Supporting Information for JMC

Anti-Fouling Magnetic Nanoparticles For siRNA Delivery

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Experimental part.

MATERIAL

2,2'-azobisisobutyronitrile (AIBN) was recrystallized twice from methanol prior to use. Oligoethylene glycol methyl ether acrylate (OEG-A) (number average molecular weight M_n = 450 g/mol, PDI = 1.02, Aldrich) and *N,N*'-dimethyaminoethyl acrylate (DMAEA) were filtered through an alumina column to remove the inhibitor prior to use. Bovine serum albumin (BSA, purity 90%) and Bradford reactant were purchased from Aldrich. All chemicals were used as received, unless otherwise specified. Carbon disulfide (CS_2, 99%+, Aldrich), diethyl ether (99%, Ajax), *n*-hexane (95%, Ajax), dichloromethane (99%, Ajax), *N,N*-Dimethylformamide (DMF, 99%, Ajax), tetrahydrofurane (THF), triethylamine (99%, Aldrich), acetone (99%, Ajax), *N,N*-dimethylacetamide (DMAc, 99%, Aldrich), 3-mercaptopropionic acid (99+, Aldrich), tris(2-carboxyethyl)phosphine hydrochloride (TCEP, 99%, Aldrich), and silica gel (Fluka, 150-200 nm). The centrifuge filters (Amicon® Ultra-15, MWCO 50 000 Da and 100 000 Da) were purchased from Millipore Corporation. Membranes for dialysis (MWCO 3 000, 12-14 000, 25 000 and 50 000 Da) were purchased from Fisher Biotec (Cellu SepT4, regenerated cellulose-Tubular membrane).

Small interfering RNA (siRNA, sense strand: 5'-ThioMC6-D-rGrCrU rGrArC rCrCrU rGrArA rGrUrU rCrArU rCrUrU-3'; antisense strand: 5'-rGrArU rGrArA rCrUrU rCrArG rGrGrU rCrArG rCrUrU-3') specific for enhanced green fluorescent protein (eGFP) mRNA, RNAse-free water and HEPES (4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid) buffer (pH 7.5) were purchased from Integrated DNA Technologies.

CHARACTERIZATIONS

NMR Spectroscopy. ^{1}H, ^{31}P and ^{13}C NMR spectra were recorded using Bruker ACF300 (300 MHz for ^{1}H NMR) or ACF500 (500 MHz for ^{1}H NMR) spectrometers. D_2O, DMSO-D_6 or $CDCl_3$ were used as solvents. DMAEA and OEG-A monomer conversions were determined via ^{1}H NMR spectroscopy, comparing the signal area from the vinyl protons (δ ~ 5.4-6.3 ppm, 3H/mol) to the signal area from the methylene oxide (δ ~ 3.6 ppm, 30 H/mol) and methyl adjacent to amine (δ ~ 2.8 ppm, 6H/mol) groups, respectively for OEG-A and DMAEA.

Mass Analysis. Electrospray-ionization mass spectrometry (ESI-MS) experiments were performed using a Thermo Finnigan LCQ Deca ion trap mass spectrometer (Thermo Finnigan, San Jose, CA). The instrument was calibrated with caffeine, MRFA, and Ultramark 1621 (all from Aldrich) in the mass range 195-1822 Da. All spectra were acquired in positive ion mode over the mass to charge range, *m*/*z*, 100-2000 with a spray voltage of 5 kV, a capillary voltage of 44 V, and a capillary temperature of 275 °C. Nitrogen was used as sheath gas while helium was used as auxiliary gas. The sample (1mg/mL) was prepared by dissolving in a 60:40 v/v mixture of tetrahydrofurane (THF): methanol with an acetic acid concentration of 0.4 mM. 56 Spectra were recorded in positive ion mode with an instrumental resolution of 0.1 Da. All reported molecular weights were calculated via the program package CS ChemDraw 6.0 and were monoisotopic. The theoretical molecular weight over charge ratios (*m*/*z*, assuming *z*+1) were calculated using the exact molecular mass of the predominant isotope within the structure.

Gel permeation Chromatography (GPC). Gel permeation chromatography (GPC) was conducted using *N,N*-dimethylacetamide [DMAc; 0.03% w/v LiBr, 0.05% 2, 6–di-Butyl-4-methylphenol (BHT)] or aqueous solutions (deionized water containing sodium azide) as mobile phases. Aqueous GPC was performed using Shimadzu modular system comprising a DGU-12A solvent degasser, on LC-10AT

pump, a CTO-10A column oven, and a RID-10A refractive index detector and a SPD-10A Shimadzu UV Vis detector (flow rate: 0.8 mL/min). The column system was equipped with a Polymer Laboratories 5.0 mm bead-size guard column (50 × 7.8 mm^2) followed by two PL aquagel MIXED-OH columns (8μm). Calibration was performed with PEO standards ranging from 106 to 909,500 g/mol. DMAc GPC analyses were performed using a Shimadzu modular system comprising an SIL-10AD auto-injector, a Polymer Laboratories 5.0-mm bead-size guard column (50 × 7.8 mm) followed by four linear PL (Styragel) columns (10^5, 10^4, 10^3, and 500Å) at 50 °C (flow rate = 1 mL/min) and an RID-10A differential refractive-index detector. The calibration was performed with polystyrene standards with narrow polydispersity ranging from 500 to 10^6 g/mol.

UV-vis Spectroscopy. UV-vis spectra were recorded using a CARY 300 spectrophotometer (Varian) equipped with a temperature controller.

Infrared Spectroscopy. FT-IR spectra were obtained using a Bruker Spectrum BX FT-IR system using diffuse reflectance sampling accessories.

Dynamic light scattering (DLS). Dynamic light scattering studies of the IONPs at 1 mg/mL in an aqueous 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.

Thermal gravimetric analysis (TGA). TGA of IONPs was performed using a Pyris 1 (Perkin Elmer) with a rate 5 ^oC/ min from room temperature to 500 ^oC. All the samples were preheated at 80 ^oC to remove the water traces. The weight lost was calculated between the difference between the weights at room temperature and at 500 ^oC.

Zeta-Potential. The particle zeta potential was measured by means of electrophoretic mobility using a Brookhaven ZetaPlus analyzer. A particle concentration of 0.250 mg/mL was used.

TEM Microscopies. The sizes and morphologies of the nanoparticles were observed using a transmission electron microscopy (TEM, Philips CM-200) or JEOL1400 TEM at an accelerating voltage of 200 kV or 100 kV. The particles were dispersed in water (0.1 mg/mL) and deposited onto 200 mesh, holey film, copper grid (ProSciTech).

X-ray diffraction (XRD) of the IONPs was carried out on Philips MPD (Cu Kλ, 40 mA, 45 kV) equipped with a 2D Pixcel detector, scanning on an automatic divergent slit mode at 2θ = 20-70°, step size = 0.026° and 18.69 s per step.

Specific surface area (SSA) of the as-prepared IONPs was measured on Micromeritics Tristar 3000 by means of N_2 adsorption at 77 K using the BET method. The surface area equivalent size were deduced from $d^{BET} = 6/(d \times S^{SA})$ where d = 4.87 g cm-3 is the density of maghemite phase iron oxide.

Agarose gel electrophoresis for siRNA.

siRNA or siRNA-IONP@polymer complexes (using a concentration of siRNA (10^{-5} mol/L)) and TBE buffer (10 μL, tris-borate EDTA, pH 7.4) were subjected to electrophoresis at 100 V for 30 min on a 3% agarose gel containing ethidium bromide. Gels were visualized using a Geldoc Imaging System Model (Bio-rad) under UV irradiation.

Synthesis of the RAFT agent.

The synthesis of RAFT agent was reported elsewhere.[1]

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm from TMS): 1.20 (16H, m, P-($C\underline{H}_2$)$_9$-), 1.59 (3H, d, -$C\underline{H}_3$), 2.75 (2H, t, -$C\underline{H}_2$-CO_2H), 3.50 (2H, t, -$C\underline{H}_2$-S-), 4.00 (2H, m, -$C\underline{H}_2$-O-(CO)), and 4.78 (1H, quartet, CH), 10.0 (1H, s, $CO_2\underline{H}$), 12.0 ppm (2H, -PO(OH).

FT-IR (cm^{-1}): 3300 (m, O-H), 1730 (vs, C=O), 1640 (vs, C-C), 1164 (w, P-O), 1026 (vs, P=O) and 960 (deformation, P–O).

ESI-MS: 511.1 (Na^+) (theoretical value = 511.67). However, this RAFT agent was found to partially decompose in the mass spectrometer before the ionization.

Synthesis of Iron oxide nanoparticles.

Iron oxide nanoparticles, in the form of maghemite (γ-Fe_2O_3), were synthesized rapidly via a one-step *flame spray pyrolysis* (FSP) as detailed elsewhere.[2, 3] Briefly, a solvent-based liquid precursor consisting of 0.5 M of iron 2-ethylhexanoate (Alfa Aesar, 52% in mineral spirit) in xylene (Riedel de Haen, 96%) was delivered to the FSP nozzle at 5 mL min^{-1} and dispersed by 5 L min^{-1} of O_2 (1.5 bar) to form fine droplets. Combustion of the liquid precursor was ignited by a surrounding oxy-methane ring (1.5 L min^{-1} CH_4/3.2 L min^{-1} O_2) to form the primary core flame. Within the core flame, maghemite nanoparticles were formed rapidly via the gas-to-particle route. The aerosol particles were collected on a glass fiber filter (Whatmann GF/D) with the assistance of a vacuum pump (Alcatel SD series).

RAFT POLYMERIZATIONS

***N,N*-Dimethylaminoethyl acrylate (DMAEA) polymerizations (Scheme 2A)**

Polymerizations were conducted at 70 ^oC employing ACVA as the primary radical source and 2-(2-carboy-ethyltrithiocarbonate)-propionic acid 11-(phosphonic acid)-undecnyl ether as RAFT chain transfer agent. All polymerizations with this monomer were performed directly in a dry acetonitrile (the presence of water can involve the degradation of trithiocarbonate) with an initial monomer concentration ($[M]_0$) of 1 M. An example of DMAEA polymerization follows for a feed ratio of $[DMAEA]_0/[CTA]_0/[AIBN]_0$ = 100.0/1/0.2. DMAEA (1.43 g (0.01 mol), RAFT (51.5 mg, 1.0×10^{-4}

mol), ACVA (5.6 mg, 2.0×10^{-5} mol) and dry acetonitrile (10 mL) were mixed. The reaction mixture was purged with nitrogen in an ice bath for 30 mins. It was then placed in an oil bath at 70 ^{o}C for 6 hours. The crude product was analyzed by ^{1}H NMR and DMAc GPC. The polymerization solution was partially concentrate and quickly dialyzed against water (pH = 5) for 5 hours (water was changed hourly), and then freeze-dried. The product was dissolved in ethanol and the polymer was precipitated in diethyl ether. The precipitation was repeated twice to remove any unreacted monomer or RAFT agent. The product was dried in vacuo to yield a yellow viscous product. Finally, the polymer was dissolved in water and the amine was quaternized by slowly addition of HCl in the mixture. Finally, the product was dialyzed for 2 days to remove the unreacted HCl and freeze dried.

OEG-A Polymerizations (Scheme 2B)

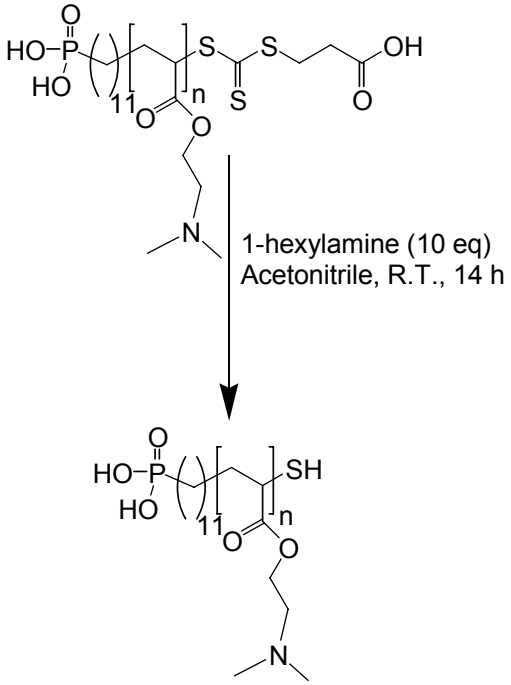
A similar procedure was followed for all the OEG-A polymerizations except for changes in the concentrations of the components used. The initial concentrations of OEG-A, RAFT agent and the initiator were 0.51, 14.57 x 10^{-3}, 2.90 x 10^{-3} M, respectively, yielding a ratio of $[OEG\text{-}A]_0$:$[RAFT]_0$:$[Initiator]_0$ of 35.0: 1.0: 0.2. OEG-A polymerizations were performed at 60 °C. P(OEG-A)s were analyzed directly using ^{1}H NMR and DMAc GPC to determine the monomer conversions and the molecular weights. The polymer samples were concentrated by partial evaporation of acetonitrile, and the P(OEG-A) was precipitated in cold diethyl ether (in an ice bath) two times to remove any non-reacted OEG-A or RAFT agent. Finally the polymer was further purified by dialysis against water using a dialysis membrane with a molecular weight cutoff of 3 500 Da. After filtration, the yellow viscous products were dried in vacuo at 40 ^{o}C for 24 hours to give a yellow-orange solid. The purified products were further analyzed by ^{1}H NMR, UV-visible, DMAc GPC and by FT-IR.

Chain extension of P(DMAEA) macroRAFT agent (Scheme 3)

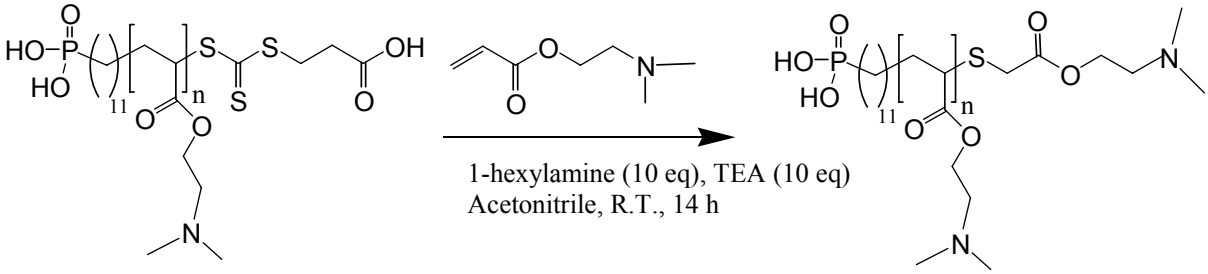
The P(DMAEA) previously obtained was used as a macroRAFT agent in the presence of OEG-A monomers. The reaction was carried out in acetate buffer (pH = 5.5) at 65 ^{o}C in the presence of ACVA. An example is given: P(DMAEA) macroRAFT agent (0.5 g, M_n = 3 500 g/mol, i.e. 1.5 × 10^{-3} mol of polymer) was diluted in acetate buffer (5 mL). ACVA (13 mg, 4.8 × 10^{-5} mol) and OEG-A (2.0 g, 4.44 × 10^{-3} mol) were dissolved in acetate buffer (5 mL). The solution was purged with nitrogen and the reaction was carried out at 70 ^{o}C for 5 hours. At the end of polymerization, the polymers were precipitated in cold diethyl ether (3 times) and then, dialyzed using MWCO = 11 000 -13 000 Daltons. Polymers were characterized by DMAc GPC and ^{1}H NMR.

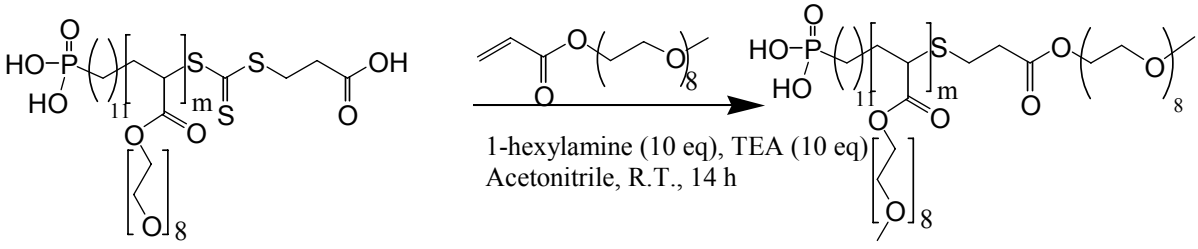
Aminolysis of the RAFT end group after polymerization (Scheme S1 and S2).

The addition of primary or secondary amine can cleave trithiocarbonate to yield a thiol end group (Scheme S1). In this paper, we exploited this technique to cleave and in-situ to cape the thiol by thiol ene reaction (Scheme S2). An example is given for modification of the trithiocarbonate end-group of P(OEG-A). P(OEG-A) (0.5 g, 4.8 × 10^{-5} mol, M_n by ^{1}H NMR = 10 300 g/mol, entry 1 of Table 1) and OEG-A (110 mg, 2.5 × 10^{-4} mol) were dissolved in acetonitrile (3 mL). The solution was purged with nitrogen for 10 min. Hexylamine (25 mg, 24.0 × 10^{-5} mol) and triethylamine (TEA, 2.4× 10^{-4} mol) in acetonitrile were added under nitrogen. The reaction was carried out for 14 hours. P(OEG-A) was purified by precipitation in cold diethyl ether. The solution was centrifuged and a viscous product was collected. The colorless viscous product was analyzed by UV-visible to confirm the absence of trithiocarbonate at 305 nm. This process was used post-polymerization to remove the RAFT functionality.



Scheme S1. Aminolysis of trithiocarbonate to yield thiol functionalized polymers.





Scheme S2. In situ aminolysis and capping of RAFT terminated polymers in the presence of monomers.

GRAFTING ONTO OF POLYMERS ON IONPs

Grafting of α-phosphonic acid, ω-dithiopyridine functionalized poly(OEG-A) to iron oxide nanoparticles. Iron oxide nanoparticles (10 mg), (diameter 8 nm) were dispersed in water (9 mL). The solution was sonicated for 2 min at 20 watts with a sonicator 3000 Misonix. 40 mg of P(OEG-A), ($M_{n\ UV\text{-}visible}$ = 10 300 g/mol, PDI = 1.18) was dissolved in water (1 mL). The solution of poly(OEG-A) was added slowly to the dispersion of iron oxide nanoparticles, and the resulting solution/dispersion was sonicated for 2 mins (Power = 30 W). The solutions were filtered to remove the unstabilized particles and centrifuged (using an Eppendorf Centrifuge 5804) for 30 mins (15 000 rpm/min). The iron oxide nanoparticles were isolated at the base of the centrifuge tube. The solutions were decanted and the particles were re-dispersed in water (10 mL) using the sonicator for 1 min (P = 20 W). The process was repeated twice. Iron oxide nanoparticles were subsequently analyzed by TGA, XPS, TEM and DLS.

METHODS

Cell culture. Cells were obtained from the Children's Cancer Institute Australia (CCIA). SHEP (Human Neuroblastoma) cells with and without expression of enhanced green fluorescent protein (eGFP) were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) without addition of antibiotics. Cells were regularly maintained in an incubator at 37 ^{o}C and 5% CO_2 humidified atmosphere.

Intracellular delivery of siRNA-IONPs@[P(OEG-A)/P(DMAEA)] complexes siRNA, from Integrated DNA Technologies at a concentration of 5 × 10^5 nM was freshly complexed with IONP@[P(OEG-A)/P(DMAEA)] (1.0 mg/mL) at a 1: 9 ratio for 20 minutes. The conjugate was then diluted with serum-free DMEM to yield a siRNA concentration of 100 nM. The reverse transfection was performed in this experiment and provided a faster process than the forward transfection. When

the siRNA-IONP@polymers complexes were ready inside a well plate, SHEP cells stably expressing eGFP) in DMEM plus 10% FBS were added into each well at a density of 1×10^5 cells/well. Control experiments were also carried out by co-transfection of 0.2% Lipofectamine™ RNAiMAX (Invitrogen) and siRNA, only siRNA, and cells without any treatment. The final concentrations of siRNA and FBS per well were 50 nM and 5%, respectively. The cell suspension in a well plate was mixed gently by rocking the plate back and forth. Thereafter, all cells were incubated without medium change for 72 hours at 37 ^{o}C in a 5% CO_2 incubator. Cells that were treated under magnetic field, were first allowed to form a monolayer on the surface of the tissue culture well plates for 3 hours and then subjected to a magnetic field using a supermagnetic plate (OZ Biosciences) for 5 hours. Cells were then incubated for the same period of time as previously mentioned. Gene silencing effect was observed by fluorescence microscopy and flow cytometry. All experiments were done in duplicate.

Fluorescence microscopy. SHEP cells were treated with different samples by reverse transfection in a 12 mm diameter cover slip contained in 4 well plates. eGFP expression in cells was observed after 3 days using an Olympus BX61 fluorescence microscope and DP-BSW image capture and control software with equal exposure time.

Flow cytometry. SHEP **c**ells grown in 4 well plates were treated with different samples as described above. After 3 days incubation, cells were prepared for fluorescence measurement. Briefly, cells were harvested by trypsinization and washed twice in PBS plus 0.5% BSA. The cells were then re-suspended in PBS plus 0.5% BSA and filtered via the cell strainer having pore size of 70 μM before analysis by a Beckman Coulter flow cytometer. SHEP cells that do not express eGFP were used as a control. The percentage of relative mean fluorescence intensity was calculated as the ratio to the eGFP positive control cells (SHEP cells, stably expressing eGFP, without any treatment) multiplied by 100.

Cell viability. The viability of cells was determined using a CellTiter Blue cell viability assay (Promega Corporation, Australia). Cells were seeded at a density of 5×10^5 cells/50 μL/well in 96 well plates. A 50 μL of each siRNA treatment was added into the cells**.** After 72 hours incubation, samples were treated following the manufacturer`s protocol. The fluorescence intensity of each well was measured at an excitation/emission wavelength of 544/590 nm using a fmax fluorescence microplate reader (Molecular Devices Corp. Sunnyvale, CA). The percentage of relative cell viability relative to the control wells without any treatment was calculated by fluorescence values of test samples divided by fluorescence values of control samples and then multiplied by 100.

Protein adsorption. IONPs (1.0 g/L) were suspended in a series of phosphate buffers (pH = 6.5, similar results was obtained at 7.4) with initial bovine serum albumin (BSA) concentrations (1 g/L). The samples were then placed on a shaker at room temperature for 14 hours to reach adsorption equilibrium. IONPs were removed by centrifugation. The supernatant BSA concentrations were determined following the Bradford method[4] using a UV-Visible spectrometer (Varian Cary 300 scan). Supernatant (10 μL) was added to Bradford reactant (3 mL), and mixed for 2 mins at room temperature (the solution turns blue in the presence of protein). The concentration of BSA was measured by absorption at 595 nm. The test was repeated three times for each sample. The average of the replicate measures was taken to determine the BSA concentration at equilibrium. The amount of BSA adsorbed was using the following equation.

$$\%\text{-BSA}_{\text{adsor.}} = 100 - (([\text{BSA}]_{\text{eq.}}/[\text{BSA}]_0) \times 100)$$

Where $[\text{BSA}]_0$ and $[\text{BSA}]_{\text{eq.}}$ correspond to BSA concentrations measured by Bradford assay without IONPs and BSA concentrations in the presence of IONPs at equilibrium, respectively.

Proton Relaxometry. Proton transverse relaxation rate measurements were carried out using a Bruker Minispec mq60 with a magnetic field strength of 1.4 T and a measuring frequency of 60MHz. All suspensions were temperature stabilized at 25 ºC prior to measurement. Proton transverse relaxation data were acquired with a multi-spin-echo sequence (TR = 10 sec, Number of echoes = 1000, inter-echo spacing = 2 msec, number of averages = 4). Proton relaxation rates were determined by fitting a mono-exponential curve to the signal versus time data. The iron concentrations were determined by ICP-AES of acid digested samples.

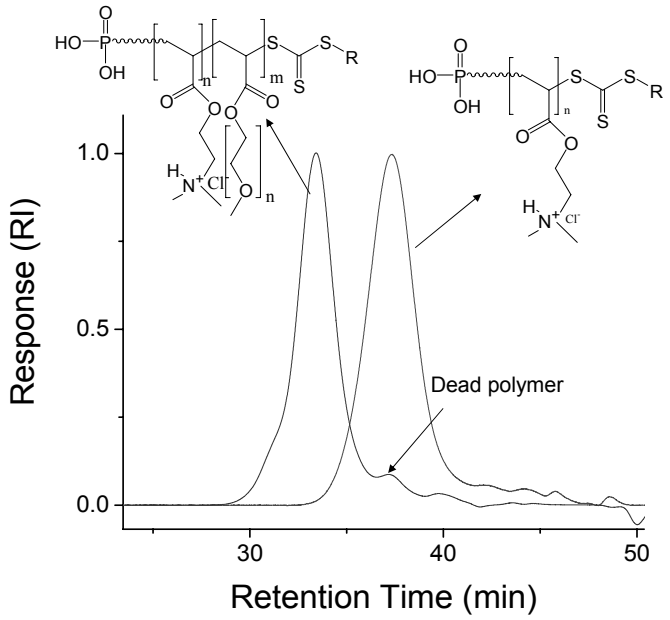


Figure S1. DMAc GPC traces of P(DMAEA)-*b*-(OEG-A) diblock polymers after purification, M_n = 13 500 g/mol, entry 7 of Table 1.

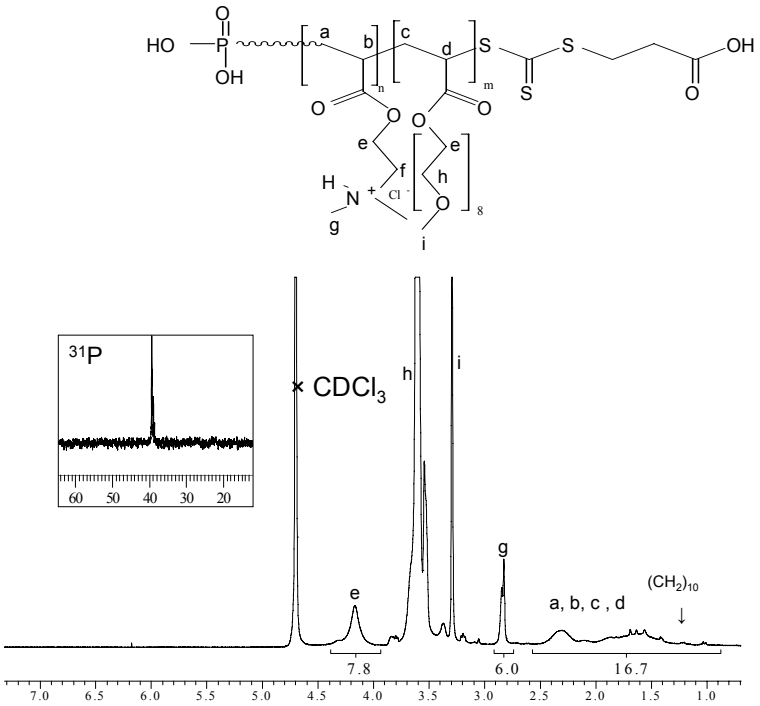


Figure S2. Typical ^{1}H NMR of P(DMAEA)-*b*-(OEG-A) diblock polymers after purification, M_n = 13 500 g/mol, entry 7 of Table 1.

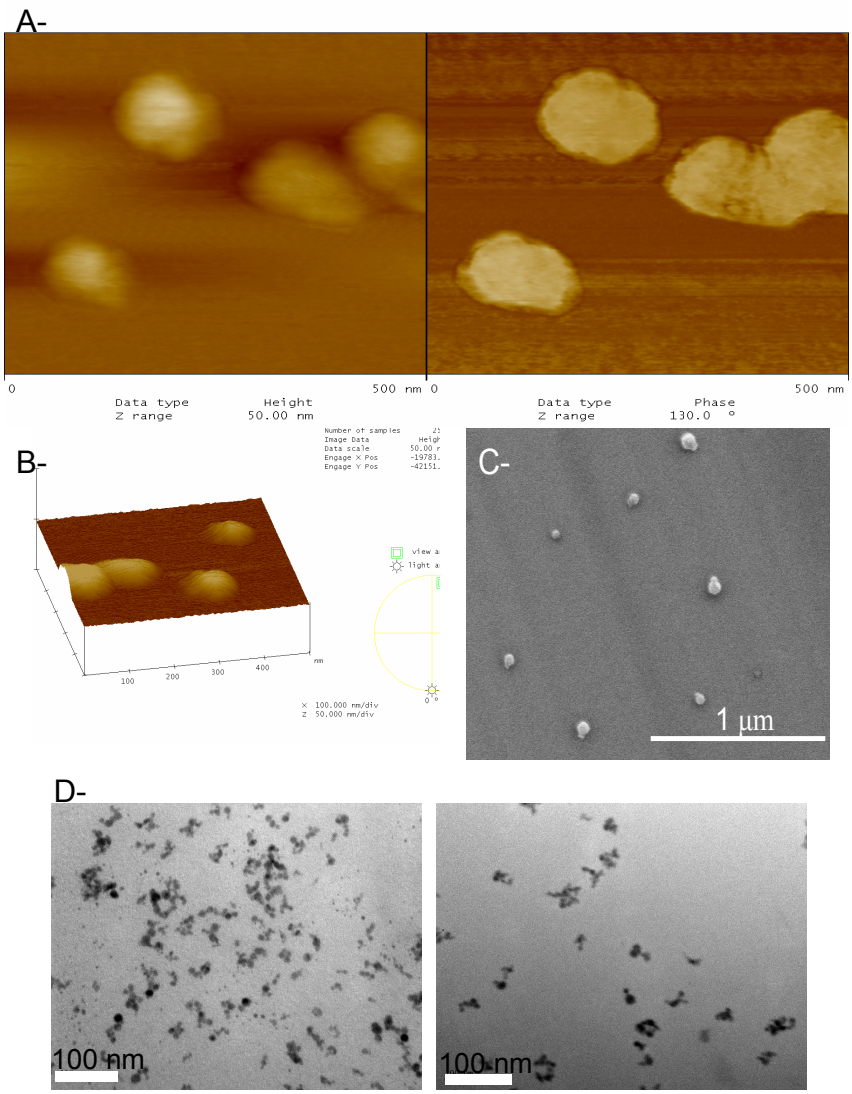


Figure S3. Atom force microscopy and SEM pictures of IONPs@[P(OEG-A)/P(DMAEA)]: 55/45 mixture of P(OEG-A) M_n 30 300 g/mol and poly(DMAEA) M_n 9 6 00 g/mol coated.: A- right: contact mode and left: phase mode; B- ^{3}D view of these particles; C- SEM pictures, D- TEM pictures of (left) IONPs@[P(DMAEA)] poly(DMAEA), M_n 9 6 00 g/mol, and right- IONPs@[P(OEG-A)/P(DMAEA)]: 55/45 mixture of P(OEG-A) M_n 30 300 g/mol and poly(DMAEA) M_n 9 6 00 g/mol coated

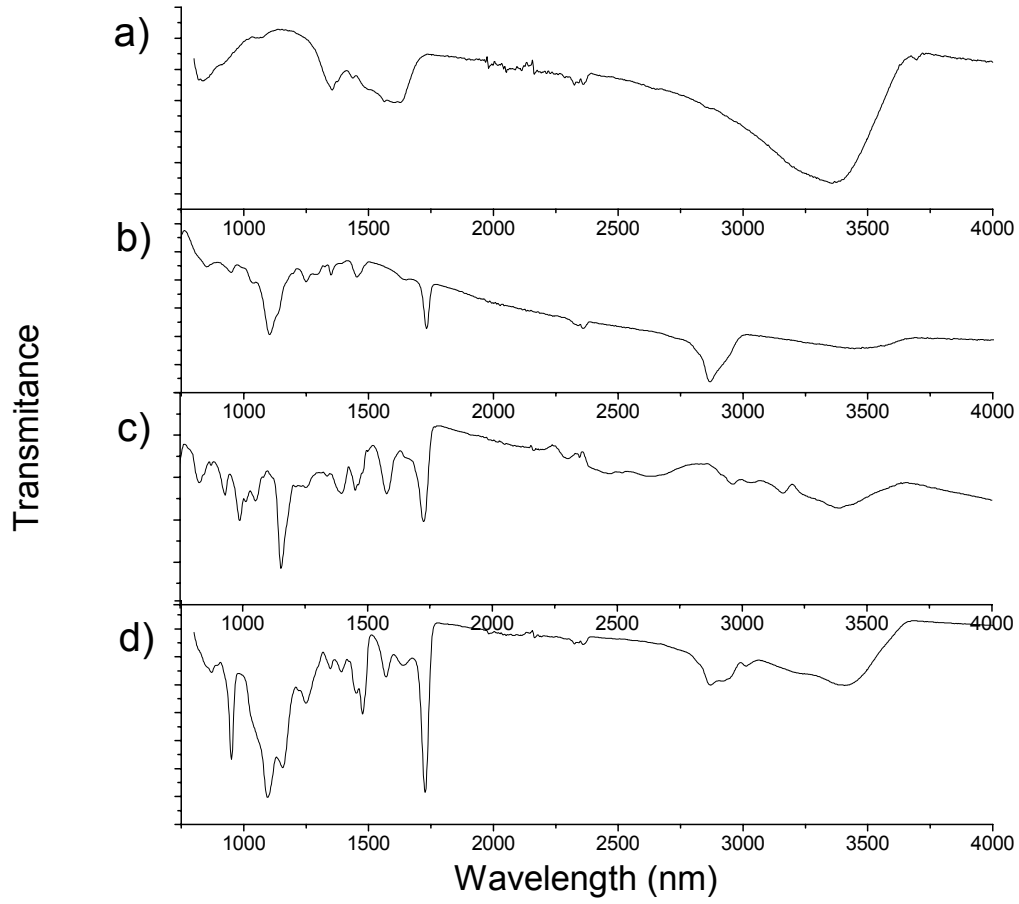


Figure S4. Surface analysis of IONPs@polymers: A- ATR analysis: a) naked "IONPs", b) purified IONP@P(OEG-A) (M_n = 10 300 g/mol), c) purified IONP@P(DMAEA) (M_n = 9 600 g/mol), d) IONPs@[P(OEG-A)/ P(DMAEA)], P(OEG-A)/ P(DMAEA) composition 45/55 wt-%, $M_n^{P(OEG-A)}$ = 30 300 g/mol, M_n^{PDMAEA} = 9 600 g/mol; B- XPS of IONPs@polymers: a) purified IONP@P(DMAEA (M_n = 9 600 g/mol), b and c) IONPs@[P(OEG-A)/ P(DMAEA)], P(OEG-A)/ P(DMAEA) composition 55/45 and 70/30 wt-%) purified IONP@P(OEG-A), $M_n^{P(OEG-A)}$ = 30 300 g/mol, $M_n^{P(DMAEA)}$ = 9 300 g/mol.

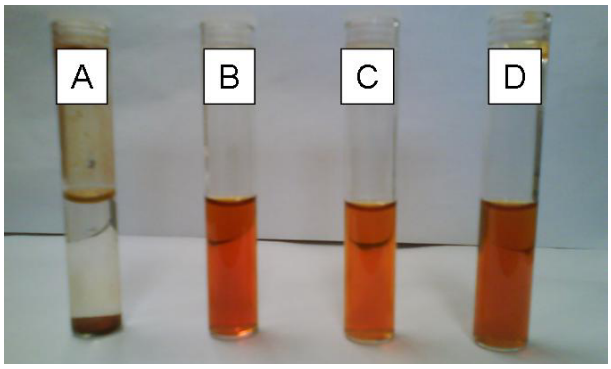


Figure S5. Pictures: (A) Uncoated IONPs; (B) IONPs@P(OEG-A), M_n 10 300 $gmol^{-1}$; (C) IONPs@P(DMAEA) M_n 9 600 g/mol ; (D) IONPs@[P(OEG-A)/P(DMAEA)]: 55/45 mixture of P(OEG-A) M_n 30 300 g/mol and poly(DMAEA) M_n 9 6 00 g/mol coated. All particles were dispersed in deionized water.

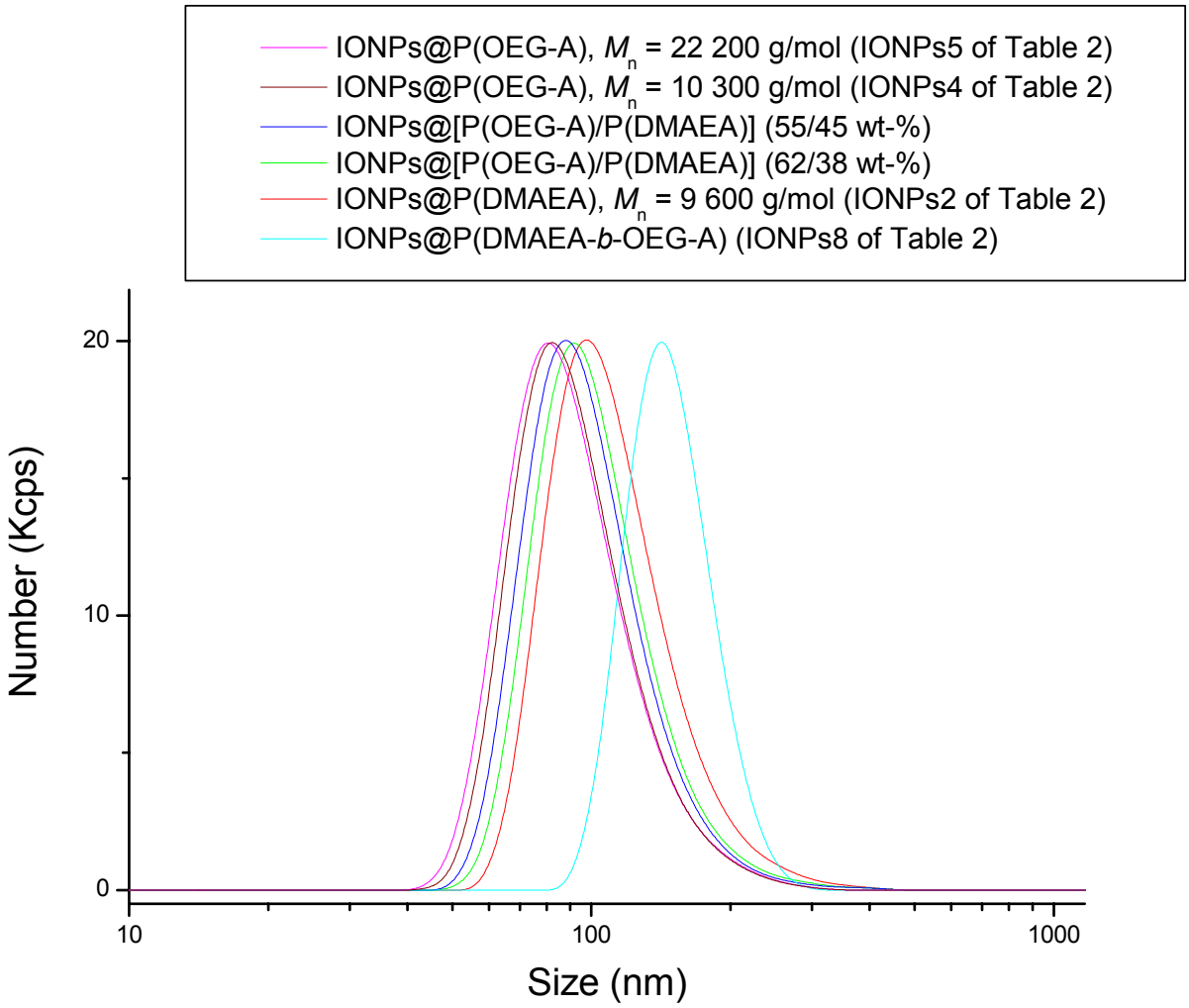


Figure S6. DLS distributions of different IONPs coated polymers, number versus size (nm). Note: the intensity for the different distribution was normalized.

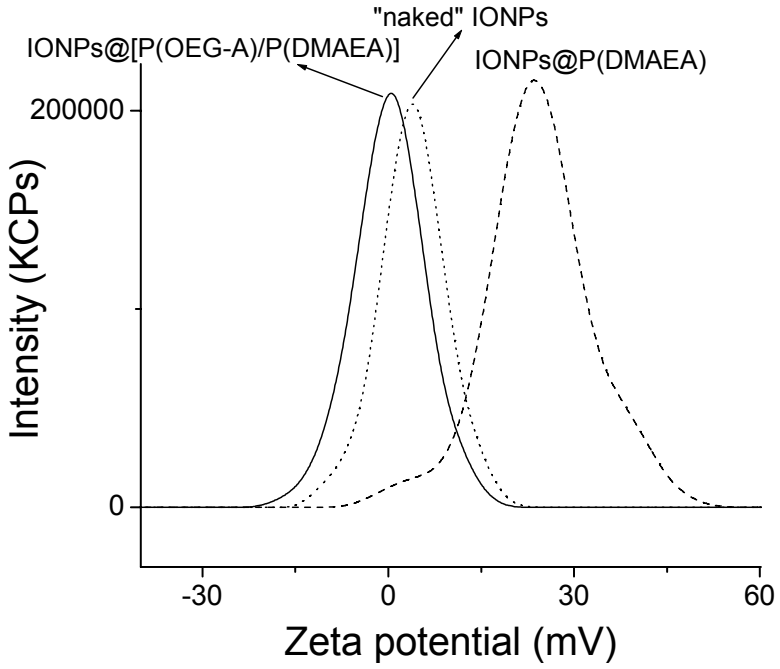


Figure S7. Zeta-potential distribution of "naked" IONPs, IONPs@P(DMAEA), with M_n = 9 600 g/mol and IONPs@[P(OEG-A)/ P(DMAEA)], P(OEG-A)/ P(DMAEA) composition 55/45, with $M_n^{P(OEG-A)}$ = 30 300 g/mol, $M_n^{P(DMAEA)}$ = 9 300 g/mol.

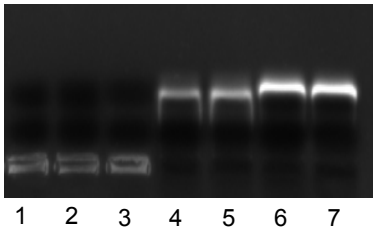


Figure S8. Agarose gel electrophoresis picture. Key: 1- siRNA-IONPs@P(DMAEA)], $M_n^{P(DMAEA)}$ = 9 300 g/mol (ratio N/P = 1.6); 2- siRNA-IONPs@P(DMAEA)/(OEG-A)], P(OEG-A)/ P(DMAEA) composition 70/30, with $M_n^{P(OEG-A)}$ = 30 300 g/mol, $M_n^{P(DMAEA)}$ = 9 300 g/mol (ratio N/P = 1.6); 3- siRNA-IONPs@P(DMAEA)/(OEG-A)], P(OEG-A)/ P(DMAEA) composition 55/45, with $M_n^{P(OEG-A)}$ = 30 300 g/mol, $M_n^{P(DMAEA)}$ = 9 300 g/mol (ratio N/P = 1.6); 4 and 5- siRNA-IONPs@P(DMAEA)/(OEG-A)], P(OEG-A)/ P(DMAEA) composition 55/45, with $M_n^{P(OEG-A)}$ = 30 300 g/mol, $M_n^{P(DMAEA)}$ = 9 300 g/mol with ratio N/P = 0.4 and 0.8, respectively; 6 siRNA complexed with "naked" IONPs; 7- only siRNA. (All the samples were prepared at the same concentration 10^{-5} mol/L)

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