

## A block copolymer-stabilized co-precipitation approach to magnetic iron oxide nanoparticles for potential use as MRI contrast agents

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### Supporting Information

#### Materials

All chemicals were used as received from Ajax and Aldrich, unless otherwise specified. Membranes for dialysis (MWCO 3,000 and 12-14,000 g/mol) were purchased from Fisher Biotec (Cellu SepT4, regenerated cellulose-tubular membrane). Monomer oligo(ethylene glycol) methyl ether acrylate with average  $M_n$  of 480 g/mol (OEGA) was used directly to synthesize P(OEGA) macroRAFT. Dimethyl(2-hydroxyethyl) phosphonate was purchased from Apollo Scientific. The monomer, dimethyl phosphonate ester ethyl acrylate (DMPEA) was synthesized from 2-hydroxyethyl dimethyl ester phosphonate and acryloyl chloride. Solketal acrylate (SolA) was synthesized from solketal (2,2-dimethyl-1,3-dioxane-4-methanol) with acryloyl chloride.

#### Characterisation

**NMR Spectroscopy.** <sup>1</sup>H and <sup>31</sup>P NMR spectra in deuterated chloroform (CDCl<sub>3</sub>), deuterated dimethyl sulfoxide (d<sub>6</sub>-DMSO) were recorded using Bruker Avance 300 (300 MHz).

All chemical shifts are quoted in parts per million (ppm), referenced to residual solvent chemical shift, <sup>1</sup>H NMR: CDCl<sub>3</sub> = 7.26, d<sub>6</sub>-DMSO = 2.50.

OEGA monomer conversion was determined *via* <sup>1</sup>H-NMR spectroscopy by the following equation:  $\alpha^{\text{OEGA}} = 1 - (\int_{5.9 \text{ ppm}} / (\int_{4.3 \text{ ppm}} / 2))$ , where  $\int$  is the peak integral of monomer (vinyl proton at 5.9 ppm, 1H and ester proton at 4.3 ppm, 2H) and the polymer (ester proton at 4.3 ppm, 2H). The degree of polymerization of the resultant P(OEGA) macroRAFT was calculated by the following equation:  $\text{DP}^{\text{OEGA}} = (\int_{4.1 \text{ ppm}} / 2) / (\int_{0.1 \text{ ppm}} / 9)$ , with  $\int_{4.1 \text{ ppm}}$  corresponds to the ester group of P(OEGA) signal at 4.1 ppm and  $\int_{0.1 \text{ ppm}}$  the trimethylsilyl-propargyl end group as a reference. The molecular weight of P(OEGA) macroRAFT was calculated by <sup>1</sup>H-NMR as follows:  $M_{n, \text{NMR}}^{\text{P(OEGA)}} = \text{DP}^{\text{OEGA}} \times \text{MW}^{\text{OEGA}} + \text{MW}^{\text{RAFT 1}}$ , with  $\text{MW}^{\text{OEGA}}$  and  $\text{MW}^{\text{RAFT 1}}$  represent the molar mass of OEGA and RAFT 1, respectively.

The conversion of the 2<sup>nd</sup> monomer used in the synthesis of diblock copolymer was summarized below, where  $\int_{5.9 \text{ ppm}}$  represent a vinyl proton from the 2<sup>nd</sup> monomer.

P(OEGA)-*b*-P(DMPEA):  $\alpha^{\text{DMPEA}} = 1 - (\int_{5.9 \text{ ppm}} / (\int_{3.7 \text{ ppm}} / 6))$

P(OEGA)-*b*-P(*t*BuA):  $\alpha^{\text{tBuA}} = 1 - (\int_{5.9 \text{ ppm}} / (\int_{1.4 \text{ ppm}} / 9))$

P(OEGA)-*b*-P(SolA):  $\alpha^{\text{SolA}} = 1 - (\int_{5.9 \text{ ppm}} / (\int_{1.3 \text{ ppm}} / 6))$

The degree of polymerization ( $\gamma$ ) of the 2<sup>nd</sup> monomer was calculated using the following equation:

P(OEGA)-*b*-P(DMPEA):  $DP^{DMPEA} = (\int_{3.7 \text{ ppm}}/6)/(\int_{0.1 \text{ ppm}}/9)$ , where  $\int_{3.7 \text{ ppm}}$  represent the dimethyl phosphonate ester protons (6H) from the 2<sup>nd</sup> monomer.

P(OEGA)-*b*-P(*t*BuA):  $DP^{tBuA} = (\int_{1.4 \text{ ppm}}/9)/(\int_{0.1 \text{ ppm}}/9)$ , where  $\int_{1.4 \text{ ppm}}$  represent the *tert*-butyl ester protons (9H) from the 2<sup>nd</sup> monomer.

P(OEGA)-*b*-P(SolA):  $DP^{SolA} = (\int_{1.3 \text{ ppm}}/6)/(\int_{0.1 \text{ ppm}}/9)$ , where  $\int_{1.3 \text{ ppm}}$  represent the isopropylidene acetal protons (6H) from the 2<sup>nd</sup> monomer.

The trimethylsilylpropargyl RAFT end-group ( $\int_{0.1 \text{ ppm}}$ ) at 0.1 ppm was used as a reference.

The molecular weight of the resultant diblock copolymer after deprotection was calculated using the degree of polymerization before deprotection ( $DP^{2^{\text{nd}} \text{ monomer}}$ ) as follows:

P(OEGA)-*b*-P(PAEA):  $M_{n, NMR} = M_{n, NMR}^{P(OEGA)} + DP^{DMPEA} \times MW^{PAEA}$ , with  $MW^{PAEA}$  represents the molar mass of phosphonic acid ethyl acrylate (PAEA).

P(OEGA)-*b*-P(AA):  $M_{n, NMR} = M_{n, NMR}^{P(OEGA)} + DP^{tBuA} \times MW^{AA}$ , with  $MW^{AA}$  represents the molar mass of acrylic acid (AA).

P(OEGA)-*b*-P(GA):  $M_{n, NMR} = M_{n, NMR}^{P(OEGA)} + DP^{SolA} \times MW^{GA}$ , with  $MW^{GA}$  represents the molar mass of glycerol acrylate (GA).

**Transmission electron microscopy.** The size of the magnetite nanoparticles were observed using a FEI Tecnai G2 20 TEM with a beam voltage of 200 kV. Samples were prepared by placing a droplet of IONPs dispersion in water on graphite-coated copper grid and draining the excess using filter paper after 20 min.

**X-ray diffraction (XRD).** The crystal characterization of the synthesized neat magnetite nanoparticles was performed using Philips PANalytical Xpert X-ray Multipurpose Diffraction System at 40 mA and 45 kV using monochromated Cu-K $\alpha$  radiation ( $\lambda = 1.541 \text{ \AA}$ , step size = 0.01, 0.02 or 0.05, time per step = 10 or 20 s/step). The crystal size of the magnetite nanoparticles was determined by Scherrer equation (below), where  $K$  is the shape factor (0.9 for spherical particles),  $\lambda$  is the X-ray wavelength,  $B$  is the peak width at  $2\theta = 36^\circ$  and  $d$  is the particle size.

$$B(2\theta) = \frac{K \cdot \lambda}{d \cdot \cos \theta}$$

**Infrared Spectroscopy.** ATR-FTIR spectra of the polymeric nanoparticles were obtained using a Bruker Spectrum BX FTIR system using diffuse reflectance sampling accessories. The spectrophotometer was equipped by tungsten halogen lamp and Si/Ca beam splitter. Spectra were obtained at regular time intervals in the MIR region of 4000 – 500  $\text{cm}^{-1}$  at a resolution of 4  $\text{cm}^{-1}$  (640 scans) and analysed using OPUS software.

**Dynamic light scattering (DLS).** Dynamic light scattering studies of the magnetite nanoparticles at 0.1-0.2 mg/mL  $\text{Fe}_3\text{O}_4$  in water, 0.1M NaCl aqueous solution and 0.1M phosphate buffer containing 0.1 mM bovine serum albumin (BSA) were conducted using a Malvern Instruments Zetasizer Nano ZS instrument equipped with a 4 mV He-Ne laser operating at  $\lambda = 633 \text{ nm}$ , an avalanche photodiode detector with high quantum efficiency, and an ALV/LSE-5003 multiple tau digital correlator electronics system.

**Thermal gravimetric analysis (TGA).** Thermogravimetric analysis (TGA) of the magnetite nanoparticles was performed on a Perkin-Elmer Thermogravimetric Analyzer (Pyris 1 TGA). Pre-dried samples were heated from room temperature to 700°C at a constant temperature increase of 20°C min<sup>-1</sup> using air as the furnace gas. The weight loss was calculated through the difference between the weights at room temperature and at 600°C. The weight loss of the synthesized neat magnetite nanoparticles was determined between 20% and 50%. The grafting density was estimated with the assumption that the nanoparticles are spherical using the weight loss and the surface area of IONPs ( $S_{IONP}$ ) according to the following equation:

$$\text{Grafting Density (nm}^{-2}\text{)} = \frac{(\text{weight loss}/M_{n, \text{polymer}}) \times N_a}{m_{IONP} \times S_{IONP}}$$

$M_{n, \text{polymer}}$  corresponds to the molecular weight of diblock copolymer determined by <sup>1</sup>H-NMR.  $N_a$  is Avogadro's number and  $m_{IONP}$  is the mass of polymer coated IONPs used for the TGA analysis (e.g. mass of nanoparticles = initial mass before TGA analysis – weight loss). The specific surface area of IONPs ( $S_{IONP}$ ) was calculated from the particle size obtained by XRD and TEM following this relationship  $d_{\text{TEM, XRD}} = 6/(\rho \times S_{IONPs})$ , where  $\rho = 5.17 \text{ g cm}^{-3}$  is the density of neat magnetite phase iron oxide.

## Polymer synthesis

### MacroRAFT synthesis

Poly(oligoethylene glycol acrylate) or **P(OEGA)** was synthesized using azobisisobutyronitrile (AIBN) as the initiator, acetonitrile as the solvent by using reversible addition-fragmentation chain transfer (RAFT) polymerization with 3-(trimethylsilyl)prop-2-yn-1-yl 2-(((3-Propionic acid)thio) carbonothioyl)thio)propanoate) or TSPPA as the RAFT chain transfer agent **1**. OEGA (13.3 g, 27.76 mmol), RAFT **1** (0.694 mmol), azobisisobutyronitrile or AIBN (0.069 mmol) and acetonitrile (14 mL) were mixed and heated at 70°C for 5 hours. The degree of polymerization of P(OEGA) was calculated to give  $n = \sim 29$  units at the monomer conversion of  $\sim 70\%$ . The molecular weight was determined by <sup>1</sup>H-NMR using the trimethylsilylpropargyl terminal group of the RAFT agent at 0.1 ppm as a reference. The dispersity determined by SEC was 1.2.

### Block copolymer synthesis

**P(OEGA)-*b*-P(AA)** was prepared using RAFT polymerization. The homopolymer P(OEGA) was used as the macro initiator (P(OEGA) macroRAFT) and the acetonitrile as the solvent. For the polymerization of P(OEGA)-*b*-poly(*tert*-butyl acrylate): P(OEGA) (1 g, 0.071 mmol), *tert*-butyl acrylate (1.43 mmol), AIBN (0.0071 mmol) and acetonitrile (1.5 mL) were added to a schlenk flask. The flask was purged with N<sub>2</sub> during 30 min at 0°C. Then the flask was immersed in an oil preheated at 70°C. The polymerization was allowed to proceed for 6 h under stirring. The solution was dialyzed (molecular weight cut off or MWCO 12000-14000 g/mol) against water to remove the unreacted monomer. The number of repeat units ( $y$ ) of the second block was set up with 20 units. POEGA-*b*-poly(acrylic acid) (PAA) was obtained by treating P(OEGA)-*b*-poly(*tert*-butyl acrylate) with 32% HCl: dioxane (1:10, v/v). P(OEGA)-*b*-P(*t*BuA) (0.055 mmol), 0.4 mL HCl (32%) (4.0 mmol) and dioxane (4 mL) were mixed in a bottle at 100°C for 3 h. The resulting P(OEGA)-*b*-P(AA) was dialyzed (MWCO 6000-8000 g/mol) against water and then dried in a vacuum oven.

**P(OEGA)-*b*-P(PAEA)** was synthesized using a similar procedure, and obtained by deprotection of P(OEGA)-*b*-P(DMPEA). For the polymerization of the P(OEGA)-*b*-P(DMPEA): P(OEGA) macroRAFT (1 g, 0.071 mmol), dimethyl phosphonate ester ethyl acrylate or DMPEA (1.43 mmol), AIBN (1.2 mg, 0.0071 mmol) and acetonitrile (2 mL) were added to a schlenk flask. After purification by precipitation in 1:1 diethyl ether/petroleum spirit, P(OEGA)-*b*-P(DMPEA) (0.047 mmol) was treated with 0.37 mL SiMe<sub>3</sub>Br (2.82 mmol) and anhydrous dichloromethane (2.5 mL). After 3h of reaction, the solvent was evaporated followed by the addition of 2 mL of methanol. The solutions were dialyzed (MWCO: 6000-8000 g/mol) against water to give the resultant block copolymer P(OEGA)-*b*-P(PAEA).

**P(OEGA)-*b*-P(GA)** or P(OEGA)-*b*-poly (glycerol acrylate) was obtained after deprotection of P(OEGA)-*b*-P(solketal acrylate) or P(OEGA)-*b*-P(SolA). For the polymerization of P(OEGA)-*b*-P(SolA), 0.29 g (1.43 mmol) of solketal acrylate was used in combination with P(OEGA) macroRAFT (1 g, 0.071 mmol), AIBN (1.2 mg, 0.0071 mmol) and acetonitrile (2 mL). After purification by precipitation in 1:1 diethyl ether/petroleum spirit, the resultant P(OEGA)-*b*-P(SolA) was deprotected by using 6 M HCl-THF (1:9, v/v). Therefore, P(OEGA)-*b*-P(SolA) (0.028 mmol), 0.3 mL 6 M HCl (1.86 mmol) and THF (2.8 mL) were mixed together. The resulting solution was dialyzed (MWCO 6000-8000 g/mol) against water and then dried into a vacuum oven.

The number of repeating units (*m*) of the second block or DP<sup>2<sup>nd</sup> monomer</sup> was calculated using <sup>1</sup>H-NMR based on the trimethylsilylpropargyl peak at 0.1 ppm as a reference. Based on the equations in the NMR spectroscopy below, the resultant degree of polymerization (*n* for OEGA and *m* for the 2<sup>nd</sup> monomer) as well as their molecular weights were summarized in **Table S1**.

**Table S1.** The degree of polymerization and molecular weights of diblock copolymers.

Polymer	Description	<i>n</i> <sup>a</sup>	<i>m</i> <sup>a</sup>	<i>M<sub>n, NMR</sub></i> (g/mol)
P(OEGA) macroRAFT	Poly(oligoethylene glycol acrylate) <sub><i>n</i></sub>	29		14,280
P(OEGA)- <i>b</i> -P(AA)	Poly(oligoethylene glycol acrylate) <sub><i>n</i></sub> - <i>b</i> -Poly(acrylic acid) <sub><i>m</i></sub>	29	15	17,000
P(OEGA)- <i>b</i> -P(PAEA)	Poly(oligoethylene glycol acrylate) <sub><i>n</i></sub> - <i>b</i> -Poly(phosphonic acid ethyl acrylate) <sub><i>m</i></sub>	29	15	15,400
P(OEGA)- <i>b</i> -P(GA)	Poly(oligoethylene glycol acrylate) <sub><i>n</i></sub> - <i>b</i> -poly(glycerol acrylate) <sub><i>m</i></sub>	29	18	16,900

Notes: *a*- determined by <sup>1</sup>H-NMR.

### Preparation of polymer coated IONPs formulation

The magnetic polymeric-nanoparticles were prepared by chemical co-precipitation of Fe<sup>2+</sup> and Fe<sup>3+</sup> in the mol ratio of 1:2 in the presence of the diblock copolymer. For the stock solution of the iron salts, 0.233 g of iron (III) chloride hexahydrate and 0.086 g of iron (II) chloride tetrahydrate were mixed in 20 mL of de-ionized water to give a concentration of 0.065 M or 9.75 mg/mL of FeCl<sub>3</sub> and FeCl<sub>2</sub>. 2 mL of the iron salt stock solution (19.5 mg of FeCl<sub>3</sub> and FeCl<sub>2</sub>) was mixed with a 2 mL of aqueous solution of diblock copolymer at different weight e.g. 20 mg diblock copolymer was mixed with 2 mL of the iron salt solution for a polymer-to-iron (polymer:Fe) ratio (w/w) of 1:1 (**Table S2**). The mixture was incubated at 40°C for 30 minutes prior to the addition of 70 μL of 28% (w/w) ammonium hydroxide

(0.063 g, 0.58 mmol) followed by sonication for 30 minutes. The weight of the diblock copolymer was adjusted to achieve higher or lower polymer:Fe ratio. After incubation at room temperature, the mixture was dialyzed (MWCO 12000-14000 g/mol) against water to remove the iron salts and base. The polymer coated IONPs were washed 3 times with deionized water to remove unreacted materials and impurities. IONPs dispersion with a range of color from orange to black was obtained depending on the polymer:Fe ratio of respective diblock copolymer. For P(OEGA)-b-P(GA) functionalized IONPs, the mixture of diblock copolymer and iron salts was pre-heated at 80°C in water for 30 minutes before the addition of base.

**Table S2.** Experimental setup of *in situ* co-precipitation of IONPs using 19.5 mg of FeCl<sub>3</sub> and FeCl<sub>2</sub> salts mixture (0.13 mmol Fe ion in 2 mL de-ionized water) at different concentration of diblock copolymers. The molar ratio between Fe (iron) ions and the anchoring groups (phosphonic acid PAEA, carboxylate AA and glycerol GA) was calculated.

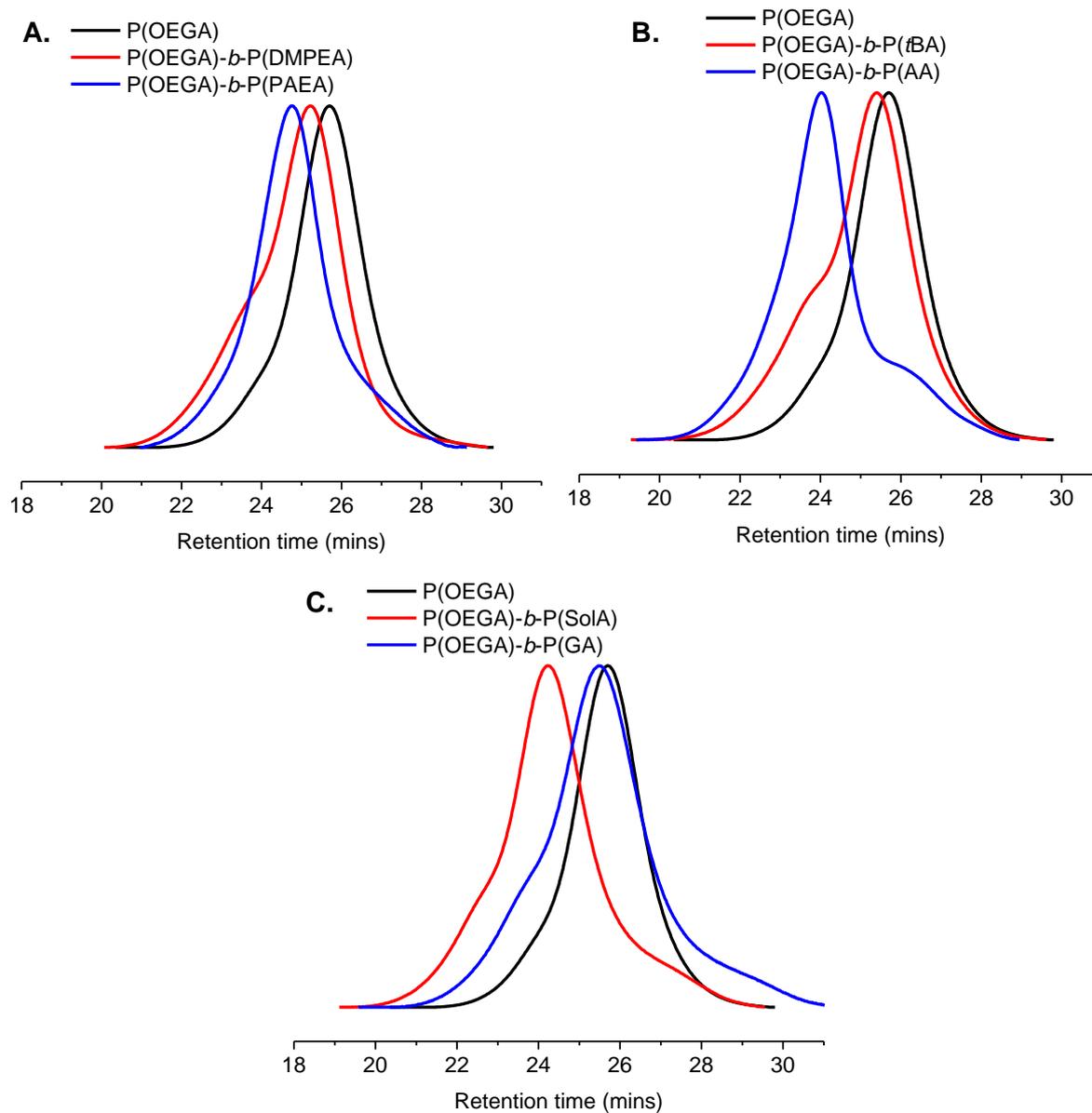
Polymer : Fe salts ratio	Diblock copolymer (mg)	Fe ion : anchoring groups PAEA <sup>a</sup>	Fe ion : anchoring groups AA <sup>b</sup>	Fe ion : anchoring groups GA <sup>c</sup>
1 : 10	2	73.44	66.53	-
1 : 4	5	29.37	26.61	24.34
1 : 2	10	14.69	13.31	12.17
1 : 1	20	7.34	6.65	6.08
1 : 2	40	3.67	-	3.04
1 : 4	80	-	-	1.52

Notes: a- anchoring groups PAEA was calculated by  $DP^{DMPEA} \times (m^{P(OEGA)-b-P(PAEA)} / M_n^{NMR^{P(OEGA)-b-P(PAEA)}})$ ; b- anchoring groups AA was calculated by  $DP^{tBuA} \times (m^{P(OEGA)-b-P(AA)} / M_n^{NMR^{P(OEGA)-b-P(AA)}})$ ; c- anchoring groups GA was calculated by  $DP^{SolA} \times (m^{P(OEGA)-b-P(GA)} / M_n^{NMR^{P(OEGA)-b-P(GA)}})$ .

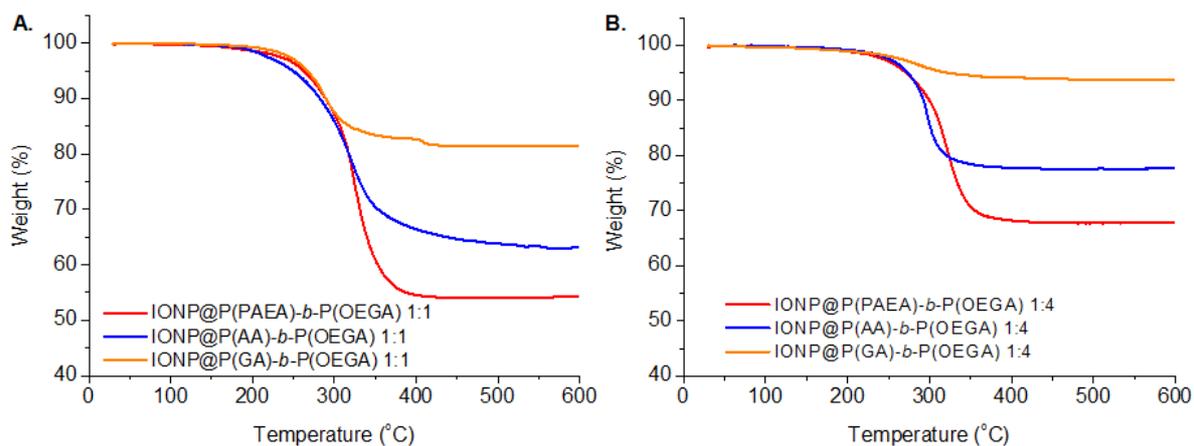
### MRI $r_2$ Relaxivity Measurement.

A 9.4T Bruker Biospin 94/20 USR equipped with a Transceiver RES <sup>1</sup>H 72mm Quad RF coil was used for T<sub>2</sub> relaxometry measurements. A dilution series for each sample (500 μl) was prepared in water and measurements were performed at 20 °C. T<sub>2</sub>-weighted images were acquired using a Multi-Slice-Multi-Echo (MSME) sequence with TR = 3000 ms and 32 echoes with echo spacing 10.686 ms, FOV = 5 x 5 cm, matrix size = 256 x 256, scan time = 12 min and slice thickness 1 mm. The net magnetizations for each sample were determined from the manually-drawn regions of interest (ROIs). The T<sub>2</sub> relaxation times were determined by fitting a mono-exponential decay equation using Paravision 5 (Bruker). The T<sub>2</sub> relaxivity was then calculated as slope from a plot of the inverse of the relaxation times (R<sub>i</sub>, i = 1,2) versus iron concentration in water. The iron concentrations [Fe] were determined by Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES) using a Perkin Elmer Optima 7300 ICP-OES spectrometer. The nanoparticles were digested overnight in highly concentrated hydrochloric acid (32%), followed by dilution in water.

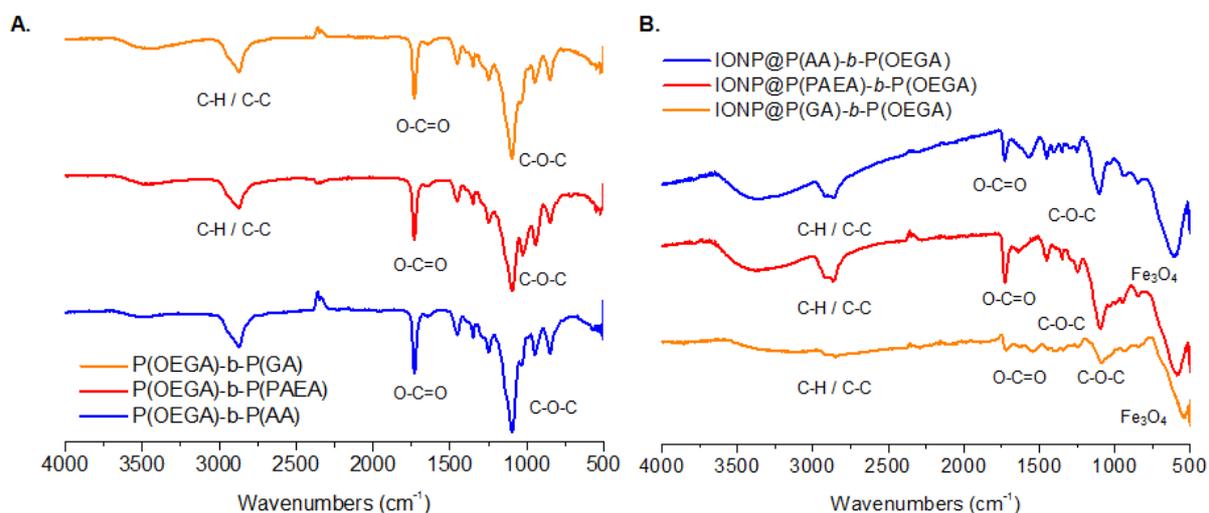
## Supplementary figures



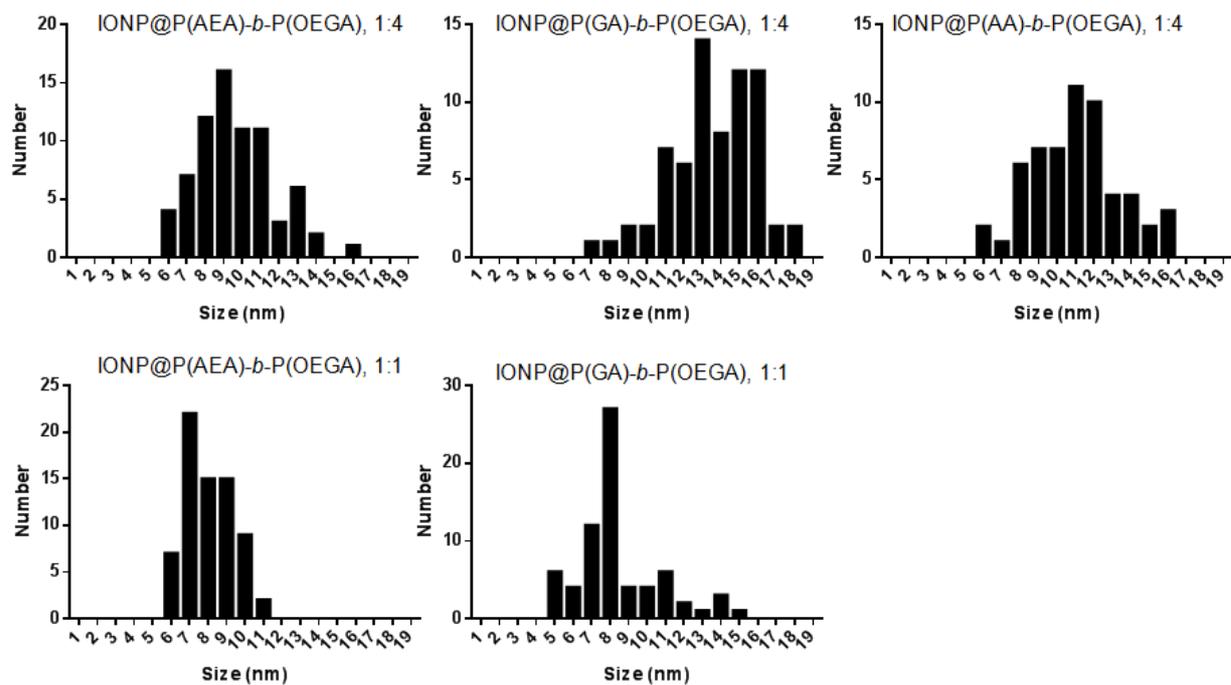
**Figure S1.** SEC of phosphonic acid (A), carboxylic acid (B) and glycerol functionalized block co-polymers before and after deprotection. The dispersities of all diblock copolymers were determined by SEC in the range of 1.2-1.3.



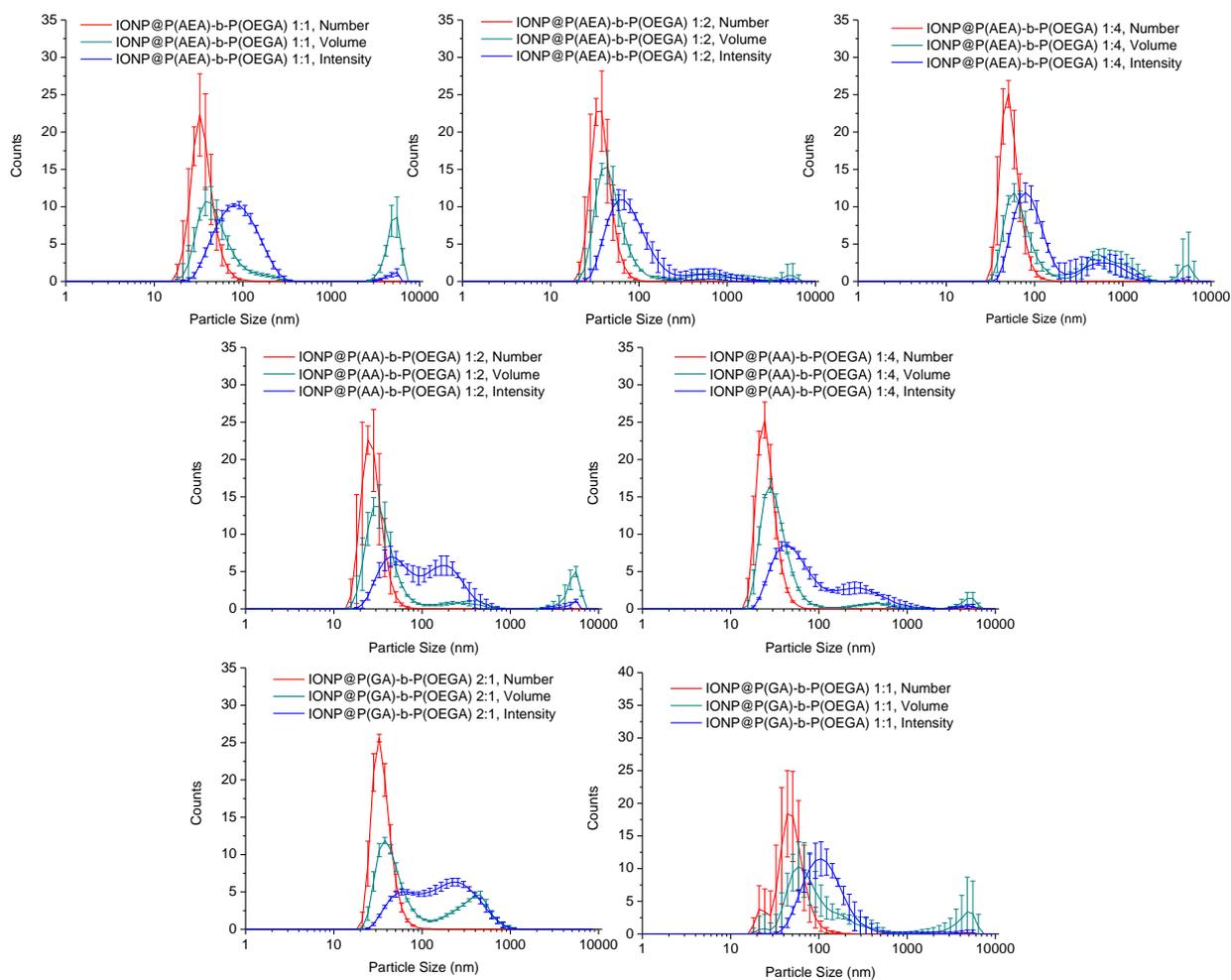
**Figure S2.** TGA results of polymer coated IONPs with different anchoring groups at 1:1 polymer-to-iron ratio (A) and at 1:4 polymer-to-iron ratio (B).



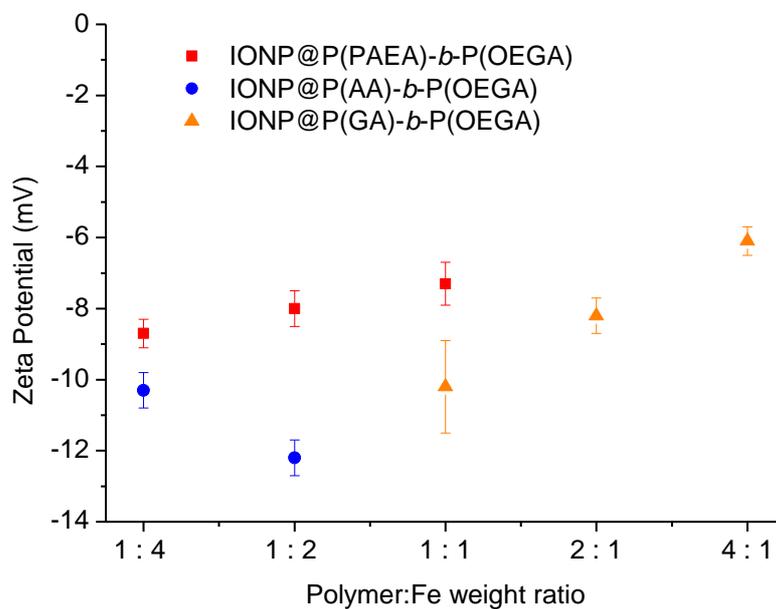
**Figure S3.** ATR-FTIR spectra of block copolymers (A) and the resultant polymer coated iron oxide nanoparticles (IONPs) after *in situ* co-precipitation and purification (B).



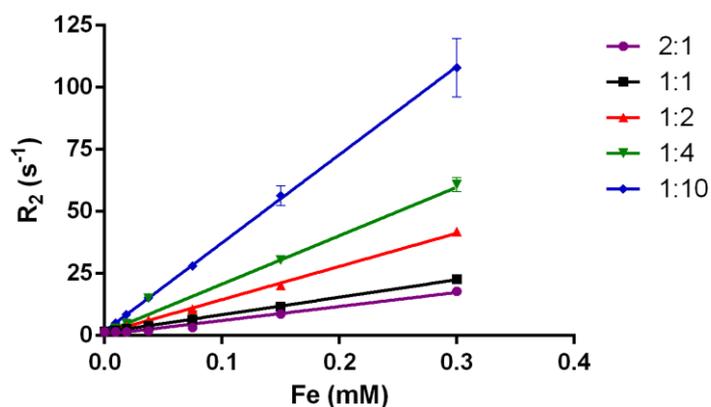
**Figure S4.** The particle sizes in TEM were determined using ImageJ. 60 to 70 particles per image were manually selected and measured using the ROI manager, followed by conversion to nm-scale using the embedded scale bar.



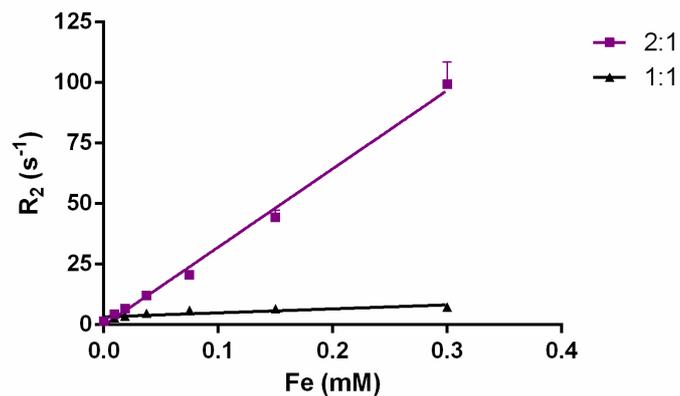
**Figure S5.** Particle size distribution of polymer coated nanoparticles  
Weighted by number, volume and intensity (DLS in water).



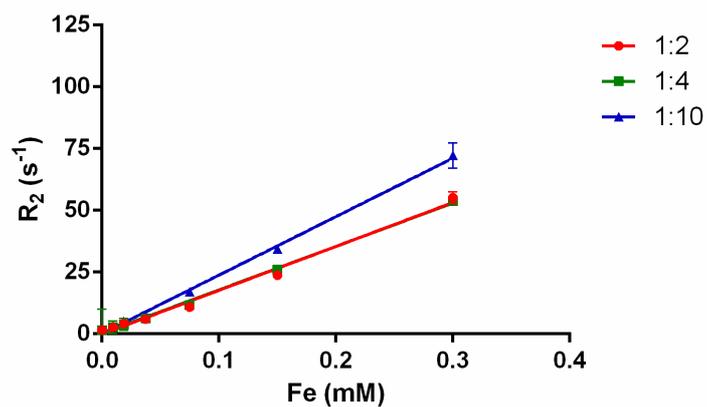
**Figure S6.** Zeta potential ( $\zeta_p$ ) measurement of polymer coated IONPs in 0.05 M NaCl aqueous solution prepared at different polymer-to-iron ratio.



**Figure S7.**  $r_2$  relaxivity measurement of IONP@P(PAEA)-*b*-P(OEGA) in water at different polymer-to-iron ratio using 9.4T MRI scanner.



**Figure S8.**  $r_2$  relaxivity measurement of IONP@P(GA)-*b*-P(OEGA) in water at different polymer-to-iron ratio using 9.4T MRI scanner.



**Figure S9.**  $r_2$  relaxivity measurement of IONP@P(PAA)-*b*-P(OEGA) in water at different polymer-to-iron ratio using 9.4T MRI scanner.