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The Stabilization and Bio-functionalization of Iron Oxide Nanoparticles using Heterotelechelic Polymers

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Experimental part complement.

Material.

All other 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), dithiodipyridine (97%, Fluka), 2-mercaptoethanol (99%, Aldrich), 3-mercaptopropionic acid (99+, Aldrich), glutathione reduced (98%, 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).

Characterizations.

NMR Spectroscopy. ^1H and ^{13}C NMR spectra were recorded using Bruker ACF300 (300 MHz) or ACF500 (500 MHz) spectrometers. D_2O, DMSO-D_6 or $CDCl_3$ were used as solvents.

NIPAAm, styrene and OEG-A monomer conversions were determined via ^1H NMR spectroscopy, comparing the signal area from the vinyl protons (δ ~ 5.4-6.3 ppm, 3H/mol for NIPAAm, δ ~ 5.3-5.8 ppm, 3H/mol for styrene, and δ ~ 5.4-6.3 ppm, 3H/mol for OEG-A) to the signal area from the isopropyl methylene (δ ~ 3.6- 3.85 ppm, 1H/mol), aromatic (δ ~ 6.2- 7.4 ppm, 5H/mol), and methylene oxide (δ ~ 3.6 ppm, 10 H/mol) groups, respectively for NIPAAm, styrene and OEG-A.

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.

Size Exclusion Chromatography (SEC). Size exclusion chromatography (SEC) 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 SEC 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: 1 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 SEC 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 ^{o}C/ min from room temperature to 500 ^{o}C. The weight lost was calculated between the difference between the weights at room temperature and at 500 ^{o}C.

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 together with a background electrolyte comprised of 50 mM KNO_3.

Microcopies. TEM and SEM. 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 or in methanol (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 Kα, 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 N2 adsorption at 77 K using the BET method. The surface area equivalent size were deduced from dBET = 6/(ρ × SSA) where ρ = 4.87 g cm-3 is the density of maghemite phase iron oxide.

Synthesis of the RAFT agent

Step 1: Synthesis of undecen-1-yl acetate (product 1). 10-Undecenol was dissolved in DCM in the

presence of triethylamine (TEA). Acetyl chloride was added at 0 ^{o}C for 1 hr and the reaction was stirred overnight at room temperature. The resulting precipitate was removed by filtration on cotton, and water (50 ml) was added. The mixture was extracted by DCM until a neutral pH was attained. The organic phase was collected and dried over $NaSO_4$. After removal of the solvent under reduced pressure, undecen-1-oate (1, Scheme 1) was isolated a colorless oil (2.82 g, yield 77%).

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm from TMS): 1.20 (14H, m, CH_2=CH-(C$\underline{H}_2$)$_7$-), 1.95 (5H, m, -CH_2-O(CO)C$\underline{H}_3$ and CH_2=CH-C$\underline{H}_2$-), 3.95 (2H, t, -C$\underline{H}_2$-O), 4.90 (2H, dd, C$\underline{H}_2$-CH-) and 5.75 (2H, dd, CH_2-C$\underline{H}$-). The spectrum is given in Figure S1.

FT-IR (cm^{-1}): 1740 (vs, C=O), 1640 (vs, C-C).

Step 2: Synthesis of dimethylphosphonate-11-acetyloyloxyundecanol (product 2).

Undecen-1-yl acetate (8.42 g, 39.00 mmol) and dimethylhydrogenophosphonate (58.5 mmol) were added to a round-bottom flask. The solution was degassed with nitrogen for 30 mins to remove trace oxygen. The flask (solution under nitrogen) was then placed in an oil bath at 130 ^{o}C. *Tert*-butyl peroxide was added slowly, to mitigate the exothermic reaction. The reaction mixture was stirred at 135 ^{o}C for 16 hours until complete consumption of the double bond. The excess of dimethylhydrogenophopshonate was distilled at 130 ^{o}C under low pressure. The crude product was purified using a silica column with a solvent mixture of ethyl acetate and hexane (60/40 v-%). The solvent was removed to obtain a colorless viscous product.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm from TMS): 1.20 (20H, m, P-(C$\underline{H}_2$)$_{10}$-), 1.95 (3H, m, -CH_2-O(CO)C$\underline{H}_3$), 3.60 (3H, d, (C$\underline{H}_3$)$_2$-O-) and 3.95 (2H, t, -C$\underline{H}_2$-O). The spectrum can be seen in Figure S1.

^{31}P NMR (300 MHz, $CDCl_3$): 35.43 ppm.

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

Step 3: Synthesis of dimethylphosphonate undecan-11-ol *(product 3).*

Dimethyl (acetyloyloxyundecan)-phosphonate (43×10^{-3} mol) and potassium hydroxide (KOH) (2.4 g,

43×10^{-3} mol, 8 mol-%) were mixed with methanol (50 ml) and stirred (under a condenser) at 60 ^{o}C for 18 h. The disappearance of the peak at 1.95 ppm in ^{1}H NMR, characteristic of C$\underline{H_3}$C(O) group, and of the band at 1730 cm^{-1} (C=O) in FT-IR were verified.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm from TMS): 1.20 (20H, m, P-(C$\underline{H_2}$)$_{10}$-), 2.60 (1H, s, -CH$_2$-O$\underline{H}$), 3.5 (2H, m, -C$\underline{H_2}$-OH), and 3.60 (3H, d, (C$\underline{H_3}$)$_2$-O-P). The spectrum can be seen in Figure S1.

^{31}P NMR (300 MHz, $CDCl_3$): 35.43 ppm.

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

Step 4: Synthesis of product 2-bromo-propionic acid 11-(dimethoxy-phosphonyl)-undecnyl ether (***4***).

Dimethylphosphonate undecan-11-ol (product 3, 5.92 g, 20.0 mmol) was dissolved in DCM (50 mL). Bromopropionic bromide (15.94 g, 43.2 mmol) was added slowly for 1 hr at 0 ^{o}C and subsequently stirred for 16 h at room temperature. The triethylamine bromide was filtered and washed several times with DCM (50 mL). The product was purified by extraction, twice with deionized water (20 mL), three times with acidic water (pH 2), and three times by basic solution (pH 9). The organic phase was dried over anhydrous sodium sulfate (Na_2SO_4) and then isolated using a rotary evaporator. The resulting yellow oil was purified by silica gel column chromatography (ethylacetate/hexane: 60/40, volume-%) (Yield = 70%).

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm from TMS): 1.20 (20H, m, P-(C$\underline{H_2}$)$_{10}$-), 1.8 (3H, d, C$\underline{H_3}$-CH), 3.60 (3H, d, (C$\underline{H_3}$)$_2$-O-P), 4.00 (2H, m, -C$\underline{H_2}$-O-(CO)), and 4.20 (2H, m, -C$\underline{H}$-Br). The spectrum is given in Figure S1.

^{31}P NMR (300 MHz, $CDCl_3$): 35.43 ppm.

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

Step 5: Synthesis of product 2-(2-carboy-ethyltrithiocarbonate)-propionic acid 11-(dimethoxy-phosphonyl)-undecnyl ether (**5**)

Initially, 2-mercaptopropionic acid was dissolved in diethyl ether. Then triethylamine was

added slowly at 0 ^{o}C, subsequently, carbon disulfide was added slowly. The solution was stirred at room temperature for 14 h to yield an orange precipitate. The solvent was removed. The crude product was precipitated two times in diethyl ether, which yielded an orange colored viscous product. This product was used without further purification. ^{1}H NMR confirmed the expected structure.

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm from TMS): 1.20 (18H, t, $C\underline{H}_3$ of TEA), 2.50 (2H, t, -$C\underline{H}_2$-S-), 2.70 (2H, t, -$C\underline{H}_2$-COO^-), 2.90 (12H, q, N-$C\underline{H}_2$- of TEA).

Product 4 (5.92 g, 20.0 mmol) was dissolved in DCM (50 mL) and stirred with a 2-fold excess of dithiocarboxysulfanyl propionic acid salt (15.94 g, 43.2 mmol) for 16 h at room temperature. The triethylamine bromide was filtered and washed several times with DCM (50 mL). The product was purified by extraction, two times with deionized water (20 mL), and three times with acidic water (pH 2). The organic phase was dried over anhydrous sodium sulfate ($NaSO_4$) and then isolated by rotary evaporation. The resultant yellow oil was purified by silica gel column chromatography (ethylacetate/hexane: 60/40, volume-%) (Yield: 70%).

^{1}H NMR (300 MHz, $CDCl_3$), δ (ppm from TMS): 1.20 (20H, m, P-$(C\underline{H}_2)_{10}$-), 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-), 3.60 (3H, d, $(C\underline{H}_3)_2$-O-P), , 4.00 (2H, m, -$C\underline{H}_2$-O-(CO)), and 4.78 (1H, quartet, CH), 10.0 (1H, s, $CO_2\underline{H}$). The spectrum is given in Figure S1.

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: 539.1 (Na^+) (theoretical value = 539.67). This RAFT agent was found to decompose in the mass spectrometer before the ionization. Molecular peak as well as assigned fragments were detected, but in concentrations lower than expected.

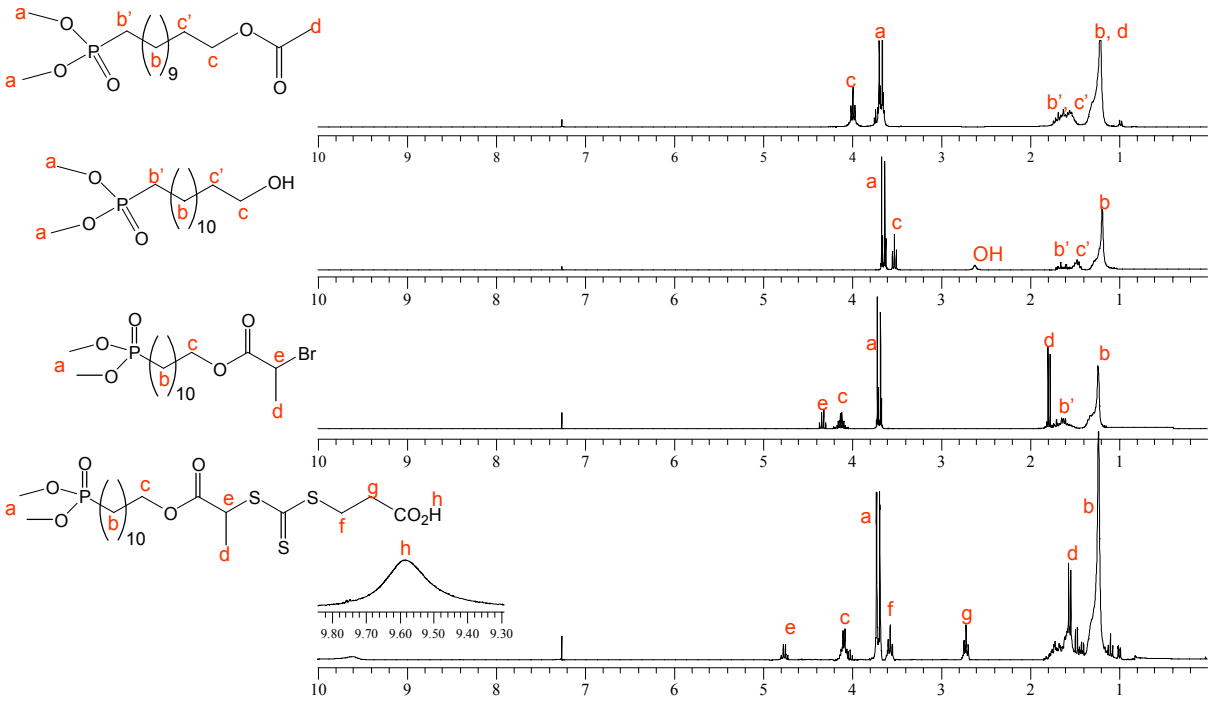

Figure S1. ^{1}H NMR of different products obtained for the several steps of synthesis of RAFT agent.

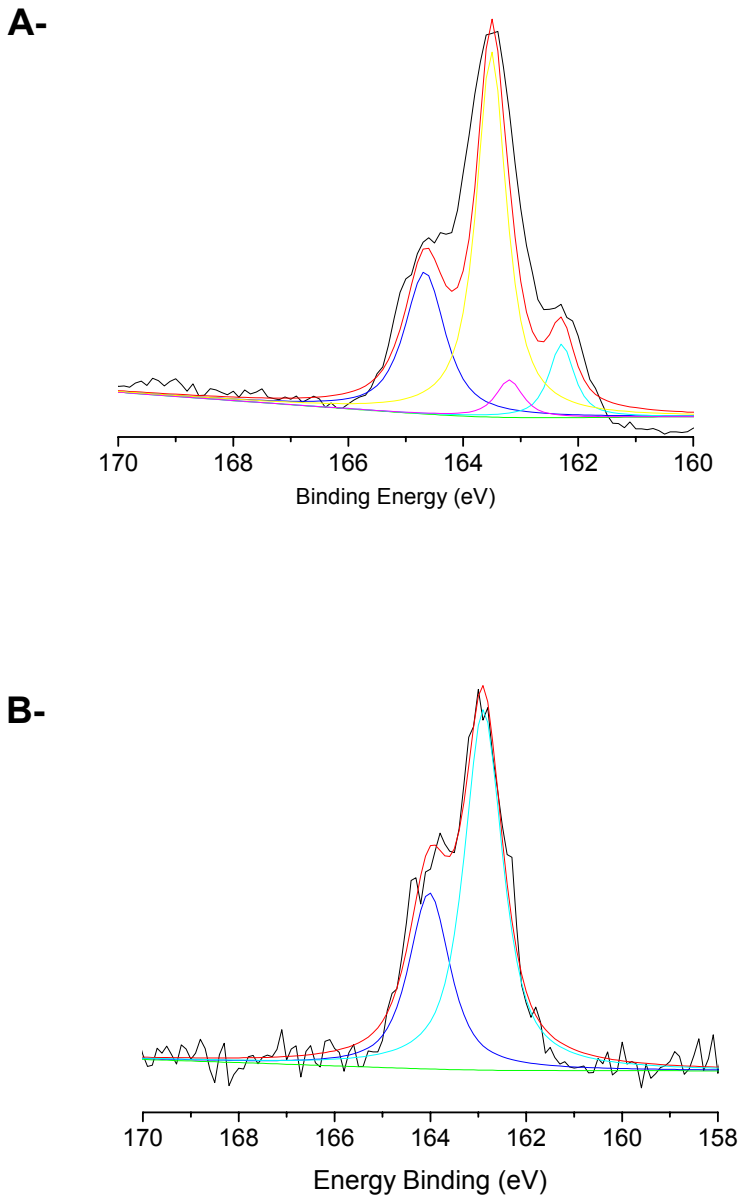

Figure S2. XPS spectra of S2p. a- polymer before reaction with dithiopyrine and b- after reaction with dithiopyridine (and purification).

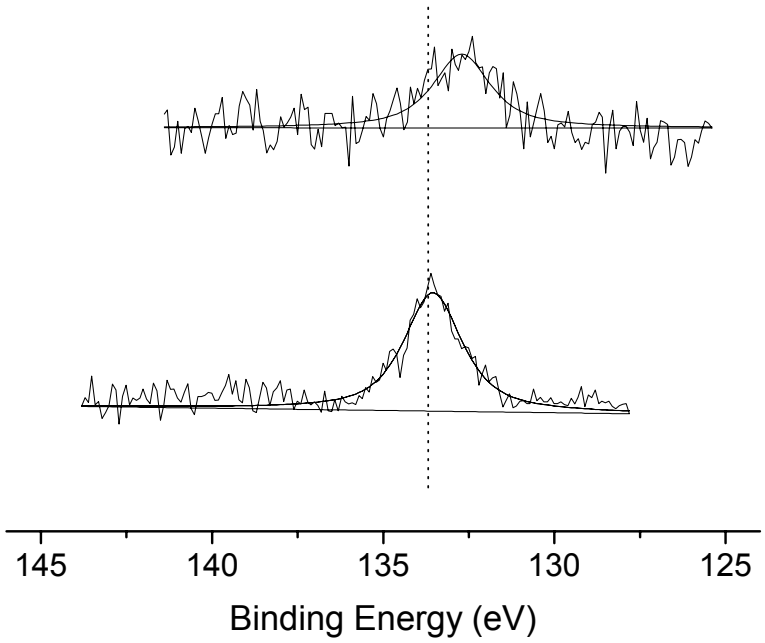

Figure S3. XPS spectra of P2p before and after attachment on iron oxide nanoparticles (Spectrum before attachment was carried on a alumina fold).

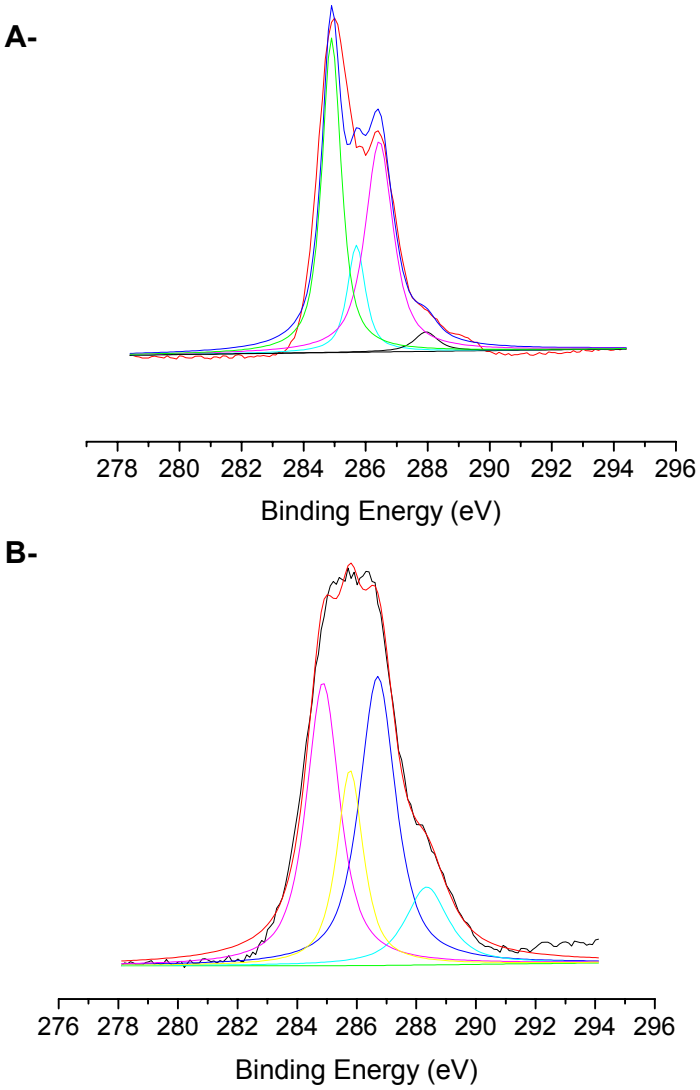

Figure S4. XPS of C1s of IONPs coating with polymer (M_n = 36 000 g/mol). A- before reaction with a peptide and after reaction with peptide.

Calculation of number of polymer chains per iron oxide nanoparticles and density.

The value was calculated in using the difference of lost weight (LW) assessed by TGA analysis at 500 ^{o}C between IONPs coated with polymers and IONPs without polymer.

Net LW = LW - LW^{IONPS}

Net LW, LW and LW^{IONPS} correspond to lost weight corresponding to polymer degradation, lost weight of IONPs coated with polymer assessed by TGA and lost weight without polymer, respectively.

Number of chains per particle (*N*).

$N = [(m^{IONPs} \times \text{Net LW (\%)} / 100) / M_n^{Polymer}] / [(m^{IONPs} \times (100 - \text{Net LW (\%)}) /100 \times V^{IONPs} \times \rho^{IONPs})]$

N, $m^{IONPs,}$ $M_n^{Polymer}$, V^{IONPs} , and ρ^{IONPs} correspond to number of chains per particle, weight of IONPs used for the measurement, molecular weight of polymer, volume of IONPs and density of IONPs (5 g/cm^3), respectively.

Density.

$d = N / S^{IONPs}$

S^{IONPs} area of IONPs surface determined by BET.