Electronic Supporting Information

Materials.

All chemicals and solvents were obtained from Sigma Aldrich and used as received, unless specified. The peptide aptamer with azide modification was synthesised by Mimotopes Australia with peptide sequence as follows: SPWPRPTY.

Synthesis of Hyperbranched Polymer (HBP) core (P1). Polyethyleneglycol monomethylether methacrylate (PEGMA) (10 mmol, 4.39 mL), trifluoroethyl acrylate (TFEA) (2.5 mmol, 0.31 mL), ethylene glycol dimethacrylate (EGDMA) (0.64 mmol, 0.12 mL), RAFT agent (0.54 mmol, 218 mg), azo bisisobutyronitrile (AIBN) (0.053 mmol, 8.75 mg) and 12.5 mL dry THF were added to a 100 mL round bottom flask. The solution was then degassed with argon for 10 min and the reaction was heated to 65ºC and stirred for 24 hours.

The reaction mixture was then added dropwise to a large beaker of stirring hexane (200 mL × 3). The precipitate was dissolved in THF/water and dialyzed against water for three days using snake skin dialysis tubing (MWCO 3.5 kDa) and lyophilized.
^{1}H NMR was used to confirm the structure of the polymer and the major diagnostic peaks are assigned in Figure S1.

GPC (MALLS) M_n = 67000, PDI = 1.3
M_n (NMR) = 12000
Number of trithiocarbonate end-groups per HBP = 5.5

Diameter (DLS) = 6 ± 1.5 nm.

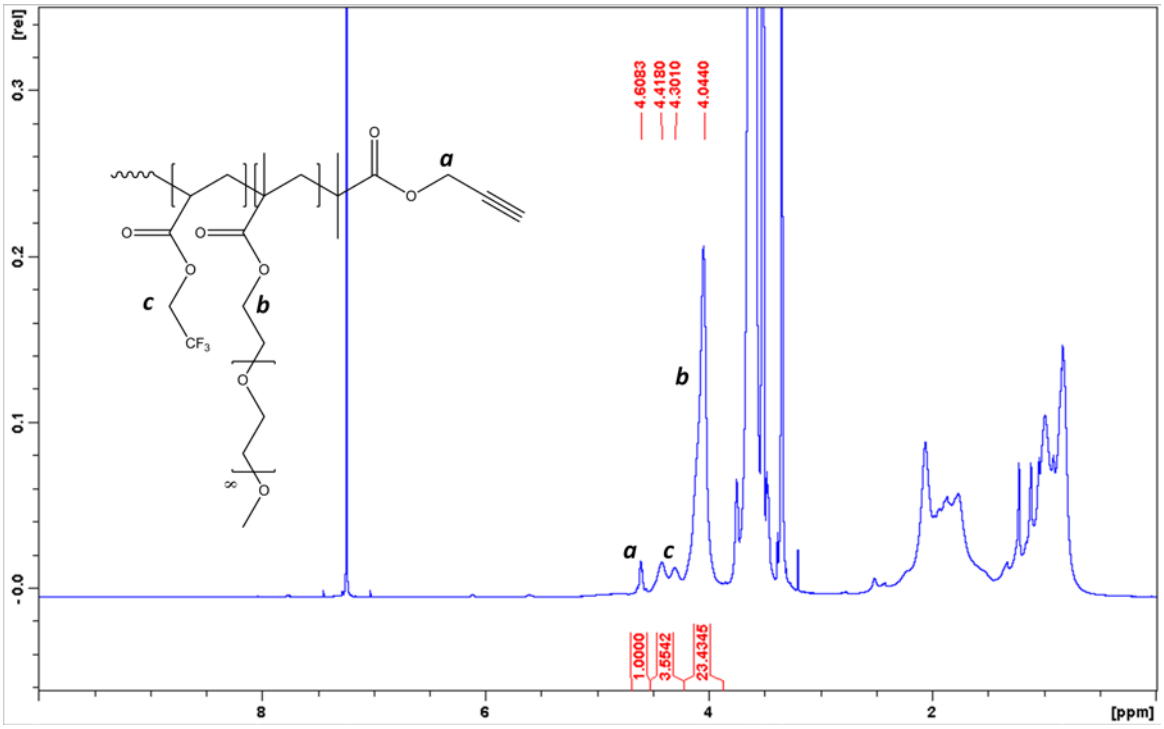

Figure S1. ^{1}H NMR spectrum of hyperbranched poly(polyethyleneglycol monomethylether methacrylate-stat-trifluoroethylacrylate) (P1).

Chain extension of core with PEG-linker (P2). P1 (0.5 g, 4.1×10^{-5} mol end-groups) was dissolved in 50% THF/H_2O (4 mL). Copper sulfate (82 µL, 0.1 M), ascorbic acid (82 µL, 0.2 M) and PEG-linker (11-azido-3,6,9-trioxundecan-1-amine) (32 µL, 1.64×10^{-4} mol) were added and the solution was degassed with nitrogen and the reaction stirred overnight at 60ºC. The product was dialyzed against water for two days using snake skin dialysis tubing (MWCO 10 kDa) and lyophilized. Successful attachment of the linker group was confirmed by appearance of ^{1}H NMR resonance at 7.7 ppm due to the vinyl proton in the triazole ring and two new resonances at 5.1 ppm and 4.5 ppm arising from the methylene protons adjacent to the vinyl group (d in Figure S2) and the nitrogen atom (f in Figure S2) of the triazole ring, respectively. The absence of the peak at 4.57 arising from the methylene protons adjacent to the alkyne group in the starting polymer (Figure S1) confirms that the click reaction proceeded to high conversion.

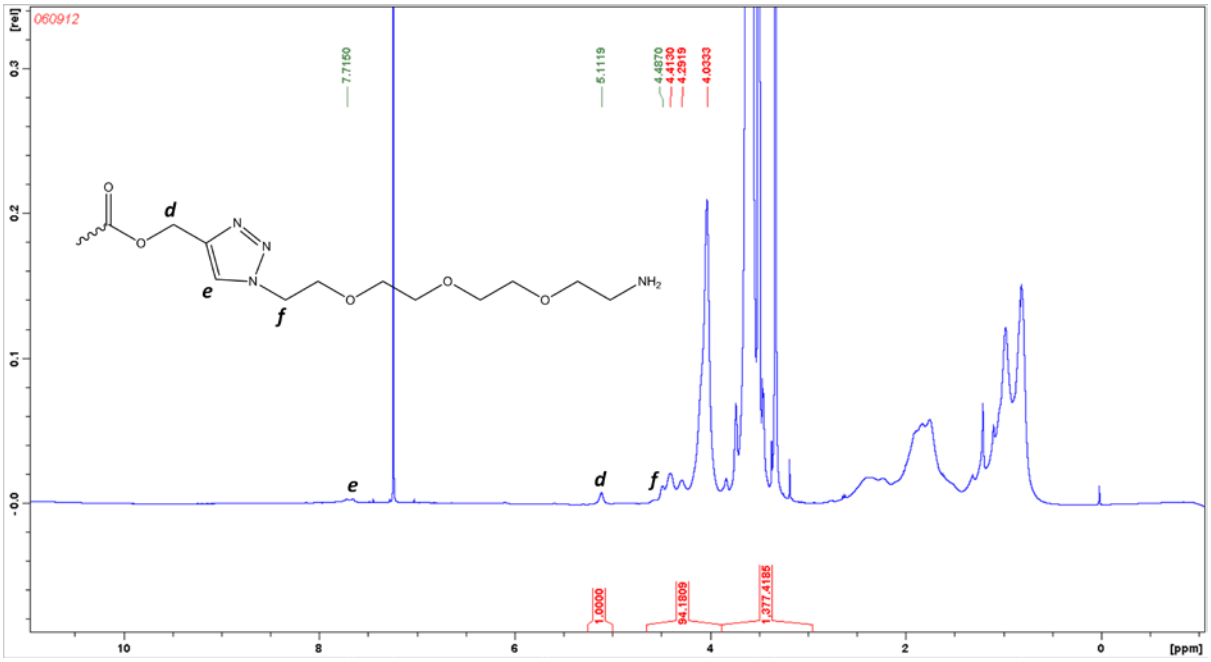

Figure S2. ^{1}H NMR spectrum of hyperbranched poly(polyethyleneglycol monomethylether methacrylate-stat-trifluoroethylacrylate) following chain extension by click chemistry with PEG-linker.

Attachment of Rhodamine B to HBP (non-conjugated control). P2 (0.1 g, 8.2×10^{-6} mol end groups) was dissolved in DMF (2 mL). RhodamineB isothiocyanate (0.85 mg, 1.6×10^{-6} mol) dissolved in DMF 0.5 mL was added and the reaction stirred overnight at room temperature. The product was dialyzed against water for two days using snake skin dialysis tubing (MWCO 10 kDa) and lyophilized.

Quantification of labelling was performed using UV-Vis spectroscopy. ($A_{polymer}$ = 0.117; $c_{polymer}$ = 1.5 x 10^{-5} M; λ_{max} = 555 nm; ε_{RITC} = 80000 $cm^{-1}M^{-1}$; RhodamineB molecules per HBP = 0.1)

Synthesis of aptamer-conjugated, RhodamineB-labelled HBP. a) P2 (0.05 g, 4.1×10^{-6} mol end groups) was dissolved in 50% THF/H_2O (4 mL). Copper sulfate (20 µL, 0.1 M), ascorbic acid (20 µL, 0.2 M) and Aptamer (azide-terminated) (2.8 mg, 2.2×10^{-6} mol) were added and the solution was degassed with nitrogen and the reaction stirred overnight at 60ºC. The product was dialyzed against water for two days using snake skin dialysis tubing (MWCO 10 kDa) and lyophilized.

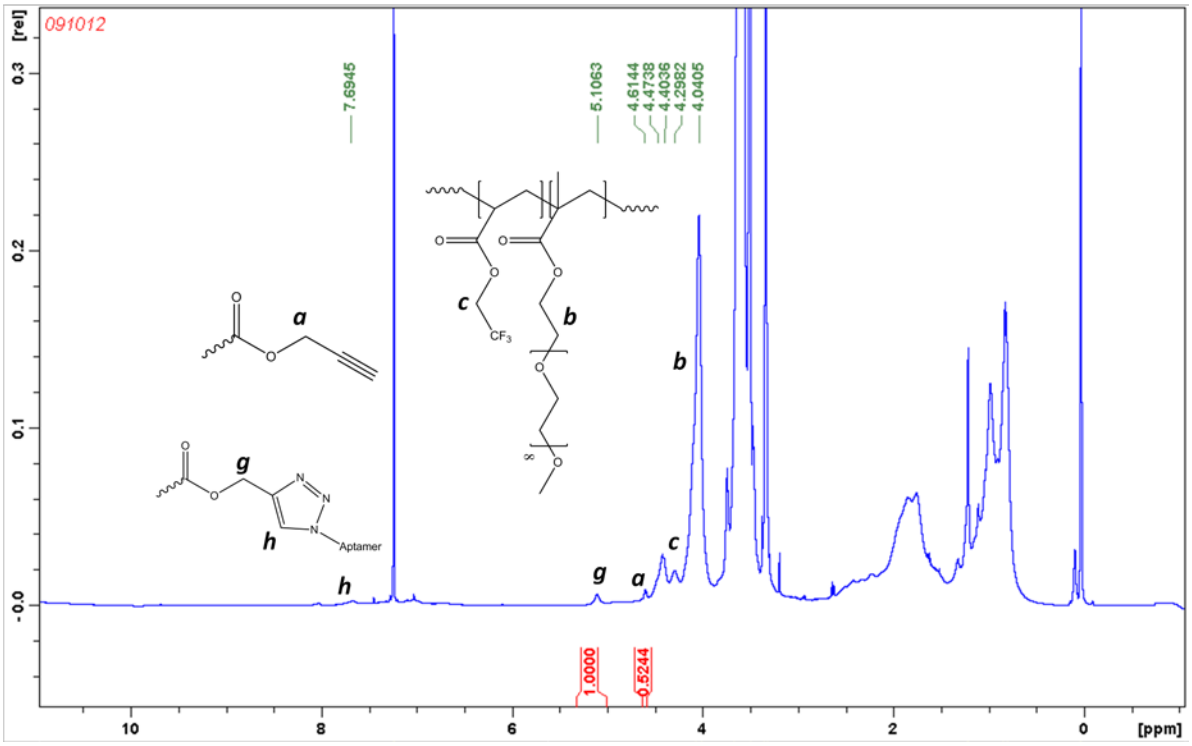

Figure S3. ^{1}H NMR spectrum of hyperbranched poly(polyethyleneglycol monomethylether methacrylate-stat-trifluoroethylacrylate) following chain extension by click chemistry with peptide aptamer.

The product was analysed by ^{1}H NMR. Successful attachment of aptamer was confirmed by the presence of the resonance at 5.1 ppm due to methylene protons adjacent to triazole ring following azide-alkyne cycloaddition (peak g in Figure S3). Approximately 2.7 aptamer peptides were attached per HBP.

b) PEG-Linker (11-azido-3,6,9-trioxundecan-1-amine) (2 µL, 1.0×10^{-5} mol) and RhodamineB isothiocyanate (0.4 mg, 7.5×10^{-7} mol) dissolved in DMF (1 mL) were stirred overnight at room temperature. To this was added the polymer obtained from part a (0.05 g, 4.1×10^{-6} mol end groups) dissolved in H_2O (1 mL), copper sulfate (20 µL, 0.1 M), ascorbic acid (20 µL, 0.2 M) and the solution was degassed with nitrogen and the reaction stirred overnight at 50ºC. The product was dialyzed against water for two days using snake skin dialysis tubing (MWCO 10 kDa) and lyophilized.

Quantification of Rhodamine labelling was performed using UV-Vis spectroscopy. ($A_{polymer}$ (555nm) = 0.438; $c_{polymer}$ = 1.5 x 10^{-5} M; ε_{RITC} = 80000 $cm^{-1}M^{-1}$; RITC molecules per HBP = 0.37).

Synthesis of folate-conjugated, NIR797-labelled HBP. P2 (0.1 g, 8.2×10^{-6} mol end groups) was dissolved in DMF (2 mL). Folic acid (2.9 mg, 6.6×10^{-6} mol) and EDC (3.1 mg, 1.6×10^{-5} mol) were dissolved in DMF (0.5 mL) and added. NIR-797 isothiocyanate (1.4 mg, 1.6×10^{-6} mol) dissolved in DMF 0.5 mL was added and the reaction stirred overnight at

room temperature. The product was dialyzed against water for two days using snake skin dialysis tubing (MWCO 10 kDa) and lyophilized.

Quantification of labelling was performed using UV-Vis spectroscopy. ($A_{polymer}$ (798nm) = 0.79; $A_{polymer}$ (363 nm) = 0.48; $c_{polymer}$ = 1.5 x 10^{-5} M; ε_{NIR797} = 94100 $cm^{-1}M^{-1}$; ε_{Folate} = 6500 $cm^{-1}M^{-1}$; NIR797 molecules per HBP = 0.56; folates per HBP = 4.9).

Characterisation techniques.

Gel permeation chromatography (GPC) was performed using a Waters 333 system fitted with an RI detector. Two Styragel HT3 columns were attached in series and eluted with THF at a flow rate of 1 mL/min at 40^{o}C. A Dawn 8-angle MALLS detector from Wyatt Technologies was used for light scattering experiments. The Astra software package for Windows was used to process the data.

^{1}H Nuclear magnetic resonance spectroscopy (^{1}H NMR) was undertaken on a Bruker Avance 500 spectrometer. $CDCl_3$ was used as solvent for the hyperbranched polymers as described in the text.

Fluorescence Imaging. C57 black mice were injected subcutaneously with ~5 x 10^5 B16 melanoma cells (100 µL, 5 x 0^6 cells/mL) on the left flank. The tumours were allowed to grow for 7 days to a visible, raised tumour mass. In a typical imaging experiment the mouse was anaesthetised with 1-2% vaporised isoflurane in an induction chamber. While maintained under anaesthesia, the mouse was injected with 30 µL of polymer solution (20 mg/mL) via the lateral tail vein and then transferred to a Carestream MS FX Pro imaging station (Carestream Health, Inc., Woodbridge CT, USA). Images were collected continuously for the first hour and the mice were anaesthetised again for each subsequent time point, up to 24 hours. Following the final time point, the animal was culled and organs were collected for *ex vivo* imaging and FACS analysis.

Red fluorescence images were collected with 540 ±10 nm excitation and 600 nm ±17.5 nm emission filter set (f-stop 2.80, 2 x 2 binning, 120 mm FOV, 30 sec exposure time). Near infrared fluorescence images were collected with 750 ±10 nm excitation, 830 nm ±17.5 nm emission filter set (f-stop 2.80, 2 x 2 binning, 120 mm FOV, 30 sec exposure time). For anatomical context, fluorescence images were co-registered with an X-ray image (f-stop 2.80, 0.2 mm aluminium filter, 120 mm FOV, 30 sec acquisition time). All images were batch exported as 16-bit TIFF images and image processing was completed using Image-J (National Institutes of Health, Bethesda, Maryland, USA) and Carestream Multispectral Software (Carestream Health, Inc., Woodbridge CT, USA). Fluorescence images were false coloured (Rhodamine B: red, NIR-797 yellow) and overlayed on the X-ray images.

Cells were analysed on a BD LSR II Analysing Flow Cytometer using DIVA software. Typically, 10000 cells were analysed per experiment.

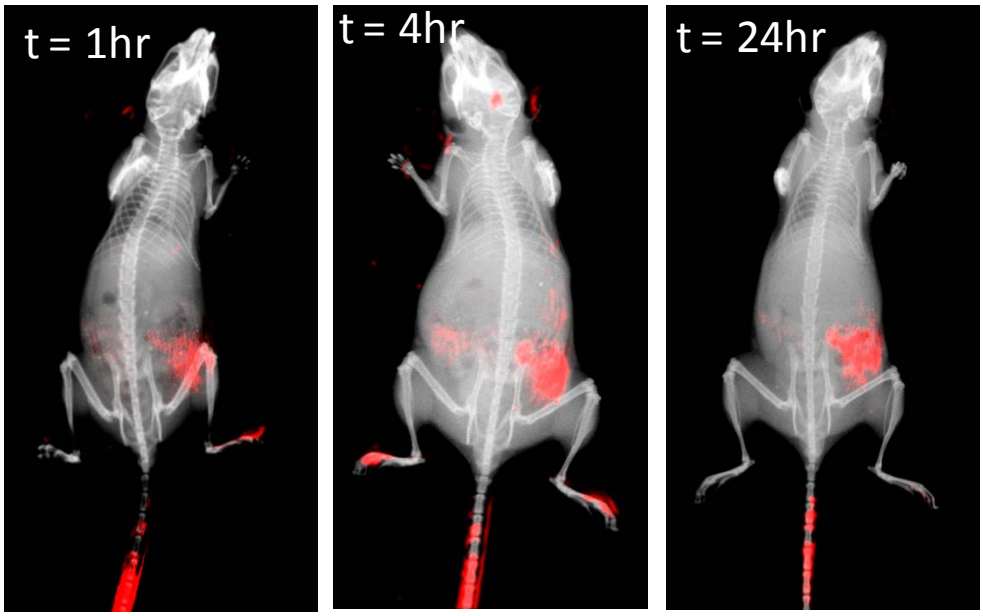

Figure S4. Fluorescence Images of aptamer-conjugated polymer at various timepoints following i.v. injection of 30 µL of aptamer-conjugated polymer solution (20 mg/mL).

***Ex vivo* analysis of organs.** Organs were surgically removed from the mice at specified time-points following iv injection of HBP. Typically, liver (l), kidneys (k), spleen (s), tumour (t), heart (h) and gut (g) were removed and placed in the Carestream imager for analysis.

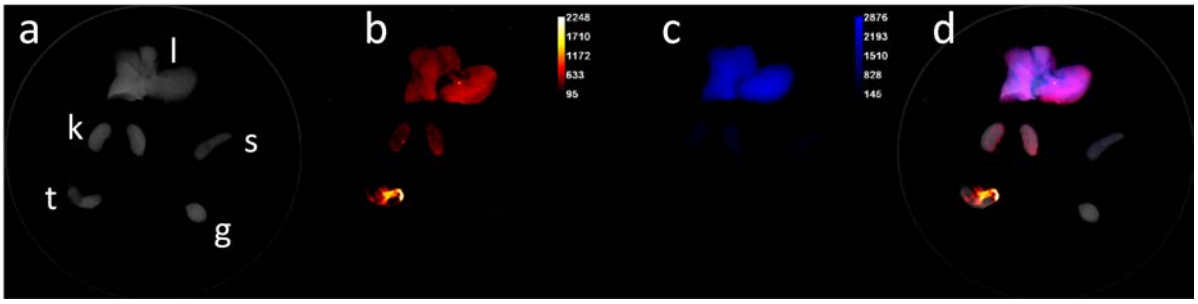

Figure S5. Fluorescence images of various organs (a) following i.v. injection of 50 µL PBS solution containing 10 mg/mL folate-conjugated HBP (c - blue) and 10 mg/mL aptamer-conjugated HBP (b - Red). The overlayed signals are shown in part d. Clearly the aptamer-targeted HBP shows much greater signal in the tumour mass than the folate-targeted polymer.