mol) + 7 mg 4,4-azobisisobutyronitrile (AIBN) (4.34 x 10^{-5} mol) and 101 mg ethyleneglycol dimethacrylate (EGDMA) (96 uL, 5.1 x 10^{-4} mol) were dissolved in 5 mL toluene and sealed in a round bottom flask fitted with a septum. The mixture was purged with argon for 15 minutes then heated to 65 ^{o}C for 72 hours. Upon completion of the reaction, the polymer was precipitated in hexane and the viscous oil dried under vacuum. The polymer was characterized by GPC-MALLS and ^{1}H NMR (Table S1). ^{1}H NMR ($CDCl_3$): 0.9-1.2 ppm (br m, CH backbone); 1.17-1.47 ppm (SCH2*C11H20*); 1.7-2 ppm (br m, *CH2*CH); 2.2 ppm (s, N(*CH3*)2); 2.62 ppm (br t, CH2*CH2*N); 3.4 ppm (t, S*CH2*CH2); 4.1 ppm (br t, *CH2*CH2N); 4.4 ppm (br q, *CH2*CF3). ^{19}F NMR (D2O): -73 ppm. UV-Vis (λ_{max} = 306 nm). FTIR (ATR): 3376cm^{-1} NH stretch; 2772-2950 cm^{-1} CH stretch; 1728 cm^{-1} C=O stretch; 1181 cm^{-1} CF stretch; 1155 cm^{-1} COC ester stretch.

Removal of RAFT end-group from P1. The RAFT end-groups were removed using an approach reported by Perrier et al. [ref. 9 in text]. Briefly, 0.3 g P1 (2.5 x 10^{-5} mol, 8.25 x 10^{-5} mol RAFT groups – see table S1) and 271 mg 2,2′-Azobis(2-methylpropionitrile) (AIBN 1.65 x 10^{-3} mol) were heated to 65 ^{o}C in 5 mL THF with stirring for 24 hours. The polymer was precipitated into hexane three times, followed by extensive dialysis against DMF:water (50:50). The final product was a colourless, viscous oil. Yield: 0.21 g.
UV-VIS analysis showed the absence of absorption at 306 nm. The ^{1}H NMR spectrum is very similar to that for P1, except that the broad triplet is no longer visible at 3.4 ppm (the singlet due to the new end-groups arising from the methylpropionitrile end-groups is masked by the polymer peaks at ~ 1.5 ppm). ^{19}F NMR (D2O): -73 ppm. FTIR (ATR): 3376cm^{-1} NH stretch; 2772-2950 cm^{-1} CH stretch; 1728 cm^{-1} C=O stretch; 1181 cm^{-1} CF stretch; 1155 cm^{-1} COC ester stretch.

Synthesis of alkyne-terminated PDMAEA-stat-tFEA (P2). Procedure as above but alkyne-terminated RAFT agent (174 mg, 4.34 x 10^{-4} mol) was employed in place of 2-dodecylsulfanyl thiocarbonyl sulfanyl-2-methyl propionic acid. The NMR spectrum and assignments of the hyperbranched polymer endgroups in $CDCl_3$ is shown in Figure S3; important peaks have been highlighted. ^{1}H NMR ($CDCl_3$): 0.9-1.2 ppm (br m, CH backbone); 1.17-1.47 ppm (SCH2*C11H20*); 1.7-2 ppm (br m, *CH2*CH); 2.2 ppm (s, N(*CH3*)2); 2.62 ppm (br t, CH2*CH2*N); 3.4 ppm (t, S*CH2*CH2); 4.1 ppm (br t, *CH2*CH2N); 4.4 ppm (br q, *CH2*CF3); 4.65 ppm (br, *CH2*CCH). ^{19}F NMR (D2O): -73 ppm. UV-Vis (λ_{max} = 306 nm). FTIR (ATR): 3376cm^{-1} NH stretch; 2772-2950 cm^{-1} CH stretch; 1728 cm^{-1} C=O stretch; 1181 cm^{-1} CF stretch; 1155 cm^{-1} COC ester stretch.

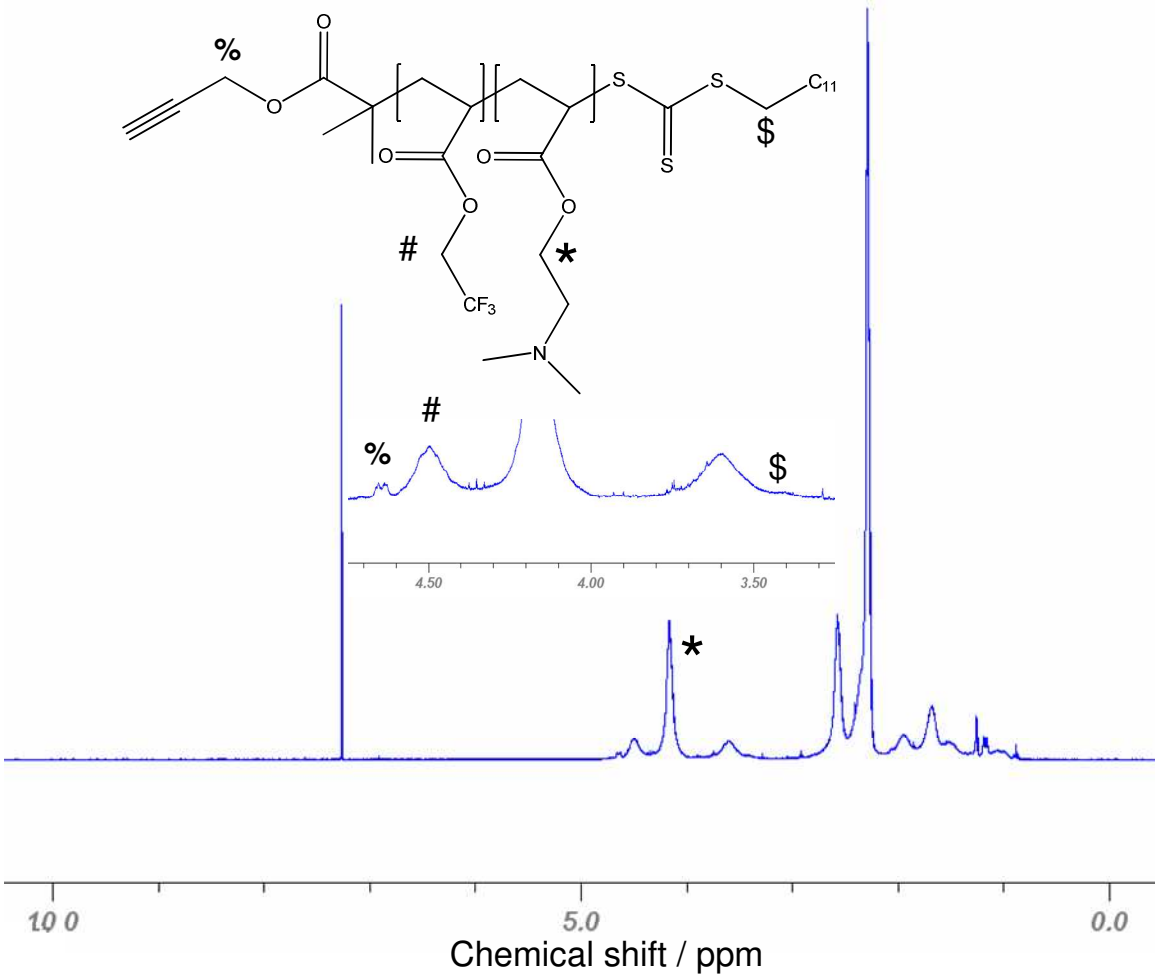

Figure S3. NMR spectrum of P2 in $CDCl_3$. Methylene groups on DMAEA and tFEA are shown, along with the methylene end-group adjacent to the alkyne-terminus.

Chain extension of Polymer 1 with PEGMA (P3). 0.5 g P1 (~ 1.5 x 10^{-4} mol, ~ 6 x 10^{-4} mol RAFT units) and 2 g PEGMA-475 was dissolved in 5 mL THF containing 2.5 mg AIBN. The solution was degassed with argon and then reacted for 24 hours at 65 ^{o}C. The polymer was precipitated in hexane and dialyzed against water for 5 days to remove unreacted monomer. The polymer was freeze-dried yielding a slightly yellow, but

viscous oil. (dn/dc = 0.044) ^{1}H NMR: Ratio of OCH2 peak in DMAEA backbone (4.1 ppm) and (OCH2CH2)n peak in PEGMA (3.6 ppm) was used to determine number of PEGMA chains per molecule (P3, Table S1); ^{1}H NMR ($CDCl_3$) 0.9-1.2 ppm (br m, CH backbone); 0.9ppm (br, CH2C*H3*); 1.17-1.47 ppm (SCH2*C11H20*); 1.7-2 ppm (br m, *CH2*CH); 2.2 ppm (s, N(*CH3*)2); 2.62ppm (br t, CH2*CH2*N); 3.35ppm (s, OCH3); 3.5-3.6ppm (m, C*H2*C*H2*O); 3.95ppm (br t, COOC*H2*CH2); 4.1ppm (br t, *CH2*CH2N); 4.4 ppm (br q, *CH2*CF3). ^{19}F NMR (D2O): -73 ppm. UV-Vis (λ_{max} = 306 nm). FTIR (ATR): 3551 cm^{-1} NH stretch; 2867 cm^{-1} CH stretch; 1726 cm^{-1} CO stretch; 1573 cm^{-1} NH bending; 1155 cm^{-1} COC ester stretch; 1096 cm^{-1} COC ether stretch.

Table S1 Molecular properties of hyperbranched polymers

Polymer	[a]M_n	[a]M_w	[a]PDI	[b]M_n(NMR)	[c]# End-groups
P1	12.2	21.8	1.8	3.7	3.3
P2	12.6	22.1	1.8	3.1	4.1
P3	31.4	113.2	3.6	8.7	3.6

[a]SEC-MALS in dimethylacetamide (dn/dc = 0.044); [b]NMR ratio of RAFT end-groups to DMAEMA backbone peaks; [c]Mn (MALS)/Mn(NMR)

Click reaction between mannose-azide and alkyne-terminated polymer (P4). 0.5 g P2 (~6 x 10^{-4} mol alkyne groups), 150 mg mannose azide (6 x 10^{-4} mol), 8 mg $CuSO_4$ (6 x 10^{-5} mol), 6 mg sodium ascorbate (3 x 10^{-5} mol) was dissolved in 6 ml solvent (50 % MeOH in H_2O). The reaction was stirred in the fume cupboard at room temperature for 48 hours. The solution was freeze-dried and the polymer dissolved in THF and passed through a basic alumina column. The solvent was evaporated and the polymer dialyzed against water for 7 days. The click reaction was monitored by FTIR. Quantitative conversion of the alkyne groups was observed after the reaction by monitoring the C≡C vibration at 2100 cm^{-1} which was not visible upon completion of the reaction. ^{1}H NMR was also used to observe conversion by comparing peaks at 8.1 ppm, 5.1 ppm and 3.38 ppm as shown in Figure S4. Peaks at 8.1 (^) and 5.1 (%) ppm show successful click coupling, while the integration of the peak at 3.3 ppm ($) matches that of the peak at 5.1 ppm and is double that of the peak at 8.1 ppm suggesting quantitative coupling. Mannose peaks span the region 3.45 – 4.4 ppm and are generally masked by DMAEA and tFEA peaks. ^{1}H NMR (d6-DMSO): 0.9-1.2 ppm (br m, CH backbone); 1.17-1.47 ppm (SCH2*C11H20*); 1.7-2 ppm (br m, *CH2*CH); 2.2 ppm (s, N(*CH3*)2); 2.62 ppm (br t, CH2*CH2*N), 3.38ppm (t, SC*H2*CH2), 3.45ppm (m, 2H, OCHHCH2 + H5), 3.62-3.72ppm (H-4, H-3, H'-6), 3.79-3.85ppm (m, H-2, H-6", H-7"), 3.92 – 4.42ppm (CH2C*H2C*N, C*H2*CH2CN, H-1), 4.1 ppm (br t, *CH2*CH2N); 4.4 ppm (br q, *CH2*CF3). 5.11ppm (S, 2H, OCH2N), 8.10ppm (s, 1H, NC*H*C). ^{19}F NMR (D2O): -73 ppm. FTIR (ATR) 3376cm^{-1} NH stretch, 2772-2950 cm^{-1} CH stretch, 1728 cm^{-1} C=O stretch, 1181 cm^{-1} CF stretch, 1155 cm^{-1} COC ester stretch,

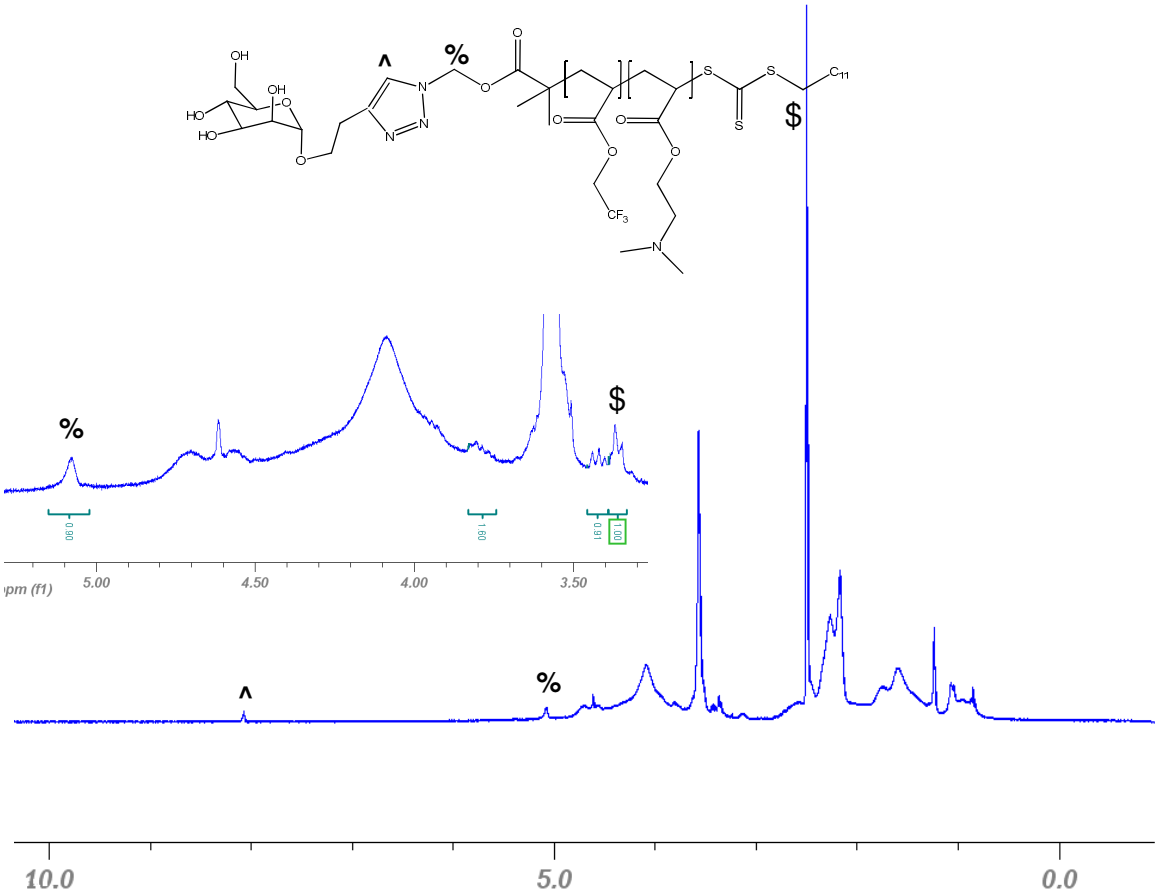

Figure S4. ^{1}H NMR spectrum of P4 in d_6-DMSO. Peak at 8.1 ppm (^) and 5.1 ppm (%) show successful click coupling. The inset shows the expanded region of the mannose peaks and the RAFT end-group; the methylene adjacent to the thiocarbonylthio-group is marked with $.

Cytotoxicity: MTS assay. 3T3 cells were seeded into 96 well plates, and were allowed to grow to around 90 % confluence. Polymer (100 uL) dissolved in medium (DMEM (Invitrogen) + 10% foetal bovine serum (FBS; Moregate) with 2mM glutamine (Invitrogen)) to various concentrations was then added to each well. Medium in the absence of polymer was used as the control. A non-toxic polymer (PEG400) was used for comparison. Plates were incubated at 37 ^{0}C for 24 or 48 hours. After this time, 20 uL MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) solution (CellTitre 96® Aqueous one) was added to each well, and plates were left to incubate at 37 ^{0}C for a further 3 hours. Following incubation the plates were immediately transferred to a plate reader (Infinite M200 Tecan)) and read at 490 nm, with reference at 650 nm. Each experiment was conducted twice on different days and each experiment gives averages of at least 3 measurements. Figure S5 shows the concentration of live cells for each polymer, normalised to cell proliferation in the absence of polymer. In general, the cytotoxicity of P1 before and after end-group removal is significant compared to the PEG400 standard (F-test, $p < 0.05$, $n = 6$).

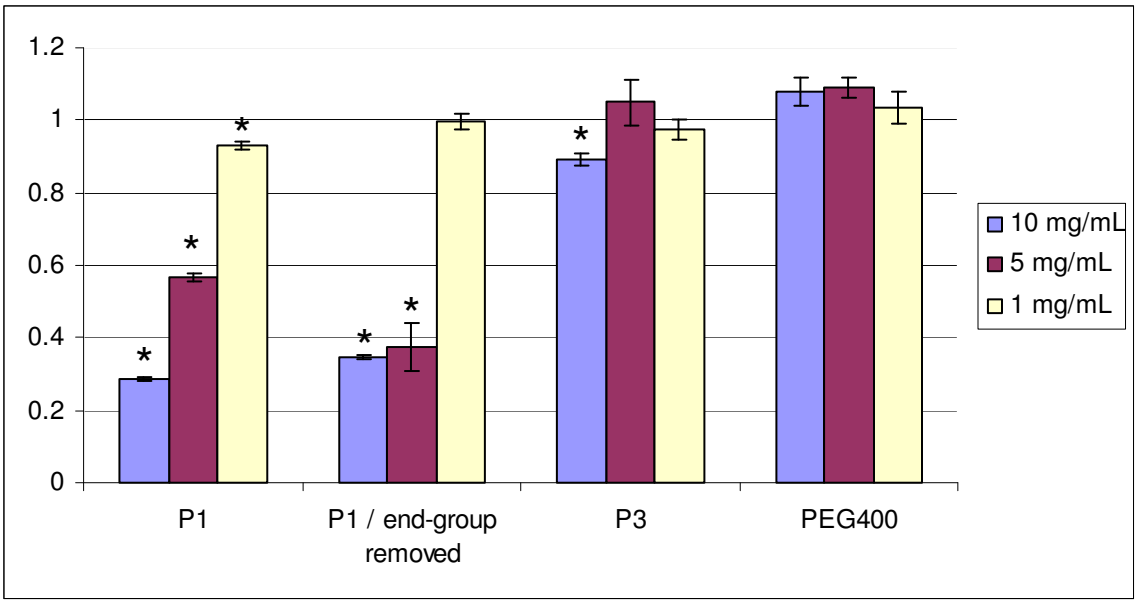

Figure S5. Graph outlining cell proliferation after 24 hour exposure as measured by MTS test (mean ± SD, n=3). Y-axis is normalized to cell proliferation in absence of polymer. *$p < 0.05$ vs cells after exposure to PEG400 (n = 6); error bars are ± SD.

Mannose-binding assay. Concanavalin A (10 mg) was dissolved in freshly prepared assay buffer (0.1 M tris-HCl, 90 μM NaCl, 1 mM Ca^{2+} and 1 mM Mn^{2+}, pH 7.5, 10 mL). The test polymer was dissolved using the same buffer (10 mg/mL). Equal volumes of Concanavalin A and polymer solutions (350 μL of each) were mixed using a pipette for 5-10 seconds and transferred to a 1 mL quartz cuvette (1 cm path length); absorbance (A) at 550 nm was measured with time over 10 minutes. For the case of P2, no change in absorbance was observed over a period of 1 hour. However, polymer 4 rapidly became turbid and an increase in absorbance was observed. This absorbance decreased over time as the aggregated particles fell from solution. Bovine serum albumen (BSA) was used as a control protein having no specific receptor mediated binding for mannose. P4 showed no specific increase in absorbance over 1 hour in the presence of BSA. Figure S6 shows the change in absorbance as a function of time for P4 in the presence of MBL or BSA and P2 in the presence of MBL.

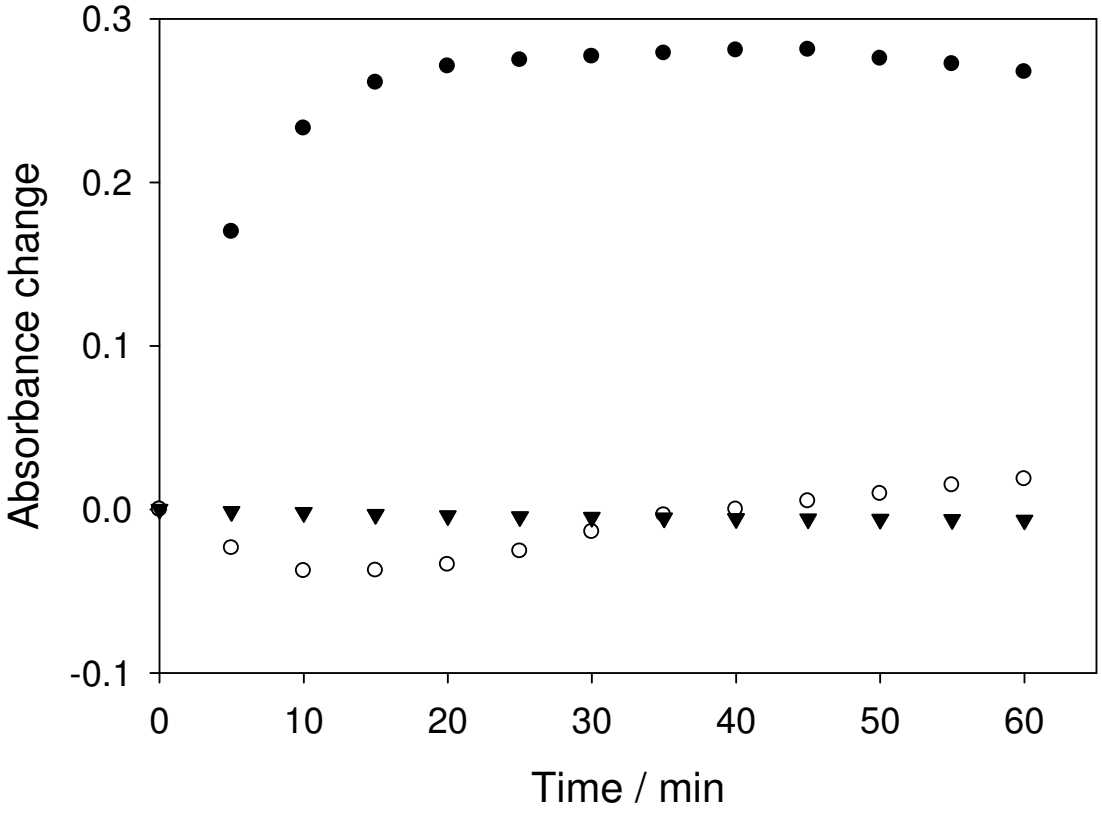

Figure S6. Comparison of the change in absorbance of P2 and P4 in the presence of MBL (open circles and filled circles, respectively). P4 in the presence of a protein that shows no specific binding to mannose (BSA) is also included as a reference (filled triangles).

In vivo images of P3 following intravenous injection.

The non-toxic, chain-extended polymer P3 was also tested for *in vivo* imaging efficacy. P3 was much larger in size and had a PEG-containing shell around the DMAEMA core. PEGylation is well-known to increase circulation time by the EPR effect. Whereas P1 quickly cleared to the bladder where it was in sufficient concentration to image, the location of P3 was less clear due to distribution throughout the vasculature. Figure S7 shows the ^{19}F MRI taken under the same conditions as that for P1 (described in the characterization section) 2 hours following injection.

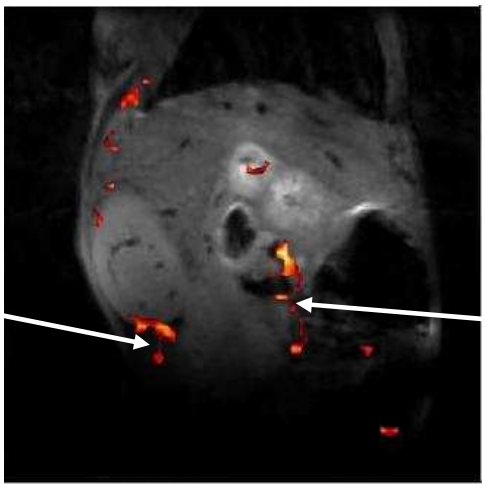

Figure S7. Frontal slice of mouse body 2 hours following injection of P3 into tail-vein. High-resolution proton image is shown in greyscale, with ^{19}F image overlayed. MRI acquisition conditions are the same as those described for imaging P1 in the characterization section.

References.

1. Lai, J. T.; Filla, D.; Shea, R. *Macromolecules* **2002,** 35, (18), 6754-6756.
2. Quemener, D.; Davis, T. P.; Barner-Kowollik, C.; Stenzel, M. H. *Chemical Communications (Cambridge, United Kingdom)* **2006**, (48), 5051-5053.
3. Geng, J.; Mantovani, G.; Tao, L.; Nicolas, J.; Chen, G.; Wallis, R.; Mitchell, D. A.; Johnson, B. R. G.; Evans, S. D.; Haddleton, D. M. *Journal of the American Chemical Society* **2007,** 129, (49), 15156-15163.