Electronic Supplementary Material (ESI) for Polymer Chemistry.

Electronic Supplementary Information for:

Effective macrophage delivery using RAFT copolymer derived nanoparticles

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EXPERIMENTAL

Characterisation. Gel permeation chromatography (GPC) was performed on a Waters Alliance system equipped with an Alliance 2695 Separations Module (integrated quaternary solvent delivery, solvent degasser and autosampler system), a Waters column heater module, a Waters 2414 RDI refractive index detector, a Waters PDA 2996 photodiode array detector (210 to 400 nm at 1.2 nm) and 4 × Agilent PL-Gel columns (3 x PL-Gel Mixed C (5 µm) and 1 x PL-Gel Mixed E (3 µm) columns), each 300 mm × 7.8 mm^2, providing an effective molar mass range of 200 to 2 × 10^6). Tetrahydrofuran (THF) high purity solvent (HPLC grade) was pre-filtered through aluminium oxide (90 active neutral, 70-230 mesh) with 0.45 µm filter, and 0.1 g l^{-1} 2,6-di-tert-butyl-4-methylphenol (BHT) was added as inhibitor. The filtered THF containing BHT was purged slowly with nitrogen gas and used as an eluent with a flow rate of 1 ml min^{-1} at 30 °C. Number (M_n) and weight average (M_w) molar masses were evaluated using Waters Empower-3 software. The GPC columns were calibrated with low dispersity polystyrene standards (Polymer Laboratories) ranging from 580 to 7,500,000 g mol^{-1} and molar masses are reported as polystyrene equivalents. A 3rd-order polynomial was used to fit the log M_p vs. time calibration curve, which was near linear across the molar mass ranges.

Proton nuclear magnetic resonance (^{1}H-NMR) (400 MHz) spectra were recorded using a Bruker AV400 spectrometer at 25 °C in deuterated chloroform ($CDCl_3$) to determine monomer to polymer conversions and copolymer compositions, trioxane was used as an internal standard. M_n calculated *via* NMR end-group analysis: RAFT agent (δ1.25, br s, 18 protons) integrated against BMA (δ3.90, br s, 2 protons) and PEGMA-475 (3.64, br s, 28 protons) peaks.

Dynamic Light Scattering measurements were performed on a Beckman Coulter DelsaNano C Particle Analyzer. BRAND 1.5 ml semi-micro polystyrene cuvettes were used.

A Wyatt Technology Optilab rEX refractive index detector was used to determine refractive index increment (dn/dc) values and multi-angle light scattering measurements were taken using a Wyatt

Technology DAWN HELEOS detector. The solvent used for both sets of measurements was aqueous 100 mM $NaNO_3$ with 200 ppm NaN_3 at a flow rate of 0.800 ml min^{-1}.

Materials. Butyl methacrylate (BMA) was purchased from Acros Organics. Methacrylic acid (MAA) and poly(ethylene glycol) methyl ether methacrylate (PEGMA-475) monomers were purchased from Sigma-Aldrich. Inhibitors were removed from the BMA and PEGMA-475 monomers by passing them through an aluminium oxide (Sigma-Aldrich) column prior to use. Inhibitors were removed from MAA *via* distillation. RAFT agent: 4-cyano-4[(dodecylsulfanylthiocarbonyl)sulfanyl] pentanoic acid and *N*-acryloxysuccinimide (NAS) were synthesised according to reported literature procedures.[1,2] The initiator 1,1'-azobis(cyclohexanecarbonitrile) (ACHN) was purchased from Sigma-Aldrich and used as received.

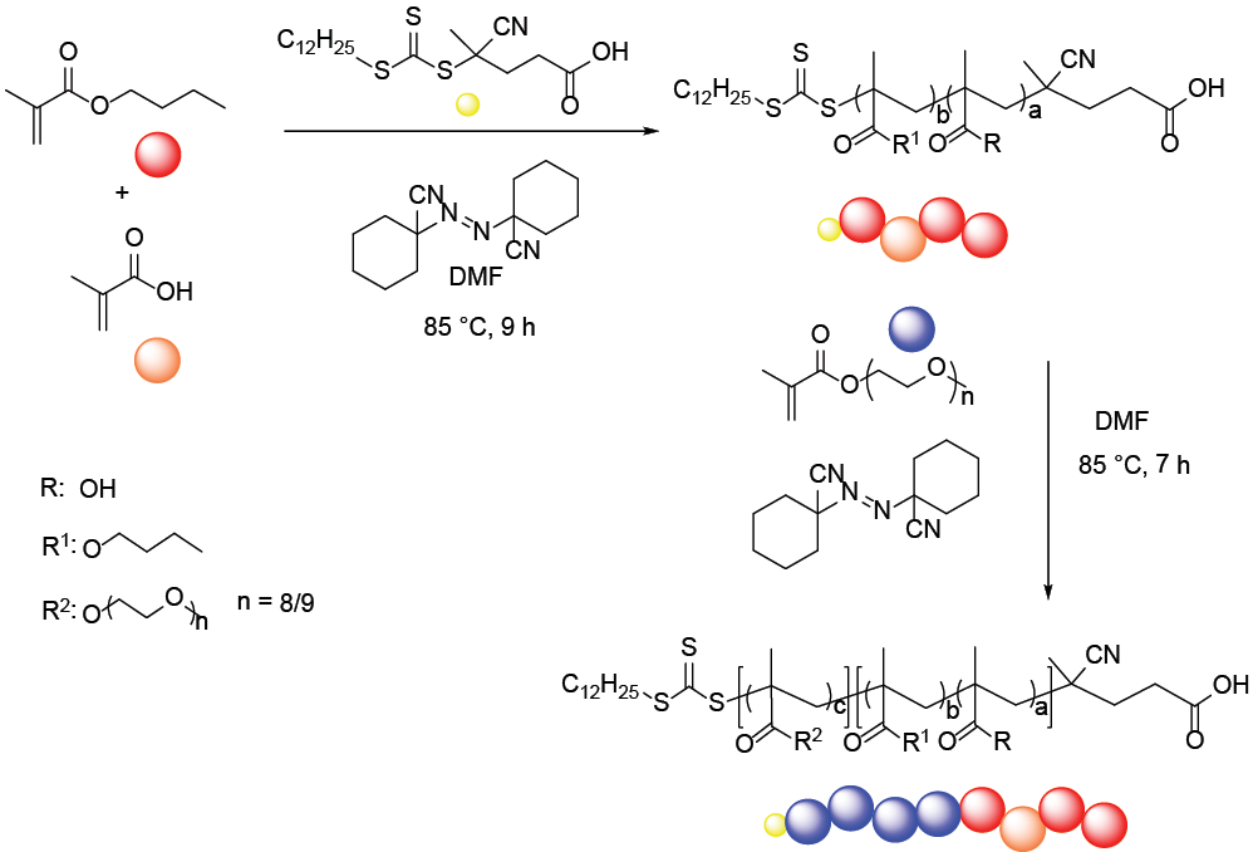
General Methods. Polymers were purified *via* dialysis. Dialysis tubing with a 3,500 MWCO and an average flat width of 54 mm was used, purchased from Spectrum Labs. Reaction mixture containing synthesised polymer was pipetted into the dialysis tubing and dialysis tubing closures, purchased from Spectrum Labs, were placed at each end. Dialysis tubing containing the solution was then placed in 1 l deionised water for 48–72 h. Water was replaced every 24 h.

Automated parallel synthesiser. Parallel synthesis reactions were carried out using a Chemspeed Swing-SLT automated parallel synthesiser. The synthesiser was equipped with a glass reactor block consisting of 16 reaction vessels (13 ml) with thermal jackets connected in series through the reaction block and connected to a heating/cooling system (Hüber, -90 °C to 140 °C). In addition, all reaction vessels were equipped with cold-finger reflux condensers (~7 °C). Mixing was achieved by vortex agitation (up to 1400 rpm). Liquid transfers were handled by a 4-needle head (4-NH) capable of four simultaneous sample transfers. The 4-NH was connected to a reservoir bottle (degassed DMF solvent) for needle rinsing after each liquid transfer step. This DMF solvent reservoir was degassed *via* continuous sparging with nitrogen and was also utilised to prime the tubing lines of the 4-NH. When experiments were performed, the synthesiser was maintained under an inert atmosphere by supplying a constant flow of nitrogen into the

hood of the synthesiser. A nitrogen atmosphere was also applied to reactors and stock solutions at all times. Prior to the experiments, the reaction vessels were cooled to -90 °C and subjected to 10 cycles of vacuum (2 min each) and filling with nitrogen (2 min each) to ensure the elimination of oxygen. After this pre-treatment, the RAFT polymerisation experiments were performed.

Automated synthesis of quasi-block copolymers.

Scheme S1. Quasi-block copolymerisation reaction



Copolymer formed in the first step of the reaction acts as a macroRAFT agent in the block copolymerisation, a = 4–81, b = 27–198, c = 11–32. ACHN was used as the initiator in both steps of the reaction. Square parentheses represent copolymer blocks.

BMA, MAA, PEGMA-475, DMF and DMF solutions of ACHN and the RAFT agent, 4-cyano-4[(dodecylsulfanylthiocarbonyl)sulfanyl] pentanoic acid were all degassed for approx. 30 min *via* sparging with nitrogen before being loaded into the pre-programmed automated synthesiser (quantities in Table S1). The Chemspeed automated synthesiser transferred the programmed quantities of BMA, MAA,

initiator, RAFT agent and DMF into the reaction vessels. The Chemspeed was then sealed to air and filled with nitrogen and before reactors were heated to 85 °C while under vortex (approx. 300 rpm). The temperature of the reflux condensers on top of the reactors was set to 7 °C. Polymerisation reactions were considered to have started once the reaction temperature had been reached in the apparatus. The reactions were heated for 9 h before being cooled to 20 °C. Aliquots of 200 µl were taken from the reaction mixtures. The aliquots were removed using the automated liquid handling system and place into NMR tubes and size exclusion chromatography (SEC) vials (75 µl each) to determine the monomer conversion and molecular weight at the end of the reactions. NMR and SEC samples were then prepared by the automated liquid handling system on the automated synthesiser by adding the necessary solvents (deuterated chloroform and tetrahydrofuran respectively). PEGMA-475 and a second portion of ACHN (to keep the initiator concentration consistent in both reactions, see Table S1 for quantities) were then added to the reaction vessels. The reactions were heated to 85 °C for a further 7 h before cooling to 20 °C. Aliquots of 200 µl were taken at the end of the reaction for NMR and SEC analysis. Polymers were purified *via* dialysis and isolated *via* lyophilisation.

Bench synthesis of quasi-block copolymers. BMA, MAA, ACHN, RAFT agent 4-cyano-4[(dodecylsulfanylthiocarbonyl)sulfanyl] pentanoic acid and trioxane (approximately 10 mg) were dissolved in DMF. Reaction quantities can be found in Table S1. The solution was then transferred to a Young's tap Schlenk flask and the mixture was degassed *via* the freeze-pump-thaw method (3 × 20 min cycles). After the solution had warmed to room temperature it was heated at 85 °C for 9 h. Upon completion of the reaction, the mixture was allowed to cool before PEGMA-475, ACHN and DMF (Table S1) were added to the reaction vessel. The mixture was then degassed again, *via* the freeze-pump-thaw method (3 x 20 min cycles) and then allowed to warm to room temperature before being heated at 85 °C for 7 h. Upon cooling the quasi-block copolymers were purified *via* dialysis and then isolated *via* lyophilisation.

Synthesis of pure block copolymers. Pure block copolymers were synthesised in the same fashion as quasi-block copolymers with the exception that after synthesis of the p(BMA-*co*-MAA) first block, the reaction mixtures were purified *via* dialysis for 48 h and then lyophilised. The copolymer macroRAFT agents were then dissolved in DMF and PEGMA-475 and ACHN (quantities in Table S1) were added prior to degassing *via* the freeze-pump-thaw method (3 × 20 min cycles) in preparation for the second reaction.

Table S1. Millimoles of reagents used to synthesise block copolymers.

Sample	BMA	MAA	RAFT	ACHN	DMF	PEGMA	ACHN(2)
(1)	3.72	1.86	0.15	0.02	32.43	1.57	7.00×10^{-3}
(2)	4.50	0.50	0.15	0.02	32.43	1.57	7.00×10^{-3}
(3)	4.50	0.50	0.15	0.02	32.43	1.57	7.00×10^{-3}
(4)	4.21	1.05	0.15	0.02	32.43	1.57	7.00×10^{-3}
(5)	4.50	0.50	0.15	0.02	32.43	1.57	7.00×10^{-3}
(6)	4.50	0.50	0.10	9.62×10^{-3}	22.57	1.52	4.81×10^{-3}
(7)	4.50	0.50	0.07	7.12×10^{-3}	22.57	1.50	3.56×10^{-3}
(8)	4.50	0.50	0.15	0.02	22.57	1.57	7.43×10^{-3}
(9)	9.00	1.00	0.15	0.02	32.43	1.57	7.00×10^{-3}
(10)	4.50	0.50	0.15	0.02	22.57	3.14	7.43×10^{-3}
(11)	3.72	1.86	0.15	0.02	32.43	1.57	7.00×10^{-3}
(12)	4.21	1.05	0.15	0.02	32.43	1.57	7.00×10^{-3}
(13)	4.50	0.50	0.10	7.22×10^{-3}	22.57	1.52	4.81×10^{-3}
(14)	4.50	0.50	0.15	0.02	32.43	1.57	7.00×10^{-3}
(15)	4.50	0.50	0.15	0.02	32.43	1.57	7.00×10^{-3}
(16)	4.50	0.50	0.07	5.34×10^{-3}	22.57	1.50	3.56×10^{-3}
(17)	18.00	2.00	0.15	0.02	32.43	3.14	7.00×10^{-3}
(18)	4.50	0.50	0.15	0.02	32.43	1.57	7.00×10^{-3}
(19)	4.50	0.50	0.15	0.02	22.57	4.70	7.43×10^{-3}
(20)	4.50	0.50	0.07	7.12×10^{-3}	22.57	0.75	3.56×10^{-3}
(21)	4.50	0.50	0.15	0.02	32.43	1.57	7.00×10^{-3}
(22)	4.50	0.50	0.15	0.02	32.43	1.57	7.00×10^{-3}

Quantities (millimoles) used in reactions to synthesise library of block copolymers.

Table S2. Percentage conversion values and yields for synthesised block copolymers.

Sample	% conversion BMA-co-MAA block	% conversion PEGMA block	% Yield
(1)	60	57	59
(2)	61	57	59
(3)	61	57	59
(4)	59	67	63
(5)	52	67	59
(6)	79	57	68
(7)	75	52	64
(8)	83	86	84
(9)	73	67	71
(10)	97	95	96
(11)	71	67	69
(12)	58	67	62
(13)	76	51	63
(14)	75	86	80
(15)	75	76	76
(16)	72	48	60
(17)	47	38	44
(18)	69	86	77
(19)	86	70	74
(20)	72	67	70
(21)	64	76	70
(22)	61	57	60

Percentage conversion values calculated from ^{1}H NMR end group analysis and integration against an internal standard (trioxane).

Fluorescent labelling and crosslinking.

Four methods; A, B, C and D were used to incorporate NAS and MRhB monomers into the synthesised block copolymers**.** Method A involved the synthesis of a rhodamine triblock copolymer: p(BMA-*co*-MAA)-*b*-p(PEGMA-475)-*b*-p(NAS-*co*-MRhB). In method B the tetrablock copolymer, p(BMA-co-MAA)-*b*-p(PEGMA-475)-*b*-p(NAS)-*b*-p(MRhB) was synthesised. Method C was the synthesis of a rhodamine triblock copolymer: p(BMA-*co*-MAA)-*b*-p(PEGMA-475-*co*-NAS)-*b*-p(MRhB) and method D, the synthesis of a rhodamine diblock copolymer: p(BMA-*co*-MAA)-*b*-p(PEGMA-475-*co*-NAS-*co*-MRhB). Method A was used for the majority of polymers and is detailed below, the same quantities and standard polymerisation procedures were used in methods B, C and D. Method A: p(BMA-*co*-MAA)-*b*-p(PEGMA-475) block

copolymer macroRAFT agent (20 µmol), NAS (60 µmol), MRhB (1 µmol) and ACHN (2 µmol) were dissolved in DMF (1.5 ml, 19.5 mmol). Due to the small quantity of ACHN required, larger amounts were weighed out and diluted. The reaction mixtures were transferred to a Young's tap Schlenk flask and then degassed using the freeze-pump-thaw method (3 x 20 min cycles) before being heated at 85 °C for 7 h. The products were purified *via* dialysis in water and then lyophilised to isolate the product.

To crosslink the micelles; polymers (7 µmol) were dissolved in H_2O (500 ml) above the criticial micelle concentration (see page S13). The polyetheramine, JEFFAMINE D230 was added (2 mg, 10 µmol) under nitrogen and the reaction was stirred for 20 h at room temperature. A diamine: polymer molar ratio of 7: 10 was used to give a diamine: NAS ratio of approximately 2: 1. Due to solubility issues methanol had to be added to some reaction mixtures prior to the addition of JEFFAMINE. In such instances methanol was removed once the polymer had dissolved and did not affect crosslinking.

Encapsulation of DAPI.

Crosslinked micelle particles (3 mg) were dissolved in water (2.3 ml) to give a 1.3 mg/ml solution and 4',6-diamidino-2-phenylindole solution (DAPI) (720 µl, 5 mg/ml) was added. Reaction mixture was vortexed for 1 min and then stirred for 24 h at room temperature in the absence of light. Micelle particles were purified *via* dialysis in water for 48 h.

LIVE SUBJECT STATEMENT: All experiments were performed in compliance with the National Health and Medical Research Council of Australia's guidelines and were approved by the Monash Animal Research Platform ethics committee.

Mice. Cells isolated for this work were scavenged from C57Bl/6 mice utilised in experiments approved by the MARP animal ethics committee. Mice were bred at Monash Animal Services (Monash University, Clayton, Australia) and ranged in age from 6–12 weeks. Assays were carried out on different days with

cells harvested from different mice. Four mice were used per experiment and the cells were pooled before being labelled and then divided into incubation tubes.

Isolation of bone marrow cells. The femur, tibia and iliac crest were removed from the hind legs of mice. The bones were cleaned of muscle and fat and then crushed in phosphate buffer saline containing 2% fetal bovine serum (PBS-2% FBS) using a mortar and pestle. The cells were washed with PBS-2% FBS, centrifuged at 400 × gravity at 8 °C and the supernatant decanted.

Isolation of peritoneal cells. The outer skin was removed from the area surrounding the peritoneal cavity and PBS-2% FBS (10 ml) was slowly injected. The area was gently massaged to dislodge the maximum number of cells before the cell suspension was removed. Approximately 2×10^6 cells were obtained per mouse.

Flow cytometry and cell sorting. Fluorescence activated cell sorting was performed using an Influx Cell Sorter (BD Biosciences) equipped with 4 lasers: 450 nm, 488 nm, 561 nm and 635 nm. A 70 µm nozzle was used. Cells were sorted at 25,000 cells sec^{-1}. Flow cytometry analysis was performed on a LSR II (BD Biosciences) with 7 solid state lasers: 355 nm 405 nm, 488 nm, 532 nm, 561 nm, 592 nm and 628 nm. Flowjo X was used to analyse all flow cytometry data.

The internalisation of particles was determined by rhodamine fluorescence detected in cells. Rhodamine was excited with the 561 nm laser and fluorescence emission was detected with a 564–606 nm band pass filter. The delivery of DAPI to cells was determined by DAPI fluorescence detected in cells. DAPI was excited with the 355 nm laser and detected with a 425–475 nm band pass filter.

Antibody labelling. Cells were immunolabelled for cell sorting and flow cytometry with an antibody cocktail containing Gr-1-FITC, CD11b-APCCy7 and F4/80-AF647. When fluorescence microscopy was used, cells were also labelled with CD45-BV510 to assist in identifying hematopoietic cells. Antibody details for cell sorting and analysis are displayed in Table S3.

Table S3. Antibodies used for cell sorting

Antibody	Description	Clone	Conc. (μg/ml)	Supplier
Gr-1-FITC	FITC anti-mouse Ly-6G and Ly-6C (Gr-1)	RB6-8C5	0.25	BD Pharmigen
CD11b-APC-Cy7	APC-Cy7 Rat anti-mouse CD11b	M1/70	0.50	BD Pharmigen
F4/80-AF647	Alexa Fluor 647 anti-mouse F4/80	BM8	10	Biolegend

Antibodies used at concentrations displayed.

Sorting strategy. Sorted populations of macrophages (Gr-1$^-$, Mac-1$^+$, F4/80$^+$ cells) and non-macrophages (Gr-1$^-$, Mac-1$^-$, F4/80$^-$ cells) were collected from the sort, gates outlined in Figure S1. It should be noted that peritoneal macrophages were GR-1^{low} as opposed to GR-1$^-$. Peritoneal macrophages also had much higher levels of F4/80 than bone marrow macrophages.

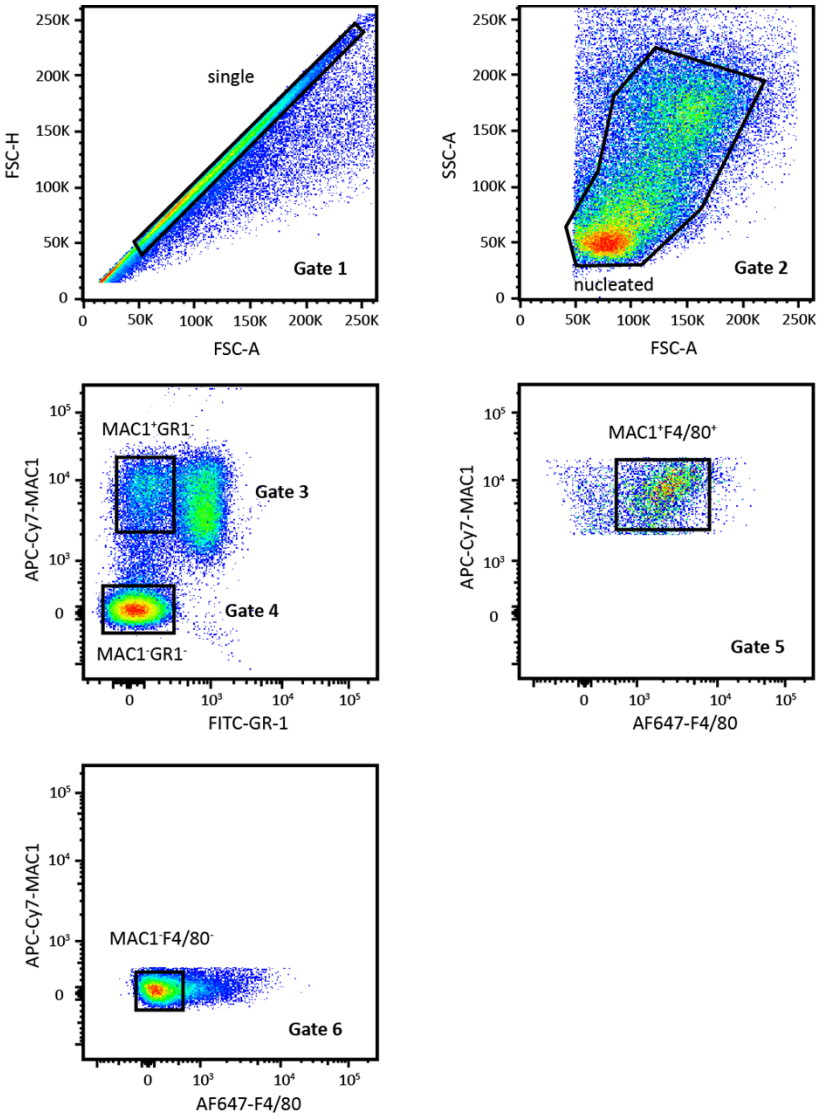


Figure S1. Gating strategy for both macrophage and non-macrophage populations. Each dot on a graph represents one event, i.e. a cell. Gate 1 represents single cells and doublets and aggregates. Gate 2 represents nucleated cells and excludes cell debris. Gates 3 represents Mac-1^{+}/Gr-1^{-} cells and Gate 4 represents Mac-1^{-}/Gr-1^{-} cells. Gate 5 represents Mac-1^{+}/Gr-1^{-}/F4/80^{+} macrophages. Gate 6 represents Mac-1^{-}/Gr-1^{-}/F4/80^{-} non-macrophage population.

GENERAL PROCEDURE FOR CELL INTERNALISATION STUDIES

Cell internalisation assay. Sorted cell populations: A solution of particles (200 µl, 100 µg/ml) in PBS 0.5 % bovine serum albumin (PBS 0.5% BSA) were added to a suspension of 2×10^{5} sorted cells (in 1 ml PBS-2%

FBS) (giving final particle concentrations of 17 μg/ml) and the resultant mixture incubated at 30 min at 37 °C. Cells incubated with 2 μm Pacific Blue labelled latex beads, known to be phagocytosed only by macrophages, were used to confirm the presence of functional macrophages. Cells incubated with PBS 0.5% BSA in the absence of nanoparticles were used as negative controls. Following incubation, cells were washed and resuspended in propidium iodide (PI; 0.1 μg/ml) in PBS-2% FBS at a density of approximately 50×10^6 cells/ml for flow cytometric analysis as described above.

Unsorted cell populations: Whole bone marrow cells or peritoneal cells were immunolabelled as described above, washed and resuspended to 200,000 cells/ml in PBS-2% FBS. Solutions of particles dissolved in PBS 0.5% BSA (500 μl, 100 μg/ml) were added to 500,000 cells (2.5 ml in PBS-2% FBS) (giving final particle concentrations of 17 μg/ml) and the resultant mixture incubated for 30 min at 37 °C. Controls used were the same as described above for sorted cells. Following incubation, cells were washed and then resuspended in PI as described above for flow cytometric analysis.

Fluorescence microscopy. Fluorescence microscopy images were acquired using an Olympus BX51 microscope. After FACS analysis, cells were transferred to 2 ml eppendorf tubes, centrifuged at 400 × gravity, dry pelleted and resuspended in 5 μl of VECTASHIELD Antifade Mounting Medium (Vector Laboratories). Cell suspensions were transferred to Thermo Scientific Superfrost microscope slides with 20 mm × 20 mm glass coverslips prior to imaging.

EFFECT OF CONCENTRATION ON MICELLE FORMATION

Different concentrations, ranging from 7.8×10^{-7} to 5.0×10^{-5} mol dm^{-3}, of p(BMA-*co*-MAA)-*b*-p(PEGMA-475) in water were made up and analysed *via* dynamic light scattering (DLS) and the pendant drop method to determine the critical micelle concentration (CMC). Pendent drop method surface tension measurements were carried out on a KRUSS Drop Shape Analysis System DSA 10, using water as the liquid. Drops were slowly pushed from the needle and the drop pendent was captured by the camera before the drop left the needle. Images were analysed using Drop Shape Analysis software and interfacial tension

(IFT) values were obtained. 6 × drops were analysed and then averaged for each sample concentration. The concentrations used for the pendant drop analysis and DLS were: 100, 75, 50, 25, 13, 6.3, 3.1, 1.6, and 0.78 μmol dm^{-3}.

Table S4. Comparison of sample concentration and micelle size.

Sample concentration (mol dm^{-3})	Size of micelle formed (nm)	Range in micelle size (nm)
5.0×10^{-5}	30	27-34
2.5×10^{-5}	34	31-37
1.3×10^{-5}	32	28-36
6.3×10^{-6}	53	41-64
3.1×10^{-6}	70	61-80
1.6×10^{-6}	56	42-70
7.8×10^{-7}	115	53-178

DLS analysis showed that micelles formed at concentrations as low as 7.8×10^{-7} mol dm^{-3}, however, micelle size remained constant when the concentration was 1.3×10^{-5} mol dm^{-3} or above. Micelles formed at concentrations lower than 1.3×10^{-5} mol dm^{-3} were considerably larger. The range in micelle size also increased as the concentration was decreased below 1.3×10^{-6} mol dm^{-3}, indicating instability. It was therefore concluded that stable micelles formed at concentrations higher than 1.3×10^{-5} mol dm^{-3}. Data obtained using the pendant drop method supported this conclusion (Figure S3).

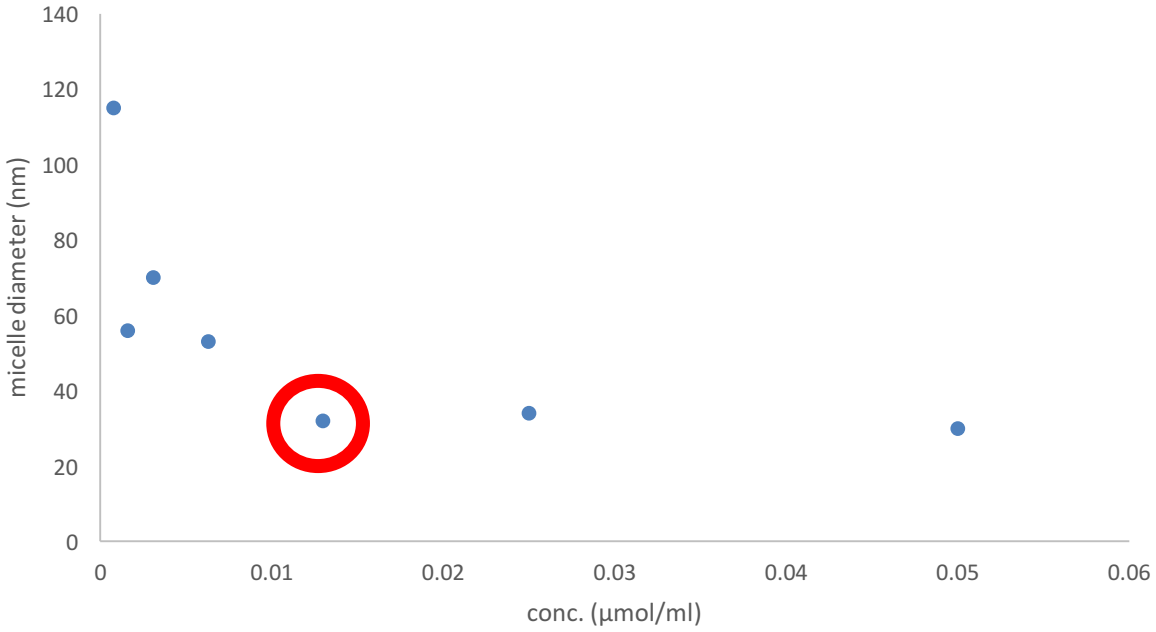


Figure S2. Plot showing micelle size *vs* polymer concentration. Change in gradient at 0.013 mmol dm^{-3} indicated the CMC.

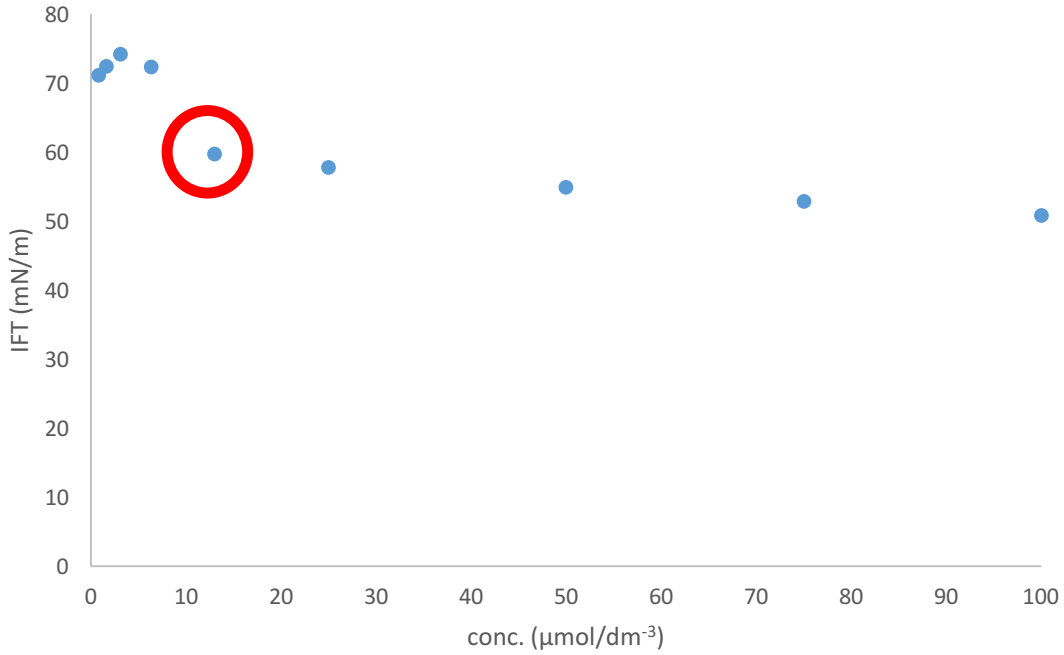


Figure S3. Plot of interfacial tension (IFT) measurement against polymer concentration. Change in gradient at 1.3×10^{-5} mol dm^{-3} indicated the CMC.

EFFECT OF TEMPERATURE ON MICELLE FORMATION

0.5 mg ml^{-1} solutions of quasi-block copolymers p(BMA-*co*-MAA)-*b*-p(PEGMA-475) in 10 mmol NaCl were analysed using DLS. Micelle size at 25, 30, 35, 40, 45, 50, 55, 60, 65 and 70 °C was measured to determine the stability of micelles when heated.

Table S5. Effect of temperature on micelle size.

Temperature (°C)	Micelle A diameter (nm)	Micelle B diameter (nm)	Micelle C diameter (nm)
25	16	25	36
30	17	26	35
35	17	24	42
40	17	25	36
45	18	23	34
50	17	23	34
55	18	23	40
60	17	23	37
65	18	23	35
70	17	23	35

Micelle A: QB p(BMA-*co*-MAA)-*b*-p(PEGMA-475)-*b*-p(NAS)-*b*-p(MRhB), 9: 1 BMA: MAA. Micelle B: QB p(BMA-*co*-MAA)-*b*-p(PEGMA-475-*co*-NAS), 9: 1 BMA: MAA. Micelle C: PB p(BMA-*co*-MAA)-*b*-p(PEGMA-475)-*b*-p(NAS-*co*-MRhB), 4: 1 BMA: MAA.

Table S6. Comparison between M_n of polymer blocks and micelle size.

Sample	Total M_n (Da)	M_n of BMA/MAA block (Da)	BMA:MAA ratio	M_n of PEGMA block (Da)	Micelle diameter (nm)
(1)	5,900	3,000	2: 1	2,900	25
(2)	5,900	3,100	9: 1	2,800	16
(3)	5,900	3,100	9: 1	2,800	16
(4)	6,300	3,000	4: 1	3,300	27
(5)	5,900	2,600	9: 1	3,300	36
(6)	10,200	5,900	9: 1	4,300	42
(7)	12,700	7,500	9: 1	5,200	59
(8)	8,400	4,200	9: 1	4,200	37
(9)	10,600	7,300	9: 1	3,300	124
(10)	14,400	4,900	9: 1	9,500	47
(11)	6,900	3,500	2: 1	3,400	24
(12)	6,200	2,900	4: 1	3,300	25
(13)	9,500	5,700	9: 1	3,800	57
(14)	8,000	3,700	9: 1	4,300	26
(15)	7,600	3,800	9: 1	3,800	40
(16)	11,900	7,200	9: 1	4,700	52
(17)	13,200	9,400	9: 1	3,800	143
(18)	7,700	3,400	9: 1	4,300	29
(19)	14,700	4,300	9: 1	10,400	49
(20)	10,600	7,200	9: 1	3,400	54
(21)	7,000	3,200	9: 1	3,800	42
(22)	6,000	3,100	9: 1	2,900	16

Higher M_n values generally resulted in larger micelles. M_n calculated *via* NMR end-group analysis of the RAFT agent (δ1.25, br s, 18 protons) integrated against BMA (δ3.90, br s, 2 protons) and PEGMA-475 (3.64, br s, 28 protons) peaks. Percentage MAA was too low to be accurately determined *via* end group analysis. MAA conversion was calculated from T_0 and T_x time points (MAA: δ5.97, br s) using an internal standard (trioxane, δ4.96, s). Micelle diameter was measured using DLS at 25 °C.

EFFECT OF RAFT END-GROUP REMOVAL ON MICELLE SIZE

The RAFT end group was removed from a number of quasi-block copolymers to analyse the effect that this would have on the size of the micelle particle formed, Table S7). The disappearance of the peak from the hydrocarbon chain on the RAFT agent (δ 1.25) in the ^{1}H NMR indicated that the RAFT group was successfully removed from all samples. Micelle size did not vary significantly once the RAFT end group had been removed.

Table S7. Comparison of micelles formed from polymer chains with and without the RAFT end group attached.

Sample	BMA:MAA ratio	MW of polymer (Da)	Micelle diameter when RAFT end group attached	Micelle diameter when RAFT end group removed
1	9:1	11,900	**124 nm** (99-174)	**139 nm** (128-151)
2	9:1	17,900	**129 nm** (68-182)	**140 nm** (136-144)
3	9:1	25,900	**123 nm** (70-151)	**140 nm** (119-191)
4	2:1	11,700	**25 nm** (15-47)	**22 nm** (18-25)
5	2:1	13,600	**159 nm** (146-169)	**175 nm** (149-229)
6	2:1	24,800	**92 nm** (64-111)	**133 nm** (130-136)

Average micelle diameters shown in bold, the range is shown in parentheses.

Table S8. Behaviour of polymers in a variety of solvents pre and post crosslinking reaction.

	Solvent				
Polymer	**Water (nm)**	**Hexane (nm)**	**THF (nm)**	**Isopropanol (nm)**	**Toluene (nm)**
p(BMA-*co*-MAA)-*b*-p(PEGMA-*co*-NAS)	27	No micelles formed	No micelles formed	No micelles formed	No micelles formed
Crosslinked p(BMA-*co*-MAA)-*b*-p(PEGMA-*co*-NAS)	49	Sample insoluble	90	96	No micelles formed
p(BMA-*co*-MAA)-*b*-p(NAS)-*b*-p(PEGMA)	22	No micelles formed	No micelles formed	No micelles formed	No micelles formed
Crosslinked p(BMA-*co*-MAA)-*b*-p(NAS)-*b*-p(PEGMA)	31	Sample insoluble	130	119	No micelles formed
p(BMA-*co*-MAA)-*b*-p(PEGMA)-*b*-p(NAS-*co*-MRhB)	16	Sample insoluble	No micelles formed	No micelles formed	No micelles formed
Crosslinked p(BMA-*co*-MAA)-*b*-p(PEGMA)-*b*-p(NAS-*co*-MRhB)	36	Sample insoluble	88	121	No micelles formed

Average diameter of micelle particles formed given in nm, measured using DLS at 25 °C.

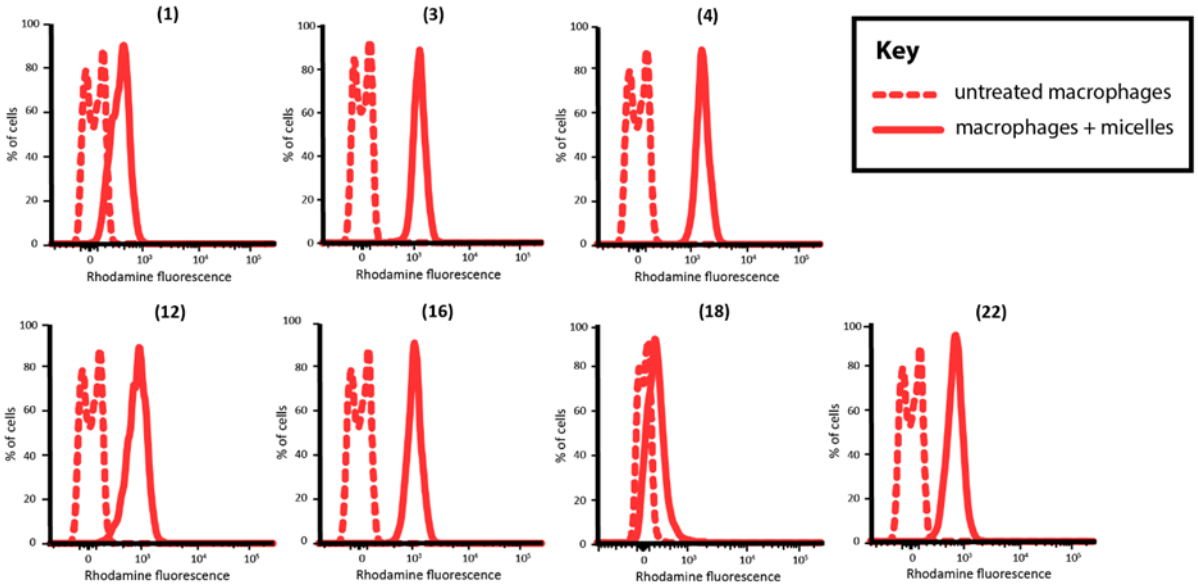


Figure S4. FACS histograms showing the internalisation of different particles by macrophages. Particle details given in the main text, Table 2.

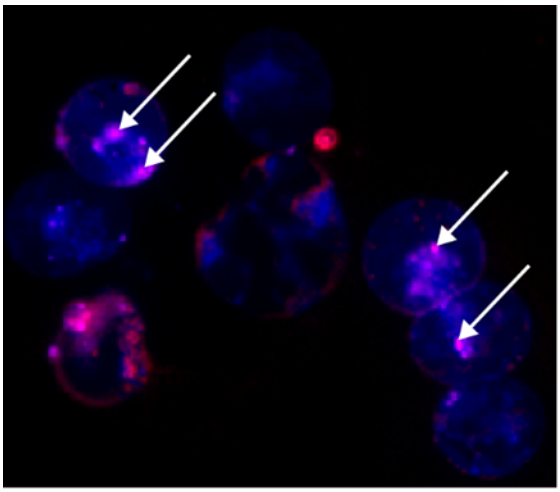


Figure S5. Fluorescent microscope image of uptake of sample (10) by primary bone marrow macrophages purified by FACS; rhodamine B labelled nanoparticles (pink) can be seen to have been internalised by bone marrow macrophages (blue).

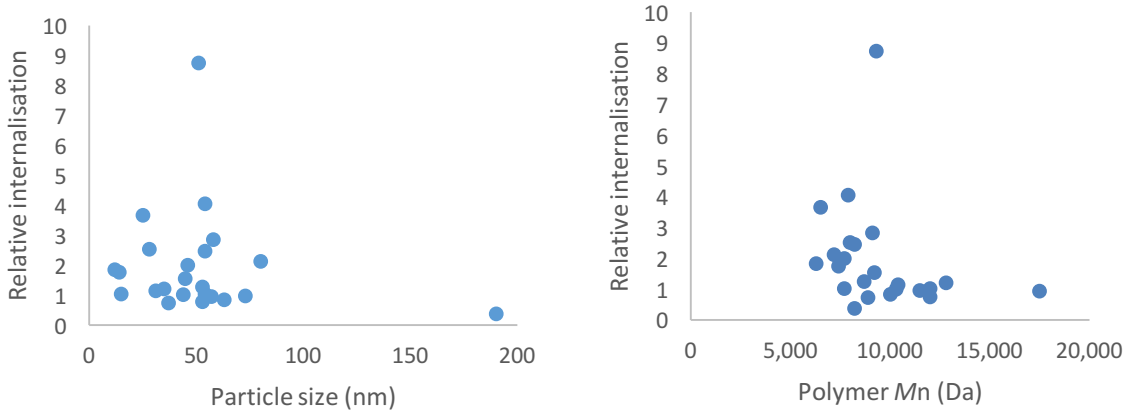


Figure S6. Plots of particle size (left) and polymer M_n (right) against internalisation of particles by macrophages. Relative internalisation was calculated using MCF values obtained from FACS results, which were normalised in terms of particle rhodamine fluorescence. Relative internalisation = MCF/relative particle fluorescence (**Table S9**).

Table S9. Particle fluorescence at 250 µg/ml, displayed in relative fluorescence units.

Particle	Fluorescence (RFU)
(1)	1,692
(2)	433
(3)	2,447
(4)	3,479
(5)	1,947
(6)	754
(7)	595
(8)	1,002
(9)	2,194
(10)	1,238
(11)	318
(12)	512
(13)	854
(14)	1,335
(15)	2,197
(16)	706
(17)	508
(18)	784
(19)	821
(20)	657
(21)	2,765
(22)	2,312

Particle fluorescence was calculated from a concentration *vs* fluorescence plot of each sample. The excitation and emission wavelengths used were: λ_{ex}: 555 nm, λ_{em}: 575 nm.

Table S10. Fluorescence and internalisation details of particles of interest.

Sample	Relative polymer fluorescence	No. polymer chains per particle	Relative particle fluorescence	MCF	Normalised particle internalisation
(3)	7.54	44	332	3,280	9.90
(4)	9.05	46	416	13,400	32.3
(10)	4.56	37	169	2,000	11.9
(11)	0.980	18	18.0	1,770	98.4
(15)	6.94	89	618	6,520	10.6

All fluorescence and endocytosis values displayed in relative fluorescence units (RFU). Relative polymer fluorescence is the relative fluorescence per mole of polymer and relative particle fluorescence is the relative fluorescence per mole of particles. The number of polymer chains per particle was determined using multi-angle light scattering (MALS). Relative particle fluorescence = relative polymer fluorescence × no. polymer chains per particle. Normalised particle internalisation = macrophage MCF/ relative particle fluorescence. Macrophage MCF values are from peritoneal macrophages.

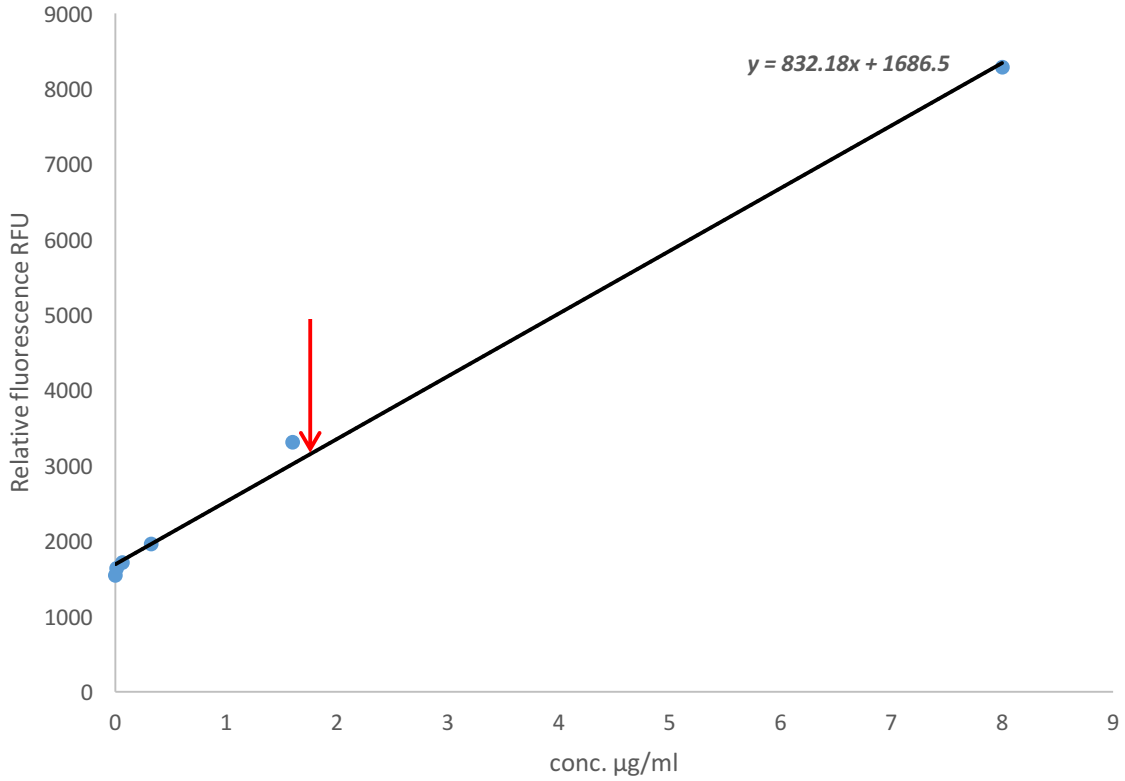


Figure S7. Plot of DAPI fluorescence *vs* concentration. Line of best fit used to determine concentration of DAPI encapsulated inside nanoparticles. Red arrow highlights the concentration encapsulated by nanoparticle (11); 2.00 µg/ml.

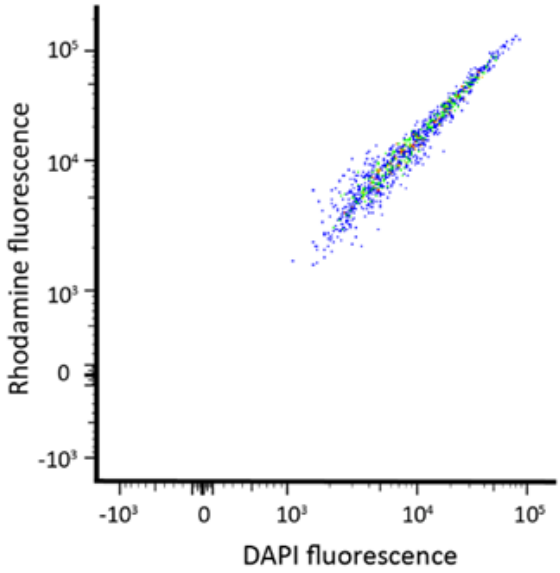


Figure S8. Plot of rhodamine fluorescence against DAPI fluorescence for peritoneal macrophages containing internalised particles. There is a strong linear correlation between the number of particles internalised and the amount of DAPI delivered.

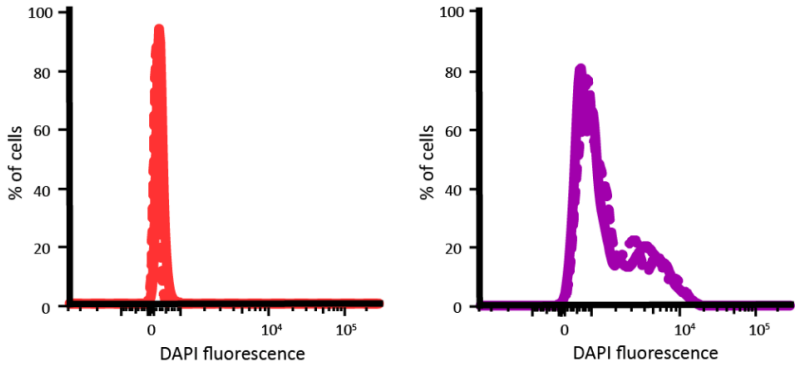


Figure S9. DAPI fluorescence of DAPI treated cells (solid line) compared to untreated cells (dashed line), showing free DAPI is unable to stain cells in the absence of nanoparticle encapsulation. Bone marrow macrophages (red) and peritoneal macrophages (purple) were both tested.

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