Supporting Information

Dendritic maleimide functionalization of core-shell ($\gamma$-Fe$_2$O$_3$/polymer) nanoparticles for efficient bio-immobilization

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1) General information: materials and characterization methods

Materials

Solvents and starting materials were obtained commercially from Acros, Alfa Aesar or Aldrich (reagent grade) and were used without further purification. The solvents were purchased anhydrous (DMF, DMSO, Dioxane, THF, CHCl₃, EtOAc, AcOH) or were dried according to standard procedures over dehydrating agents and distilled under an inert atmosphere (CH₂Cl₂: CaH₂; MeOH: CaH₂; Toluene: Na/benzophenone). 300 nm γ-Fe₂O₃/Polymer core-shell superparamagnetic nanoparticles (Carboxyl-Adembeads), COOH: 350 µmol.g⁻¹ of particles, were purchased from Ademtech SA ; 15 nm Streptavidin Gold Nanoparticles (SA-Au NPs) from BBI ; 20 nm Amino PEGylated Gold Nanoparticles (H₂N-Au NPs) from Polysciences Inc.; and Thiol-PEG-Biotin (HS-PEG-Biot), from Polypure.

General characterization methods for organic compounds

The ¹H and ¹³C spectra were recorded on Bruker Avance 300 FT NMR spectrometer (¹H: 300.13 MHz, ¹³C: 75.46 MHz) or on Bruker Avance II 400 FT NMR spectrometer (¹H: 400.13 MHz, ¹³C: 100.62 MHz). Chemical shifts, δ, are represented in part per million (ppm) and coupling constants, J, in Hertz (Hz). The following abbreviations were used to explain the multiplicities: s = singulet, d = doublet, t = triplet, q = quartet, and m = multiplet.

Mass spectra were performed on a QStar Elite Mass Spectrometer equipped with an electrospray ionization (ESI) source (operated in a positive or negative ion mode).

Elemental analyses were carried out in elemental analysis department at CNRS-Vernaison, France. For column chromatography, Merck silica gel 60 (230–400 mesh) was used.

ATR FTIR experiments

The ATR spectra were recorded on a Thermo-Scientific Nicolet iS50 FT-IR spectrometer equipped with a DLaTGS detector (KBr window) using the diamond iS50 ATR accessory. Each spectrum was obtained in the 4000-400 cm⁻¹ spectral range, at a resolution of 4 cm⁻¹, by coadding 500 scans.

UV-Vis experiments

UV-Vis spectra were recorded between 200 and 800 nm using a PerkinElmer UV/Vis spectrophotometer Lambda 650.
Transmission Electron Microscopy (TEM)

The microscopy was done at the Bordeaux Imaging Center - Bordeaux University a Core facility of the French network "France Bio Imaging". The help of Sabrina LACOMME and Etienne GONTIER is acknowledged. TEM images were obtained on Electronic Microscope HITACHI H7650, (120 Kv, high resolution, equipped with a GATAN 11MPx camera). The samples were prepared by deposition of MNPs dispersed in ethanol or water (5-10 µL, 0.25-0.5 mg/mL) on carbon coated copper grids and dried in the air.

Zeta Potential

Zeta Potential measurements were carried out on Horiba Scientific nanoparticle analyzer SZ-100. The measurements were performed at 25°C for diluted aqueous suspension of MNPs at pH varying from 3.5 to 9.0.
2. Synthesis of precursors and dendron D

Scheme S1. Synthesis of dendron D.

**Synthesis of compound 1**

Maleic anhydride (30.0 g, 0.31 mol) was suspended in 150 mL of toluene, the suspension was warmed up to 80°C and 33 mL of furan (0.46 mol, 1.3 eq.) were added. The turbid solution was stirred for 6 h and then cooled to room temperature without stirring. White crystals, which precipitated out of solution after 1 h, were collected by vacuum filtration, washed with petroleum ether (2×30 mL) and dried under vacuum to yield compound 1 (58 %) as small white needles.

**1H NMR** (400 MHz, DMSO) δ 6.58 (s, 2H, CH=CH), 5.35 (s, 2H, CHO), 3.31 (s, 2H, CH).

**13C NMR** (101 MHz, DMSO) δ 171.53 (s, 2C, C=O), 136.85 (s, 2C, CH=CH), 81.64 (s, 2C, CHO), 49.08 (s, 2C, CH).

**FT IR (cm⁻¹)** = 1858, 1780 (νC=O five membered ring anhydrides); 921, 878, 849, 822 (maleic anhydride ring deformation).

**Synthesis of compound 2**

Compound 1 (10.00 g, 60.19 mmol) was dispersed in 20 mL of MeOH and the suspension cooled to 0 °C. Then, a solution of ethanolamine (3.6 mL, 60.19 mmol, 1 eq.) in 20 mL of MeOH was added dropwise during 10 min. The resulting clear yellow solution was stirred first, for 5 min. at 0 °C, then 30 min. at room temperature, and finally refluxed for 4 h. At the end of reaction the flask was cooled to room temperature, and after 2 h the product began to crystallize. The mixture
was stored in the freezer overnight, and the precipitated crystals were collected by vacuum filtration (yield: 44 %).

\[ 1^1H \text{ NMR (300 MHz, CDCl}_3 \] \( \delta \) 6.51 (s, 2H, CH=CH), 5.26 (s, 2H, CH), 3.70 (dt, \( J = 10.0, 3.5 \) Hz, 4H, -CH\(_2\)-N-, HO-CH\(_2\)), 2.88 (s, 2H, CH), 2.40 (s, 1H, -OH).

\[ 1^3C \text{ NMR (75 MHz, CDCl}_3 \] \( \delta \) 176.89 (s, 2C, N=), 136.62 (s, 2C, CH=CH), 81.08 (s, 2C, OCH), 60.30 (s, 1C, -CH\(_2\)-OH), 47.60 (s, 2C, CH), 41.84 (s, 1C, -CH\(_2\)-N). MS (ESI, m/z) [M+Na]" cac. = 232.0580, found = 232.0587

FT IR (cm\(^{-1}\)) = 3473 (\nu OH), 2932 (\nu\(_s\) CH\(_3\)), 2866 (\nu\(_s\) CH\(_2\)); 1768, 1683 (\nu\(_c\)=O cyclic imides in five membered ring); 918, 875, 851 (maleic anhydride ring deformation).

**Synthesis of compound 3**

A mixture formed of compound 2 (2.00 g, 9.56 mmol, 1 eq. in 40 mL of freshly distilled CH\(_2\)Cl\(_2\)) and anhydrous TEA (2.2 mL, 15.5 g, 15.3 mmol, 1.6 eq.) was cooled to 0 °C and 1 mL of acryloyl chloride (1.13 g, 12.43 mmol, 1.3 eq.) were added dropwise. The mixture was stirred at room temperature overnight under argon atmosphere. The solvent, excess of acryloyl chloride and TEA were evaporated under vacuum. The resulting residue was re-solubilized in 150 mL of CH\(_2\)Cl\(_2\), washed with brine (3×100 mL), dried over MgSO\(_4\), filtrated and concentrated in vacuum. Then, the obtained residue was purified by column chromatography on silica gel (AcOEt 100%) to yield compound 3 (60 %) as white crystals.

\[ 1^1H \text{ NMR (300 MHz, CDCl}_3 \] \( \delta \) 6.51 (s, 2H, CH=CH), 6.37 (d, \( J = 17.3 \) Hz, 1H, CH\(_2\)=), 6.06 (dd, \( J = 17.3, 10.5 \) Hz, 1H, -CH\(_2\)-), 5.83 (d, \( J = 11.9 \) Hz, 1H, CH\(_2\)=), 5.25 (s, 2H, CH), 4.30 (t, \( J = 5.4 \) Hz, 2H, -O-CH\(_2\)), 3.80 (t, \( J = 5.4 \) Hz, 2H, -CH\(_2\)-N), 2.86 (s, 2H, CH).

\[ 1^3C \text{ NMR (75 MHz, CDCl}_3 \] \( \delta \) 176.07 (s, 2C, -N-(C=O)-), 165.84 (s, 1C, -C=O-O-), 136.66 (s, 2C, CH=CH), 131.41 (s, 1C, CH\(_2\)=), 128.07 (s, 1C, =CH-), 81.02 (s, 2C, -CH\(_2\)-O), 60.81 (s, 2C, -O-CH\(_2\)), 47.56 (s, 2C, -CH-), 37.89 (s, 2C, -CH\(_2\)-N).

MS (ESI, m/z) [M+Na]^+ cac. = 286.0685, found = 286.0692

EA cac.: C, 59.31; H, 4.98; N, 5.32; O, 30.39; found: C, 59.64; H, 5.03; N, 5.27; O, 29.66

FT IR (cm\(^{-1}\)) = 2907 (\nu\(_s\) CH\(_3\)); 1773, 1690 (\nu\(_c\)=O cyclic imides in five membered ring); 1723 (\nu\(_c\)=O ester); 914, 873, 850, 824 (maleimide ring deformation).

**Synthesis of compound 4**

A solution of di-tert-butyl dicarbonate (11.25 g, 0.05 mol, 1 eq. in 125 mL of anhydrous dioxane) was added dropwise to a solution of 2,2-(ethyleneoxy)bis(ethylamine) (44 mL, 45.75 g, 0.31 mol, 6 eq. in 125 mL of anhydrous dioxane). The mixture was stirred at room temperature overnight under argon atmosphere. Afterwards, the solvent was evaporated, the residue was solubilized in 400 mL of CH\(_2\)Cl\(_2\) and washed with H\(_2\)O (3×200 mL; to remove the excess of 2,2-(ethyleneoxy)bis(ethylamine)). The organic phase was dried over MgSO\(_4\), filtrated and
concentrated under vacuum. A column chromatography on basic alumina was then performed (AcOEt 100%) to yield compound 4 (88 %) as pale yellow oil.

1H NMR (300 MHz, CDCl$_3$) δ 5.15 (s, 1H, NH), 3.59 (s, 4H, -O-CH$_2$-CH$_2$-O-), 3.55 - 3.47 (m, 4H, -O-CH$_2$-), 3.29 (dd, J = 10.4, 5.2 Hz, 2H, CH$_2$-NH-), 2.86 (t, J = 5.2 Hz, 2H, -CH$_2$-NH$_2$), 1.57 (s, 2H, -NH$_2$), 1.42 (s, 9H, -CH$_3$).

13C NMR (101 MHz, CDCl$_3$) δ 156.08 (s, 1C, O-(C=O)-NH), 79.18 (s, 1C, C$_q$), 73.28 (s, 2C, -O-CH$_2$-), 70.26 (s, 2C, -O-CH$_2$-CH$_2$-O-), 41.70 (s, 1C, -CH$_2$-NH$_2$), 40.38 (s, 1C, -CH$_2$-NH$_2$), 28.46 (s, 3C, CH$_3$).

MS (ESI, m/z) [M+H]$^+$ calc. – 249.1818, found – 249.1808

EA calc.: C, 53.20; H, 9.74; N, 11.28; O, 25.77; found: C, 52.73; H, 9.61; N, 10.84; O, 26.35

FT IR (cm$^{-1}$) = 3361 (ν$_{NH}$ NH$_2$); 2974 (ν$_{as}$ CH$_3$); 2926 (ν$_{as}$ CH$_2$); 2866 (ν$_s$ CH$_2$); 1704 (ν$_C$=O O(C=O)NH); 1518 (ν$_{CN}$, δ$_{NH}$ C-N-H) amide II.

**Synthesis of compound 5**

To a solution of compound 4 (6.00 g, 24.16 mmol in 100 mL freshly distilled MeOH), 22 mL of methyl acrylate (20.8 g, 241.6 mmol, 10 eq.) were added. The mixture was stirred for 3 days at 50 °C. At the end of reaction the excess of methyl acrylate and MeOH was evaporated under vacuum to yield compound 5 (quantitatively) as yellow oil.

1H NMR (300 MHz, CDCl$_3$) δ 5.07 (s, 1H, NH), 3.64 (s, 6H, -O-CH$_2$), 3.56 (s, 4H, -O-CH$_2$-CH$_2$-O-), 3.51 (t, J = 5.4 Hz, 4H, -O-CH$_2$-), 3.28 (dd, J = 10.4, 5.2 Hz, 2H, -CH$_2$-NH-), 2.81 (t, J = 7.1 Hz, 4H, -N-CH$_2$-), 2.66 (t, J = 6.1 Hz, 2H, -CH$_2$-N-), 2.44 (t, J = 7.1 Hz, 4H, -CH$_2$-COO), 1.41 (s, 9H, -CH$_3$).

13C NMR (75 MHz, CDCl$_3$) δ 173.03 (s, 2C, -(C=O)-O-), 156.09 (s, 1C, -(C=O)-NH), 79.22 (s, 1C, C$_q$), 70.43 (d, J = 10.1 Hz, 2C, -O-CH$_2$-CH$_2$-O-), 69.79 (s, 2C, -CH$_2$-O-), 53.36 (s, 1C, -CH$_2$-N-), 51.63 (s, 2C, -O-CH$_3$), 50.04 (s, 2C, -N-CH$_2$), 40.48 (s, 1C, -CH$_2$-NH$_2$), 32.68 (s, 2C, -CH$_2$-COO-), 28.52 (s, 2C, -CH$_3$).

MS (ESI, m/z) [M+H]$^+$ calc. – 421.2544, found – 421.2554; MS [M+Na]$^+$ calc. – 443.2431, found – 443.2441

EA calc.: C, 54.27; H, 8.63; N, 6.66; O, 30.44; found: C, 54.66; H, 8.54; N, 6.60; O, 30.64

FT IR (cm$^{-1}$) = 3374 (ν$_{NH}$ O(C=O)NH); 2976 (ν$_{as}$ CH$_3$); 2951 (ν$_{as}$ CH$_2$); 2867 (ν$_s$ CH$_2$); 1735 (ν$_{C=O}$ ester); 1711 (ν$_{C=O}$ O(C=O)NH); 1513 (ν$_{CN}$, δ$_{NH}$ C-N-H) amide II.

**Synthesis of compound 6**

To a solution of compound 5 (11.00 g, 26.16 mmol in 100 mL freshly distilled MeOH), 140 mL of ethylene diamine (126g, 2.09 mol, 80 eq.) were added. The mixture was stirred for 5 days at 25 °C. At the end of reaction the excess of ethylene diamine and MeOH was evaporated under vacuum to yield compound 6 (quantitatively) as a very viscous brown oil.

1H NMR (400 MHz, CDCl$_3$) δ 7.46 (s, 2H, -NH$_2$), 5.65 (s, 1H, -NH$_2$), 3.56 – 3.47 (m, 8H, -CH$_2$-O-CH$_2$-CH$_2$-O-CH$_2$-), 3.24 (dd, J = 11.7, 5.8 Hz, 6H, -CONH-CH$_2$-), 2.81 – 2.69 (m, 8H, N-CH$_2$-, -CH$_2$-NH$_2$), 2.62 (t, J = 5.3 Hz, 2H, -CH$_2$-N), 2.33 (t, J = 6.2 Hz, 4H, -CH$_2$-CONH-), 2.03 (s, 4H, -NH$_2$), 1.40 (s, 9H, -CH$_3$).
was bubbled through the solution during 25 min. At the end of the reaction, the solvent, was evaporated and then the solution volume was reduced twice. After two days the excess precipitated gluey mass was separated from the solution by solubilization in a minimal volume of toluene while the starting material was solubilized in a minimal volume of CH₂Cl₂ and added dropwise to a large volume of toluene, and then the solution volume was reduced twice. After two days the precipitated gluey mass was separated from the solution by decantation and the procedure was repeated again (at least three times). By this procedure compound 7 was obtained in 46 % yield.

To a solution of compound 6 (1.81 g, 3.80 mmol in 20 mL of anhydrous DMF) a solution of compound 3 (8.00 g, 30.39 mmol, 8 eq. in 40 mL of anhydrous DMF) was added. The reaction mixture was stirred at 40°C during 5 days. At the end of reaction, the solvent was evaporated and the residue was purified relying on the difference of solubility of the mixture in toluene. The compound 7 is not soluble in toluene while the starting material (compound 3) added in a large excess is. Thus, the residue was solubilized in a minimal volume of CH₂Cl₂ and added dropwise to a large volume of toluene, and then the solution volume was reduced twice. After two days the precipitated gluey mass was separated from the solution by decantation and the procedure was repeated again (at least three times). By this procedure compound 7 was obtained in 46 % yield.

MS (ESI, m/z) [MH⁺] calc. – 477.3395, found – 477.3398; MS [MNa⁺] calc. – 499.3240, found – 499.3243

EA calc.: C, 52.92; H, 9.31; N, 17.63; O, 20.14; found: C, 52.12; H, 9.20; N, 16.88 O, 21.15

FT IR (cm⁻¹) = 3295 (νNH C=O)NH); 2970 (νas CH₂); 2929 (νas CH₂); 2866 (νs CH₂); 1702 (νC=O O(C=O)NH); 1644 (νC=O) amide I; 1537 (νCN, δNH C-N-H) amide II.

Synthesis of compound 7

To a solution of compound 6 (1.81 g, 3.80 mmol in 20 mL of anhydrous DMF) a solution of compound 3 (8.00 g, 30.39 mmol, 8 eq. in 40 mL of anhydrous DMF) was added. The reaction mixture was stirred at 40°C during 5 days. At the end of reaction, the solvent was evaporated and the residue was purified relying on the difference of solubility of the mixture in toluene. The compound 7 is not soluble in toluene while the starting material (compound 3) added in a large excess is. Thus, the residue was solubilized in a minimal volume of CH₂Cl₂ and added dropwise to a large volume of toluene, and then the solution volume was reduced twice. After two days the precipitated gluey mass was separated from the solution by decantation and the procedure was repeated again (at least three times). By this procedure compound 7 was obtained in 46 % yield.

Synthesis of dendron D

Compound 7 (3.00g) was solubilized in freshly distilled CH₂Cl₂ (40 mL) and gaseous HCl was bubbled through the solution during 25 min. At the end of the reaction, the solvent, t-butanol and excess of HCl were evaporated under vacuum. Then, the residue was solubilized in 100 mL of
H₂O, washed with CH₂Cl₂ (3×100 mL) and dried in vacuum to yield dendron D (77 %) as a yellow-orange very viscous oil.

¹H NMR (300 MHz, D₂O) δ 6.65 (s, 8H, CH=CH), 5.32 (s, 8H, CH), 4.36 (t, J = 5.1 Hz, 8H, -(C=O)O-CH₂-), 3.94 (d, J = 4.4 Hz, 2H, O-CH₂-), 3.83 (dd, J = 9.4, 4.7 Hz, 10H, -CH₂-O-, -CH₂-N-), 3.78 (s, 4H, -O-CH₂-CH₂-O-), 3.72 – 3.65 (m, 4H, -(C=O)NH-CH₂_), 3.55 (dd, J = 13.8, 6.9 Hz, 14H, -N-CH₂-, -CH₂-N), 3.43 (t, J = 5.7 Hz, 4H, -CH₂-N), 3.28 – 3.22 (t, 2H, -CH₂-NH₂), 3.16 (s, 8H, CH), 2.91 (d, J = 6.4 Hz, 12H, -CH₂-(C=O)NH-), 1.89 (s, 8C, -N(C=O)₂), 172.81 (s, 2C, (C=O)NH), 172.61 (s, 4C, (C=O)O), 136.56 (s, 8C, C=CH), 81.16 (s, 8C, CH), 69.79 (d, J = 19.5 Hz, 2C, -O-CH₂-CH₂-O-), 66.54 (s, 1C, -CH₂-O-), 64.09 (s, 1C, -O-CH₂_), 61.88 (s, 4C, (C=O)O-CH₂_), 53.09 (s, 2C, -CH₂-N), 50.56 (s, 4C, -O-CH₂-CH₂-O-), 39.17 (s, 1C, -CH₂-NH₂), 37.11 (s, 4C, -CH₂-N), 34.53 (s, 2C, -CH₂-(C=O)NH-), 28.77 (s, 4C, -CH₂-(C=O)O-), 28.23 (s, 2C, -CH₂-(C=O)NH-).

MS (FD, m/z) [M+H]⁺ calc. – 1429.6051, found – 1429.6008

EA (M×5H₂O×4HCl) calc: C, 49.04; H, 6.17; Cl, 8.52; N, 8.41; O, 27.86; found: C, 48.76; H, 6.16; Cl, 9.58; N, 8.72; O, 26.92.

FT IR (cm⁻¹) = 3389 (νₘ NH₂); 2956 (νₘ CH₂); 2866 (ν, CH₂); 1772, 1693 (νC=O cyclic imides in five membered ring); 1735 (νC=O ester); 1551 (νCN, δNH C-N-H) amide II; 1184 cm⁻¹ (C-N-C stretch); 914, 877, 852, 824 (maleimide ring deformation)
3. General grafting and maleimide deprotection procedures

**Grafting of dendron D on core-shell γ-Fe\textsubscript{2}O\textsubscript{3}/Polymer MNPs**

First, 5 mg of γ-Fe\textsubscript{2}O\textsubscript{3}/Polymer MNPs were dispersed in 1 mL of 50 mM MES/0.3 % PF solution.\textsuperscript{[a]} Then, 2.85 mg of EDC\textsuperscript{[b]} (corresponding to 8.5 eq. per equivalent of available COOH groups on γ-Fe\textsubscript{2}O\textsubscript{3}/Polymer MNPs, which is \textasciitilde 350 µmol COOH/g) solubilized in 1 mL of 50 mM MES/0.3 % PF solution and 5.99 mg of NHS\textsuperscript{[c]} (3.5 eq. per equivalent of EDC quantity) solubilized in 1 mL of 50 mM MES/0.3 % PF solution were added. Subsequently, 10 equivalents of dendron D (per equivalent of available COOH groups on γ-Fe\textsubscript{2}O\textsubscript{3}/Polymer MNPs) solubilized in 1 mL of 50 mM MES/0.3 % PF solution were loaded in the grafting reaction. The total reaction volume was brought up to 5 mL (corresponding to the final concentration of 1 mg of MNPs/mL of solution) and then sonicated (~10 sec, 60W). The grafting proceeded in a thermomixer (300 rpm) at 25°C for 16 h. During all the grafting period the particles remained well dispersed and no sedimentation or aggregation was observed. At the end of the grafting, MNPs were separated from the reaction mixture by magnetic decantation, washed with: MES (1×5mL), H\textsubscript{2}O (1×5mL), EtOH (3×5mL), re-dispersed in EtOH (5 mg/mL) and stored in the fridge.

**Maleimide group deprotection**

5 mg of the modified MNPs (γ-Fe\textsubscript{2}O\textsubscript{3}/Pol@D) were dispersed in 0.5 mL of anhydrous DMSO and the cleavage of furan protection proceeded in a thermomixer (300 rpm) at 99 °C during 5 h. At the end of deprotection reaction the resulting maleimide functionalized MNPs (γ-Fe\textsubscript{2}O\textsubscript{3}/Pol@D-Mal) were separated from the reaction mixture by magnetic decantation, washed with EtOH (5 × 1 mL), dispersed in EtOH (5 mL) and stored in the fridge.

\textsuperscript{[a]} MES: 4-Morpholineethanesulfonic acid and, PF: Pluronic F-127
\textsuperscript{[b]} N-Ethyl-N’-(3-dimethylaminopropyl)carbodiimide
\textsuperscript{[c]} N-hydroxysuccinimide
4. Immobilization tests

Covalent coupling of thiol modified biotin (HS-PEG-Biot) with the maleimide functionalized MNPs

1 mg of maleimide functionalized MNPs (γ-Fe₂O₃/Pol@D-Mal) was dispersed in 0.375 mL of 0.01 M PBS/0.05% Tween-20 buffer solution (adjusted to pH=7.1 with 1 M HCl solution), and 2.76 mg of HS-PEG-Biot solubilized in 0.125 mL of 0.01 M PBS[d]/0.05% Tween-20 buffer solution were added to the MNPs’ suspension. The reaction mixture was incubated in a theromixer (300 rpm) at 37°C for 16 h. During the incubation period the particles remained well dispersed and no sedimentation or aggregation was observed. At the end of incubation reaction, MNPs (γ-Fe₂O₃/Pol@D@Biot) were separated from the reaction mixture by magnetic decantation, washed with 0.01 M PBS/0.05% Tween-20 buffer solution, then re-dispersed in 1 mL of the same buffer solution and stored in the fridge.

Immobilization of 15 nm SA-Au NPs on biotin functionalized MNPs

First, the storage buffer solution (Tris buffer saline pH = 8.2, containing 1 % BSA, 15 mM NaN₃ and 30 % glycerol) of 15 nm SA-Au NPs was replaced with 0.01 M PBS/0.05% Tween-20 buffer solution (adjusted to pH = 7.1 with 1 M HCl solution) according to the following procedure: 1 mL of SA-Au NPs (13.65 µg/mL of protein, 1.4×10¹² particles/mL) was transferred into a conical tube and 1 mL of 0.01 M PBS/0.05% Tween-20 buffer solution was added. The particles were vortexed and then centrifuged during 1 h at the speed of 9000 rpm. The solution was separated from the particles with a pipet. Then, the particles were re-dispersed in 2 mL of same buffer solution, vortexed and centrifuged during 1 h at the same speed. The procedure was repeated twice and then particles were dispersed in 1.4 mL (corresponding to 1.00×10¹² part/mL) of the same buffer solution and stored in the fridge.

To 0.05 mg of biotin modified MMPs (γ-Fe₂O₃/Pol@D@Biot), 2.00×10¹¹ SA-Au NPs (15 nm) dispersed in 0.2 mL of 0.01 M PBS/0.05% Tween-20 buffer solution (pH = 7.1) were added (corresponding to 4.0×10¹² SA-Au NPs/mg of functionalized MNPs). The reaction mixture was incubated in a thermomixer (300 rpm) at 37°C for 16 h. and no sedimentation or aggregation was observed during this period. Afterwards, MNPs were separated from the reaction mixture by magnetic decantation and the solutions of SA-Au NPs were analyzed by UV-Vis spectroscopy. The resulting MNPs (γ-Fe₂O₃/Pol@D@Biot@SA-Au) were washed with water (5×1 mL), then re-dispersed 0.2 mL of water, and stored in the fridge.

[d] PBS: Phosphate buffered saline
Incubation of biotin functionalized MNPs with 20 nm $H_2N$-PEG-Au NPs

First, 20 nm $H_2N$-Au NPs were dispersed in 0.01 M PBS/0.05% Tween-20 buffer solution (adjusted to pH = 7.1 with 1 M HCl solution) according to the following procedure: 0.2 mL of $H_2N$-Au NPs’ aqueous solution (corresponding to $6.8 \times 10^{12}$ $H_2N$-Au NPs) were transferred into a conical tube and 2 mL of 0.01 M PBS/0.05% Tween-20 buffer solution was added. The dispersion was vortexed and then centrifuged during 40 min. at the speed of 8500 rpm. Then, the solution was separated from the particles with a pipet. The particles were then dispersed in 2 mL of same buffer solution, vortexed and centrifuged during 40 min at the same speed. The procedure was repeated one more time and then particles were dispersed in 6.8 mL of buffer solution and stored in the fridge ($1 \times 10^{12}$ particles/mL).

Subsequently, 0.05 mg of biotin functionalized MNPs ($\gamma$-$Fe_3O_3/Pol@D@Biot$) were incubated with $2.00 \times 10^{11}$ $H_2N$-PEG-Au NPs of 20 nm dispersed in 0.2 mL of 0.01 M PBS/0.05% Tween-20 buffer solution (pH = 7.1). The incubation proceeded in a thermomixer (300 rpm) at 37°C during 16 h. At the end of the incubation time, MNPs were separated from the reaction mixture, and the solutions of $H_2N$-PEG-Au NPs were removed, while MNPs were washed with water ($5 \times 1$ mL), then re-dispersed 0.2 mL of water, and stored in the fridge.

Incubation of maleimide functionalized MNPs with 20 nm $H_2N$-PEG-Au NPs

To 0.05 mg of native or maleimide functionalized MMPs ($\gamma$-$Fe_3O_3/Pol@D-Mal$), were added $2.00 \times 10^{11}$ $H_2N$-PEG-Au NPs (20 nm) dispersed in 0.2 mL of 0.01 M PBS/0.05% Tween-20 buffer solution (adjusted to pH = 7.1 with 1 M HCl solution). The reaction mixtures were incubated in a thermomixer (300 rpm) at 37°C for 16 h. No sedimentation or aggregation was observed during this period. Afterwards, MNPs were separated from the reaction mixture, and the solutions of $H_2N$-PEG-Au NPs were analyzed by UV-Vis spectroscopy, while MNPs were washed with water ($5 \times 1$ mL), then re-dispersed 0.2 mL of water, and stored in the fridge.
5. Characterization of the compounds

*Compound 1*

$^1$H NMR (300 MHz, DMSO) δ 6.58 (s, 2H, CH=CH), 5.35 (s, 2H, CH-O), 3.31 (s, 2H, CH).

$^{13}$C NMR (101 MHz, DMSO) δ 171.53 (s, 2C, C=O), 136.85 (s, 2C, CH=CH), 81.64 (s, 2C, CHO), 49.08.
Compound 2

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 6.51 (s, 2H, CH=CH), 5.26 (s, 2H, CH), 3.70 (dt, J = 10.0, 3.5 Hz, 4H, -CH$_2$N-), HO-CH$_2$), 1.88 (s, 2H, CH$_2$), 2.40 (s, 1H, -OH).

$^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 176.89 (s, 2C, N-(C=O)), 136.62 (s, 2C, CH=CH), 81.08 (s, 2C, OCH), 60.30 (s, 1C, -CH$_2$-OH), 57.60 (s, 2C, CH), 41.84 (s, 1C, -CH$_2$-N).
**Compound 3**

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 6.51 (s, 2H, CH=CH), 6.37 (d, $J = 17.3$ Hz, 1H, CH$_2$), 6.06 (dd, $J = 17.3$, 10.5 Hz, 1H, CH$_2$), 5.83 (d, $J = 11.9$ Hz, 1H, CH$_2$), 5.25 (s, 2H, CH$_2$), 4.36 (t, $J = 5.4$ Hz, 2H, O-CH$_2$), 3.80 (s, 2H, CH$_2$-N), 2.86 (s, 2H, CH)$_2$.

**Compound 5**

$^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 176.07 (s, 2C, -O-(C=O)-O), 165.84 (s, 1C, -(C=O)-O-), 156.66 (s, 2C, CH=CH), 131.41 (s, 1C, CH$_2$), 128.07 (s, 1C, -CH$_2$), 81.02 (s, 2C, -CH$_2$-O), 60.81 (s, 2C, -O-CH$_2$), 47.56 (s, 2C, -CH$_2$), 37.89 (s, 2C, -CH$_2$-N).
**Compound 4**

$^1$H NMR (500 MHz, CDCl$_3$) δ 5.15 (s, 1H, NH), 3.59 (s, 4H, -O-CH$_2$-CH$_2$-O-), 3.55 - 3.17 (m, 9H, -O-CH$_2$-), 2.20 (dd, $J = 10.4, 5.2$ Hz, 2H, CH$_2$-NH$_2$), 2.86 (t, $J = 5.2$ Hz, 2H, -CH$_2$-NH$_2$), 1.57 (s, 2H, -NH$_2$), 1.42 (s, 9H, -CH$_3$).

$^{13}$C NMR (121 MHz, CDCl$_3$) δ 160.08 (s, 1C, O-(C=O)-NH-), 79.18 (s, 1C, C$_{\beta}$), 73.28 (s, 2C, -O-CH$_2$-), 70.26 (s, 2C, -O-CH$_2$-CH$_2$-O-), 41.70 (s, 1C, -CH$_2$-NH$_2$), 39.38 (s, 3C, -CH$_2$-NH$_2$), 28.46 (s, 3C, CH$_3$).
Compound 5

\[ \text{H NMR (300 MHz, CDCl}_3\] \delta 5.07 (s, 1H, NH), 3.64 (s, 6H, -O-CH}_3), 3.56 (s, 4H, -O-CH}_3-CH}_2-O-), 3.51 (q, J = 5.4 Hz, 2H, -O-CH}_2-), 3.28 (dt, J = 10.4, 5.2 Hz, 2H, -CH}_2-NH-), 2.81 (t, J = 7.1 Hz, 4H, -N-CH}_2), 2.66 (q, J = 6.1 Hz, 2H, -CH}_2-N), 2.34 (t, J = 7.1 Hz, 4H, -CH}_2-COO), 1.41 (s, 9H, -CH}_3)

\[ \text{C NMR (75 MHz, CDCl}_3\]
\[ \delta 173.63 (s, 2C, -C=O), 156.09 (s, 1C, -C=O)-O), 156.09 (s, 1C, -C=O)-O), 156.09 (s, 1C, -C=O)-O), 156.09 (s, 1C, -C=O)-O), 89.22 (s, 1C, C=O), 70.43 (d, J = 10.1 Hz, 2C, -O-CH}_2-CH}_2-O-), 69.79 (s, 2C, -CH}_3-), 53.36 (s, 1C, -CH}_2-N), 51.63 (s, 2C, -O-CH}_3), 50.04 (s, 2C, -N-CH}_2), 40.48 (s, 1C, -CH}_2-NH-), 32.68 (s, 2C, -CH}_2-COO), 28.52 (s, 2C, -CH}_3)

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Compound 6

\[^1^H\text{NMR}\ (400\text{ MHz, CDCl}_3)\delta\ 7.46\ (s, 2H, -NH), 5.65\ (s, 1H, -NH), 3.56 - 2.47\ (m, 9H, -CH_2-CH_2-CH_2-O-CH_2-OCH_3), 3.24\ (dd, J = 11.7, 5.8\text{ Hz}, 6H, -CONH, CH_3), 2.81 - 2.60\ (m, 3H, N-CH_2-CH_2-NH_2), 2.62\ (t, J = 5.3\text{ Hz}, 2H, -CH_2-N), 2.33\ (t, J = 6.2\text{ Hz}, 4H, -CH_2CONH-), 2.03\ (s, 4H, -NH_2), 1.40\ (s, 9H, -CH_3)\.

\[^1^3^C\text{NMR}\ (151\text{ MHz, CDCl}_3)\delta\ 171.93\ (s, 2C, -C\equivO\text{NH}), 155.26\ (s, 1C, -O(C\equivO)\text{NH}), 153.22\ (s, 1C, C\equivO), 69.32\ (s, 2C, -O-CH_2-CH_2-O-), 68.34\ (s, 2C, -O-CH_2-), 52.77\ (s, 1C, CH_2-N), 50.01\ (s, 2C, N-CH_2-), 41.14\ (s, 1C, -CONH, CH_3), 40.86\ (s, 2C, -CH_2-NH_2), 39.18\ (s, 2C, -CONH,CH_2-), 33.36\ (s, 2C, -CH_2CONH), 27.52\ (s, 3C, -CH_3)\.
**Compound 7**

$^1$H NMR (500 MHz, CDCl₃) δ 6.51 (s, 6H, CH=CH₂), 5.24 (s, 6H, CH), 4.20 (s, 6H, -C(=O)O-CH₂), 3.73 (t, J = 5.3 Hz, 8H, -CH₂-N), 3.51 (t, J = 4.6 Hz, -CH₂-O-CH₂-), 2.87 (s, 6H, -CH₃), 2.50 (s, J = 5.8 Hz, 6H, -CH₂-N), 1.43 (s, 9H, -CH₃).
**Dendron D**

$^1$H NMR (300 MHz, D$_2$O) $\delta$ 6.65 (s, 8H, CH$_2$-CH), 5.32 (s, 8H, CH$_2$), 4.36 (t, $J$ = 5.1 Hz, 8H, -CH$_2$-O), 3.94 (d, $J$ = 4.4 Hz, 2H, OH, -CH$_2$), 3.83 (dd, $J$ = 9.4, 4.7 Hz, 10H, -CH$_2$-O, -CH$_2$-N), 3.78 (s, 4H, -CH$_2$-CH$_2$-O), 3.72 - 3.65 (m, 4H, -CH$_2$-O-NH-CH$_2$), 3.55 (dd, $J$ = 13.8, 6.9 Hz, 14H, -N-CH$_2$-N, -CH$_2$-N), 3.43 (s, $J$ = 5.7 Hz, 4H, -CH$_2$-N), 3.28 - 3.22 (t, 2H, -CH$_2$-NH$_2$), 3.16 (s, 8H, CH), 2.91 (dd, $J$ = 6.4 Hz, 12H, -CH$_2$-(C=O)NH$_2$, -CH$_2$-(C=O)O$^-$)

$^{13}$C NMR (75 MHz, D$_2$O) $\delta$ 179.31 (s, 8C, -N(C=O)$_2$), 172.81 (s, 2C, (C=O)O$^-$), 172.64 (s, 4C, (C=O)O$^-$), 136.56 (s, 8C, CH$_2$-CH), 81.16 (s, 8C, CH$_2$), 69.79 (d, $J$ = 19.5 Hz, 2C, -O-CH$_2$-CH$_2$-O$^-$), 68.54 (s, 1C, -CH$_2$-O$^-$), 64.09 (s, 1C, -O-CH$_2$-CH$_2$-O$^-$), 51.88 (s, 4C, (C=O)O$^-$-CH$_2$), 53.69 (s, 2C, -CH$_2$-N), 52.91 (s, 1C, -CH$_2$-N), 49.85 (s, 8C, N-CH$_2$), 49.14 (s, 2C, N-CH$_2$), 47.54 (s, 8C, CH$_2$), 39.17 (s, 1C, -CH$_2$-NH$_2$), 37.11 (s, 4C, -CH$_2$-N), 34.55 (s, 2C, -CH$_2$-(C=O)NH$_2$), 28.77 (s, 4C, -CH$_2$-(C=O)O$^-$), 28.23 (s, 2C, -CH$_2$-(C=O)NH$_2$)
6. Figures

Fig. S1. ATR FTIR spectra of $\gamma$-Fe$_2$O$_3$/Pol@D and $\gamma$-Fe$_2$O$_3$/Pol@D-Mal in the A) 1830 - 1620 cm$^{-1}$ and B) 950 - 800 cm$^{-1}$ spectral ranges, after subtraction of the native $\gamma$-Fe$_2$O$_3$/Pol-COOH data.

Fig. S2. ATR FT-IR spectrum of dendron D.
Fig. S3. TEM image of native $\gamma$-Fe$_2$O$_3$/Polymer MNPs.

Fig. S4. pH effect on the surface charge of native $\gamma$-Fe$_2$O$_3$/Pol-COOH MNPs (solid line in black) and maleimide functionalized $\gamma$-Fe$_2$O$_3$/Pol@D-Mal MNPs.
**Fig. S5.** Field dependence of magnetization at different temperatures for $\gamma$-Fe$_2$O$_3$/Pol-COOH (A, C) and $\gamma$-Fe$_2$O$_3$/Pol@D-Mal (B, D), where $M$ is the magnetization of the sample and $H$ is the applied magnetic field. Temperature dependence of the magnetization saturation (E) and of the coercive field (F) for $\gamma$-Fe$_2$O$_3$/Pol-COOH (blue curves) and $\gamma$-Fe$_2$O$_3$/Pol@D-Mal (red curves).
**Fig. S6.** Calibration plot (A) and calibration curve (B) of 15 nm SA-Au NPs. Absorption spectrum of the 15 nm SA-Au NPs’ solution, recovered after the immobilization reactions (C) and (D) dependence of Absorbance at 526 nm as function of 15 nm SA-Au NPs number left in solution after the immobilization reaction.

**Fig. S7.** TEM image of $\gamma$-Fe$_2$O$_3$/Pol@D-Mal MNPs recovered after their incubation with 20 nm H$_2$N-PEG-Au NPs.