Supporting information for "Poly[2-(methacryloyloxy)ethylphosphorylcholine]-coated iron oxide nanoparticles: synthesis, colloidal stability and evaluation for stem cell labelling".

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1. Materials and instrumentation

1.1 Materials

Sodium oleate (>97 %) was purchased from TCI. Molday ION Rhodamine B (CL-50Q02-6A-50) was purchased from Biopal, Inc. All the other chemicals were purchased from Sigma-Aldrich. All chemicals were used as received without any further purification. All aqueous solutions were prepared with ultrapure water from a Milli-Q system (Millipore, resistivity 18.2 MΩcm at 25 °C).

1.2 Instrumentation

Dynamic light scattering (DLS) was carried out using a Viscotek 802-100 Dynamic Light Scattering Analyzer. Samples were ran at 20 °C in the appropriate solvent. Measurements were carried out using Malvern OmniSIZE software 3.0.

Powder X-ray diffraction (PXRD) was carried out using a Panalytical X'pert Pro multipurpose diffractometer with Co $K\alpha_1$ radiation (λ = 1.789010 Å). PXRD patterns were assigned using the JCPDS database.

Transmission electron microscopy (TEM). TEM images were collected using a FEI 120kV Tecnai G2 Spirit BioTWIN TEM operating at an accelerating voltage of 100 kV. Samples were prepared on 200 mesh hexagonal copper grids with a carbon sputter coated Formvar substrate. For staining of polymer coating, a 3 % solution of phosphotungstic acid (PTA) was used. Measurements of nanoparticles were carried out using ImageJ software.

Fourier-transform infrared (FT-IR) spectroscopy was carried out using a Bruker Tensor 27 plate reading FT-IR. Samples were prepared with KBr to form a pellet for single sample analysis. For each sample, 20 scans in the region from 650 to 4000 cm^{-1} were accumulated with a resolution of 4 cm^{-1}.

Thermogravimetric analysis (TGA) was carried out using a TA Instruments Q5000IR TGA under air atmosphere at 100 mL/min. For samples not containing polymer, a 5−10 mg sample was heated to 120 °C at 10 °C/min and kept at 120 °C for 20 min to remove all adsorbed solvent. The sample was

then heated to 600 °C at 10 °C/min and kept at 600 °C for 20 min. For samples containing polymer, a 5−10 mg sample was heated to 120 °C at 20 °C/min and kept at 120 °C for 20 min to remove all adsorbed solvent. The sample was then heated to 1000 °C at 20 °C/min and kept at 1000 °C for 40 min. Weight loss was calculated between weights measured at 120 °C and at final temperature.

Gel permeation chromatography (GPC) was carried out using a Viscotek GPCmax 2001 triple-detection GPC in 10 % methanol in deionised water eluent at 50 °C. PEO narrow and calibration standards were used.

^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) was carried out using a Bruker Avance 400 MHz spectrometer. Chemical shifts were measured in parts per million (ppm) relative to residual protic solvent. Polymer samples for NMR analysis were prepared in deuterated methanol (CD_3OD).

Micromass LCT mass spectroscopy (MS) was used to confirm the composition of initiators synthesised for ATRP reactions.

Elemental analysis. The carbon, hydrogen and nitrogen content of samples were analyzed with a Thermo EA1112 Flash CHNS-O Analyzer. The measurement taken for the elemental content (%) was an average of four measurements.

Electrophoretic Mobility (ζ-Potential) ζ-Potential measurements were recorded on a Malvern Zetasizer Nano ZS with SPION samples in deionised water or PBS pH 7.4.

UV-Vis Spectroscopy was carried out on a Thermo 2000c Nanodrop UV-Vis spectrophotometer. Absorbance of SPIONs dispersed in PBS pH 7.4 in a polypropylene cuvette with pathlength of 1 cm. The sample volume used was ≥1 mL and was measured at a fixed wavelength of 400 nm. Samples were kept at their measured temperature between measurements.

Magnetic Resonance Imaging (MRI) measurements were carried out on a Bruker Avance III MR scanner at 7 Tesla. Prior to measurement Fe content was measured via ICP-OES. Samples were prepared in in 1% low melting temperature agarose with final concentrations of 0.25, 0.12 and 0.06 mM Fe. Imaging was carried out using a sequence based on RARe (Rapid Acquisition with Relaxation Enhancement)[1] pulse sequence at base echo times TE = 11, 33, 55, 77 and 99 ms and at a repetition time TR = 5 s. Relaxation time T_2 was calculated based on an exponential fit of the signal intensity (S) as function of echo time (TE) using the following equation:

$$S = S_0 \exp\left(\frac{-TE}{T_2}\right) + S'$$

Where: S_0 and S' are signal intensities at TE = 0 and infinity, respectively.

Superconducting quantum interference device (SQUID) magnetometry measurements were carried out on an MPMS SQUID 7T Quantum Design magnetometer. Samples were measured at 2 T under a helium atmosphere at 300 K. Samples were prepared as follows: dry samples (1-5 mg) were contained and fixed in a size 4 gelatin capsule which were then placed inside the middle of a plastic drinking straw.

2. Experimental and Characterisation

2.1 Preparation of monodisperse oleic acid coated iron oxide (Fe_3O_4) nanoparticles

2.1.1 Preparation of iron(III) oleate complex

Iron(III) chloride hexahydrate (10.8 g ($Fe_3Cl_3.6H_2O$, 40 mmol, Aldrich, 98 %)) and sodium oleate (36.5 g (120 mmol, TCI, 97 %)) were dissolved in a solvent mixture of deionised water (60 mL), ethanol (80 mL) and hexane (140 mL). The mixture was heated under reflux at 70 °C for 4 hours. The resulting upper dark red organic layer containing iron(III)-oleate was washed three times with 30 mL deionised water in a separating funnel. The remaining organic layer was dried over anhydrous magnesium sulphate and hexane removed via rotary evaporation. FT-IR bands found at the following wavenumbers: 722, 1302, 1441, 1526, 1586, 1712, 2854, 2925 and 3006 cm^{-1}. Elemental analysis: theoretical C: 72.05, H: 11.08; experimental average C: 70.73, H: 11.69. TGA weight loss: 89.99%.

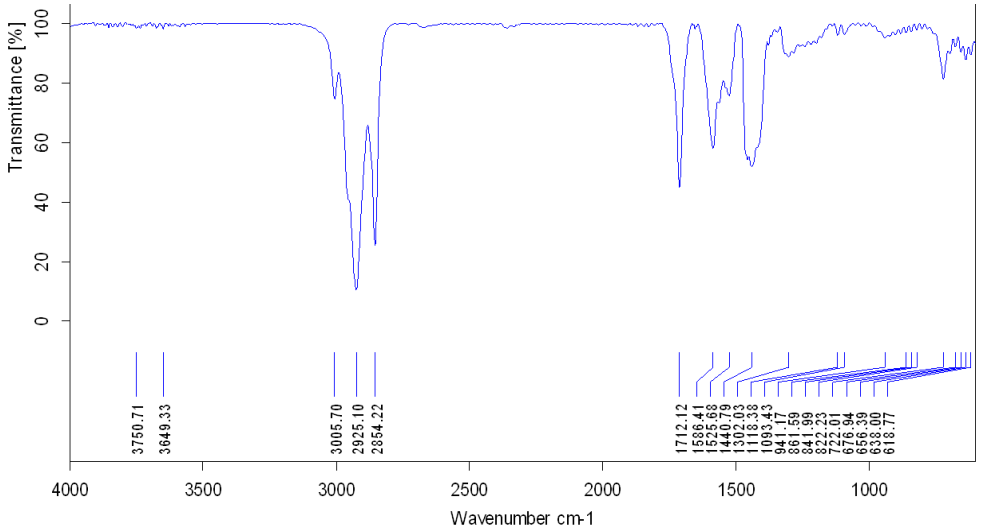


Figure S1: FT-IR spectrum of iron(III) oleate; C=O stretching peak at 1712 cm^{-1} characteristic to the iron(III)oleate complex.

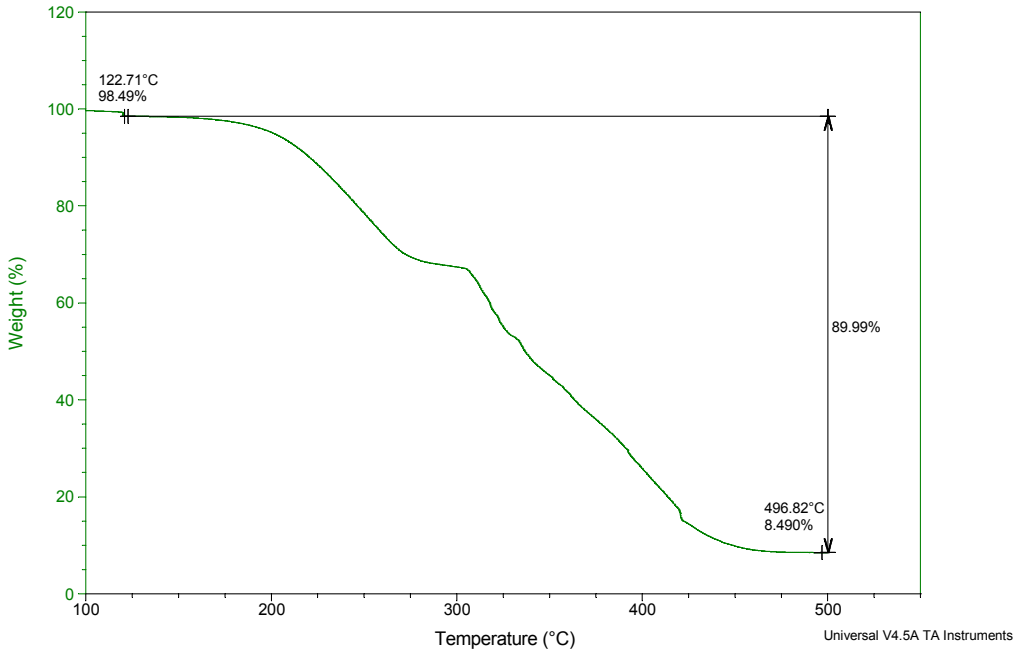


Figure S2: TGA spectrum of iron(III) oleate: total weight loss 89.99 %.

2.1.2 Synthesis of monodisperse oleic acid coated iron oxide nanoparticles

Monodisperse oleic acid coated iron oxide nanoparticles were synthesised as reported.[2] 7.0 g (8 mmol) of the iron-oleate complex synthesised as described above and 1.10 g of oleic acid (4 mmol, Aldrich, 90 %) were dissolved in 38.88 g of 1-octadecene (Aldrich, 90 %) at room temperature. The reaction mixture was heated to 320 °C maintaining a heating rate of 3.3 °C min^{-1}, and then kept at 320 ^{o}C for 30 minutes. The resulting solution was then cooled to room temperature, and 100 mL of ethanol was added to the black solution to precipitate the oleic acid coated iron oxide nanoparticles. The nanoparticles were separated using a NdFeB magnet (Magnet Sales; size, 10 mm D 5 mm H; strength ~1.18 T) and stored as a solution of known concentration in chloroform in the fridge. FT-IR bands were found at the following wavenumbers: 1384, 1466, 2361, 2853 and 2923 cm^{-1}. DLS measurements OA-SPIONs in THF: $D_{h,\ intensity}$ = 13.9 ± 2.8 nm, $D_{h,\ mass}$ = 12.4 ± 2.4 nm and $D_{h,\ number}$ = 10.6 ± 1.5 nm.

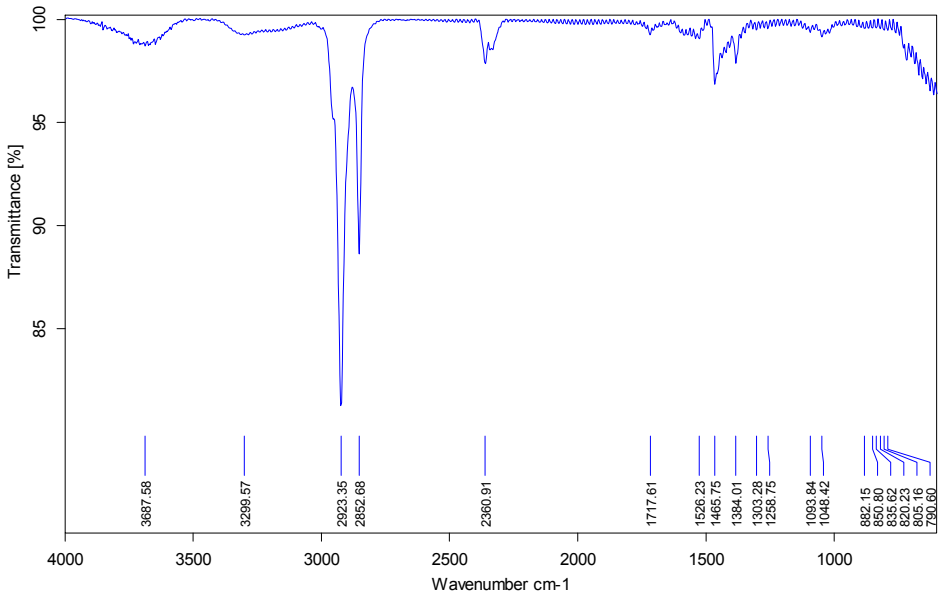


Figure S3: FT-IR spectrum of oleic acid coated iron oxide nanoparticles.

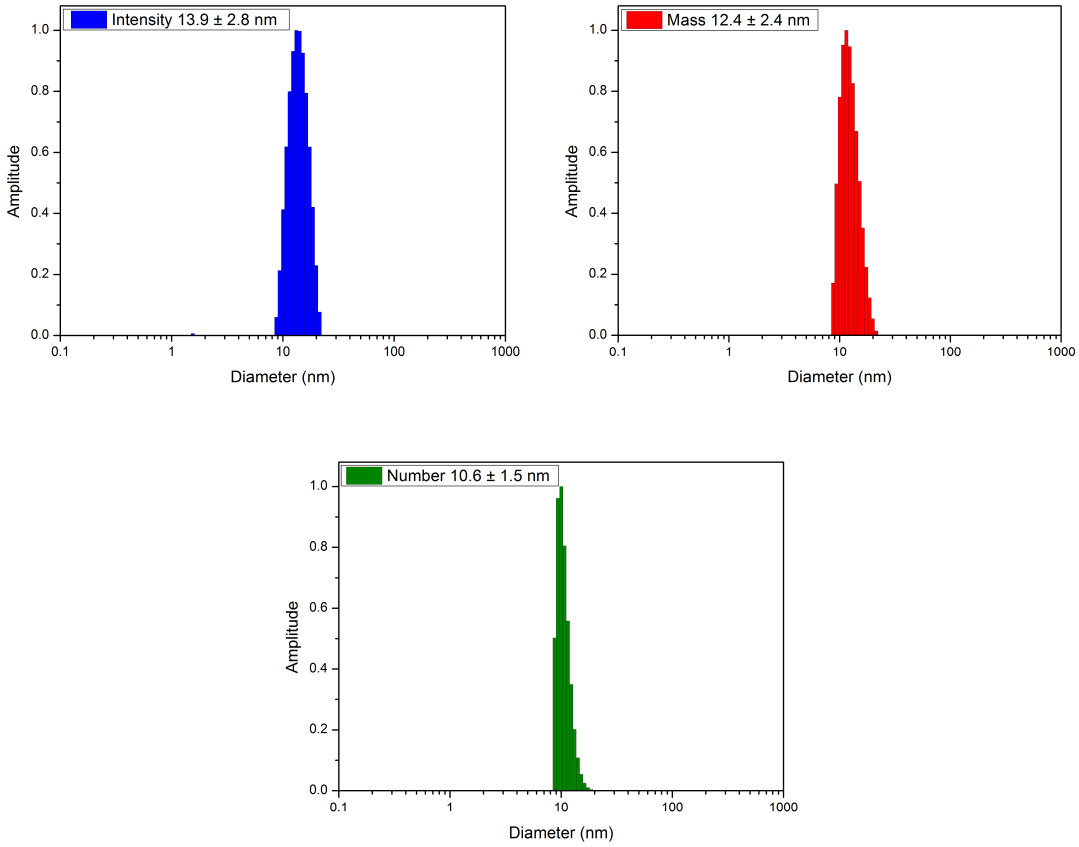


Figure S4: DLS distributions for oleic acid coated SPIONs in THF

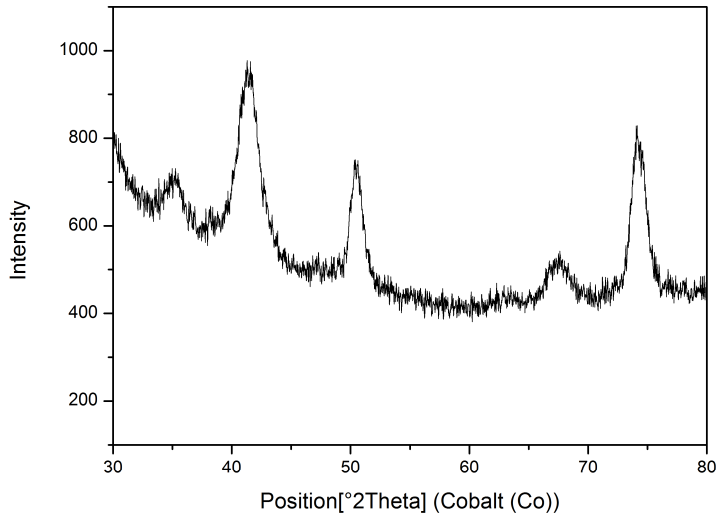
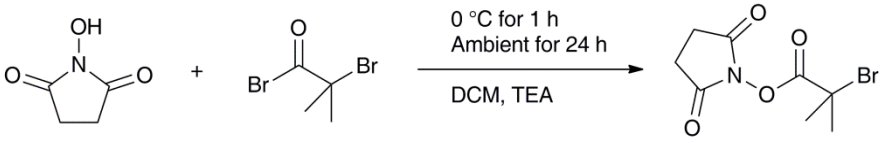


Figure S5: PXRD plot of oleic acid SPIONs .The observed XRD pattern showed good agreement with the reference pattern for magnetite (ICDD no. 00-019-0629) and indicates the material is crystalline. Average particle size diameter measured 7.63 nm as derived from the Scherrer equation.

2.2 Preparation of *N*-succinimidyl functional MPC homopolymer.

2.2.1 Synthesis of *N*-succinimidyl-2-bromoisobutyrate initiator for ATRP



SchemeS1: Scheme for the synthesis of *N*-succinimidyl-2-bromoisobutyrate (NSBriB) initator.

In a 1 L round bottom flask, dry dichloromethane (DCM, 500 mL) was added and cooled to 0°C under a nitrogen atmosphere. Under magnetic stirring, *N*-hydroxysuccinimide (NHS, 2.980 g, 26 mmol) and triethylamine (TEA, 7.1 mL) were added. The mixture was left to stir under nitrogen until all the NHS had dissolved. 2-Bromoisobutyryl bromide (3.60 mL, 29 mmol) was then added to the mixture dropwise and the mixture was left to mix overnight and warm to room temperature. The TEA salts were filtered from the mixture and the remaining organic solution washed with 0.1 M HCl and 0.1 M Na_2CO_3 solutions. The DCM was then removed *via* rotary evaporation to leave an oil which was then precipitated over cold hexane. A fluffy cream solid was obtained and dried in a desiccator overnight with a yield of 90% (~5g wet).[3] ^{1}H-NMR (400 MHz, chloroform-*d*, ppm): δ 2.2 (s, 6 H, CH_3), 2.9 (s, 4 H, CH_2). ^{13}C-NMR (100 MHz, chloroform-*d*, ppm): δ 168.9, 167.9, 31.1, 26.0. Elemental Analysis: expected C 36.39%, H 3.82%, N 5.30%, actual C 36.38%, H 3.81%, N 5.29%. Micromass LCT mass spectrometry expected (found): 264.07 g mol^{-1} (264.0 g mol^{-1}).

2.2.2 ATRP of MPC using *N*-succinimidyl-2-bromoisobutyrate (NSBriB) initiator

A typical ATRP reaction was carried out using molar ratios of [MPC]/[NSBriB]/[CuCl]/[bipy] = 40:1:1:2.1 in a solvent mixture ratio 2:3 (v/v) of methanol/DMSO. In a dry 100 mL round bottom flask, MPC monomer (5.00 g, 16.93 mmol, 40 equiv) was dissolved in degassed methanol (9.0 mL) over ice. In a separate flask, NSBriB initiator (0.112 g, 0.423 mmol, 1 equiv) was dissolved in DMSO (13.5 mL) with bipy ligand (0.139 g, 0.889 mmol, 2.1 equiv) and Cu(I)Cl (0.042 g, 0.423 mmol, 1 equiv). After purging both flasks with nitrogen for 30 min, the initiator/catalyst mixture in DMSO was added to the stirred MPC solution under nitrogen at 22 °C. The reaction mixture was left for 24 hours to ensure full conversion. After 24 h, ^{1}H NMR analysis indicated that more than 99% MPC had been polymerised (disappearance of vinyl signals at δ 5.5−6.0). On exposure to air, the reaction solution turned blue, indicating aerial oxidation of the Cu(I) catalyst. The resulting NS−MPC homopolymer was mixed with Dowex Marathon MSC ion-exchange resin to remove Cu(II)Cl. The ion-exchange resin was then removed by filtration and remaining solution was rotary evaporated to remove methanol and precipitated into THF to remove DMSO. The precipitated polymer was then dried under vacuum and stored in the fridge in a sealed drying tube. DP_n = 29 (^{1}H-NMR in CD_3OD), M_n(NMR) = 9,000 g mol^{-1}, M_n(GPC) = 10,100 g mol^{-1} and M_w/M_n = 1.076.

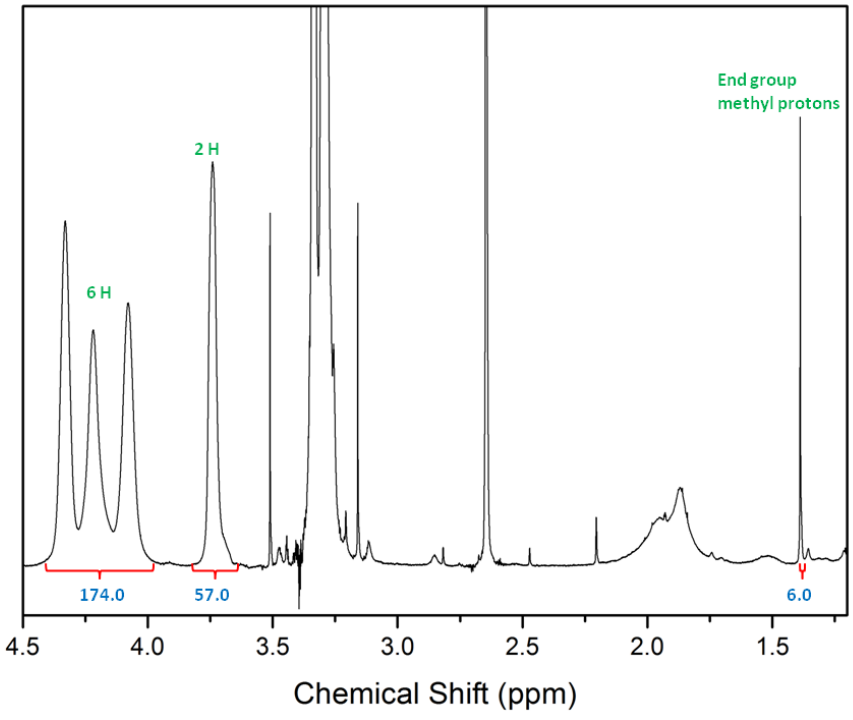


Figure S6: ^{1}H-NMR in CD_3OD spectrum of NS-pMPC_{29}

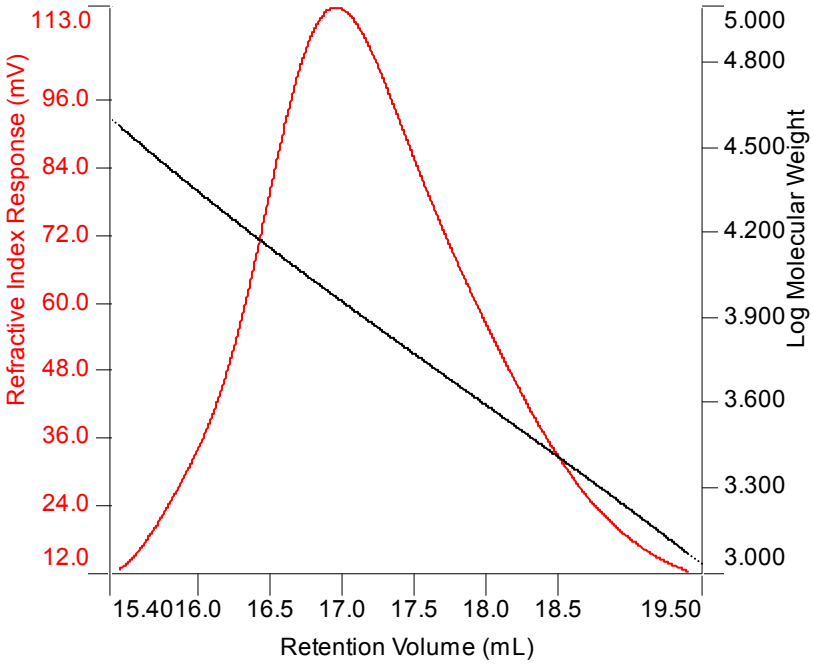


Figure S7: GPC chromatograph of NS-pMPC$_{29}$

2.3 Ligand exchange reaction of oleic acid coated iron oxide nanoparticles with (3-amino)propyltrimethoxysilane (APTMS)

In a 40 mL vial, dried toluene (30 mL, dried over 4Å molecular sieves) and dried oleic acid coated iron oxide nanoparticles (4 mL of solution in dried toluene at 30.723 mg mL^{-1}) was added. The vial was then sealed using an air tight rubber suba seal and the air space above the solvent in the vial replaced by nitrogen. The sealed vial was then sonicated using an ultrasonic bath (Fisher Scientific, model FB15051, frequency = 30-40 kHz) for 5 minutes to ensure nanoparticles were fully dispersed in the solvent. APTMS (90 µl, excess) was then injected into the sealed vial during another minute of sonication. The vial was then left to mix by a rotary mixer over a 72 hour period at 25 °C. After this time, the solid was recovered using a NdFeB magnet. The supernatant was then decanted and the particles washed three times *via* sonication for one minute and centrifuged at 2,907 G for 5 minutes with 40 mL aliquots of toluene followed by three times *via* sonication for one minute and centrifugation at 7,441 G for 10 minutes with 15 mL aliquots of ethanol. The particles were then re-suspended in the appropriate amount of ethanol for storage at a concentration of 2 mg mL^{-1}. Zeta potential of the amino silane coated SPIONs (A-SPIONs) in DIW and PBS pH 7.4 were of +25.6 ± 0.4 mV and +15.3 ± 0.8 mV respectively. A dry sample of A-SPIONs was prepared for elemental and thermogravimetric analysis (TGA) by removing ethanol *via* rotary evaporation from a 5 mL sample which was then dried in the oven at 70 °C for 2 hours. FT-IR bands were found at the following wavenumbers: 799, 1040, 1097, 1384, 1557, 2361, 2851, 2920 and 3416 cm^{-1}.

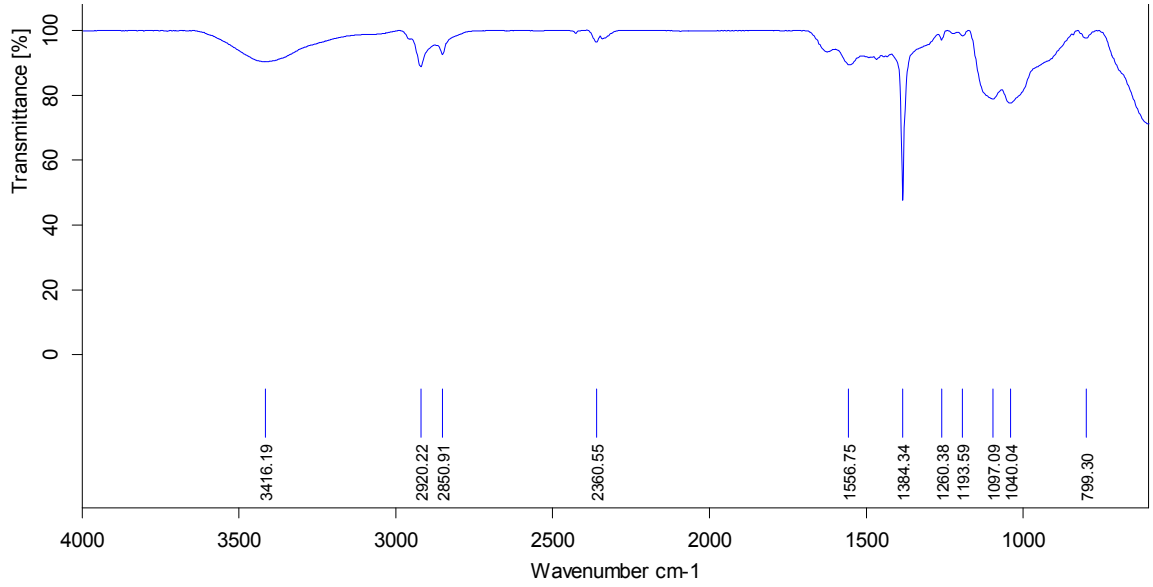


Figure S8: FT-IR spectrum of A-SPIONs.

The footprint of the tridentate amino silane ligand has been quoted as a surface area of 0.073 nm^2.[2, 4, 5] From this, we can calculate the theoretical number of ligands for one mono-layer on the surface of a 7.63 nm diameter nanoparticle.

Surface area (SA) of a 7.63 nm SPION:

$$SA_{SPION} = 4\pi r^2$$

$$= 182.89 \text{ nm}^2$$

Predicted number of APTMS ligands that can fit as one monolayer on a 7.63 nm SPION (L_1):

$$L_1 = \frac{SA_{SPION}}{Footprint_{APTMS}}$$

$$= 2505 \text{ ligands}$$

Based on a 100% magnetite SPION, it is possible to calculate the number of (Fe_3O_4) units per SPION.

$$Mass\ of\ one\ SPION = M_{SPION} = \rho_{Fe_3O_4} x \left(\left[\frac{4}{3}\pi r^3\right] x10^{-21}\right)$$

$$= 1.20 \text{ x } 10^{-18} \text{ g}$$

$$N_{Fe_3O_4} = \frac{M_{SPION} \times N_A}{MW_{Fe_3O_4}} = 3121$$

Thus the molar composition of A-SPION with one monolayer of APTMS would be $(Fe_3O_4)_1(APTMS)_{0.8}$.

Elemental and thermogravimetric analyses were used to determine the actual composition of A-SPION. Table S1 summarises experimental elemental analysis results, TGA weight loss and calculations of weight contributions from APTMS, residual oleic acid and SPION core.

Table S1: Experimental and calculated elemental analysis and TGA weight loss values for dried A-SPION.

		Actual experimental values	Calculated from Nitrogen content			
			APTMS	Residual Oleic Acid	Total values	SPION (as Fe_3O_4)
Elemental analysis	C	**23.60**	11.78	11.82	**23.6**	
	H	**4.30**	2.64	*1.84*	***4.48***	
	N	**4.58**	4.58		**4.58**	
	O		15.69	1.75		11.26
	Si		9.18			
	Fe					29.46
	Total weight %		43.87	15.41		40.72
TGA	Weight loss	**34.88**	19	15.41	**34.41**	

From the experimental procedure the nitrogen content of the A-SPION product could only be attributed to APTMS ligands reacted onto the surface. Using the weight percentage measured for nitrogen, it was possible to calculate the carbon and hydrogen contribution from APTMS. An excess of carbon and hydrogen was observed compared to the expected values for a pure APTMS coverage. The excess carbon and hydrogen values matched those calculated for oleic acid (but not toluene), so, the contaminant was considered to be residual oleic acid.

Using the results calculated from elemental analysis, the expected TGA weight loss was calculated as 34.41% which matched the experimental value of 34.88%, thus, confirming the composition extrapolated from elemental analysis.

The weight composition of A-SPION was determined as (Fe_3O_4), 40.72%; (APTMS), 43.87% and (Oleic acid), 15.41%. The molar composition of A-SPION was determined as $(Fe_3O_4)_1(C_3H_8NSiO_3)_{1.8}(C_{18}H_{34}O_2)_{0.3}$. The amount of APTMS on the A-SPION corresponded to 2.21 equivalents of the value calculated earlier for a monolayer so the number of APTMS ligands on the surface (L_{exp}) was estimated at 5541.

2.4 Amidation reaction of NS-pMPC$_{29}$ homopolymer with amino silane coated iron oxide nanoparticles.

Using the number of APTMS ligands per A-SPION calculated above, the amount of polymer required for complete amidation reaction can be predicted.

Amount of pMPC required for a monolayer per 10 mg of A-SPIONs

$$= \left[\left(\frac{1}{\left[\rho_{Fe_3O_4} x \left(\left[\frac{4}{3}\pi r^3\right] x10^{-21}\right)\right] + \left[\left(\frac{L_{exp}}{N_A}\right) xMW_{reacted\ APTMS}\right]}\right)/100\right] x \left[\frac{L_{exp}}{N_A} xMW_{pMPC}\right]$$

$$= 0.462 \text{ g}$$

It is expected that not all amino groups on the A-SPION are available for reaction due to unfavourable conformations. Furthermore, it is expected that not all amino groups available could be reacted with a pMPC chain due to steric hindrance. The amount of $pMPC_{29}$ used for amidation reaction was chosen as 0.9 equivalents of all amino groups on the particle, which was expected to be in excess of actually available groups.

In a 15 mL vial, NS-$pMPC_{29}$ (0.41 g, 0.9 equiv.) was dissolved in methanol (5 mL). To this solution, amino silane coated nanoparticles (5 mL, pre-sonicated solution dispersed in ethanol, 2 mg mL^{-1}) were added under bath sonicator set at 40 °C and further sonicated for 1 h. The reaction mixture was then left to rotary mix for 48 h at 22 °C before dialysis into deionised water. The dialysis solution was changed with deionised water (4 L) five times over the course of a period of 8 h. The dialysis bag was then left in the final change of deionised water (4 L) for 15 h before concentrating using a 50,000 MWCO Corning Spin-X UF centrifugal concentrator. The concentrated solution of $pMPC_{29}$-NPs was then passed through a Sephadex G25 gel filtration chromatography column in phosphate buffered saline (PBS) pH 7.4 to remove any unreacted polymer. To prepare dry samples for TGA and elemental analysis, the $pMPC_{29}$-NPs were dialysed back into deionised water to remove PBS salts prior to freeze drying. Zeta potential in DIW and PBS at pH 7.4 was found to be +3.0 ± 0.3 mV and -2.26 ± 0.12 mV respectively. Elemental analysis C: 38.35, H: 7.57, N: 3.92. The sample was wet due to the highly hygroscopic pMPC polymer. Weight composition of the sample was determined as (Fe_3O_4), 5.1%; (APTMS/pMPC-coating), 82.7% and (Water), 12.2%. Further determination of the composition of the coating of pMPC-SPION was achieved using TGA data, see below. TGA total weight loss 93.42%. TEM measurements core diameter = 7.8 ± 1.7 nm, polymer-coated particle diameter = 16.6 ± 2.2 nm. DLS measurements in PBS pH 7.4: $D_{h,\ intensity}$ = 20.1 ± 2.1 nm, $D_{h,\ mass}$ = 19.4 ± 2.0 nm, $D_{h,\ number}$ = 18.4 ± 1.7 nm.

A sample of freeze dried $pMPC_{29}$-SPION was taken for TGA analysis and measurement of iron content based on previously published methods.[6] The TGA data revealed a weight loss of 10.47% between 20 and 120 °C which was attributed to adsorbed water due to the highly hygroscopic nature of pMPC. The Fe content was found to be 0.0360 mg/mg of sample. Considering a Fe_3O_4 SPION, the iron oxide content of the sample was calculated as 0.0498 mg Fe_3O_4/mg of sample. Using this data it was possible to calculate the weight fraction of coating, f_c, in pMPC-SPION.

$$Coating\ weight\ fraction = f_c = \frac{mass\ of\ coating}{overall\ \text{pMPC-SPION}\ mass}$$

$$= \frac{mass\ dry\ sample(from\ TGA) - mass\ of\ SPION\ core(from\ titration)}{mass\ dry\ sample(from\ TGA)}$$

$$f_c = \frac{(1 - 0.1047) - 0.0498}{(1 - 0.1047)} = 0.9444$$

Using a different method to express f_c, it was possible to calculate the *coverage* or fraction of all amino groups on the particle reacted with a polymer chain. The calculations are as follows:

$$Coating\ weight\ fraction = f_c = \frac{mass\ of\ coating}{overall\ \text{pMPC-SPION}\ mass}$$

$$= \frac{mass_{pMPC-ligand} + mass_{unreacted\ APTMS-ligands}}{mass_{SPION\ core} + mass_{pMPC-ligand} + mass_{unreacted\ APTMS-ligand}}$$

With $mass_{\text{pMPC-ligand}}$, the total mass of pMPC ligand reacted on a particle, see Figure S9 for structure

$$mass_{pMPC-ligand} = N_{reacted} \times \frac{MW_{pMPC-ligand}}{N_A}$$

With $mass_{\text{unreactedAPTMS-ligand}}$, the mass of unreacted APTMS ligand on a particle, see figure S9 for structure

$$mass_{unreacted\ APTMS-ligand} = N_{unreacted} \times \frac{MW_{APTMS-ligand}}{N_A}$$

With $N_{reacted} = L_{exp} \times coverage$ and $N_{unreacted} = L_{exp} \times (1 - coverage)$

The expression of the coating weight fraction could then be developed:

$$f_c = \frac{(L_{exp} \times coverage)\frac{MW_{pMPC-ligand}}{N_A} + L_{exp} \times (1 - coverage)\frac{MW_{APTMS-ligand}}{N_A}}{M_{SPION} + (L_{exp} \times coverage)\frac{MW_{pMPC-ligand}}{N_A} + L_{exp} \times (1 - coverage)\frac{MW_{APTMS-ligand}}{N_A}}$$

The equation could be rearranged to calculate the coverage

$$coverage = \frac{(1 - f_c)MW_{APTMS-ligand} - \frac{f_c \times M_{SPION} \times N_A}{L_{exp}}}{(f_c - 1) \times (MW_{pMPC-ligand} - MW_{APTMS-ligand})}$$

The values of the parameters required to calculate the coverage are as such:

$$f_c = 0.9444$$

$$M_{SPION} = \rho_{Fe_3O_4} x \left(\left[\frac{4}{3}\pi r^3\right] x10^{-21}\right) = 1.20\ 10^{-18}\text{g}$$

$$L_{exp} = 5541$$

$$MW_{APTMS-ligand} = 134.19\ \text{g mol}^{-1}$$

$$MW_{pMPC-ligand} = 8846\ \text{g mol}^{-1}$$

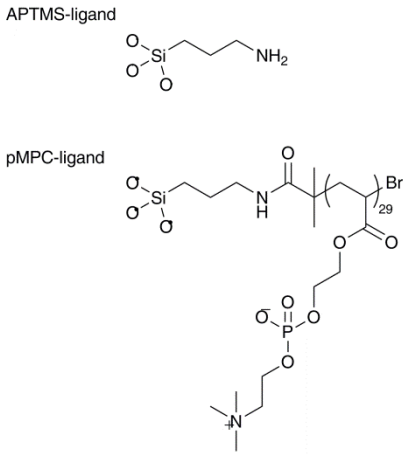


Figure S9: Structure of surface reacted APTMS ligand and pMPC ligand.

Thus, the coverage was calculated as 0.2389. 23.89% of all amino groups were reacted with a $pMPC_{29}$ polymer chain resulting in 1324 polymer chains attached to the SPION surface based on 5541 amino groups on the A-SPION. Taking into account the amount of water present in this particular sample, the molar composition of $pMPC_{29}$-SPION could be expressed as:
$(Fe_3O_4)_1(C_3H_8NSiO_3)_{1.4}(Br\text{-}pMPC_{29}\text{-}C_7H_{13}NSiO_3)_{0.4}(H_2O)_{27}$.

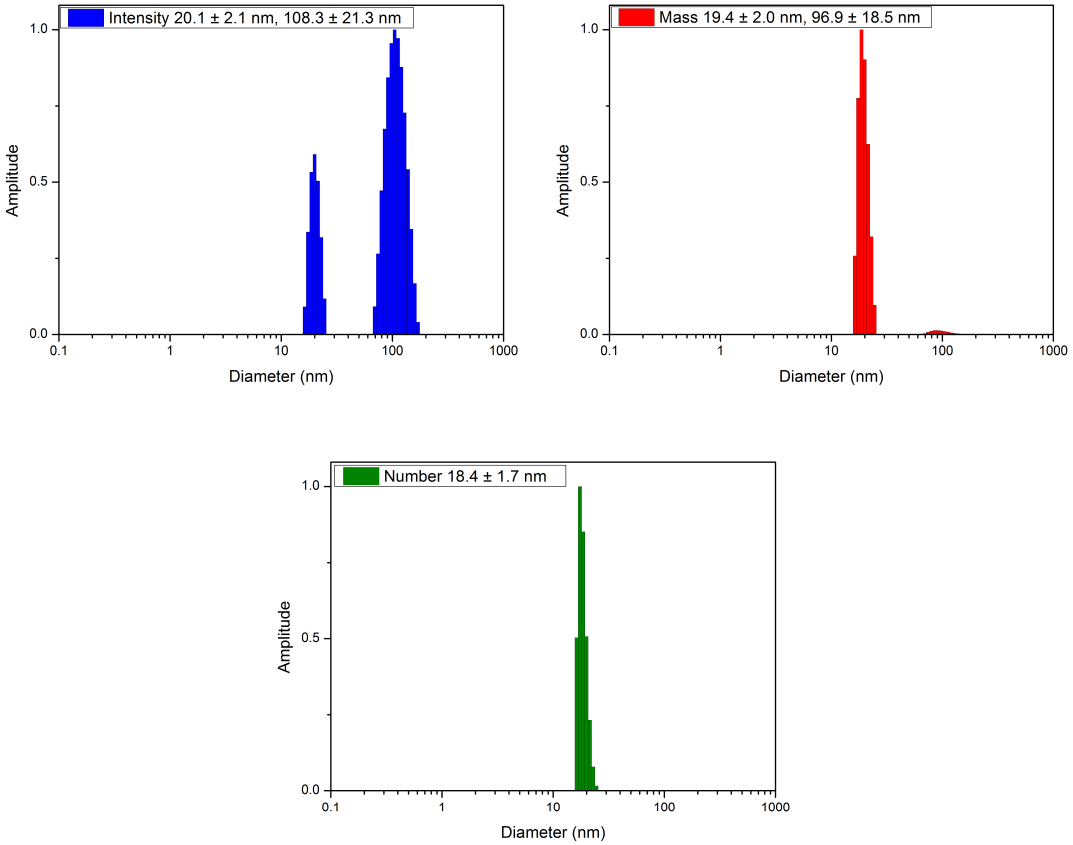


Figure S10: DLS distributions for $pMPC_{29}$-SPIONs in PBS pH 7.4.

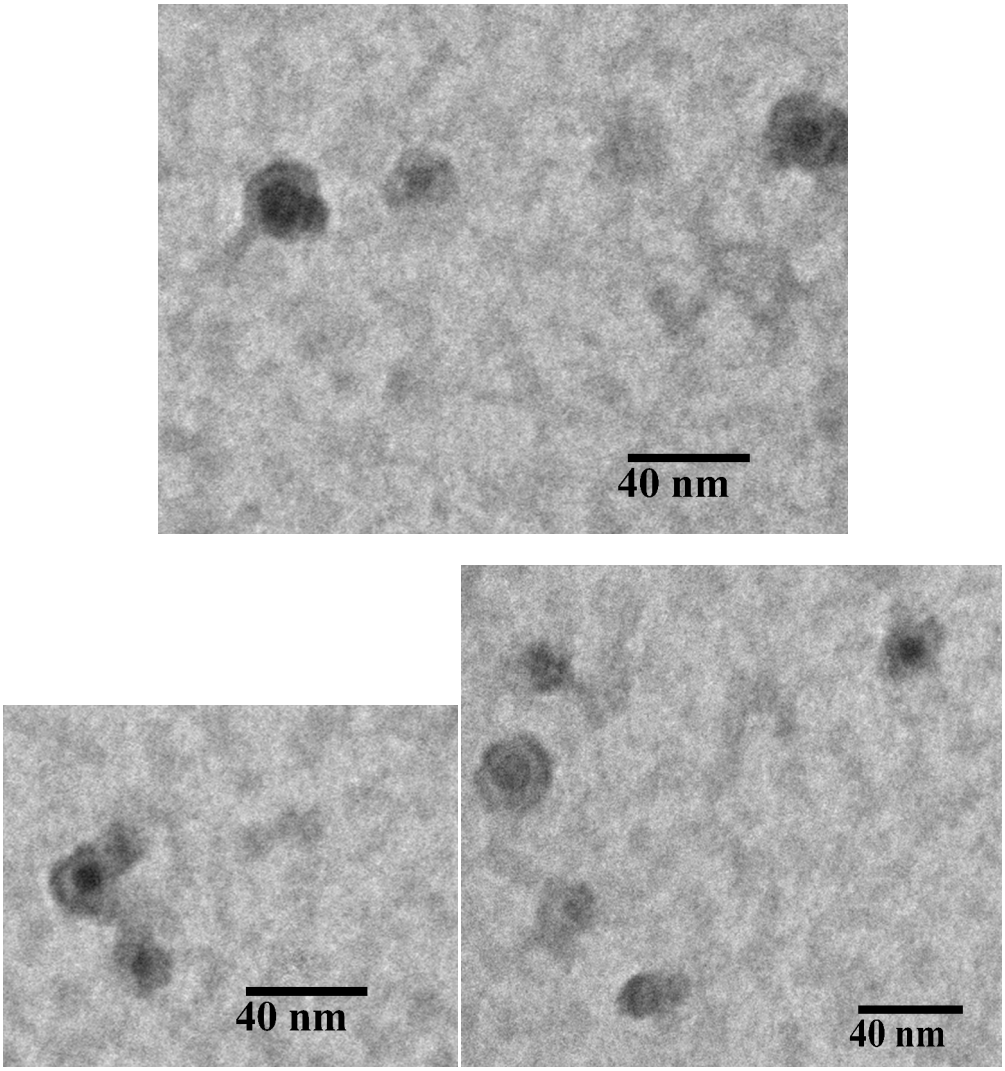


Figure S11: Additional TEM images of $pMPC_{29}$-SPIONs stained with 3% PTA solution demonstrating the consistency of polymer coverage and core-shell architecture across the sample.

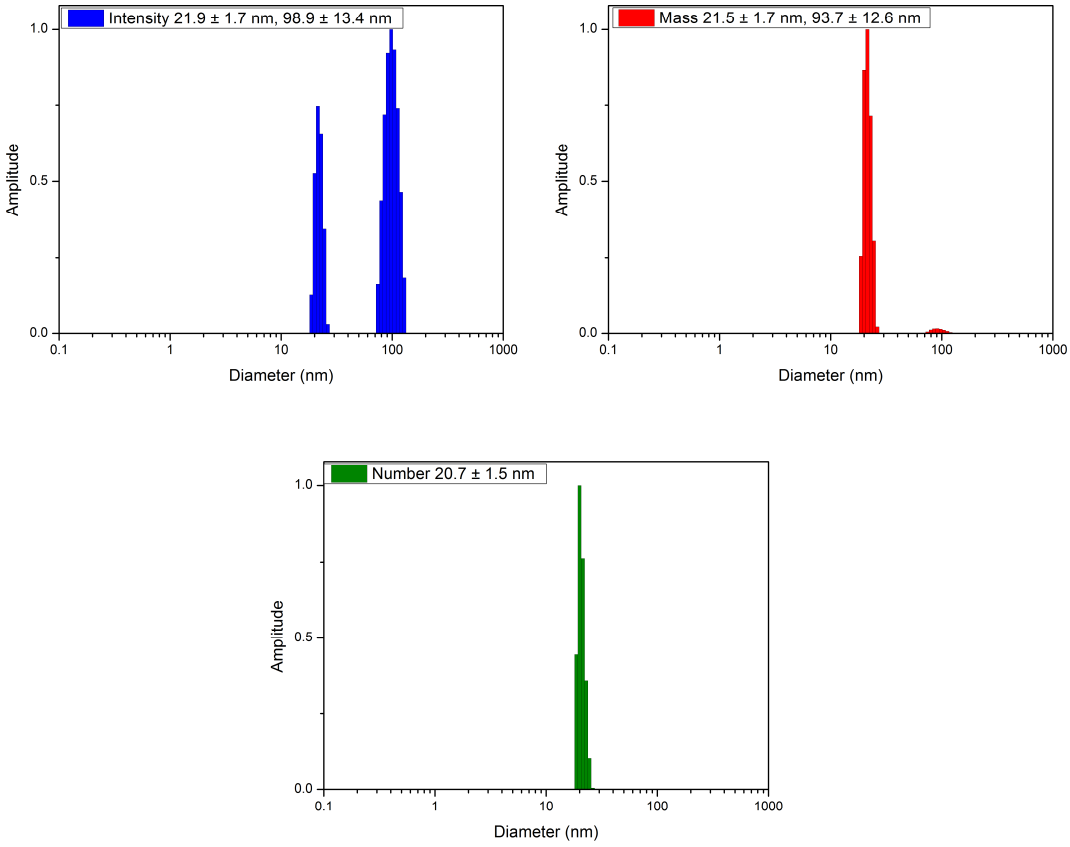
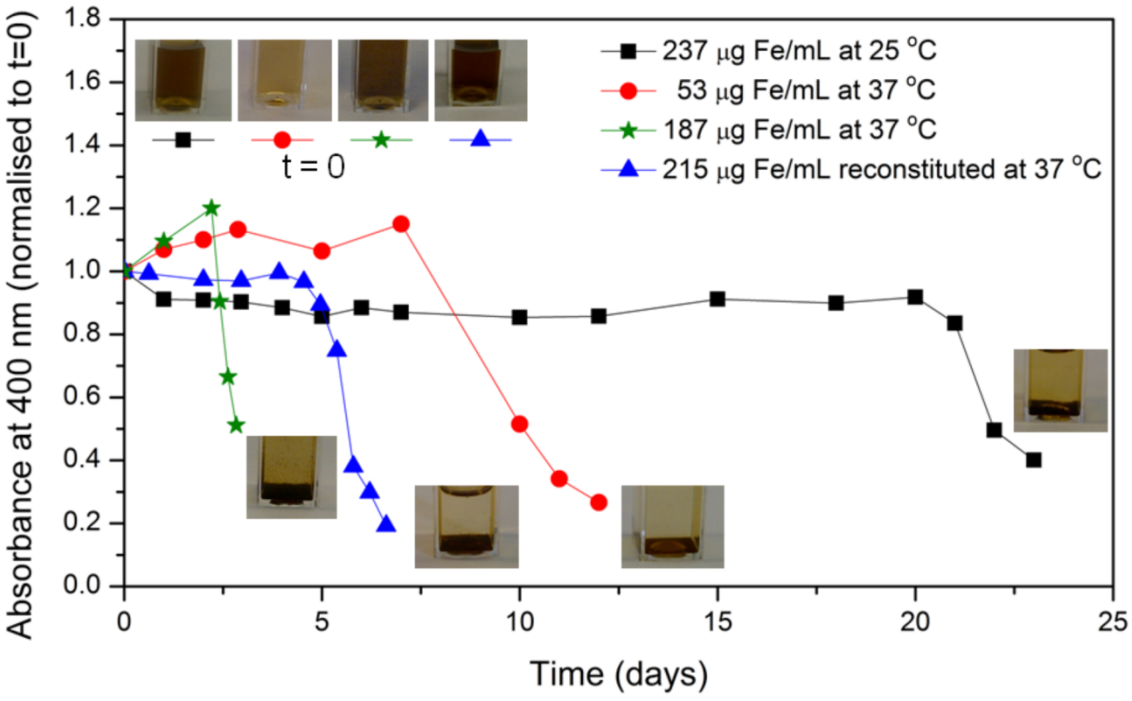


Figure S12: DLS distributions for re-dispersed freeze dried $pMPC_{29}$-SPIONs in PBS pH 7.4.

2.5 *Stability studies*



2.6 *In vitro studies*

A kidney-derived murine stem cell line[7] was used as for uptake and cytotoxicity studies. Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma) supplemented with 10% fetal bovine serum (FBS; PAA), 1% L-glutamine (Sigma) and 100 U/mL of penicillin and streptomycin (Sigma). In all cases, cells were grown under standard culture conditions (37 °C, 5% CO_2).

2.6.1 Cytotoxicity

Cells were seeded in 96 well plates and allowed to grow overnight. The culture medium was then replaced with fresh medium containing the nanoparticles at a range of concentrations (0-50 µg(Fe)/mL) and the cells were allow to grow for a further 24h. The wells were then washed with PBS to remove excess nanoparticles and replaced with fresh medium containing the CCK-8 reagent (Sigma) according the manufacturer's specifications. After 2h, the absorbance of each well was measured in a microplate reader at 450 nm, where the absorbance intensity at this wavelength is directly proportional to the number of viable cells in the well. Experiments were performed in triplicate and results were normalised to control conditions (cells that were not exposed to nanoparticles). A negative control where cells were exposed to 0.1% Triton-X-100 instead of the nanoparticles was used in each experiment.

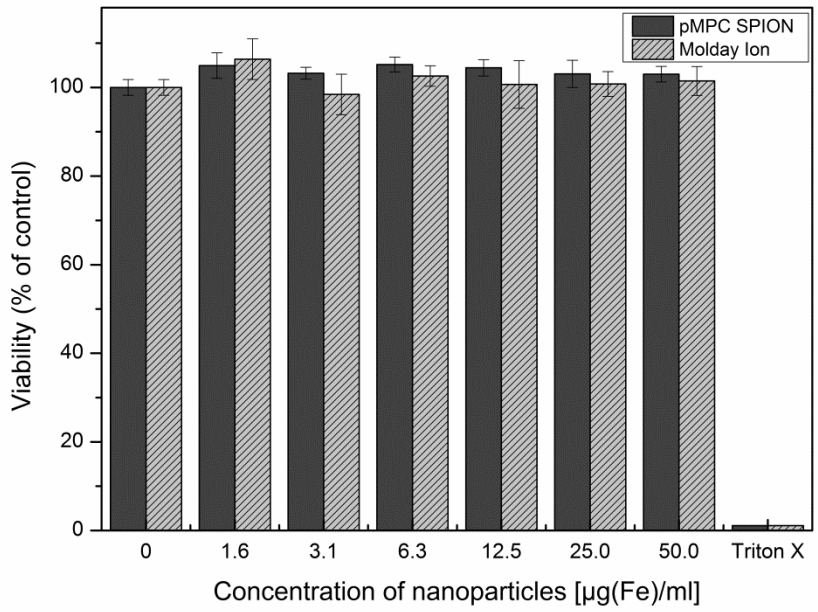


Figure S13: Relative viability of cells exposed to a range of concentrations of pMPC coated SPIONs or Molday Ion nanoparticles. The cells were incubated in culture medium containing the nanoparticles for 24 h followed by viability measurement with CCK-8 reagent. Results are plotted as the relative viability in respect to control conditions as a function of nanoparticle concentration and error bars represent the standard deviation from three replicates.

2.6.2 Particle degradation

Degradation of the nanoparticles in a model of lysosomal environment was based on procedures that have been previously described.[8, 9] Nanoparticles (1 µg, Fe basis) were added to microtest tubes containing buffers at physiological or acidic pH and incubated at 37 °C for up to 8 days. The buffers consisted of PBS supplemented with 20 mM of sodium citrate at a pH of 7.14, 5.5 or 4.5, the last two adjusted with hydrochloric acid. At several time points, a sample was taken for measurement of free iron ions in solution. The measurement was carried out by adding 20% volume of a freshly prepared colorimetric reagent consisting of 6.5 mM ferrozine and 10 mM ascorbic acid.[6] After color development for 30 minutes, the sample was transferred to a 96-well plate and the absorbance at 590 nm measured in a microplate reader. The absorbance value was compared to that of a calibration curve obtained from dilute solutions of an iron standard (TraceCERT, Sigma) to obtain the amount of free irons in solution. The value obtained was normalized to the amount of particles (Fe) added to each tube (1 µg) yielding the dissolved fraction.

2.6.3 Magnetic Measurements

Magnetisation curves were obtained for OA-SPION and pMPC-SPION resulting in magnetic saturations of 53.50 emu/g and 33.20 emu/g respectively showing typical superparamagnetic behaviour with minimal remanence and coercivity.

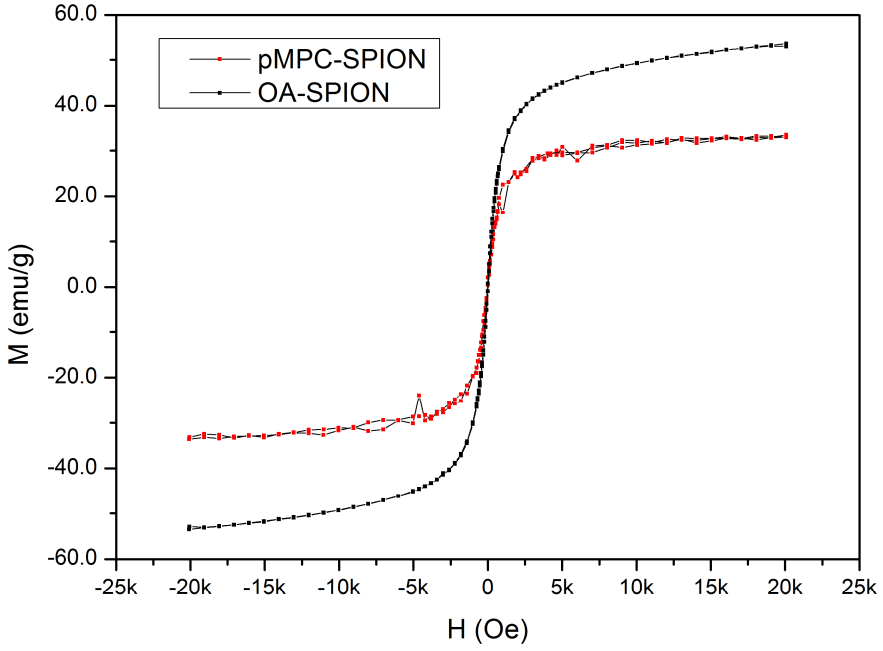


Figure S14: Magnetisation-field magnetic hysteresis curves for OA-SPION and pMPC-SPION at 300K. The 2 T magnetisation for OA-SPION is 53.50 emu/g and pMPC-SPION 33.20 emu/g

2.6.4 Cell Uptake

Cells were sub-cultured in 3.5 cm dishes at a density that yielded 40-50% confluence on the following day. At this time point, the culture medium was replaced with fresh medium containing the nanoparticles at a concentration of 5-50 µg(Fe)/mL and cells were allowed to grow for a further 24h.

After the incubation period, cells were washed twice with phosphate buffered saline (PBS) to remove excess SPIONs from the medium, fixed with 4% paraformaldehyde for 10 minutes and the finally washed with PBS to remove excess fixative. For imaging nanoparticles taken up by cells those were stained with the Iron Stain Kit (Sigma), which consists of a Prussian Blue staining for iron deposits and a Pararosaniline counterstain, followed by image acquisition under an optical microscope..

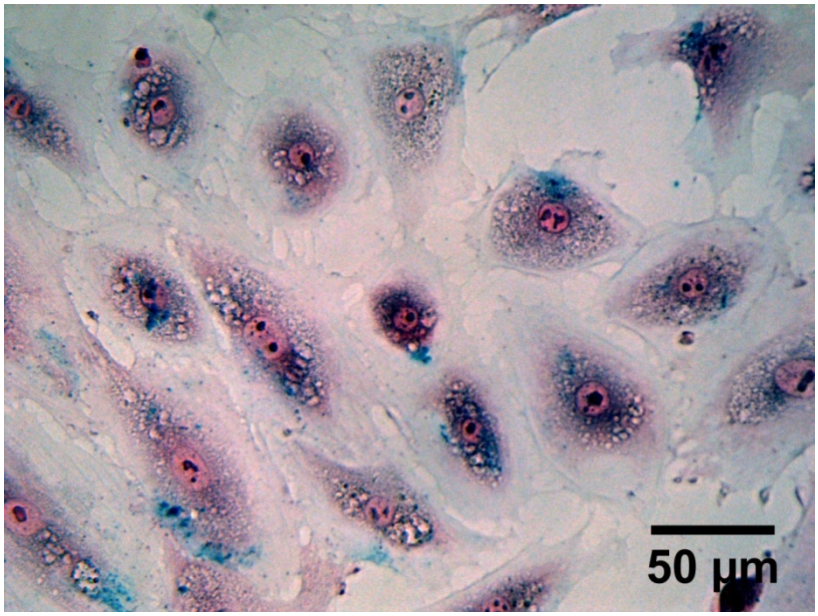


Figure S15: Light microscopy image: Cells fixed and stained after exposure to re-dispersed freeze dried $pMPC_{29}$-SPIONs (50 µg(Fe) mL^{-1}) for 24 h.

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