Electronic Supplementary Information

Magnetic nanoparticles with diblock glycopolymer shells give lectin concentration-dependent MRI signals and selective cell uptake

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Experimental Section

Materials

Copper wire (diameter = 0.25 mm) was activated by washing in sulfuric acid for 10 min, and exhaustively rinsed with MiliQ Water, acetone and dried under nitrogen. The copper was used immediately after acid treatment. The ligand, *tris*(2-(dimethylamino)ethyl)amine (Me_6TREN) was synthesized according to literature procedures[1] and stored under nitrogen prior to use. Monomer oligo(ethylene glycol) methyl ether acrylate with average M_n of 480 g/mol (OEGA) were received from Sigma-Aldrich and prior to reaction were de-inhibited by passing the monomers through a column filled with basic alumina. In the synthesis of 1-(2-propargyl)-D-mannose and glucose, H_2SO_4-silica catalyst were prepared and used according to the literature procedure.[2] 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucose, diethyl (3-bromopropyl) phosphonate, concanavalin A and its FITC-conjugates were used as received from Sigma-Aldrich. Dialysis membrane (MWCO 3,500) was obtained from Spectrum Laboratories (Cellu SepT4, regenerated cellulose-tubular membrane). All other reagents and solvents were obtained at the highest purity available from Sigma-Aldrich and used without further purification unless otherwise stated.

Characterizations

NMR Spectroscopy. ^{1}H, ^{31}P and ^{13}C NMR spectra were recorded using Bruker DPX-300 and DPX-400 (400 MHz) spectrometers. $CDCl_3$, d_6-DMSO and MeOD were used as solvents. All chemical shifts are quoted in parts per million (ppm), referenced to residual solvent frequencies (^{1}H NMR: $CDCl_3$ = 7.26, d_6-DMSO = 2.50, MeOD = 3.31 and ^{13}C NMR: $CDCl_3$ = 77.16, d_6-DMSO = 39.52). For ^{31}P NMR spectra, ^{31}P resonances were externally referenced to 85% H_3PO_4 in D_2O at 0.00 ppm. OEGA Monomer conversion (α^{OEGA}) and GA conversion (α^{GA}) were determined by ^{1}H NMR using the following equation:

$\alpha^{OEGA} = 1 - \int_{6.5\ ppm} / [\int_{6.5\ ppm} + (\int_{4.1\ ppm}/2)]$, with $\int_{6.5\ ppm}$ corresponds to the integrals of acrylate group (CH=) and $\int_{4.1\ ppm}$ the ester group of P(OEGA) signal at 4.1 ppm.

$\alpha^{GA} = 1 - \int_{6.5\ ppm} / [\int_{6.5\ ppm} + \int_{4.3\ ppm}]$, with $\int_{6.5\ ppm}$ corresponds to the integrals of acrylate group (CH=) and $\int_{4.3\ ppm}$ corresponds to one proton from the ester group of P(GA) signal at 4.3 ppm.

Theoretical DP of the glycopolymer (number of sugar unit) was calculated from their precursor P(glycidyl acrylate) (DP^{GA}) using signals at 4.3 or 2.9 ppm.

$DP^{OEGA} = [(\int_{4.1\ ppm}/2)/(\int_{0.9\ ppm}/6)]$, with $\int_{4.1\ ppm}$ corresponds to the ester group of P(OEGA) signal at 4.1 ppm and $\int_{0.9\ ppm}$ the isobutyryl end group.

$DP^{GA} = [(\int_{4.3\ ppm})/(\int_{0.9\ ppm}/6)]$, with $\int_{4.3\ ppm}$ to one proton from the ester group of P(GA) signal at 4.3 ppm and $\int_{0.9\ ppm}$ the isobutyryl end group.

The triazole signal at 8.0 ppm (or the glycosidic anomeric signal at 5.5 ppm) of the resultant glycopolymer was used for calculation of the copper azide-alkyne click reaction yield, using the following equation: yield (%) = $[((DP^{GA} + DP^{OEGA}) \times \int_{8.0ppm}) / DP^{GA} \times (\int_{4.1\ ppm}/2)] \times 100$, with $\int_{8.0ppm}$ and $[\int_{4.1ppm}$ correspond to the integrals of signals at 8.0 and at 4.1 ppm.

Size Exclusion Chromatography (SEC) Size exclusion chromatography (SEC) was implemented using a Varian 390-LC system in *N,N'*-dimethylacetamide (DMF) (1 g/L LiBr) at 50 ^{o}C equipped with refractive index and vixcometry detectors, 2 x PLgel 5 mm mixed-D columns (300 x 7.5 mm), 1 x PLgel 5 mm guard column (50 x 7.5 mm). Narrow linear poly(methyl methacrylate) standards in range of 200 to 2.0 x 10^6 g/mol were used to calibrate the system. All samples were filtered through 0.45 μm filters. Chromatograms were processed using Cirrus 2.0 software (Polymer Laboratories).

Electron Spray Ionization-Mass Spectrometer. ESI-MS was recorded on a Thermo Finnigan LCQ Deca quadrupole ion trap mass spectrometer (Thermo Finnigan, San Jose, CA), equipped with an atmospheric pressure ionization source operating in the nebulizer assisted electrospray mode and was used in positive ion mode.

Infrared Spectroscopy. ATR-FTIR spectra of the sugars and block co-polymers were recorded on a Bruker VECTOR-22 FTIR spectrometer using a Golden Gate diamond attenuated total reflection cell. ATR-FTIR spectra of the glycopolymer functionalized magnetite 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 cm^{-1} at a resolution of 4 cm^{-1} (640 scans) and analyzed using OPUS software.

Dynamic light scattering (DLS). Dynamic light scattering studies of the magnetite nanoparticles at 0.2 mg/mL in water or HEPES saline buffer solution (with and without Concanavalin A) were conducted using a Malvern Instruments Zetasizer NaNo ZS instrument equipped with a 4 mV He-Ne laser operating at λ = 633 nm, an avalanche photodiode detector with high quantum efficiency, and an ALV/LSE-5003 multiple tau digital correlator electronics system.

Fluorescence Spectroscopy. Fluorescence emission spectra were recorded using an Agilent fluorescence spectrophotometer.

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 ambient temperature to 700°C at a constant temperature increase of 20°C min-1 using air as the furnace gas. The weight loss was calculated through the difference between the weights at ambient temperature and at 600°C. The weight loss of the synthesized neat magnetite nanoparticles was determined at around ~5%. The grafting density was estimated using the weight loss (*loss-wt*) and the surface area of IONPs determined by BET according to the following equation:

$$\text{Grafting Density } (\text{nm}^{-2}) = \frac{\left(loss - wt/Mn_{polymer}\right) \times N_a}{m_{NP} \times S_{NP}}$$

$M_{n,\ \text{polymer}}$ corresponds to the weight of polymer grafted. N_a is Avogadro's number and m_{NP} is the mass of polymer grafted magnetite nanoparticles used for the TGA analysis (e.g. mass of nanoparticles = initial mass before TGA analysis – loss of weight).

Specific surface area (S_{NP}). Specific Surface Area of the synthesized magnetic nanoparticles was measured on Micromeritics Tristar 3000 by means of N_2 adsorption and desorption at 77 K using the BET isotherm method. These neat magnetite nanoparticles were dried under vacuum at 150°C for 3h before the measurement. The resultant specific surface area (S_{NP}) of neat magnetite nanoparticles was comparable with S_{NP} deduced from particle size obtained from XRD measurement. Based on the equation $L_{NP\ by\ XRD} = 6/(\rho \times S_{NP})$ where ρ = 5.17 g cm^{-3} is the density of neat magnetite phase iron oxide, the particle size of magnetite nanoparticles was estimated around 10.4 – 10.7 nm, which resulted in Specific Surface Area of around 105.48 ± 0.83 m^2/g.

Transmission electron microscopy. The size of the magnetite nanoparticles was observed using a FEI Tecnai G2 20 TEM with a beam voltage of 200 kV. Samples were prepared by placing a droplet of a 0.2 mg mL^{-1}

nanoparticles solution in water or THF 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 PANanalytical Xpert X-ray Multipurpose Diffraction System at 40 mA and 45 kV using monochromated Cu-Ka radiation (λ =1.541 Å, 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), λ is the X-ray wavelength, *B* is the peak width at 2θ = 36° and *L* is the particle size, which was estimated around 10.4 nm.

$$B(2\theta) = \frac{K.\lambda}{L.Cos\ \theta}$$

X-ray photoelectron spectrometer (XPS). The chemical surface composition of the nanoparticles synthesized was determined by X-ray Photoelectron Spectroscopy (XPS) using an EscaLab 220-IXL from VG Scientific. The instrument was operated with a monochromated Al-Ka radiation at 1486.60 eV and a power source of 120 W. A spot size of 0.5 mm in diameter with a pass energy of 100 eV was used for wide scans and a pass energy of 20 eV was used for narrow scans of particular elemental peaks. The materials were exposed to air during transfer to the instrument. Survey scans were carried out over 1200–0 eV binding energy range with 1.0 eV steps and a dwell time of 100 ms. Narrow higher resolution scans were run with 0.2 eV steps and 250 ms dwell time. Base pressure in the analysis chamber was 1.0 x 10^{9} Torr and during sample analysis 1.0 x 10^{8} Torr. The data were analyzed using XPS peak 4.1 software.

Synthetic Procedures

Synthesis of Magnetite Nanoparticles. 40 mL of 2 M $FeCl_3.6H_2O$ (0.08 mol, 21.6 g) in 1 M HCl and 20 mL of 2 M $FeCl_2.4H_2O$ (0.04 mol, 9.3 mg) in 1 M HCl were mixed in 1 L round-bottomed flask, and the mixture diluted to 0.6 L with de-ionized water. 125 mL of N_2 pre-purged 28% NH_4OH solution was then slowly added into the solution of iron chloride and the mixture was vigorously stirred for 30 min. The formation of a black precipitate of magnetite was immediately observed, and the precipitate was then collected by applying a magnetic force. The magnetic nanoparticles were extensively washed with deionized water to remove unreacted materials and impurities (yield 85%).

Synthesis of 1-(2-propargyl) D-mannose 1-(2-Propargyl) D-mannose was prepared according to the procedure reported by Mukhopadhyay *et al.*[2,3] A suspension solution of D-mannose (4.5 g, 25 mmol), propargyl alcohol (7.5 mL, 125 mmol) and H_2SO_4-silica (130 mg) was stirred at 65 °C until all reagents completely dissolved (3 h). Stirring was continued at 65 °C for an additional 3 h after complete dissolution. After cooling to ambient temperature, a part of the reaction mixture (8.00 g) was transferred to a flash column and eluted with $CHCl_3$–MeOH (8:1) to remove the excess propargyl alcohol. 1-(2-Propargyl) D-mannose was obtained as white solid after drying under vacuum (Yield: 30%). ^{1}H and ^{13}C-NMR revealed formation of the desired glycoside anomeric mixture with α anomer as the major product. FT-IR ν: 3347 (OH), 3285 (C≡C–H), 2118 (C≡C) cm^{-1}. ESI-MS m/z: calculated for $C_9H_{14}O_6$ ($M + Na^+$), 241.1; found, 241.1.

For detailed characterization, a part of the product was per-*O*-acetylated using acetic anhydride. A suspension solution of 1-(2′-propargyl) D-mannose (218 mg, 1.00 mmol), acetic anhydride (3.0 mL, 32.0 mmol), H_2SO_4-silica (6 mg) was stirred at ambient temperature for 48 h. After completion of the reaction, the solution was diluted with CH_2Cl_2 (20 mL) and washed sequentially with saturated aq. $NaHCO_3$ solution and water twice. The extract was dried over $MgSO_4$ and concentrated under vacuum. The crude mixture was then purified by column chromatography using hexane-ethyl acetate (8:1) as eluent. A ratio of 8:1 between 2,3,4,6-tetra-*O*-acetyl-1-(2-propargyl)-α-D-mannose and 2,3,4,6-tetra-*O*-acetyl-1-(2-propargyl)-β-D-mannose was obtained after drying under vacuum (Yield: 87%). ESI-MS m/z: calculated for $C_{17}H_{22}O_{10}$ ($M + Na^+$), 409.1; found, 409.0.

2,3,4,6-Tetra-*O*-acetyl-1-(2-propargyl)-α-D-mannose

^{1}H NMR (400 MHz) in $CDCl_3$: δ 5.34 (dd, 1H, H-3), 5.25–5.32 (m, 2H, H-2, H-4), 5.02 (d, 1H, H-1), 4.28 (dd, 1H, H-6a), 4.27 (d, 2H, C*H*$_2$–C≡CH), 4.10 (dd, 1H, H-6b), 3.98–4.05 (m, 1H, H-5), 2.47 (t, 1H, CH_2–C≡C*H*), 2.16, 2.10, 2.03, 1.98 (4s, 12H, 4 × $COCH_3$). ^{13}C NMR (300 MHz) in $CDCl_3$: δ 170.7, 170.0, 169.9, 169.8 (4 × $COCH_3$), 96.3 (C-1), 78.0 (CH_2–C≡*C*H), 75.7 (CH_2–*C*≡CH), 69.4, 69.1, 69.0, 66.1, 62.4 (C-6), 55.0 (*C*H_2–C≡CH), 20.9, 20.8 (2), 20.7 (4 × $COCH_3$).

2,3,4,6-Tetra-*O*-acetyl-1-(2-propargyl)-β-D-mannose

^{1}H NMR (400 MHz) in $CDCl_3$: δ 5.49 (dd, 1H, H-3), 5.06–5.31 (m, 2H, H-2, H-4), 4.95 (d, 1H, H-1), 4.35–4.45 (m, 2H, C*H*$_2$–C≡CH), 4.31 (dd, 1H, H-6a), 4.16 (dd, 1H, H-6b), 3.66–3.74 (m, 1H, H-5), 2.48 (t, 1H, CH_2–C≡C*H*), 2.18, 2.10, 2.04, 1.99 (4s, 12H, 4 × $COCH_3$). ^{13}C NMR (400 MHz) in $CDCl_3$: δ 170.8, 170.4, 170.1, 169.7(4 × $COCH_3$), 95.7 (C-1), 77.9 (CH_2–C≡*C*H), 76.1 (CH_2–*C*≡CH), 72.6, 71.2, 68.8, 66.0, 62.4 (C-6), 55.9 (*C*H_2–C≡CH), 20.9 (2), 20.8, 20.7 (4 × $COCH_3$).

Synthesis of 1-(2-propargyl) D-Glucose. 1-(2-propargyl) D-glucose was prepared according to the procedure reported by Mukhopadhyay *et al.*[2,3] A suspension solution of D-glucose (4.5 g, 25 mmol), propargyl alcohol (7.5 mL, 125 mmol) and H_2SO_4-silica (130 mg) was stirred at 65 °C until all reagents completely dissolved (3 h). Stirring was continued at 65 °C for an additional 3 h after complete dissolution. After cooling to ambient temperature, a part of the reaction mixture (8.00 g) was transferred to a flash column and eluted with $CHCl_3$–MeOH (8:1) to remove the excess propargyl alcohol. 1-(2-propargyl) D-glucose was obtained as yellowish syrup after drying under vacuum (yield 60%). FT-IR ν: 3347 (OH), 3285 (C≡C–H), 2118 (C≡C) cm^{-1}. ESI-MS m/z: calculated for $C_9H_{14}O_6$ ($M + Na^+$), 241.1; found, 241.1.

For detailed characterization, a part of the product was per-*O*-acetylated using acetic anhydride. A suspension solution of 1-(2′-propargyl) D-glucose (218 mg, 1.00 mmol), acetic anhydride (3.0 mL, 32.0 mmol), H_2SO_4-silica (6 mg) was stirred at ambient temperature for 48 h. After completion of the reaction, the solution was diluted with CH_2Cl_2 (20 mL) and washed sequentially with saturated aq. $NaHCO_3$ solution and water twice. The extract was dried over $MgSO_4$ and concentrated under vacuum. The crude mixture was then purified by column chromatography using hexane-ethyl acetate (8:1) as eluent. A ratio of 2:1 between 2,3,4,6-tetra-*O*-acetyl-1-(2-propargyl)-α-D-glucose and 2,3,4,6-tetra-*O*-acetyl-1-(2-propargyl)-β-D-glucose was obtained after drying under vacuum (Yield: 60%). ESI-MS m/z: calculated for $C_{17}H_{22}O_{10}$ (M + Na+), 409.1; found, 409.1.

2, 3, 4, 6-Tetra-*O*-acetyl-1-(2′-propargyl)-α-D-glucose

^{1}H NMR (400 MHz) in $CDCl_3$: δ 5.47 (t, 1H, H-3), 5.27 (d, 1H, H-1), 5.07 (t, 1H, H-4), 4.89 (dd, 1H, H-2), 4.20-4.30 (m, 3H, C*H*$_2$-C≡CH, H-6a), 3.92-4.13 (m, 2H, H-5, H-6b), 2.44 (t, 1H, CH_2-C≡C*H*), 2.08, 2.06,2.01, 2.00 (4s, 12H, 4 × $COCH_3$). ^{13}C NMR (400 MHz) in $CDCl_3$: δ 170.8, 170.2, 170.1, 169.7 (4×*C*OCH_3), 94.7 (C-1), 78.3 (CH2-C≡*C*H), 75.4 (CH2-*C*≡CH), 70.5, 70.0, 68.5, 67.9, 61.8 (C-6), 55.56 (*C*H2-C≡CH), 20.8 (3), 20.7 (4 × CO*C*H_3).

2, 3, 4, 6-Tetra-*O*-acetyl-1-(2′-propargyl)-β-D-glucose

^{1}H NMR (400 MHz) in $CDCl_3$: δ 5.22 (d, 1H, H-3), 4.95-5.13 (m, 2H, H-2, H-4), 4.72 (d, 1H, H-1), 4.22-4.38 (m, 3H, C*H*$_2$-C≡CH, H-6a), 4.02-4.12 (m, 1H, H-6b), 3.69-3.75 (m, 1H, H-5), 2.46 (t, 1H, CH_2-C≡C*H*), 2.07, 2.04, 2.01, 1.99 (4s, 12H, 4×$COCH_3$) ppm. ^{13}C NMR (400 MHz) in $CDCl_3$: δ 170.4, 169.7, 169.5 (2) (4×*C*OCH_3), 98.2 (C-1), 78.3 (CH_2-C≡*C*H), 75.6 (CH_2-*C*≡CH), 72.9, 72.0, 71.1, 68.4, 61.9(C-6), 56.0(*C*H_2-C≡CH), 19.7, 19.6 (3) (4×CO*C*H_3).

Synthesis of Initiator diethyl 3-(2-(2-bromoisobutyryloxy)ethylthio)propyl phosphonate **3**. 1.14 g of potassium *tert*-butoxide (10.13 mmol) was added to a 15 mL solution of 0.79 g (10.13 mmol) 2-mercaptoethanol in isopropanol. 5 mL solution of diethyl (3-bromopropyl) phosphonate **1** (2.5 g, 9.65 mmol) in isopropanol was added subsequently. The mixture was stirred at ambient temperature for 15 hours.

The solvent of the reaction mixture was removed under reduced pressure, followed by extraction using ethyl acetate (100 mL). After washing with water (200 mL), the organic phase was dried over $MgSO_4$ followed by removal of solvent under reduced pressure. Diethyl 3-(2-hydroxyethylthio)propyl phosphonate 2 was obtained as colorless oil and was used directly in the next step without intermediate purification. ^{1}H NMR (400 MHz) in $CDCl_3$ δ 4.05-3.99 (m, 4H), 3.65 (t, 2H), 2.65 (t, 2H), 2.55 (t, 2H), 1.82-1.78 (m, 4H), 1.22 (t, 6H). ^{31}P NMR (300 MHz) in $CDCl_3$: δ 33 ppm.

The precursor **2** (2 g, 7.8 mmol) was dissolved in 15 mL of anhydrous methylene chloride followed by the addition of 1.2 mL triethylamine (0.87 g, 8.57 mmol). After 15 minutes of stirring, 1.2 mL (1.9 g, 8.2 mol) of 2-bromoisobutyryl bromide was slowly added to the reaction mixture at 0°C and the reaction was held at ambient temperature overnight; then, the white precipitate was filtered off, and the clear filtrate was extracted with (2 x 100 mL) saturated sodium bicarbonate in water, followed by (1 x 100 mL) water. The solution was then dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by column chromatography (1:1 – 3:1 ethyl acetate / n-hexane) to give 1.9 g of colorless oil of diethyl 3-(2-(2-bromoisobutyryloxy)ethylthio)propyl phosphonate 3 (yield 60%): ^{1}H NMR (300 MHz) in $CDCl_3$ δ 4.25 (t, 2H), 4.06-4.01 (m, 4H), 2.72 (t, 2H), 2.61 (t, 2H) 1.88 (s 6H), 1.80 (m, 4H), 1.26 (t, 6H). ^{13}C NMR (300 MHz) in $CDCl_3$ δ 171.4 (C=O), 64.8 ($COOCH_2CH_2S$), 61.6 ($P(OCH_2CH_3)_2$), 55.6 ($BrC(CH_3)_2$), 32.6 ($PCH_2CH_2CH_2S$), 30.7 ($BrC(CH_3)_2$), 29.7 ($COOCH_2CH_2S$), 25.2 ($PCH_2CH_2CH_2S$), 22.6 ($PCH_2CH_2CH_2S$), 16.4 ($P(OCH_2CH_3)_2$). ^{31}P NMR (300 MHz) in $CDCl_3$ δ 33 ppm. ESI-MS m/z: calcd for $C_{13}H_{26}BrO_5PS$ ($M + Na^+$), 429.04 and 427.04; found, 429.1 and 427.1.

Cu(0) mediated polymerization of OEGA using phosphonate initiator **3**. 2 mg of $CuBr_2$ (0.0089 mmol), 2.12 g (4.43 mmol, 25 eq.) of oligoethylene glycol (OEGA), 72 mg of phosphonate bearing initiator **3** (0.177 mmol), 4 mL DMSO and 5.8 µL (0.021 mmol) of *tris*[2-(dimethylamino)ethyl]amine or Me_6TREN, were added to a 25 mL reaction vial. The reaction mixture was purged with N_2 for 30 min. Then, ~5 mm of copper wire or Cu(0) was added under N_2. The reaction was carried out at 25°C under stirring. Samples were taken throughout the polymerization for a kinetic study. Monomer conversion was determined using ^{1}H NMR by the following ratio: $\alpha^{OEGA} = 1 - \int_{6.5\ ppm} / [\int_{6.5\ ppm} + (\int_{4.1\ ppm}/2)]$, with $\int_{6.5\ ppm}$ corresponds to the integrals of acrylate group (CH=) and $\int_{4.1\ ppm}$ the ester group of P(OEGA) signal at 4.1 ppm. The molecular weight distribution was determined by *N*,*N*-dimethylformamide GPC. After overnight reaction (about 15 h), the reaction mixture was diluted with water and dialyzed using cellulose-tubular membrane MWCO 3,500 g/mol with water. The polymerization product was then freeze-dried to give P(OEGA). The molecular weight of the resultant P(OEGA) can be calculated by ^{1}H-NMR using the following equation:

$M_n^{P(OEGA)} = [(\int_{4.1\ ppm}/2)/(\int_{0.9\ ppm}/6)] \times MW^{OEGA} + MW^{initiator\ 3}$, with $\int_{0.9\ ppm}$ corresponding to integral of the terminal isobutyryl group at 0.9 ppm. The theoretical molecular weight was calculated using the following

equation: $M_n^{\text{Theo.}} = \alpha^{\text{OEGA}} \times [\text{OEGA}]_0/[\mathbf{3}]_0 \times \text{MW}^{\text{OEGA}} + \text{MW}^{\text{initiator 3}}$, with $[\text{OEGA}]_0$ and $[\mathbf{3}]_0$ correspond to the initial OEGA and initiator 3 concentration, respectively.

Deprotection of phosphonate bearing P(OEGA). 0.6 g of phosphonate bearing P(OEGA) was dissolved in 1 mL of anhydrous methylene chloride in a vial. Subsequently, 0.14 mL (1.0 mmol) of bromotrimethylsilane was added in a dropwise manner, and the mixture was stirred at ambient temperature for 3 h. The solvent and volatile residues were removed then by evaporation, and the silylated intermediate was cleaved by adding an excess of methanol (1 mL). The mixture was stirred at ambient temperature for another 12 h, and the reaction mixture was then dialyzed using cellulose-tubular membrane MWCO 3,500 g/mol with methanol. After removal of solvent and drying under vacuum, the polymer with terminal phosphonic acid P(OEGA) was obtained.

Cu(0) mediated sequential polymerization of OEGA and glycidyl acrylate using phosphonate initiator **3**. 2.2 mg of $CuBr_2$ (0.01 mmol), 2.4 g (5 mmol, 25 eq.) of OEGA, 81 mg of phosphonate bearing initiator **3** (0.2 mmol), 6 mL DMSO and 6.5 μL (0.024 mmol) of *tris*[2-(dimethylamino)ethyl]amine or Me_6TREN, were added to a 25 mL reaction vial. The reaction mixture was purged with N_2 for 30 min. Then, ~5 mm of copper wire or Cu(0) was added under N_2. The reaction was carried out at 25°C under stirring for 15h. 0.5 mL of DMSO solution containing 0.16 g of glycidyl acrylate or GA (1.2 mmol) and 3.2 μL (0.024 mmol) of Me_6TREN was added subsequently. The reaction mixture was continued to stir overnight at ambient temperature. Monomer conversion was determined using ^{1}H NMR by the following ratio: $\alpha^{\text{GA}} = 1 - \int_{6.5\text{ ppm}}/[\int_{6.5\text{ ppm}} + \int_{4.3\text{ ppm}}]$, with $\int_{6.5\text{ ppm}}$ corresponds to the integrals of acrylate group (CH=) and $\int_{4.3\text{ ppm}}$ corresponds to one proton from the ester group of P(GA) signal at 4.3 ppm. The molecular weight distribution was determined by *N*,*N*-dimethylformamide GPC. After overnight reaction (total about 40 h), the reaction mixture was dialyzed using cellulose-tubular membrane MWCO 3,500 g/mol with acetone. The polymerization product was then dried to give 2 g of P(OEGA)-*b*-P(GA) with $M_{n,\text{ SEC}}$ = 13,200 g/mol and PDI = 1.16. The molecular weight of the resultant P(OEGA) was calculated by ^{1}H-NMR using the following equation:

$M_n^{\text{P(OEGA)-}b\text{-P(GA)}} = [(\int_{4.1\text{ ppm}}/2)/(\int_{0.9\text{ ppm}}/6)] \text{ x } \text{MW}^{\text{OEGA}} + [(\int_{4.3\text{ ppm}})/(\int_{0.9\text{ ppm}}/6)] \times \text{MW}^{\text{GA}} + \text{MW}^3$, with $\int_{4.3\text{ ppm}}$ corresponding to integral of the glycidyl group at 4.3 ppm (other glycidyl peak at 2.9 ppm can be used for calculation as well). By ^{1}H-NMR the estimated $\text{DP}^{\text{P(OEGA)}}$ and $\text{DP}^{\text{P(GA)}}$ are about 25 and 6 units, respectively.

Azide substitution to P(OEGA)-*b*-P(GA). 0.9 g of P(OEGA)-*b*-P(GA) (0.068 mmol) was dissolved in 6 mL DMSO followed by the addition of 0.21 g sodium azide (3.29 mmol) and 0.18 g ammonium chloride (3.29 mmol). The reaction mixture was stirred at 50°C for 15h. Subsequently, the mixture was dialyzed using cellulose-tubular membrane MWCO 3,500 g/mol with water. After freeze drying, 0.9 g of azide functionalized block co-polymer P(OEGA)-*b*-P(AZPA) with $M_{n,\text{ SEC}}$ = 14,400 g/mol and PDI = 1.18 was obtained.

Cu(I)-catalyzed cycloaddition between P(OEGA)-*b*-P(AZPA) and 1-(2-propargyl)-α-D-mannose. A mixture of 0.35 g of P(OEGA)-*b*-P(AZPA) (0.025 mmol) and 50 mg of 1-(2-propargyl)-α-D-mannose (0.23 mmol) was dissolved in 2 mL DMSO. 3.2 mg of CuBr (0.023 mmol) and 6 mg of 2,2'-bipyridine (0.045 mmol) were added subsequently. The reaction was carried out at ambient temperature for 15h. The mixture was dialyzed using cellulose-tubular membrane MWCO 3,500 g/mol with water. After freeze drying, 0.3 g of α-D-mannose functionalized block co-polymer P(OEGA)-*b*-P(N3Man) with $M_{n,\ SEC}$ = 15,800 g/mol and PDI = 1.2 was obtained.

Phosphonate deprotection of P(OEGA)-*b*-P(N3Man). 0.3 g of phosphonate bearing P(OEGA)-*b*-P(N3Man) was dissolved in 1 mL of anhydrous methylene chloride in a vial. Subsequently, 0.45 mL (3.0 mmol) of bromotrimethylsilane was added in a dropwise manner, and the mixture was stirred at ambient temperature for 15 h. High excess of bromotrimethylsilane was added due to the presence of hydroxyl groups on the sugar moieties. The solvent and volatile residues were removed then by evaporation, and silylated intermediate was cleaved by adding an excess of methanol (3 mL). The mixture was stirred at ambient temperature for another 15 h, and the reaction mixture was then dialyzed using cellulose-tubular membrane MWCO 3,500 g/mol with methanol. After removal of solvent and drying under vacuum, 0.25 g of block co-polymer with terminal phosphonic acid P(OEGA)-*b*-P(N3Man)was obtained.

Cu(I)-catalyzed cycloaddition between P(OEGA)-*b*-P(AZPA) and 2,3,4,6-tetra-*O*-acetyl-1-(2-propargyl)-α/β-D-glucose. A mixture of 0.35 g of P(OEGA)-*b*-P(AZPA) (0.025 mmol) and 85 mg of 2,3,4,6-tetra-*O*-acetyl-1-(2-propargyl)- α/β-D-glucose (0.23 mmol) was dissolved in 2 mL DMSO. 3.2 mg of CuBr (0.023 mmol) and 6 mg of 2,2'-bipyridine (0.045 mmol) were added subsequently. The reaction was carried out at ambient temperature for 15h. The mixture was dialyzed using cellulose-tubular membrane MWCO 3,500 g/mol with water and methanol. After drying, 0.25 g of α/β-D-glucose (α:β = 2:1) functionalized block co-polymer P(OEGA)-*b*-P(N3Glu4OAc) with $M_{n,\ SEC}$ = 18,400 g/mol and PDI = 1.32 was obtained.

Phosphonate and acetyl deprotection of P(OEGA)-*b*-P(N3Glu4OAc). 0.25 g of phosphonate bearing P(OEGA)-*b*-P(N3Glu4OAc) was dissolved in 1 mL of anhydrous methylene chloride in a vial. Subsequently, 0.14 mL (1.0 mmol) of bromotrimethylsilane was added in a dropwise manner, and the mixture was stirred at ambient temperature for 3 h. After solvent evaporation, and 1 mL of methanol was added followed by stirring at ambient temperature for another 15 h, and the reaction mixture was then dialyzed using cellulose-tubular membrane MWCO 3,500 g/mol with methanol. After drying under vacuum, the block co-polymer with terminal phosphonic acid P(OEGA)-*b*-P(N3Glu4OAc) was subjected to acetyl removal with 25% sodium methoxide solution (0.15 mL, 0.02 mmol) in methanol (2 mL). The reaction mixture was stirred for one hour followed by the addition of ion exchanger DOWEX 50WX8. After filtration, the solution was dialyzed in water and methanol (cellulose-tubular membrane MWCO 3,500 g/mol) to give 0.2 g of the final product P(OEGA)-*b*-P(N3Glu) with $M_{n,\ SEC}$ = 14,000 g/mol and PDI = 1.21.

Thiol-epoxy addition of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucose to P(OEGA)-*b*-P(GA). A mixture of 0.4 g of P(OEGA)-*b*-P(GA) (0.03 mmol) and 69 mg of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucose (0.2 mmol) was dissolved in 2 mL DMSO. 26 µL of triethylamine (20 mg, 0.2 mmol) was added subsequently and the reaction mixture was stirred at 50°C for 15h. The mixture was dialyzed using cellulose-tubular membrane MWCO 3,500 g/mol with water and methanol. After drying, 0.3 g of β-D-glucose functionalized block co-polymer P(OEGA)-*b*-P(SGlu4OAc) with $M_{n,\ SEC}$ = 17,800 g/mol and PDI = 1.16 was obtained.

Phosphonate and acetyl deprotection of P(OEGA)-*b*-P(SGlu4OAc). 0.3 g of phosphonate bearing P(OEGA)-*b*-P(SGlu4OAc) was dissolved in 2 mL of anhydrous methylene chloride in a vial. Subsequently, 0.14 mL (1.0 mmol) of bromotrimethylsilane was added in a dropwise manner, and the mixture was stirred at ambient temperature for 3 h. After solvent evaporation, and 1 mL of methanol was added followed by stirring at ambient temperature for another 15 h, and the reaction mixture was then dialyzed using cellulose-tubular membrane MWCO 3,500 g/mol with methanol. After drying under vacuum, P(OEGA)-*b*-P(SGlu4OAc) block copolymer was subjected to acetyl removal with 25% sodium methoxide solution (0.3 mL, 0.04 mmol) in methanol (4 mL). The reaction mixture was stirred for one hour followed by the addition of ion exchanger DOWEX 50WX8. After filtration, the solution was dialyzed in water and methanol (cellulose-tubular membrane MWCO 3,500 g/mol) to give 0.2 g of the final product P(OEGA)-*b*-P(SGlu) with $M_{n,\ SEC}$ = 14,800 g/mol and PDI = 1.2.

Sugar functionalized block co-polymer grafting 'onto' iron oxide nanoparticles. For each sample, a dispersion of iron oxide nanoparticles IONPs (12 mg) was dispersed in water (6 mL). The solution was sonicated for 15 min at 25 watts with a sonicator 3000 Misonix. A solution of 0.0095 mmol of phosphonic acid bearing sugar functionalized block co-polymer, such as P(OEGA)-*b*-P(N3Man), P(OEGA)-*b*-P(N3Glu) and P(OEGA)-*b*-P(SGlu) in 2 mL water was added drop wise to the dispersion of magnetite nanoparticles. The resulting solution/dispersion was sonicated for 15 mins (Power = 25 W), followed by incubation in a shaker overnight at 40^{o}C. The solution was filtered with a syringe filter (450 µm) to remove the un-stabilized particles and centrifuged (using an Eppendorf Centrifuge 5804) for 40 mins (14 000 rpm/min). The functionalized iron oxide nanoparticles were isolated at the base of the centrifuge tube. The supernatant was removed and the particles were re-dispersed in water using the sonicator for 10 min (P = 20 W). This washing process was repeated twice to give glycopolymer functionalized magnetite nanoparticles synthesized via grafting 'onto' approach, e.g. IONP@P(OEGA)-*b*-P(N3Man) (for α-D-mannose functionalized nanoparticles).

Magnetic Resonance Imaging Relaxivity Measurement.

Inductively Coupled Plasma – Optical Emission Spectroscopy. The iron concentrations in each polymer functionalized IONPs samples were determined by Inductively Coupled Plasma - Optical Emission

Spectroscopy (ICP-OES) using a Perkin Elmer Optima 7300 ICP-OES spectrometer. The nanoparticles (~10 µL) were digested overnight in highly concentrated hydrochloric acid (32%), followed by dilution in water (1.5 mL).

T_2 and T_1 relaxivity measurement. A 9.4T Bruker Biospin 94/20 USR equipped with a Transceiver RES ^{1}H 72mm Quad RF coil was used for T_1 and T_2 relaxometry measurements. A dilution series for each sample (500 µl) was prepared in water, and measurements were performed at 20 ºC. T_2-weighted images were acquired using a Multi-Slice-Multi-Echo (MSME) sequence with TR = 4000 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. A Rapid Acquisition Rapid Echo with Variable Repetition Time (RARE-VTR) sequence was used to acquire T_1-weighted images, with TR = 88, 470, 922, 1474, 2183, 3176, 4869, and 15000 ms, TE = 8.074 ms, FOV = 5 x 5 cm, matrix size = 128 x 128, RARE factor 2, scan time = 24 min and slice thickness 1 mm. The net magnetizations for each sample were determined from the manually-drawn regions of interest (ROIs). The T_1 relaxation times were determined by fitting a mono-exponential association equation and the T_2 relaxation times by fitting a mono-exponential decay equation using Paravision 5 (Bruker). The T_1 and T_2 relaxivity was then calculated as slope from a plot of the inverse of the relaxation times (R_i, i = 1,2) versus iron concentration in water.

Colloidal stability study of glycopolymer functionalized IONPs with bovine serum albumin.

About 20 µL of a dispersion of glycopolymer functionalized iron oxide nanoparticles (~10 mg/mL) was added to 1 mL of 0.1 M phosphate buffer solution (PBS, pH = 7.4) containing 1 mg/mL of FITC conjugate bovine serum albumin (BSA). Colloidal stability and the particle size of the nanoparticles were measured after sonication and 10 minutes of incubation by dynamic light scattering (DLS) using a Malvern Instruments Zetasizer. After 20 h of incubation at 37^{o}C in a shaker, the number-average particle size was measured again to observe aggregation of nanoparticles caused by protein adsorption.

Aliquots of 0.3 mL of the each mixture was withdrawn and centrifuged. 0.2 mL of the supernatant was collected and diluted 10 times with PBS for fluorescence measurement of unbound FITC conjugated BSA. A solution of 0.1 mg/mL FITC conjugated BSA in PBS was utilized as a reference.

Interaction study of glycopolymer functionalized IONPs with lectin (concanavalin A).

Colloidal stability of glycopolymer functionalized iron oxide nanoparticles in buffer solution of Concanavalin A. About 20 µL of a dispersion of glycopolymer functionalized iron oxide nanoparticles (~10 mg/mL) was added to 1 mL of 10 mM HEPES buffer saline solution (HSA, pH = 7.0) containing 0.15 M NaCl and 1 mM $CaCl_2$. Colloidal stability and the particle size of the nanoparticles were measured after sonication and 10 minutes of incubation by dynamic light scattering (DLS) using a Malvern Instruments Zetasizer. A solution of Concanavalin A (10 mg/mL) was added to obtain about 0.1 mg/mL of concanavalin A in each sample, which was subsequently increased to 0.4 mg/mL.

Glycopolymer functionalized IONPs binding with FITC-conjugated concanavalin A. A dispersion of glycopolymer functionalized iron oxide nanoparticles (~0.1 mg/mL) in 2 mL of HEPES buffer saline solution (HSA, pH = 7.0) was prepared for each sample. A solution of FITC-conjugated Concanavalin A was added to give about 0.3 mg/mL of Concanavalin A (~2.83 μM). The mixture was incubated for about 20 minutes at ambient temperature and subsequently centrifuged for 40 minutes (14,000 rpm). The supernatant of each sample was collected and the fluorescence emission spectrum of each sample was obtained by Agilent fluorescence spectroscopy with the excitation wavelength of 494 nm (excitation slit 5, emission slit 10). IONP@P(OEGA)-*b*-P(N3Man) was washed twice with deionized water, and subsequently was redispersed in 2 mL of an aqueous solution of 0.25 M α-D-mannose (50 mg/mL). The mixture was sonicated for 10 minutes, incubated for 20 minutes at ambient temperature and then centrifuged again. The supernatant was collected and subjected to fluorescence spectroscopy again to observe the release of Concanavalin A by free α-D-mannose.

Change in T_2 MRI relaxivity measurement of glycopolymer functionalized IONPs in the presence of concanavalin A. For each glycopolymer functionalized IONP, a stock solution containing 1.5 mM Fe (~0.1 mg/mL IONPs) was prepared in water using sonication. A series solution of Concanavalin A in tris-buffer saline solution (50 mM tris, 100 mM NaCl and 1 mM $CaCl_2$) at different concentration (Con A: 0, 5, 10, 25, 50, 100 and 200 μg/mL). 30 minutes before the MRI measurement on 9.4T Bruker Biospin 94/20 USR, 20 μL of the IONPs stock solution was added to 500 μL buffer solution containing different concentration of Concanavalin A in Eppendorf tubes. After incubation at ambient temperature, the MRI samples containing 0.12 mM of iron (Fe) were scanned. The T_2 values of IONPs/Concanavalin A suspension and IONPs in buffer (without lectin) were recorded via MRI. The largest T_2 value for each IONPs without Concanavalin A was set as 100%. The change in T_2 in the presence of Concanavalin A or ΔT_2% for each sample was calculated as the percentage of change relative to the largest T_2 value for the respective glycopolymer functionalized IONPs.

Cell Viability Assays of functionalized iron oxide nanoparticles IONP@copolymer.

The cytotoxicity of functionalized iron oxide nanoparticles was tested in vitro by a standard Alamar Blue assay, which provides a homogeneous, fluorescent method for monitoring cell viability. The assay is based on the ability of living cells to convert a redox dye (blue resazurin) into a fluorescent end product (red resorufin). Nonviable cells rapidly lose metabolic capacity and thus do not generate a fluorescent signal. The **MRC-5** or human lung fibroblast cells and **A549 cells** were seeded in a tissue culture treated 96-well plate in 100 μL medium per well at a density of 5000 cells/well and incubated for 24 h. The medium was then replaced with fresh medium containing neat and polymer functionalized iron oxide nanoparticles over a concentration range (min. 3.9 μg/mL to max. 500 μg/mL) based on the weight of **IONP@copolymers**. All samples were incubated for 72 h. Alamar Blue assay dye (20 μL) was then added to each well, and the cells were incubated for 5 h. After an incubation step, data were recorded using a fluorescence plate reader

(570ex/595em). Cell viability (%) was determined as a percentage of untreated control cells. The amount of fluorescence produced (*F*) was proportional to the number of metabolically active (viable) cells in the culture. Wells without cells was set up as the negative control for the determination of background fluorescence. Wells without polymer treatment were used as the positive control. The percentage of relative cell viability relative to the control wells without any treatment was calculated by fluorescence values of test samples (treated cells) divided by fluorescence values of control samples (untreated cells) and then multiplied by 100.

Cell uptake of glycopolymer functionalized IONPs

Cell Culture. **A549** human lung cancer cells were cultured in growth media consisting of Dulbecco's modified Eagle's medium: Nutrient Mix F-12 (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS) in a ventilated tissue culture flask T-75 and passaged every 2−3days when monolayers at around 80% confluence. The cells were used only when stable cell growth was obtained (approximately 3−4 passages). The cells were incubated at 37 °C in a 5% CO_2 humidified atmosphere. The cell density was determined by counting the number of viable cells using a trypan blue dye (Sigma-Aldrich) exclusion test. The cells were detached using 0.05% trypsin−EDTA (Invitrogen), stained using trypan blue dye, and loaded on the hemocytometer. One day prior to the treatment, the cells were seeded at required cell densities (~20,000 cells/well) on 12-well plates.

Prussian Blue Staining. Lung cancer cell lines (A549) was seeded onto 12-well plate. After incubation for 24 hours at 37^{o}C, IONP@P(OEGA), IONP@P(OEGA)-*b*-P(N3Man) and IONP@P(OEGA)-*b*-P(SGlu) were added to the plate in a concentration of 20 µg/mL IONPs per well. Cell uptake study was carried out after incubation for 24 hours. After incubation, the supernatant was removed, and cells were washed three times with PBS, treated with 10% formalin solution for 5 minutes to fix the cells, and then washed with PBS. Prussian blue staining was then performed. To each well was added a 1:1 mixture of 4% potassium ferrocyanide(II) trihydrate and 2% HCl solution (0.5 mL), and cells were incubated for 30 minutes at 37^{o}C in the dark. The Prussian blue staining images were assessed by an inverted light microscope.

Determination of iron concentration by ICP-OES. After incubation for 3, 6 and 24 hours with the nanoparticles, the cell cultures were digested with an aqueous acid mixture (10% v/v conc. HCl, 30% v/v conc. HNO_3). The A549 cell lines were incubated in 1 mL acid mixture at 60^{o}C for 4 hours. Subsequently, the solution was isolated and diluted to 10 mL, after the well was washed with DI water for several times. The samples were subjected to ICP-OES, Perkin Elmer Optima 7300 ICP-OES spectrometer for quantification of iron concentration (C_{Fe}).

Additional References:

1. M. Ciampolini and M. Nardi, *Inorg. Chem.* 1966, **9**, 41-44.
2. B. Roy and B. Mukhopadhyay, *Tetrahedron Lett.* 2007, **48**, 3783-3787.
3. Q. Zhang, S. Slavin, M. W. Jones, A. J. Haddleton and D. M. Haddleton, *Polym. Chem.* 2012, **3**, 1016-1023.

Supplementary Figures:

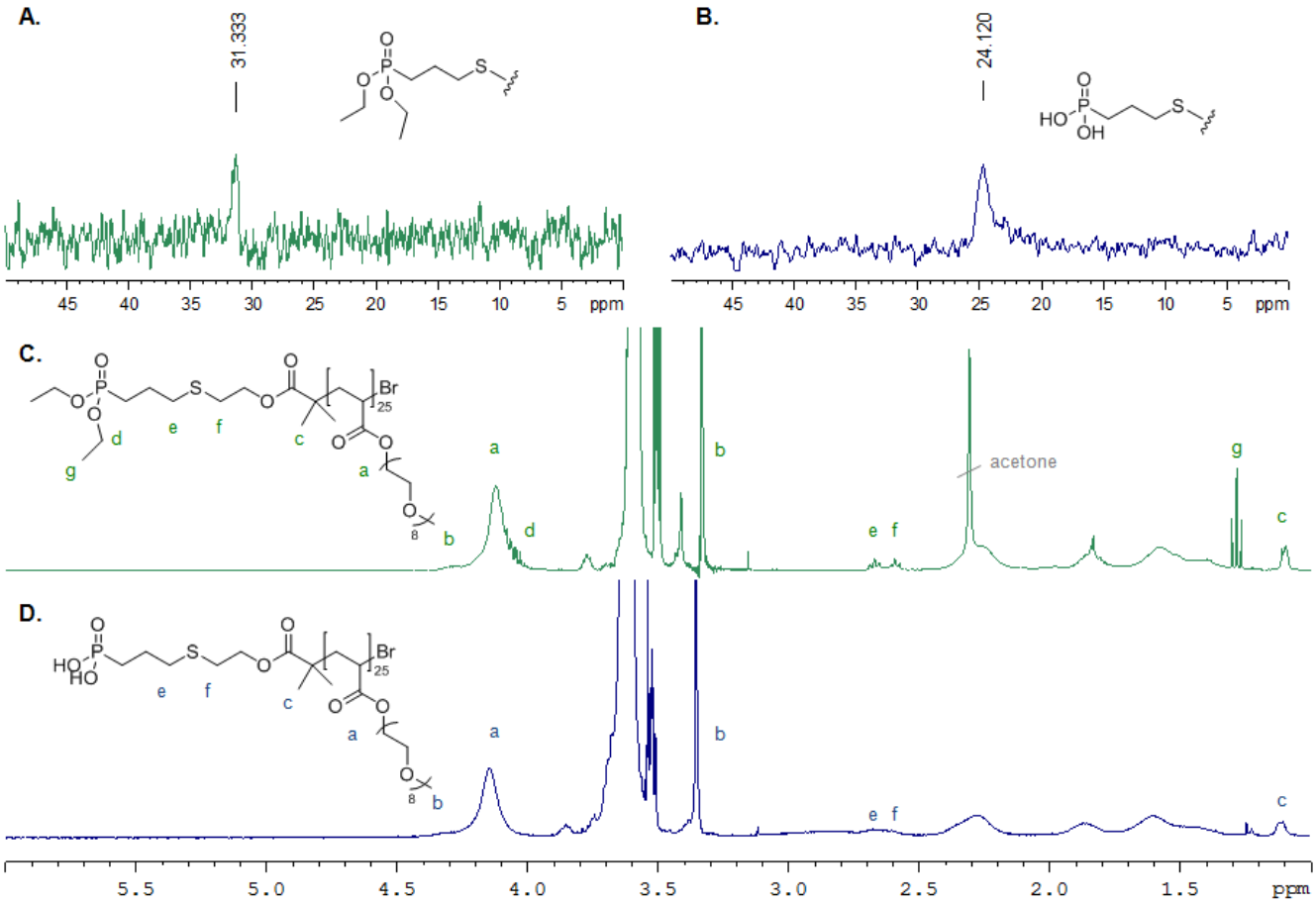

Figure S1. A- ^{31}P NMR of phosphonate bearing P(OEGA) and B- of phosphonic acid bearing P(OEGA). C- ^{1}H NMR of phosphonate bearing P(OEGA) and D- of phosphonic acid bearing P(OEGA).

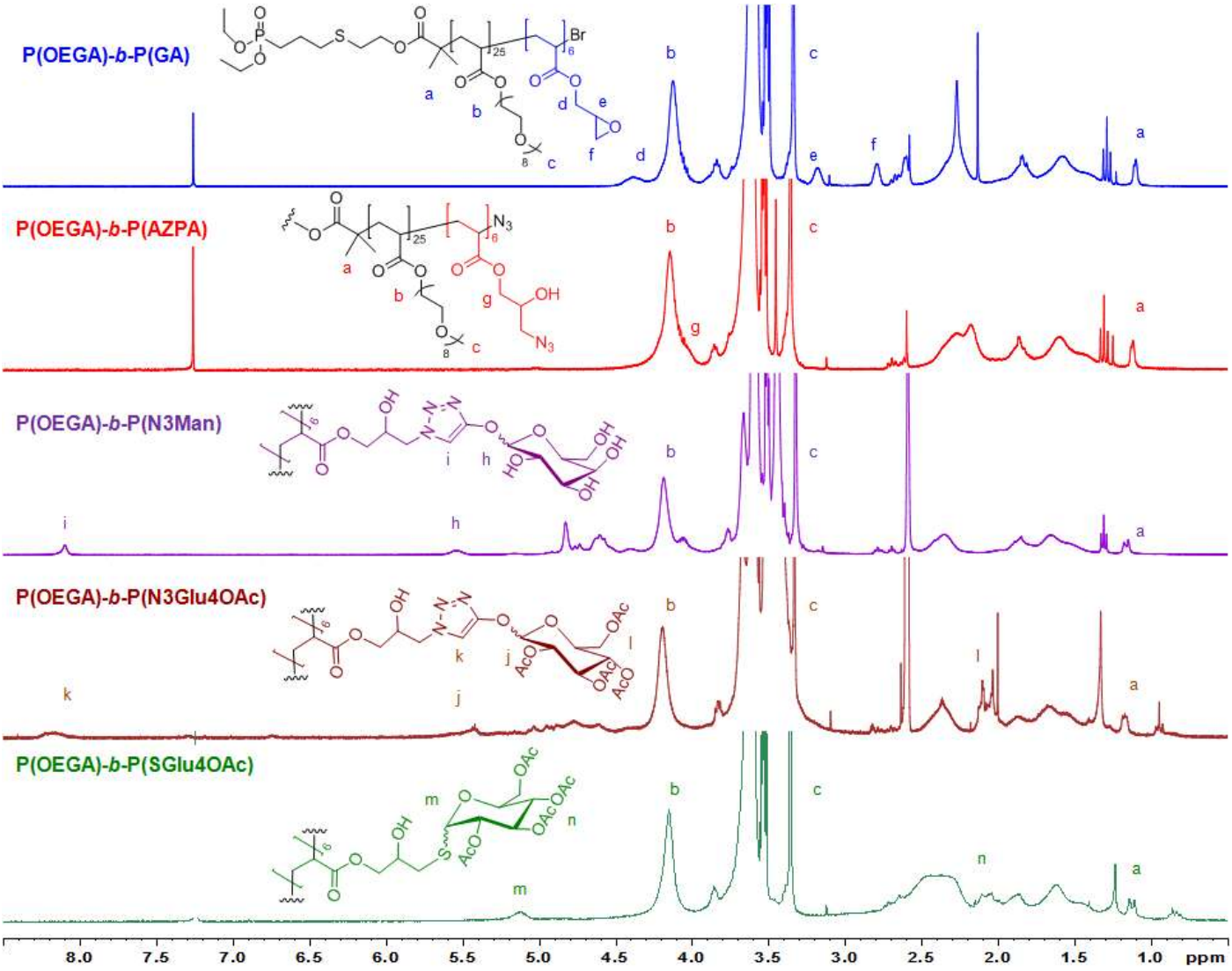

Figure S2. ^{1}H NMR of phosphonate terminated diblock glycopolymers.

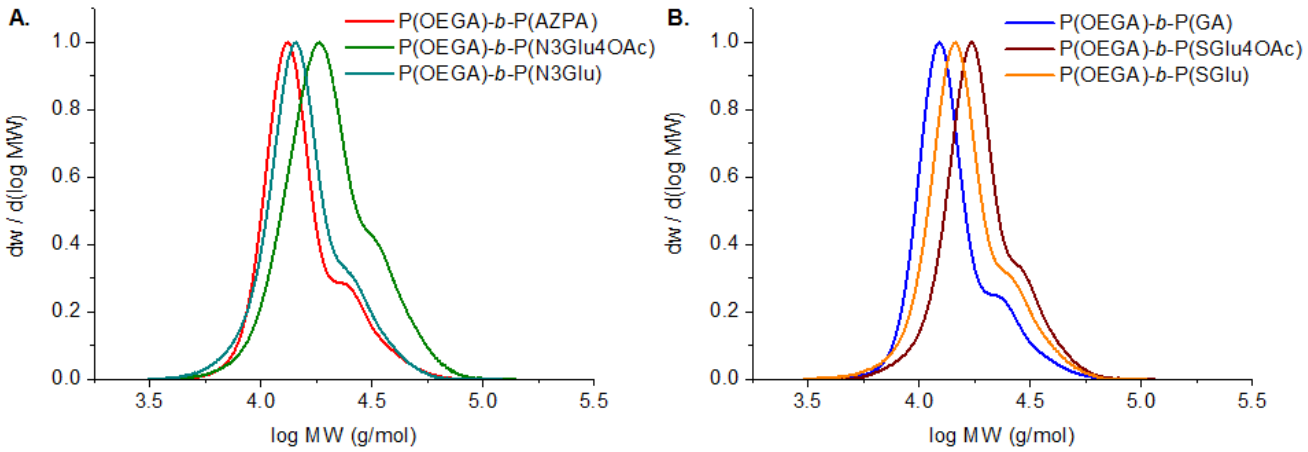

Figure S3. A- SEC of P(OEGA)-*b*-P(N3Glu) and its precursor P(OEGA)-*b*-P(AZPA). B- SEC of P(OEGA)-*b*-P(SGlu) and its precursor P(OEGA)-*b*-P(GA).

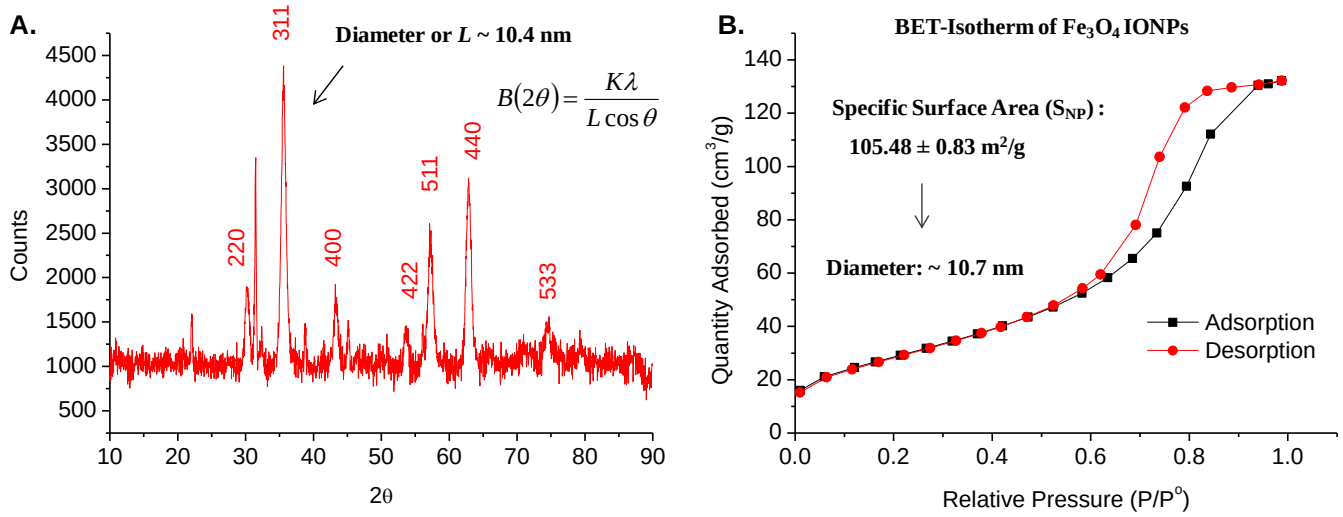

Figure S4. A- XRD of synthesized iron oxide nanoparticles indicating the spinel crystal structure of magnetite / Fe_3O_4. B- N_2 BET isotherm of synthesized magnetite / Fe_3O_4 nanoparticles for the measurement of specific surface area (S_{NP}).

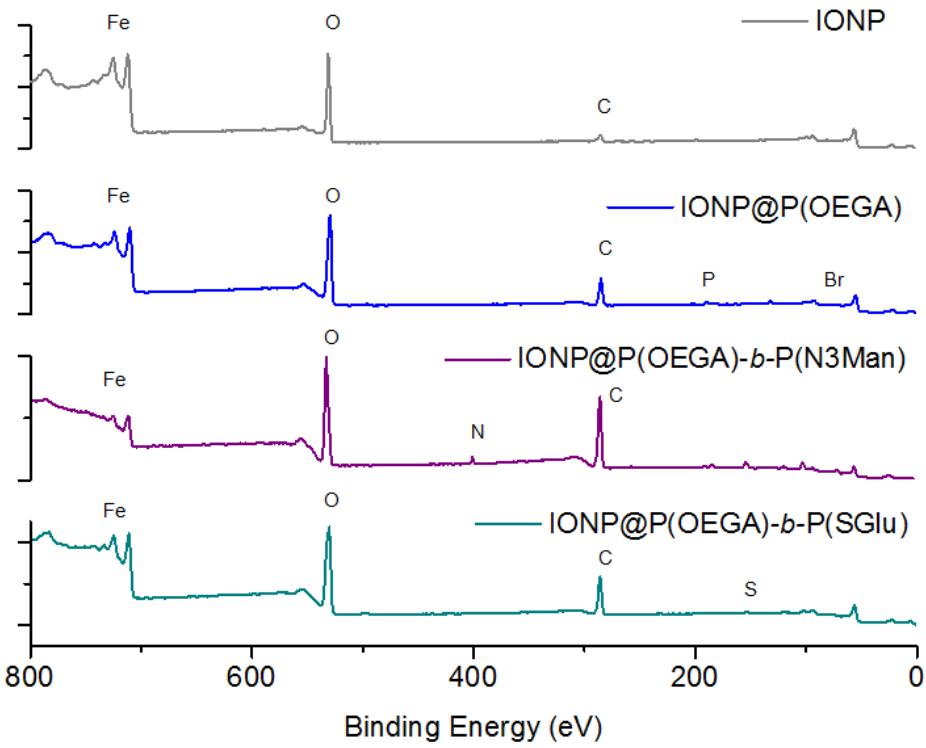

Figure S5. XPS of IONP@P(OEGA), IONP@P(OEGA)-*b*-P(N3Man) and IONP@P(OEGA)-*b*-P(SGlu).

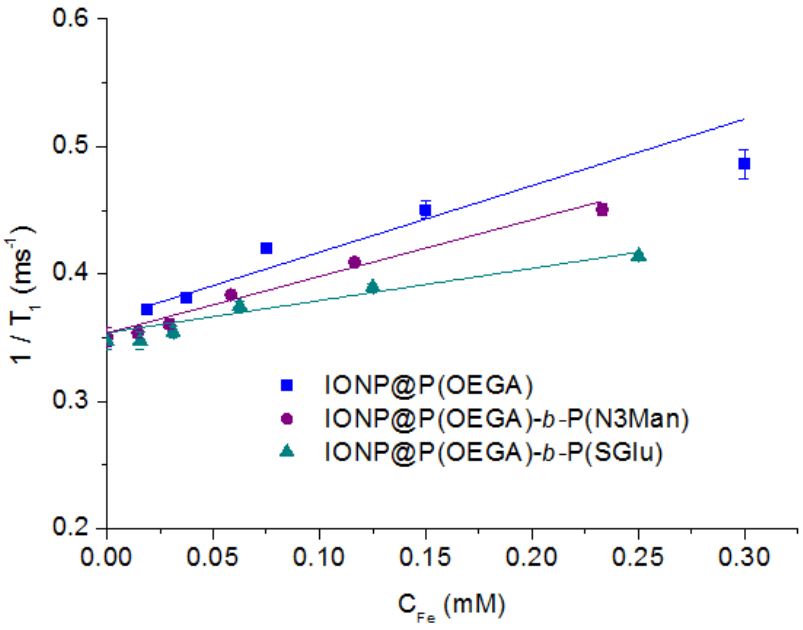

Figure S6. r_1 relaxivity of P(OEGA) and diblock glycopolymer functionalized IONPs.

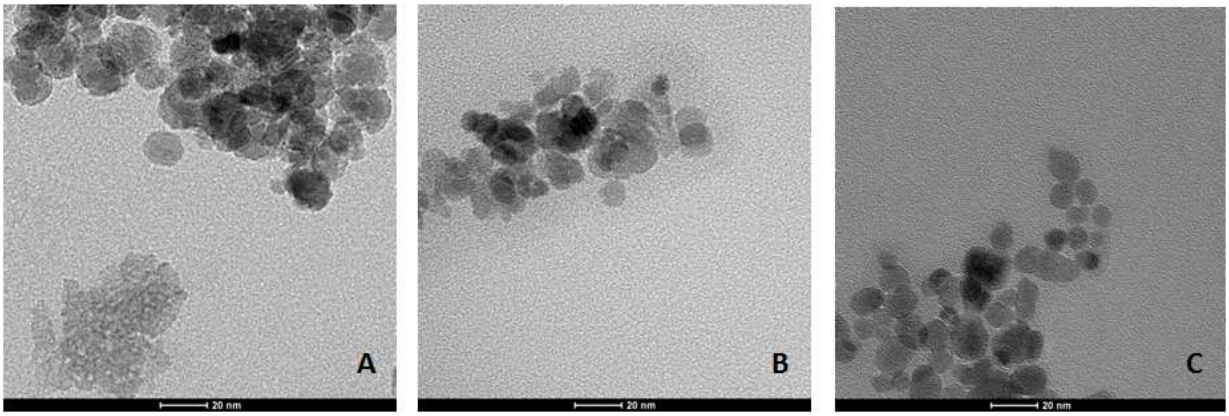

Figure S7. TEM images of A- neat IONPs; B- IONP@P(OEGA); C- IONP@P(OEGA)-*b*-P(N3Man).

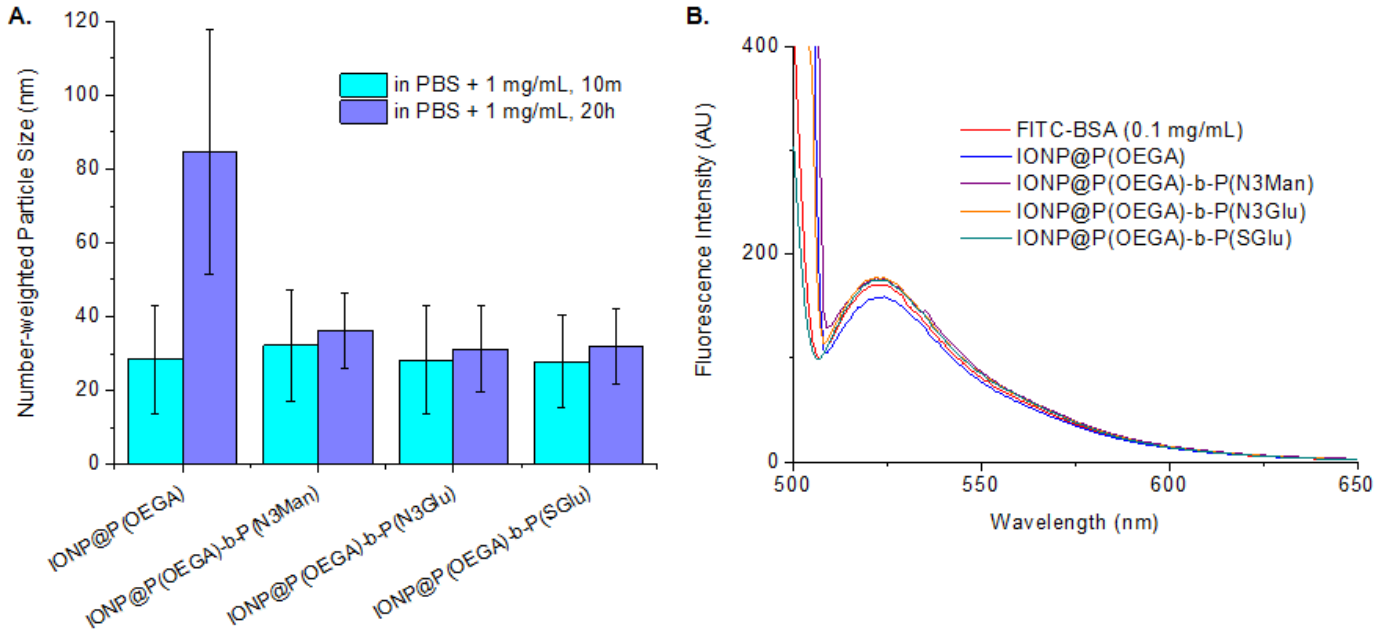

Figure S8. A- Number-weighted particle size distribution by DLS of diblock glycopolymer functionalized IONPs in phosphate buffer solution (0.1 M PBS, pH 7.4) containing 1 mg/mL BSA (bovine serum albumin). B- Fluorescence spectra of supernatants from the incubation of diblock glycopolymer functionalized IONPs with FITC-conjugated BSA (IONPs were removed by centrifugation after 20 h incubation and the supernatant was diluted 10 times), in comparison with 0.1 mg/mL FITC-BSA.

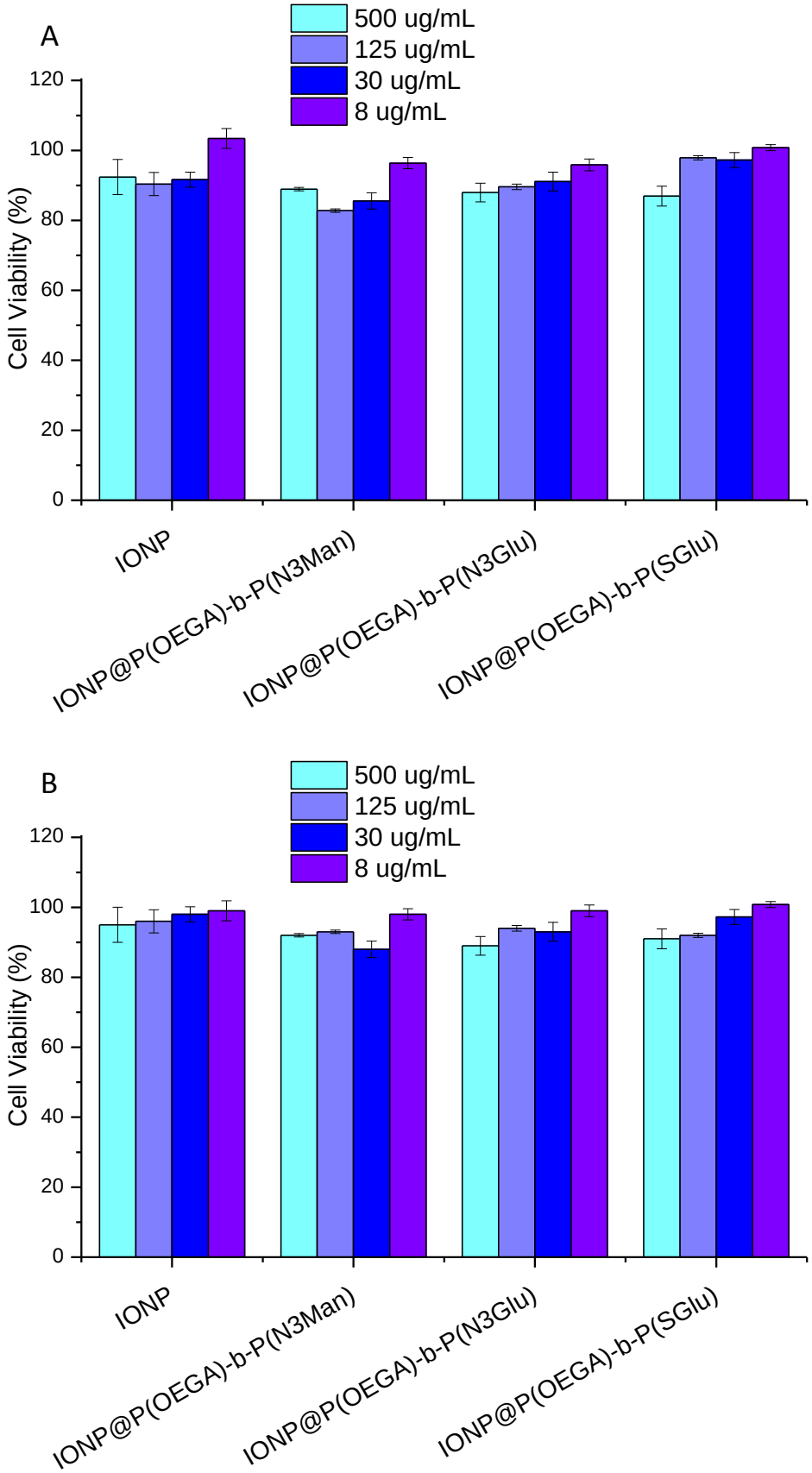

Figure S9. Cell viability for different nanoparticles (IONPs and polymer functionalized IONPs) at different concentrations using Alamar Blue assay for MRC-5 and A549 cells. Error bars correspond to the standard deviation (n = 5).

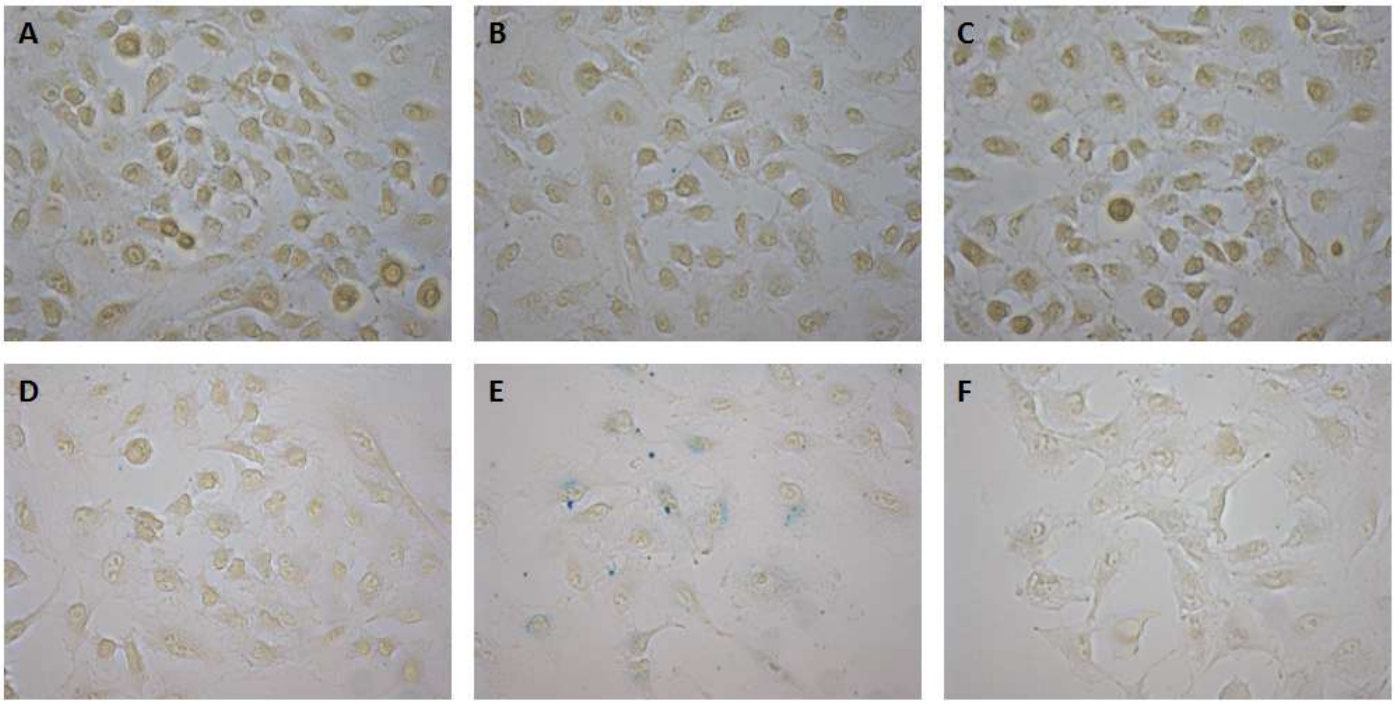

Figure S10. Prussian blue staining microscope images of A549 after 3h and 6h incubation with 20 μg/mL of (A and D) IONP@P(OEGA), (B and E) IONP@P(OEGA)-*b*-P(N3Man) and (C and F) IONP@P(OEGA)-*b*-P(SGlu), followed by removal of unbound nanoparticles and washing.