Supplementary Material (ESI) for Chemical Communications

One-step bioengineering of magnetic nanoparticles via a surface diazo transfer/azide-alkyne click reaction sequence

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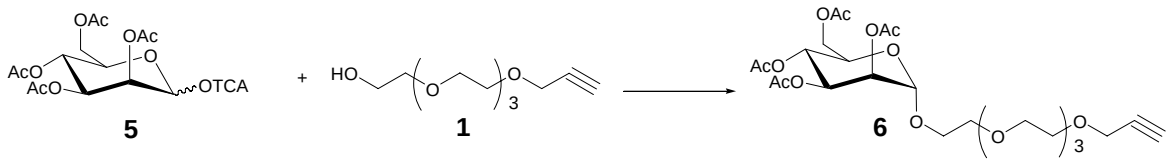
Experimental Section

Materials. γ-Fe_2O_3 nanoparticles were purchased from Alfa Aesar (Karlsruhe, Germany), while solvents and reagents for chemical modification of iron oxide nanoparticles were from Fluka (St. Gallen, Switzerland) and Riedel-de Haën (Seelze, Germany). Human serum albumin, anti-albumin antibody developed in rabbit (delipidized whole antiserum) and phosphate buffer solutions were purchased from Sigma-Aldrich (St. Louis, MO). Water was deionized and ultrafiltered by a MilliQ apparatus from Millipore Corporation (Billerica, MA). Phosphate buffer solutions were passed through hydrophilic 0.45 μm Millex-LCR Millipore filters before usage. All other reagents and solvents were used as supplied.

Methods. All chemical reactions were performed in oven-dried glassware under an inert atmosphere (nitrogen or argon) unless noted otherwise. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates (0.25 mm). Compounds were visualized by UV irradiation or dipping the plate in a cerium sulfate-ammonium molybdate (CAM) solution or sulfuric acid methanol solution. Flash column chromatography was carried out using forced flow of the indicated solvent on Kieselgel 60 (230-400 mesh). ^1H and ^{13}C NMR spectra of precursors **1-3** and their intermediates were recorded on a Bruker Avance 400 instrument (Karlsruhe, Germany) in $CDCl_3$ with chemical shifts referenced to internal standards $CDCl_3$ (7.26 ppm ^1H, 77.0 ppm ^{13}C). Signals were assigned by means of APT, ^1H-^1H COSY and ^1H-^{13}C HSQC spectra. Fourier-transformed infrared spectroscopy (FTIR) characterization has been performed both by attenuated total reflectance (ATR) and reflection-absorption spectroscopy (RAS) techniques. ATR analyses have been carried out with a Thermo Nicolet 380 spectrometer (Waltham, MA) with Smart Orbit installed, a horizontal single-reflection ATR accessory using a diamond cell, while RAS spectra were obtained on a Centaurus microscope equipped

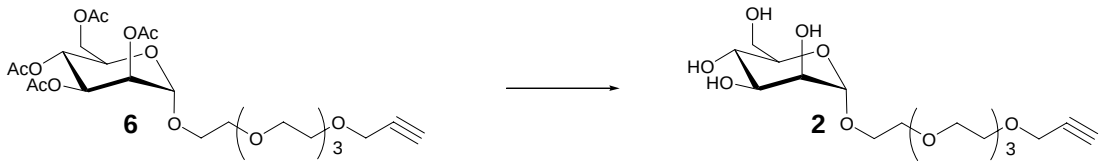
with a MCT detector which has to be cooled with nitrogen. In both cases, samples do not need any treatment before the analysis. In ATR modality, samples have been examined using the Smart accessory in the range between 4000 cm^{-1} and 400 cm^{-1} with 32 scans per analysis at a resolution of 4 cm^{-1}. In RAS modality, samples were first mixed with KBr and the powder mixture was placed on a reflective surface and examined using the Centaurus microscope in the range between 4000 cm^{-1} and 650 cm^{-1} with 64 scan per analysis at a resolution of 8 cm^{-1}. Elemental analyses were performed on a Perkin Elmer Series II CHNS/O 2400 analyser (Waltham, MA). ICP-AES measurements have been performed by a Jobin-Yvon instrument, model Ultima (Edison, NJ). HRMAS NMR experiments were carried out on a Bruker BioSpin FT-NMR Avance 500 equipped with a 11.7 T superconducting ultrashield magnet available at C.I.G.A. (Centro Interdipartimentale Grandi Apparecchiature) of the University of Milan. The HRMAS probe with internal lock is capable of performing either direct or indirect (inverse) detection experiments. In order to have the spinning side bands out of the ^1H spectrum, the best resolution and the minimum presence of rotational artifacts, MAS experiments were performed at a spinning rate of 5-8 KHz (15 kHz maximum MAS rotation available) using a 50 μL zirconia rotor. All the samples were diluted at different concentrations with deuterated solvents, such as D_2O and DMSO-d6, to find out the concentration limit to the NMR signal broadening. The sample temperature is dependent on the rotation speed and it was so varying from 303 K to 308 K. T_2 relaxation times were acquired at a temperature of 310 K using a Bruker Minispec mq20 system working with ^1H at 20 MHz magnetic field with the following parameters: CMPG sequence, 1000 echoes with a 20 ms echo time and 2 s repetition time. Samples were introduced using 10 mm NMR tubes pre-warmed in a thermostatic bath. For transmission electron microscopy characterization, the MNP dispersion was diluted to 0.01-0.1 mg/ml with 0.1 M phosphate buffer, pH 7.0, or water, and a drop was placed on a formvar/carbon coated copper grid and dried at 60°C. TEM images were obtained with the help of Dr. Nadia Santo from C.I.M.A. (Centro Interdipartimentale di Microscopia Avanzata) of the University of Milan at an EFTEM LEO 912AB microscope (Zeiss) operating at 120 kV (Leo, Oberkochen, Germany). Emission scans of Trp_{214} HSA were obtained on a Perkin Elmer LS-50B fluorescence spectrometer by excitation at 290 nm. All measurements were carried out at 20 °C and the scanning speed was 30 nm/min. The scan range was between 300 and 500 nm and spectra were corrected by subtraction of the contribution from the blank solution (0.1 M phosphate buffer solution, pH 7.0). The spectra were not corrected for instrument characteristics.

Synthesis of 3,6,9,12-tetraoxapentadec-14-ynyl 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside (6)



2,3,4,6-tetra-*O*-acetyl-α/β-D-mannopyranosyl trichloroacetimidate (**5**)[1] (470 mg, 0.96 mmol) and propargyl tetraethylene glycol **1**[2] (445 mg, 1.92 mmol) were dried under vacuum overnight. The compounds were dissolved in dry dichloromethane (5 ml) and the solution was cooled to –30 °C. A solution of trimethylsilyltriflate (0.29 ml, 1 M in dry dichloromethane) was added dropwise and the solution was let to warm to room temperature. The mixture was stirred 2 h under a nitrogen atmosphere, after which the reaction was quenched with triethylamine. The solvent was removed in vacuo and the product was purified by silica gel chromatography (hexanes/EtOAc 1/1), yielding compound **6** (352 mg, 65%) as a colorless oil. R_f 0.33 (hexanes/EtOAc 1/1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.42-5.26 (m, 3 H, H-2, H-3, H-4), 4.90 (d, *J* = 1.4 Hz, 1 H, H-1), 4.32 (dd, *J* = 12.0 Hz, *J* = 4.73 Hz, 1 H, H-6a), 4.32 (d, *J* = 2.38, 2 H, O*CH*$_2$C≡CH), 4.15-4.04 (m, 2 H, H-5, H-6b), 3.89-3.78 (m, 1 H, sugar-O-C*H*H-CH$_2$-), 3.77-3.62 (m, 15 H, sugar-O-C*H*H-C*H*$_2$-, (OC*H*$_2$C*H*$_2$)$_3$O), 2.45 (t, *J* = 2.34 Hz, 1 H, C≡C*H*), 2.18 (s, 3 H, COC*H*$_3$), 2.13 (s, 3 H, COC*H*$_3$), 2.07 (s, 3 H, COC*H*$_3$), 2.02 (s, 3 H, COC*H*$_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 97.74, 74.50, 70.75, 70.64, 70.43, 70.01, 69.61, 69.16, 69.11, 68.43, 67.42, 66.19, 62.44, 58.42, 20.72. ESI-HRMS (*m/z*) Calcd for $C_{25}H_{38}O_{14}Na$: 585.2154; Found: 585.2147.

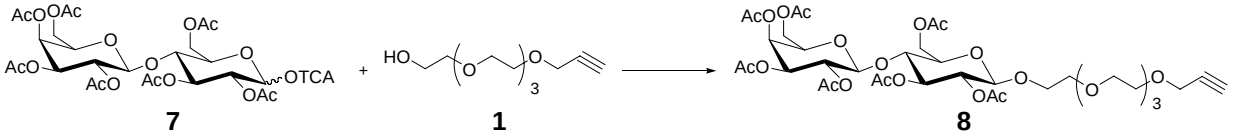
Synthesis of 3,6,9,12-tetraoxapentadec-14-ynyl α-D-mannopyranoside (2)



Compound **6** (350 mg, 0.62 mmol) was dissolved in dry MeOH (8 ml) and a freshly prepared 1 M solution of MeONa in methanol (0.25 ml, 0.25 mmol) was slowly added. Monitoring of the reaction by TLC (hexanes/EtOAc 1/1) indicated complete disappearance of starting material within 3 h. The reaction was quenched by neutralization with Amberlite IR-120 resin (H^+ form), filtration and

evaporation of the solvent under reduced pressure. The crude product was directly used for conjugation to MNP without further purifications.

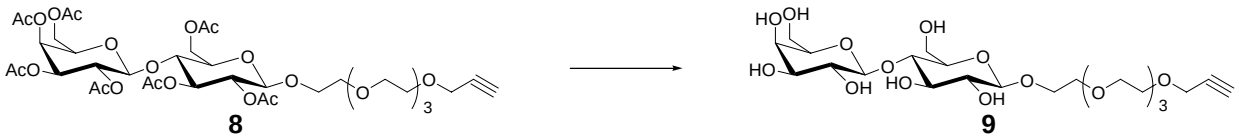
Synthesis of 3,6,9,12-tetraoxapentadec-14-ynyl (2',3',4',6'-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (8)



7 **1** **8**

(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-α/β-D-glucopyranosyl trichloroacetimidate (**7**)[3] (144 mg, 0.18 mmol) and propargyl tetraethylene glycol **1** (86 mg, 0.37 mmol) were subjected to the same procedure used for the synthesis of **6**, affording **8** (140 mg, 89%) as a colorless oil. R_f 0.25 (hexanes/EtOAc 3/7); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.37 (bd, *J* = 3.0 Hz, 1 H, H-4'), 5.22 (t, *J* = 9.25 Hz, 1 H, H-3), 5.13 (dd, *J* = 7.9, $J_{2',3'}$ = 10.43 Hz, 1 H, H-2'), 4.98 (dd, *J* = 10.41 Hz, *J* = 3.44 Hz, 1 H, H-3'), 4.91 (dd, *J* = 9.54 Hz, *J* = 8.01 Hz, 1 H, H-2), 4.59 (d, *J* = 7.96 Hz, 1 H, H-1), 4.53-4.50 (m, 2 H, H-6a, H-1'), 4.23 (d, *J* = 2.37 Hz, 2 H, OCH_2C≡CH), 4.18-4.08 (m, 3 H, H-6b, H-6'a, H-6'b), 3.96-3.87 (m, 1 H, H-5'), 3.81 (t, *J* = 9.34 Hz, 1 H, H-4), 3.76-3.61 (m, 17 H, sugar-O-CH_2-CH_2-, (OCH_2CH_2)$_3$O, H-5), 2.46 (t, *J* = 2.36 Hz, 1 H, C≡C*H*), 2.17 (s, 3 H, COCH_3), 2.15 (s, 3 H, COCH_3), 2.10 (s, 3 H, COCH_3), 2.07-2.06 (m, 9 H, COCH_3), 1.99 (s, 3 H,COCH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 100.62, 100.99, 72.78, 72.49, 71.66, 70.89, 70.59, 69.09, 66.53, 62.04, 60.49, 58.31, 20.84, 20.64, 20.84. ESI-HRMS (*m/z*) Calcd for $C_{37}H_{54}O_{22}Na$: 873.2999; Found: 873.2982.

Synthesis of 3,6,9,12-tetraoxapentadec-14-ynyl (β-D-galactopyranosyl)-(1→4)-β-D-glucopyranoside (9)

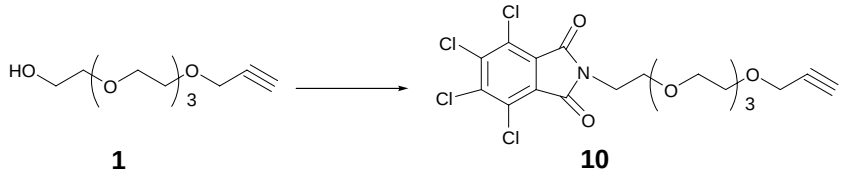


8 **9**

Compound **8** (140 mg, 0.16 mmol) was dissolved in dry MeOH (5 ml) and a freshly prepared 1 M solution of MeONa in methanol (64 μl, 0.06 mmol) was slowly added. Monitoring of the reaction by TLC (hexanes/EtOAc 3/7) indicated complete disappearance of starting material after 4 h. The reaction

was quenched by neutralization with Amberlite IR-120 resin (H^+ form), filtration and evaporation of the solvent under reduced pressure. The crude product **9** was directly used for conjugation to MNP without further purifications.

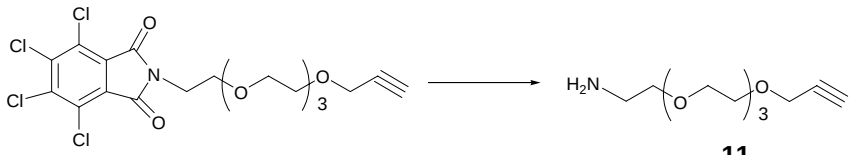
Synthesis of *N*-(3,6,9,12-tetraoxapentadec-14-ynyl)-4,5,6,7-tetrachloroisoindoline-1,3-dione (10)



1 **10**

Compound **1** (300 mg, 1.29 mmol) was dissolved in dry tetrahydrofuran (12 ml), then PPh_3 (370 mg, 1.42 mmol) and tetrachlorophthalimide (370 mg, 1.29 mmol) were added. A solution of di-*t*-butylazadicarboxylate (330 mg, 1.42 mmol) in 3 ml of dry THF was slowly added and the mixture was stirred at room temperature for 3 h. The course of the reaction was monitored by TLC (hexanes/EtOAc 1/1). The reaction mixture was quenched with water, diluted with dichloromethane and dried over Na_2SO_4. The crude product was purified by flash silica gel chromatography (hexanes/EtOAc 1/1), yielding **10** (480 mg, 75%) as a yellowish solid (found, C, 45.88; H, 3.77; N, 2.76. $C_{19}H_{19}Cl_4NO_6$ requires C, 45.72; H, 3.84; N, 2.81%). R_f 0.35 (hexanes/EtOAc 1/1); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.21 (d, *J* = 2.30 Hz, 2 H, OC*H*$_2$C≡CH), 3.93 (t, *J* = 5.68 Hz, 2 H, NC*H*$_2$CH$_2$-), 3.84 (t, *J* = 6.32 Hz, 2H, NCH$_2$C*H*$_2$-), 3.72-3.61 (m, 12 H, (OC*H*$_2$C*H*$_2$)$_3$O), 2.44 (t, *J* = 2.31 Hz, 1 H, C≡C*H*).

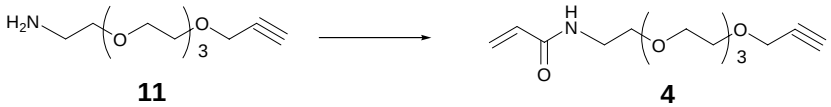
Synthesis of 3,6,9,12-tetraoxapentadec-14-yn-1-amine (11)



10 **11**

Product **10** (100 mg, 0.20 mmol) was dissolved in a solution of acetonitrile/tetrahydrofuran 3/1 (4 ml), then ethylenediamine (28 μl, 0.42 mmol) was added. The mixture was warmed to 60 °C and kept overnight at this temperature. Then, it was cooled to room temperature and filtered over a silica pad (dichloromethane/MeOH 8/2). Product **11** was used in the next step without further purification.

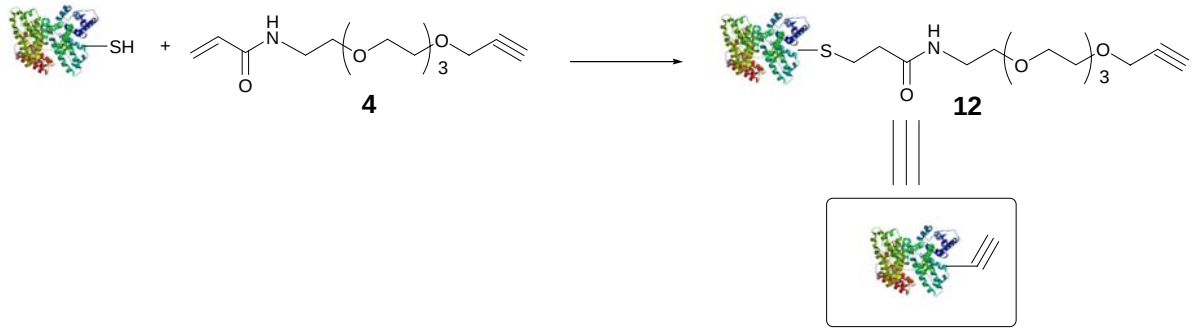
Synthesis of *N*-(3,6,9,12-tetraoxapentadec-14-ynyl)acrylamide (4)



11 **4**

Amine **11** (30 mg, 0.13 mmol) was dissolved in dry dichloromethane and the solution was cooled to 0 °C. Diisopropylethylamine (44 µl, 0.26 mmol) was added under nitrogen atmosphere, followed by acryloyl chloride (20 µl, 0.26 mmol). The reaction mixture was allowed to reach room temperature and stirred for additional 75 min. The mixture was then concentrated and the crude compound was purified by silica gel chromatography (AcOEt), obtaining **4** (20 mg, 54%) as a colorless oil (found, C, 59.07; H, 8.04; N, 4.87. $C_{14}H_{23}NO_5$ requires C, 58.93; H, 8.12; N, 4.91%); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.44 (bs, 1 H, NH), 6.38-6.08 (m, 2 H, -CH=CH*H*, -C*H*=CH_2), 5.61 (d, *J* = 9.94 Hz, 1 H, -CH=CH*H*), 4.17 (d, *J* = 1.62 Hz, 2 H, OCH_2C≡CH), 3.74-3.49 (m, 16 H, -NCH_2CH_2-, (OCH_2CH_2)$_3$O), 2.46 (bs, 1 H, C≡C*H*). The purified compound **4** was dissolved in a solution of H_2O/CH_3CN 1/1 (5 ml) and stored in vials at 4 °C.

Preparation of propargyl-conjugated protein (12)



4 **12**

HSA (133 mg, 2 µmol) was dissolved in 40 ml of deionized water, to obtain a 50 µM solution. Compound **4** (20 mg in 5 ml of H_2O/CH_3CN) was added to the protein and the mixture was gently stirred at room temperature for 12 h. The mixture was then dialyzed at 4 °C against deionized water for 5 days (water solution was changed every 12 h). The propargyl-conjugated protein **12** was then lyophilized and stored at –20 °C.

General procedure for conjugated MNP3-5

In a dried flask under nitrogen, **MNP1** (15 mg) were suspended in a mixture of dichloromethane (100 μl) and methanol (100 μl) and sonicated for 30 min. Water (100 μl) and triethylamine (20 μl) were added followed by $CuSO_4$ (1 mg dissolved in 10 μl of water). A freshly prepared solution of triflic azide in dichloromethane was added.[4] The mixture was sonicated for 2 h under nitrogen atmosphere. Sodium ascorbate (2 mg) was then added to the mixture followed by a water solution of propargyl compound **1-3** (0.13 mmol). The mixture was sonicated for additional 2 h. Conjugated **MNP3-5** were then recovered after centrifugation and washed three times with water. **MNP3-5** were dried and stored under vacuum.

Synthesis of HSA-conjugated MNP (MNP6)

In a dried flask under nitrogen, **MNP1** (15 mg) were suspended in a mixture of dichloromethane (100 μl) and methanol (100 μl) and sonicated for 30 min. PB (100 μl) and triethylamine (20 μl) were added followed by $CuSO_4$ (1 mg dissolved in 10 μl of PB). A freshly prepared solution of triflic azide in dichloromethane was added and the mixture was sonicated for 4 h under nitrogen atmosphere. Dichloromethane was then carefully removed by syringe, then sodium ascorbate (2 mg) was added to the mixture followed by addition of a PB solution of protein **12** (50 mg in 15 ml). The mixture was incubated at 37 °C for additional 24 h. Conjugated **MNP6** were recovered by a permanent magnet and washed and centrifuged 4 times with PB to remove unreacted protein. **MNP6** were then purified by filtering them through 0.45 μm pore septa and stored in vials as PB solution.

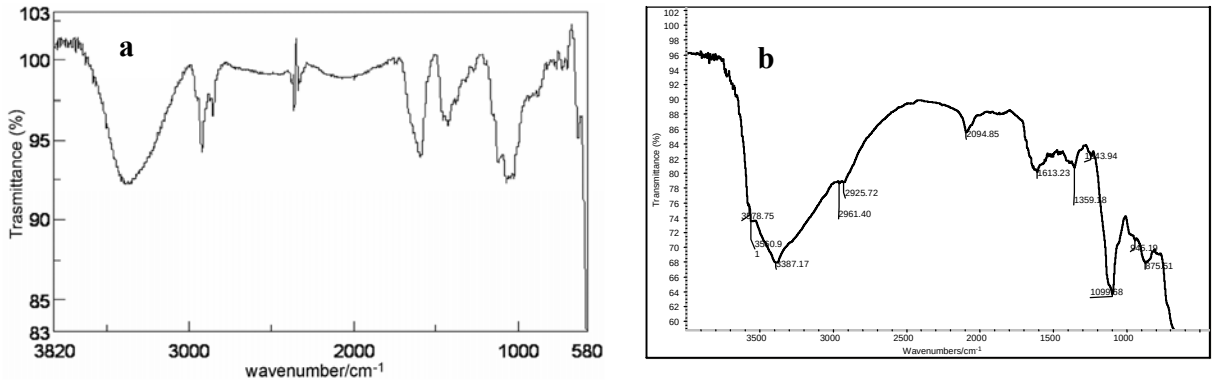


Fig. S1. a) RAS FTIR of amine magnetic nanoparticles **MNP1**; b) RAS FTIR of azido magnetic nanoparticles **MNP2**.

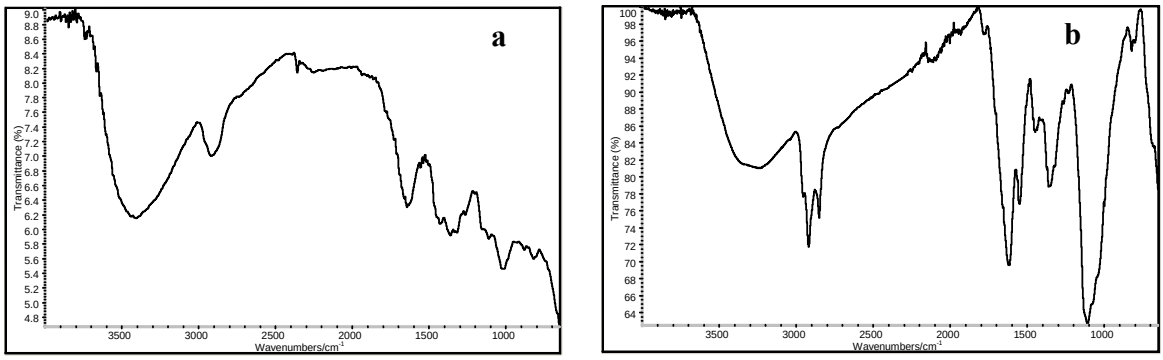


Fig. S2. RAS-FTIR of glyco-magnetic nanoparticles. a) **MNP5**; b) **MNP3.**

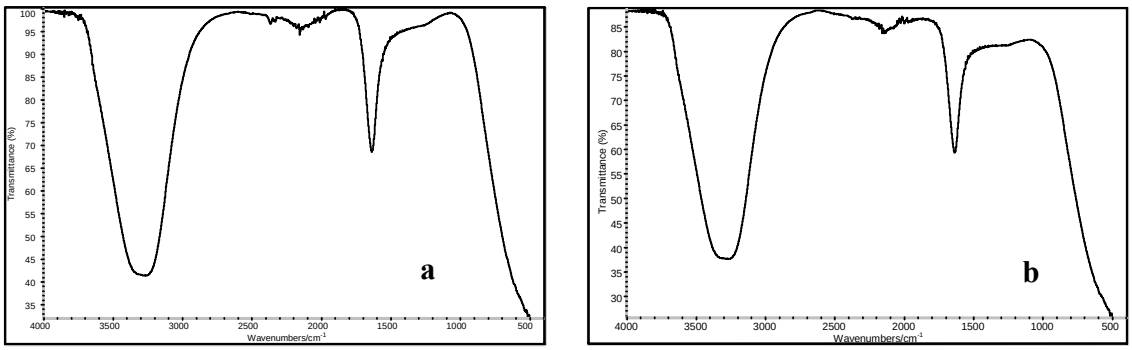


Fig. S3. a) ATR-FTIR of HSA protein in solution; b) ATR FTIR of protein-conjugated magnetic nanoparticles **MNP6**.

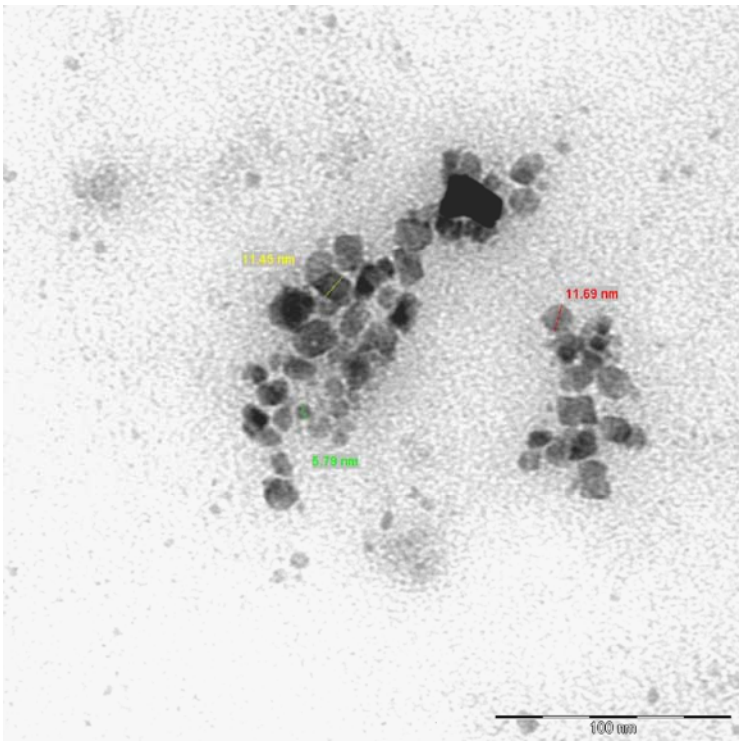


Fig. S4. Representative TEM image of lactose-derivatized magnetic nanoparticles **MNP3**.

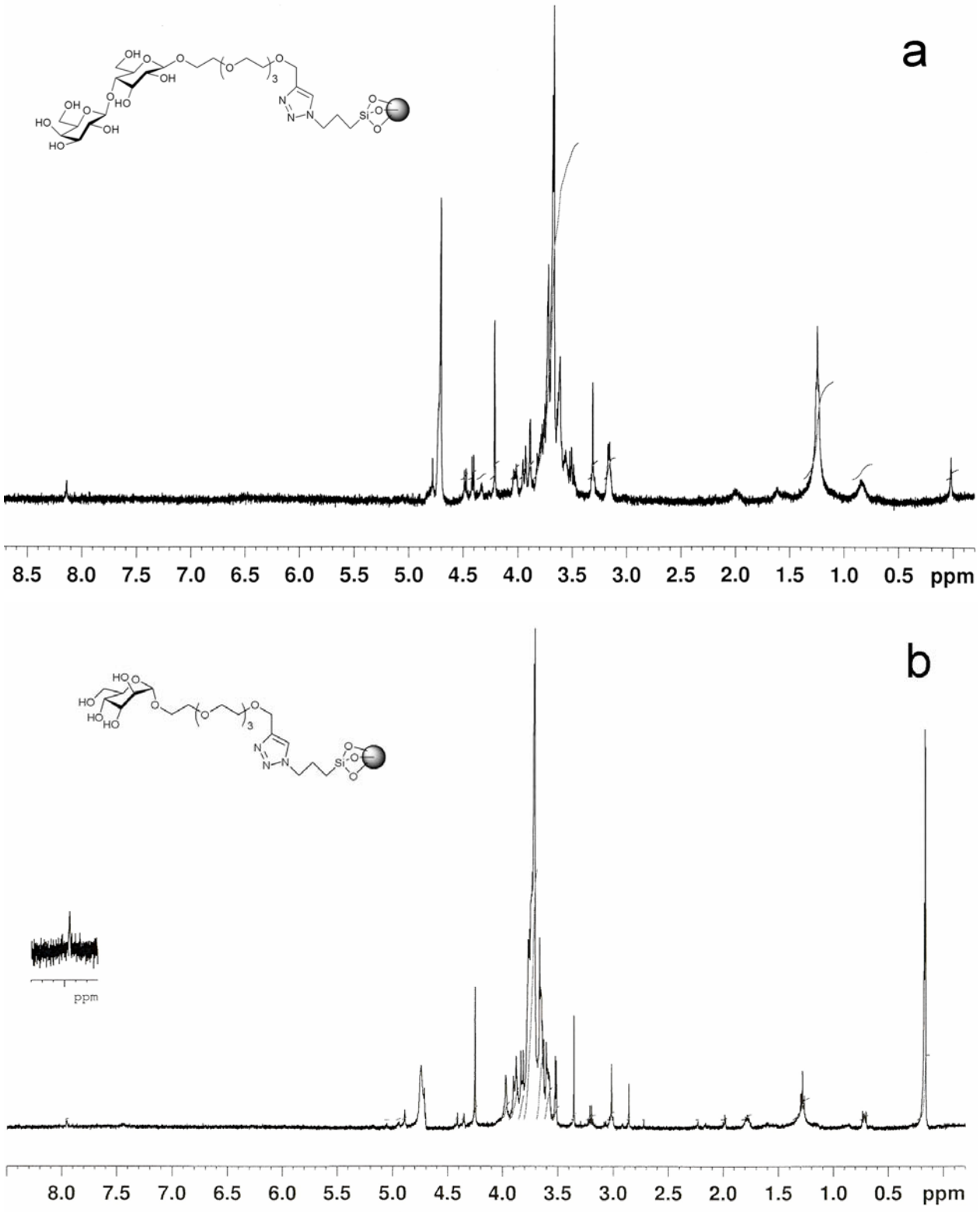


Fig. S5. ^{1}H NMR spectra of **MNP3** (a) and **MNP5** (b) in D_2O obtained by HRMAS. Triazole protons are clearly distinguishable at 8.14 ppm and 7.95 ppm, respectively.

Determination of HSA loading on magnetic nanoparticles

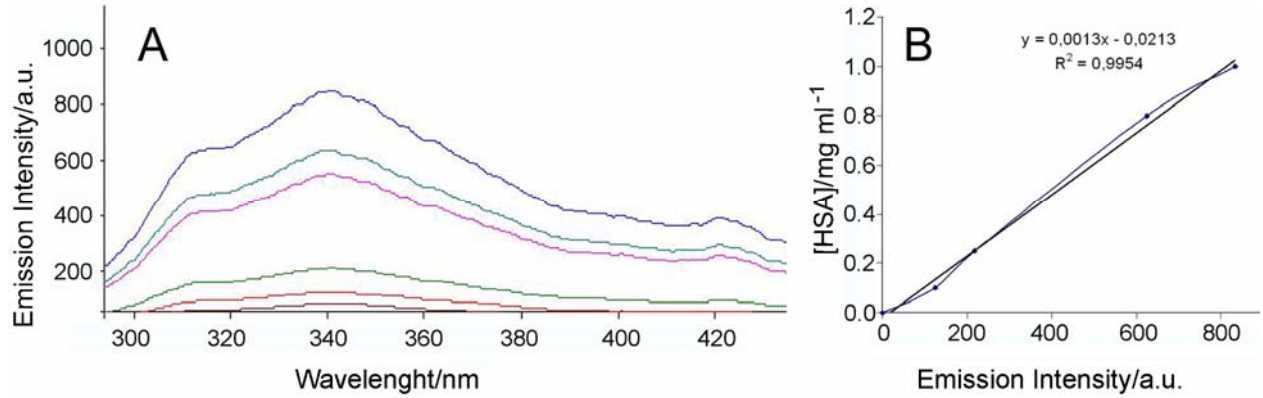


Fig. S6. A) UV-visible emission of Trp_{214} at different concentrations of HSA in 0.1 M phosphate buffer, pH 7.0: brown line = 0.1 M phosphate buffer, pH 7.0; red line = 0.1 mg/ml HSA; light green line = 0.25 mg/ml HSA; violet line = sample; dark green line = 0.8 mg/ml HSA; blue line = 1.0 mg/ml HSA. B) Standard calibration curve obtained at a λ_{em} of 340 nm by plotting increasing values of HSA concentration vs. their respective emission intensities (measurements were performed in triplicate with a mean E.I. standard deviation of ± 5 units). The measured intensity of residual protein in the supernatant mother solution was 541 units, corresponding to a HSA concentration of 0.68 mg/ml.

Each particle contains maghemite ($d = 4.90 \times 10^6$ g m^{-3}) with an average radius of 5 nm = 5×10^{-9} m, then the average volume and mass of γ-Fe_2O_3 nanoparticles are 5.24×10^{-25} m^3 and 2.57×10^{-18} g, respectively. Hence, 1 mg of γ-Fe_2O_3 contains 3.89×10^{14} particles. By determination of residual fluorescence due to HSA protein (above Fig. S6), we established that 0.32 mg of protein were immobilized onto the particle surface. Since molecular weight of HSA is ca. 6.6×10^3 g mol^{-1}, there are 4.85 10^{-9} mol/particle, corresponding to about 7.5 molecules/particle. The mean surface area spanned by a single immobilized HSA is about 35 nm^2 (previously determined in our laboratories by other methods, unpublished results), then the particle surface coating is 7.5×35 $nm^2 = 262.5$ nm^2. The surface of a single particle is $4\pi r^2 \approx 314$ nm^2, then HSA protein covers 83.6% of the overall available surface area.

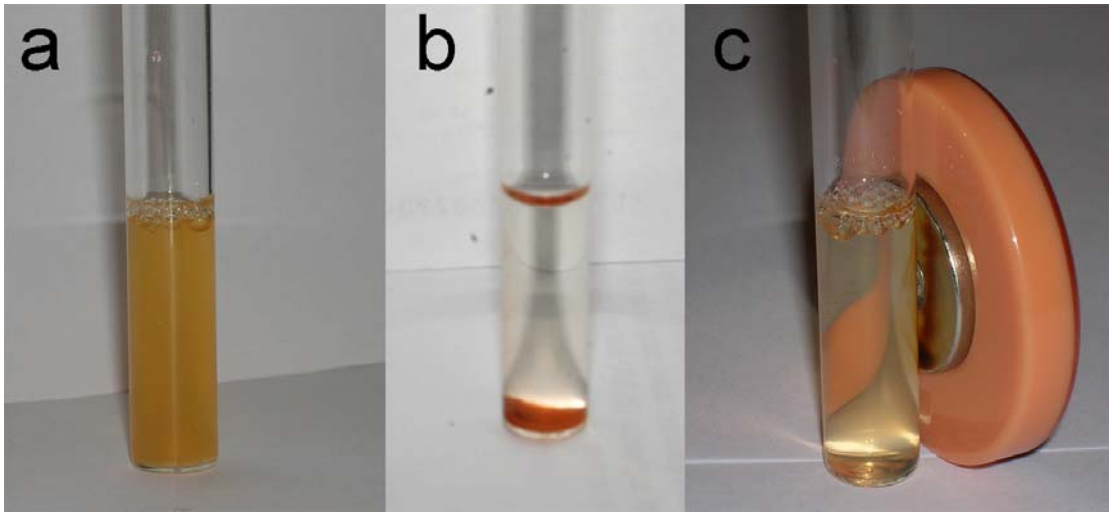


Fig. S7. Immunoprecipitation assay. A 0.02 mg ml^{-1} **MNP6** dispersion in 0.1 M of phosphate buffer (pH 7.0) was treated with 0.1 mg ml^{-1} of anti-HSA. a) Observation after 1 minute. b) Observation after 24 hrs; c) Magnetic capture of immunoprecipitate.

References

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