Supporting Information

Spatial and Temporal Control of Drug Release Through pH and Alternating Magnetic Field Induced Breakage of Schiff Base Bonds

Alexander E. Dunn[a,b], Douglas J. Dunn [a,b], Alexander Macmillan[c], Renee Whan[c], Tim Stait-Gardner[d], William S. Price[d], May Lim[a*], Cyrille Boyer[b*]

[a] ARC Centre of Excellence for Functional Nanomaterials, School of Chemical Engineering, The University of New South Wales, Sydney NSW 2052, Australia
[b] Australian Centre for NanoMedicine and Centre for Advanced Macromolecular Design, School of Chemical Engineering, The University of New South Wales, Sydney NSW 2052, Australia
[c] Biomedical Imaging Facility, Mark Wainwright Analytical Centre, The University of New South Wales, Sydney NSW 2052, Australia
[d] Nanoscale Organisation and Dynamics Group, School of Science and Health, University of Western Sydney, Penrith NSW 2751, Australia

Email: May Lim m.lim@unsw.edu.au
Cyrille Boyer cboyer@unsw.edu.au

Experimental Section

Materials

Iron (III) chloride hexahydrate (96%), iron (III) nitrate nonahydrate (99.5%), potassium hydroxide pellets and urea (99.5%) were sourced from Univar. Monosodium phosphate monohydrate (99%), nitric acid (70%) and ammonia (28%) were sourced from Analar. Tetraethoxysilane (TEOS), 300 g mol^{-1} poly(ethylene glycol) methyl ether methacrylate (Mn = 300 g mol^{-1}) (OEGMA), di(ethylene glycol) methyl ether methacrylate (95%) (DEGMA), 3-(Trimethoxysilyl)propyl methacrylate (98%) (TMSPMA), 3-vinylbenzaldehyde (97%) (VBA), tetrahydrofuran (THF), chloroform-d NMR solvent, fluorescein amine isomer 1, triethylamine (98%), acetonitrile (99.8%) bacteriological agarose, and absolute ethanol. 4,4'-Azobis(4-cyanovaleric acid) (≥75%) was sourced from Aldrich. Acetonitrile and toluene were sourced from Ajax Finechem. Type I water was produced using a Gradient Millipore integral water purification system (resistivity 18 mΩ cm^{-1}) and used in the preparation of all chemical solutions.

Synthesis of Acicular Shaped Silica Coated Magnetite Nanoparticles

Acicular hematite nanoparticles were synthesised by the forced hydrolysis of iron (III) chloride hexahydrate (0.02 M) in the presence of sodium dihydrogen orthophosphate monohydrate (0.4 mM) and catalysed by urea (0.01 M) at 100 °C for 18 h. These nanoparticles were then collected by high speed centrifugation (Allegra® X-30R with F0850 Rotor, 7,100 g) and vacuum dried for 3 days. These were then coated with silica by first suspending 30 mg of the hematite nanoparticles in 10 mL of water, followed by mixing with a solution containing 71.4 mL of ethanol, 3.14 mL of ammonia and 100.0 mL of water. Next, a tetraethoxysilane (TEOS) solution was prepared by mixing 3.00 mL of ethanol with 0.13 mL of TEOS. The nanoparticle suspension was placed in a chilled ultrasonic bath and stirred with a mechanical stirrer at the same time; TEOS solution was then added dropwise to the suspended nanoparticle solution at a rate of 3.96 mLh^{-1}. The silica coated hematite nanoparticles were collected by centrifugation, dried and reduced to magnetite in a tube furnace at 500 °C in the presence of hydrogen for 5 h.

Synthesis of P(DEGMA-*co*-OEGMA-*b*-[TMSPMA-*co*-VBA]) Copolymer and Grafting onto Silica Coated Magnetite Nanoparticles

The P(DEGMA-*co*-OEGMA) polymer was synthesised by mixing the 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid RAFT chain transfer agent, 4,4-azobis(4-cyanovaleric acid) (ACVA) initiator, poly(ethylene glycol) methyl ether methacrylate (OEGMA), di(ethylene glycol) methyl ether methacrylate (DEGMA) in acetonitrile. This mixture was then degassed and mixed for 8 h at 70 °C. The polymer was then purified using petroleum spirits. A chain extension was then carried out by mixing the dry P(DEGMA-*co*-OEGMA) polymer with 4,4-azobis(4-cyanovaleric acid) (ACVA) initiator, 3-(trimethoxysilyl)propyl methacrylate (TMSPMA) and 3-vinylbenzaldehyde (VBA) in toluene. The monomers were added in a ratio of 1:4 to form a block copolymer with 5% w/w TMSPMA and 15% w/w VBA. This mixture was then degassed and mixed for 4 h at 90 °C. The polymer was again purified by precipitation in petroleum spirits. Grafting of the polymer onto the silica coated magnetite nanoparticles was carried out by first suspending 20 mg of the nanoparticles in a solution of 30 mL of ethanol, 0.1 mL of water, 0.05 mL of hydrochloric acid and 100 mg of P(DEGMA-*co*-OEGMA-*b*-[TMSPMA-*co*-VBA]) polymer. The suspension was mixed at 25 °C for 24 h. The polymer-grafted nanoparticles were then collected using a magnet and washed three times with ethanol.

Release of Fluorescein amine through pH and Alternating Magnetic Field Induced Breakage of Schiff Bonds

Fluorescein amine was conjugated onto the polymer-nanoparticle composites by mixing 20 mg of the polymer-nanoparticle composites with 20 mg of fluorescein amine and 100 mg of triethylamine in 20 mL of acetonitrile at 25 °C for 24 h. The nanoparticles were then retrieved by magnetic separation and washed thoroughly with 0.1 M NaCl solution until no fluorescein amine was detectable in the supernatant by UV-visible spectroscopy analysis (>10 washing cycles). Water at pH 7.4 or 5.5 were prepared by the dropwise addition of potassium hydroxide solution (100 mM) and/or nitric acid (100 mM) solution to a known quantity of water. Four identical samples containing 1.75 mg mL^{-1} of fluorescein amine loaded polymer-nanoparticle conjugates were prepared (two at pH 7.4 and two at pH 5.5). One sample at each pH was held at 25 °C, while the other was exposed to an alternating magnetic field (Ambrell EASYHEAT 0224 FF CE Magnetic Induction Heater operating at 195 kHz and 90 kA/m). The release of fluorescein amine was determined from the absorbance of the supernatant at 488 nm (pH 7.4) and 480 nm (pH 5.5).

MRI Spin-Spin Relaxivity

Batches of the polymer-nanoparticle composite were prepared at concentrations between 0.00-0.10 gL^{-1} in agar. Spin-spin relaxation measurements were carried out using a Bruker Avance 11.7 T wide-bore spectrometer fitted with a mini 0.75 animal probe with triple-axis gradients (0.45 T m^{-1}). Images were taken with a field of view (FOV) of 21.1 mm by 23.4 mm, a slice thickness of 2 mm, TE/TR of 10/2500, and scan time of 8 min.

Characterisation

Transmission Electron Microscopy and Energy Dispersive X-ray Spectroscopy • Particle size, structure and properties were examined using a Transmission Electron Microscopy and Energy Dispersive X-Ray Spectroscopy (TEM and EDS, FEI Tecnai G2 20 TEM operating at 200 keV and 0.2 μA). Samples were prepared by suspending ~10 mg of dry sample in methanol and sonicating for 1 min; 10 μL of the suspension was deposited onto a Formvar coated copper grid.

Scanning Electron Microscopy • Particle morphology was also examined using Scanning Electron Microscopy (SEM, Hitachi S900 SEM operating at 4 keV). Sample stages were prepared by adhering a mica sheet to a copper sheet base with carbon tape. Samples were prepared by suspending ~10 mg of dry sample in methanol and sonicating for 1 min before dropping onto the mica stage. The sample was left to dry for 1 h before it was sputtered with chromium in an Emitech K575X Peltier Cooled Coater under an argon atmosphere.

X-ray Diffraction Crystallography • The crystalline phase of the nanoparticles was determined by X-ray Diffraction Crystallography (XRD, PANalytical Xpert Multipurpose X-ray Diffraction System) operating at 45 kV and 40 mA, with step size of 0.0263° and time per step of 77.5 s.

Vibrating Sample Magnetometry • Vibrating Sample Magnetometry (VSM, Quantum Design PPMS P525) was used to obtain the hysteresis curve of the magnetite nanoparticles. The analysis was run on a 3 mg sample at room temperature.

Ultraviolet-Visible Spectrophotometry • UV-Visible Spectrophotometry (UVV, Varian Cary 300) with temperature modulation was used to measure the Lower Critical Solution Temperature. A solution of 5 mg mL^{-1} of polymer in water was prepared and placed in a quartz cuvette. The sample was heated and then cooled at a rate of 1 °C min^{-1} from 25 °C to 65 °C whilst the absorbance was measured at 520 nm. UVV analysis was also used to determine the concentration of fluorescein amine. A known concentration of fluorescein amine at pH 5.5 and 7.4 was prepared and used to prepare serial dilution of fluorescein amine at the same pH. The absorbance at 488 nm (pH 7.4) and 480 nm (pH 5.5) were then measured to obtain the calibration curves (Figure S9).

Gel Permeation Chromatography • Gel Permeation Chromatography (GPC) was used to analyse polymer polydispersity and average chain length. Samples were prepared by dissolving 1 mL of air-dried polymer in 2 mL of N,N-dimethylacetamide (DMAc) solvent. This sample was then be filtered through a 0.45 μm filter. GPC was performed using a Shimadzu GDU-12A solvent degasser with an LC-10AT pump, RID-10A refractive index detector, and a CTO-10A column oven. The system was equipped with a Polymer Laboratories 5.0 mm bead-size guard column (50 mm×7.8 mm) followed by four 300 mm×7.8 mm linear columns.

^{1}H Nuclear Magnetic Resonance Analysis • Polymer composition was analysed using ^{1}H Nuclear Magnetic Resonance Spectroscopy (NMR, Bruker Avance III 300 MHz). Samples were prepared by dissolving 0.5 mL of air-dried polymer in 0.6 mL of chloroform-d NMR solvent. All chemical shifts were reported in ppm relative to tetramethylsilane.

Fourier Transform Infrared Spectroscopy • Fourier Transform Infrared Spectroscopy (FTIR, PerkinElmer Spotlight 400) was used to confirm the presence of polymer on the nanoparticle surface. Each spectrum is the average of 64 scans between wavenumber of 650 cm^{-1} and 4000 cm^{-1} at a resolution of 1 cm^{-1}.

X-Ray Photoelectron Spectrometer Surface Analysis • Surface elemental composition and chemical state of the nanoparticle before and after polymer grafting was determined by using X-Ray Photoelectron Spectrometer (XPS, Thermo Scientific ESCALAB250i).

Brunauer-Emmett-Teller Surface Area Analysis • The surface area of the nanoparticle was determined by Brunauer-Emmett-Teller Surface Area Analysis (BET, Micromeritics TriStar 3000). Samples were prepared by drying at 160 °C for 3 h.

Thermogravimetric Analysis • Polymer-graft density on the nanoparticle surface was quantified using Thermogravimetric Analysis (TGA, TA Instruments Q5000 IR Thermogravimetric Analyzer). The Q5000 instrument was calibrated using a calcium oxalate standard. Samples were placed on a platinum pan and the temperature was ramped at 20 °C min^{-1} until 1000 °C was reached, whereby the sample was then held for 5 min. Changes in sample weight were measured and compared with that of a polymer free control.

Dynamic Light Scattering • Dynamic Light Scattering (DLS, Malvern Zetasizer Nano ZS DLS) was used to estimate the effect of polymer grafting on the aggregate size of the nanoparticle. Samples were prepared by suspending 1 mg of sample in 50 mL of 0.006 M NaCl solution and dispersed by sonication (Misonix Sonicator S-4000, ¼ in micro-tip horn) at 30% amplitude for 6 min.

Inductive Couple Plasma-Optical Emission Spectroscopy • Nanoparticle composition was determined using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES, Perkin Elmer OPTIMA 7300). Samples were prepared by dissolving 4 mg of nanoparticles in 4 mL of aqua regia for 3 days before diluting to 20 ml for analysis.

Fluorescence-Lifetime Imaging Microscopy • Fluorescein amine-polymer-nanoparticles were examined using Fluorescence Lifetime Imaging Microscopy (FLIM, Picoquant Microtime200 Inverted Confocal Microscope). Excitation was via a fibre-coupled, pulsed laser diode operating at 470 nm (40 MHz) with a pulse width below 200 ps. The emission was collected using a 532 nm long-pass filter and a single-photon avalanche diode (SPAD) (PDM, MicroPhoton Devices) connected to time-correlated single-photon counting (TCSPC) electronics (Picoharp300, Picoquant). Lifetime images were recorded using 60×1.2 NA water-immersion objective (Olympus). Phasor analysis of FLIM was performed using SimFCS software developed at the Laboratory for Fluorescence Dyanmics, University of California at Irvine.

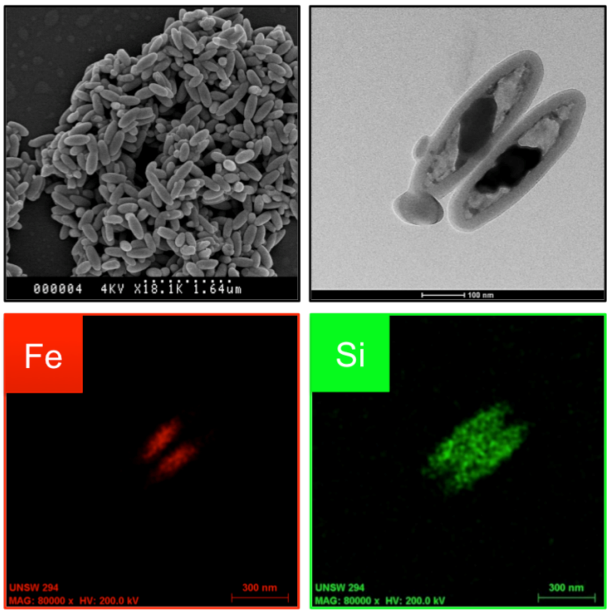

Figure S1: Scanning Electron Microscope image of acicular coated magnetite nanoparticles (top-left); Transmission Electron Microscope image of acicular coated magnetite nanoparticles (top-right); Energy Dispersive X-Ray Spectroscopy map of iron (bottom-left, red) and silicon (bottom-right, green) showing a distinct concentration of iron in the region purportedly to be magnetite, and a thin outer layer of silicon in the region purportedly to be silica.

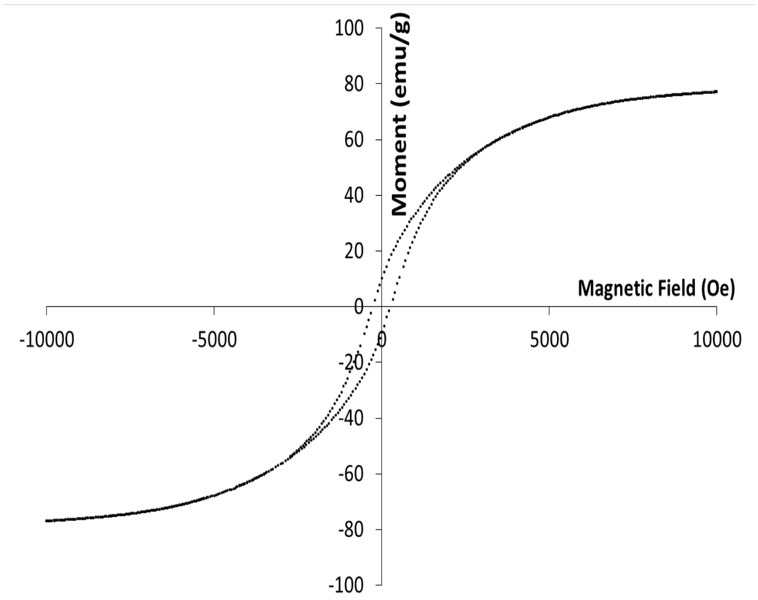

Figure S2: Magnetic hysteresis curve for “magnetic eye” nanoparticles. Room temperature magnetisation loop measurements of these nanoparticles were made using vibrating sample magnetometry (VSM). The mass composition of iron was determined using inductively couple plasma optical emission spectroscopy (ICP-OES) and the magnetisation data was corrected by excluding the non-magnetic components of the polymer-nanoparticle composite, i.e. the silica and polymer layer, which contributed 47.5% by mass of the nanoparticles. A mild hysteresis loop was observed , with a coercivity of 215 Oe. The particles exhibit a magnetic remanence and saturation magnetisation of 10 and 77 emu g^{-1}, respectively. This was attributed to the large size of the particles, which prevented single domain magnetisation, and hence the hysteresis. This property is beneficial to the magnetic heating properties of the agent by contributing hysteresis losses. The mild hysteresis from the VSM analysis suggests that this heat generation is due to both relaxational and hysteresis losses. The controllable heating properties of these polymer-nanoparticle composites were examined by exposing the nanocomposite to an alternating magnetic field (AMF). The specific absorption rate (SAR) of nanoparticles in water when exposed to an alternating magnetic field operating at 195 kHz and 90 kA/m is 308 W g^{-1} of iron.

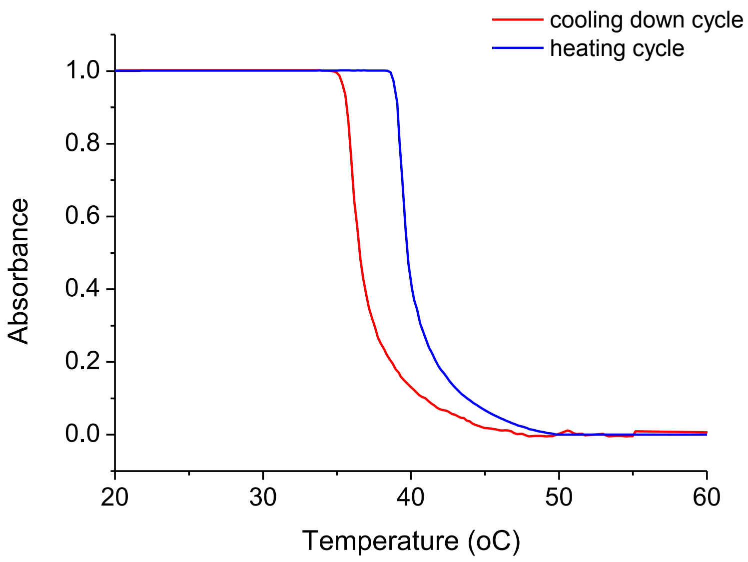

Figure S3. Thermal properties for the P(DEGMA-*co*-OEGMA-*b*-[TMSPMA-*co*-VBA]) diblock copolymers prepared in this study. UVV turbidity measurement for the heating and cooling down cycle.

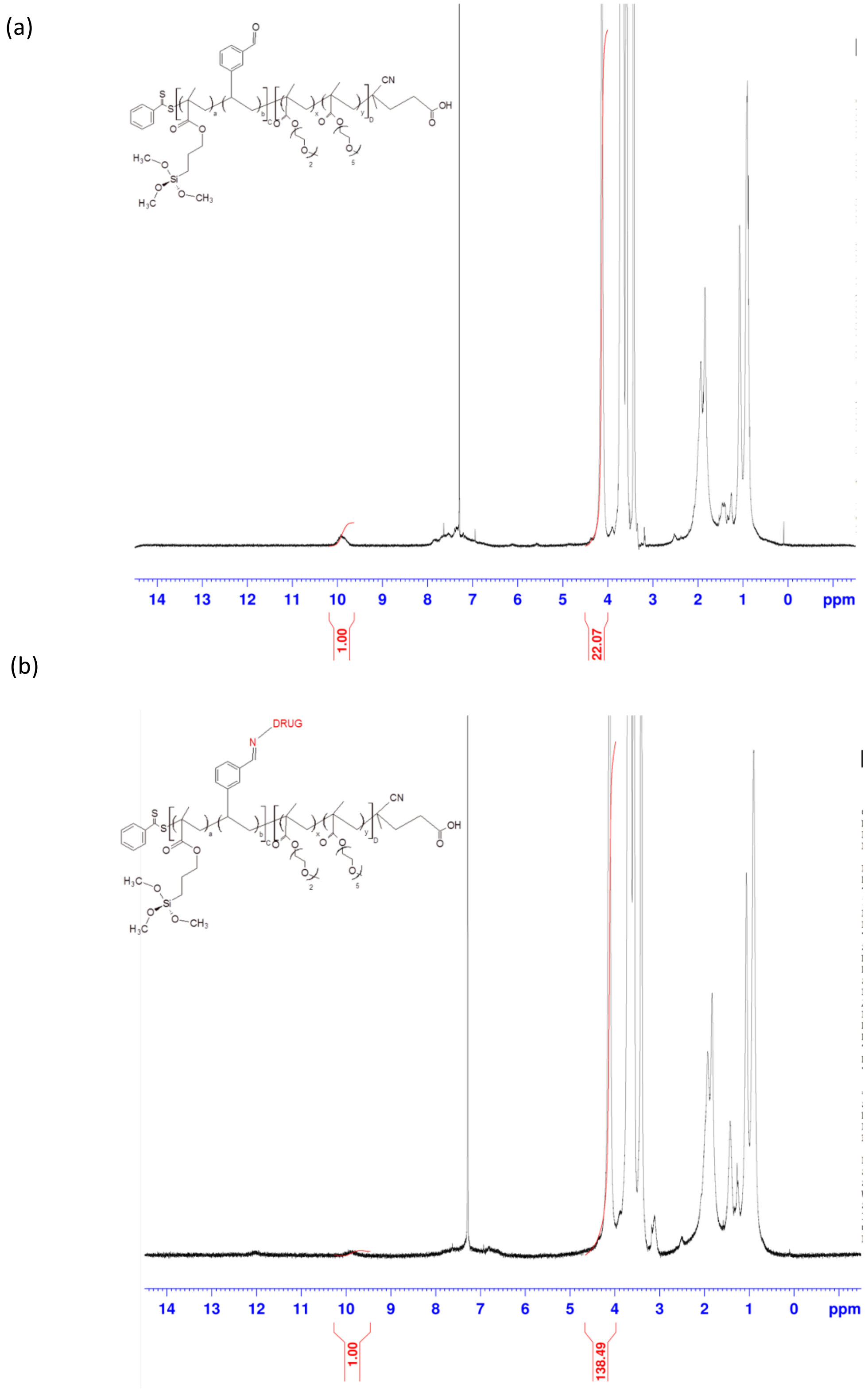

Figure S4: ^{1}H NMR spectra showing a reduction in intensity of the aldehyde peak at 10 ppm after reaction with fluorescein amine, indicating the successful formation of Schiff base bonds between P(DEGMA-*co*-OEGMA-*b*-[TMSPMA-*co*-VBA]) di-block copolymer and fluorescein amine. The additional peak at 12 ppm in b) was attributed to the acid group present on the dye molecule.

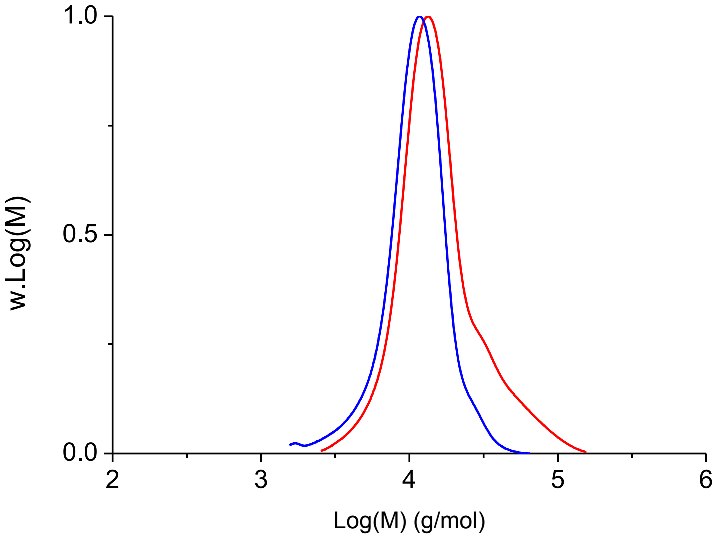

Figure S5. Molecular weight distributions of P(OEGMA-*co*-DEGMA) copolymers and P(DEGMA-*co*-OEGMA-*b*-[TMSPMA-*co*-VBA]) diblock copolymers. The additional high molecular weight should on the second block was attributed to the formation of coupled dead polymers.

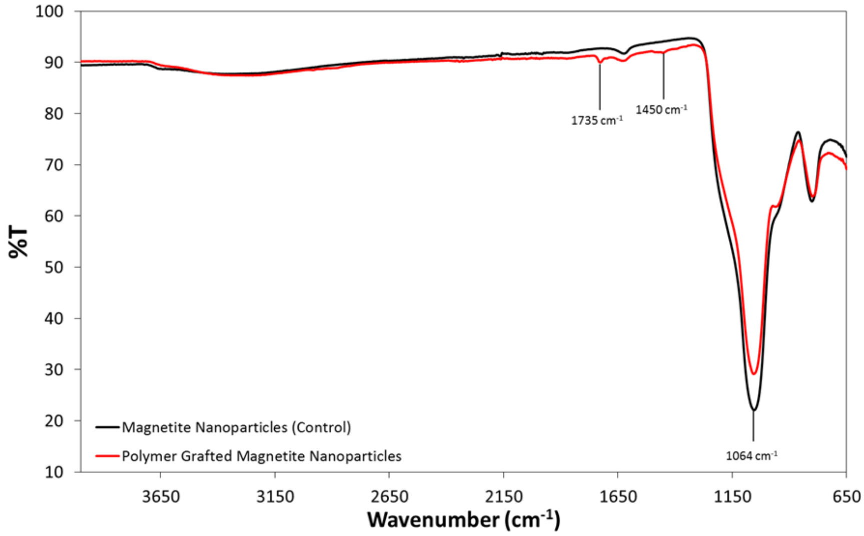

Figure S6: Fourier Transform Infrared spectra of magnetite nanoparticles (control) and polymer-grafted magnetite nanoparticles. The polymer-grafted sample (red) indicated a number of additional peaks, suggestive of successful polymer grafting, when compared to the control group (black). (1750 cm^{-1} consistent with the C=O bond, and a small aromatic peak at 1450 cm^{-1}).

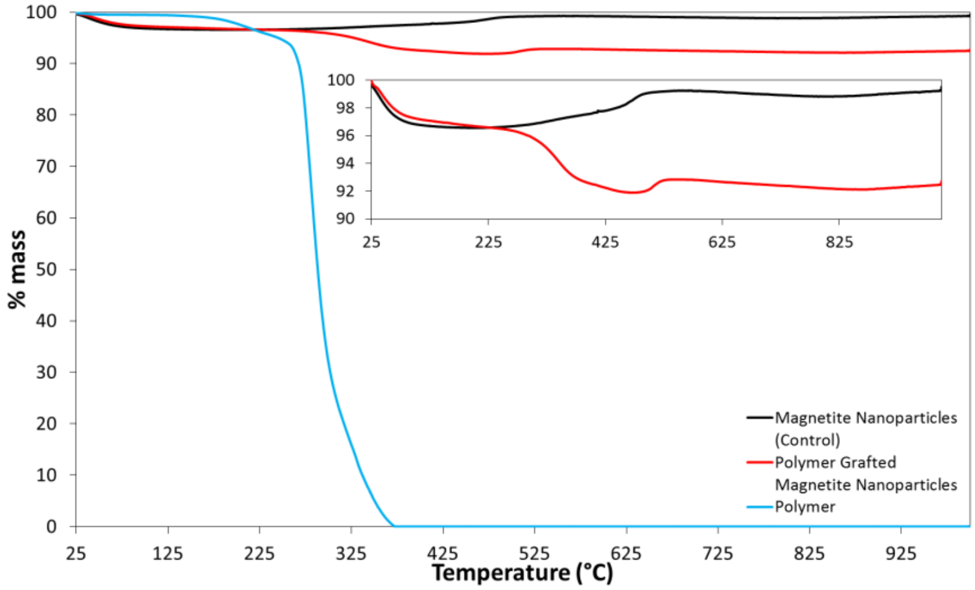

Figure S7: Thermogravimetric analysis (TGA) of the nanoparticles, polymer and polymer-grafted nanoparticles. By comparison between these results, it can be estimated that the polymer-grafted nanoparticles contain 6.82 mass % of polymer. The slight increase in the mass of the control nanoparticles was attributed to oxygen impurities in the nitrogen flow stream, which would have caused the magnetite core to oxidise and hence increase in mass.

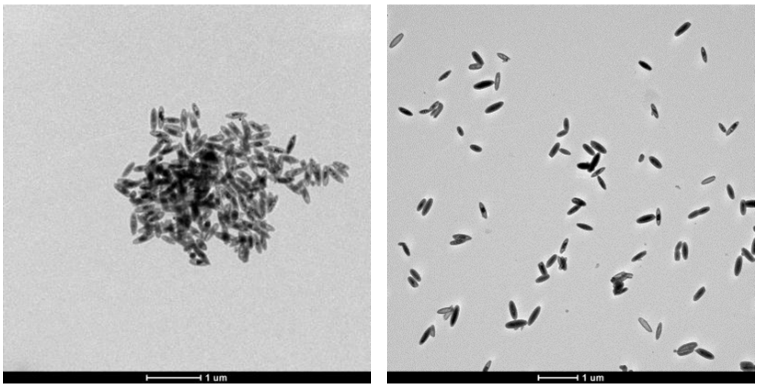

Figure S8: Transmission Electron Microscope image of acicular coated magnetite nanoparticles before (left) and after (right) grafting with the P(DEGMA-*co*-OEGMA-*b*-[TMSPMA-*co*-VBA]) di-block copolymer. Scale bars denote a length of 1 µm.

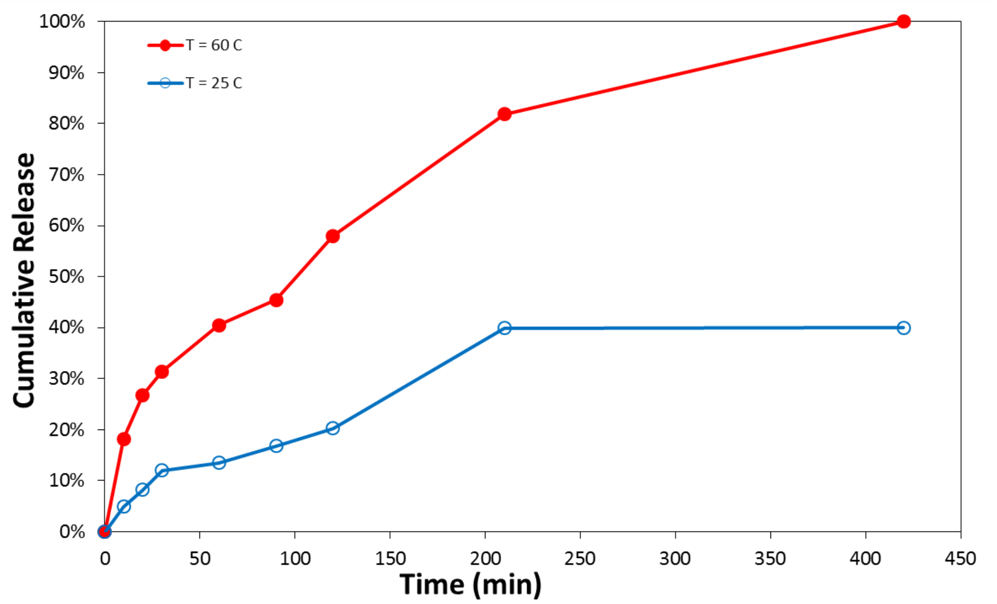

Figure S9: Release kinetics of fluorescein amine when the dye-P(POEGA-*b*-VBA) polymer conjugate was placed in a water bath set to 25 and 60 °C.

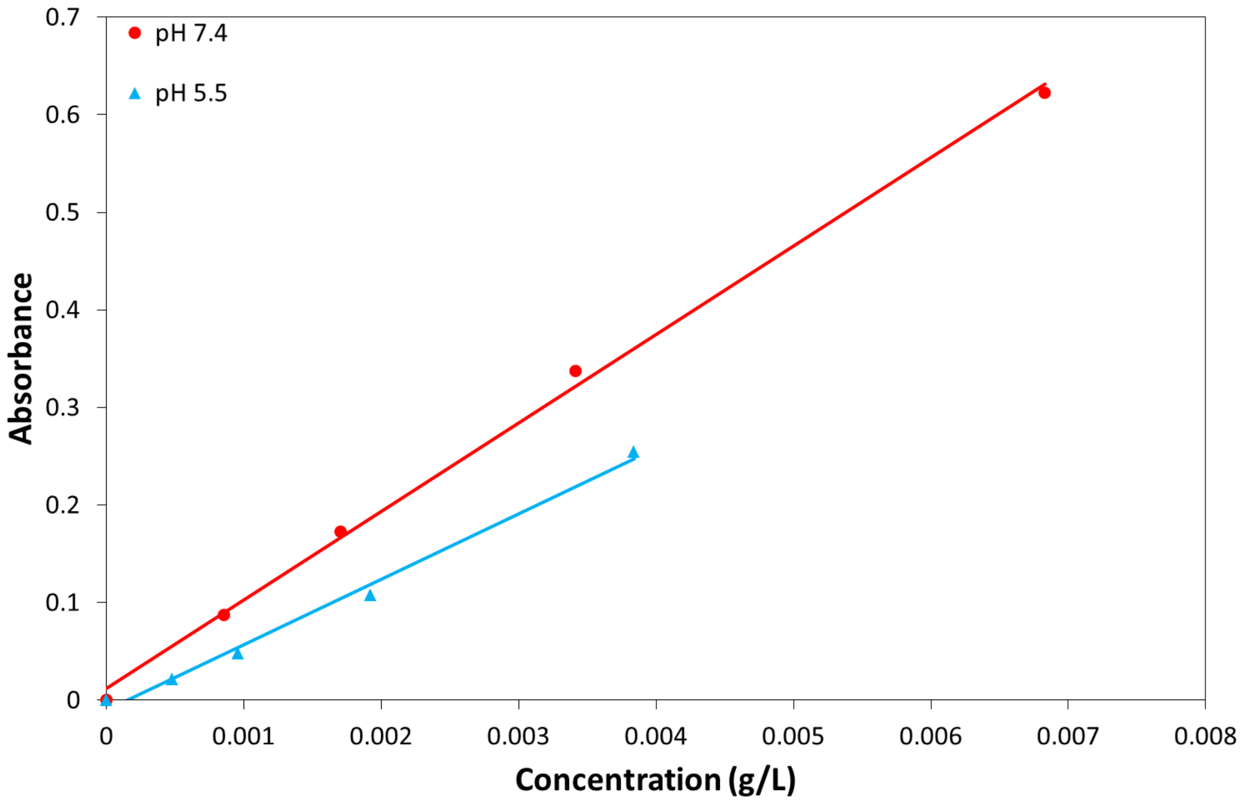

Figure S10: Calibration curve of fluorescein amine, at pH 5.5 (extinction coefficient ε = 67.2 L.mol^{-1}cm^{-1}) and 7.4 (extinction coefficient ε = 90.8 L.mol^{-1}cm^{-1}). The impacts of temperature on these extinction coefficients were assessed and deemed insignificant.

Table S1: XPS atomic percent results of bare silica-coated magnetite nanoparticles, P(DEGMA-*co*-OEGMA-*b*-[TMSPMA-*co*-VBA]), and polymer grafted silica-coated magnetite nanoparticles.

Element	**Bond**	**Binding Energy (eV)**	**Atomic %**		
			Bare Nanoparticles	**Polymer**	**Polymer-Nanoparticles**
Carbon (1s)	C-C/C-H	284.95	1.21	19.70	13.74
	C-O	286.51	0.29	43.71	19.49
	C=O	287.75	0.1	6.39	0.76
	O=C-O	288.88	-	-	3.02
Iron (2p)		710.46	0.08	-	0.11
Oxygen (1s)		532.69	63.73	30.20	42.35
Silica (2p)		103.62	34.59	-	20.52

We balance probabilities and choose the most likely.

The Hound of the Baskervilles