Electronic Supplementary Information

Covalent immobilisation of magnetic nanoparticles on surfaces via strain-promoted azide – alkyne click chemistry

Raluca M. Fratila,*[a,b] Marcos Navascuez,[a] Javier Idiago-López,[a] Maite Eceiza,[c] José Ignacio Miranda,[c] Jesús M. Aizpurua,[c] and Jesús M. de la Fuente*[a,b,d]

[a] *Institute of Materials Science of Aragón (ICMA – CSIC/University of Zaragoza), C/ Pedro Cerbuna 12, 50009, Zaragoza, Spain. E-mail: rfratila@unizar.es*
[b] *Centro de Investigación Biomédica en red en Bioingenieria Biomateriales y Nanomedicina (CIBER-BBN), Zaragoza, Spain*
[c] *José Mari Korta R&D Center, Basque Country University, UPV/EHU, P.O. 20018, Donostia-San Sebastián, Spain*
[d] *Institute of Nano Biomedicine and Engineering, Department of Instrument Science and Engineering, Shanghai Engineering Centre for Intelligent Diagnosis and Treatment Instrument. Shanghai Jiao Tong University, Dongchuan Road 800, 200240, Shanghai, PR of China. E-mail: jmfuente@unizar.es*

Experimental details

1. Materials

All commercially available reagents were used as supplied, unless otherwise stated. Iron(III) acetylacetonate, 1,2-hexadecanediol, oleic acid, oleylamine, benzyl ether, poly (maleic anhydride-alt-1 octadecene) (PMAO, MW: 30000–50000 g mol^{-1}), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), 4-aminophenyl β-D-glucopyranoside, sodium azide, tribromomethane, potassium *tert*-butoxide, 2-nitrobenzenesulfonyl chloride, diethylene glycol, tetraethylene glycol, silver tetrafluoroborate, ammonia (7M in MeOH), hexane and dry toluene were purchased from Sigma Aldrich, Acros and Merck.
α-Methoxy-ω-amino poly(ethylene glycol) (PEG MW: 750 Dalton) was purchased from Rapp Polymere GmbH. TAMRA-cadaverine was from AnaSpec. Chloroform stabilized with ethanol (Reag. Ph. Eur.), absolute ethanol (99.8 % vol.), sulfuric acid (96 % vol.) and oxygen peroxide (33% w/v) were acquired from Panreac. 11-bromoundecyltrichlorosilane was purchased from Cymit Química SL. Dibenzylcyclooctyne-PEG4-5/6-Sulforhodamine B (DBCO) was purchased from Jena Bioscience GmbH.
Acetone (99 % vol.) and *N*,*N*-dimethylformamide (DMF, 99.9% HPLC grade) were purchased from Scharlau Chemies S.A.
Buffers were prepared according to standard laboratory procedure.
Amicon centrifugal filter units (100000 MWCO cut off) were purchased from Millipore and 0.2 mm pore size 25 mm diameter cellulose acetate membrane filters were from Chmlab.
Single side polished p-type Si(100) wafers (Siegert Wafer GmbH, 100 ± 0.3 mm diameter, 525 ± 20 μm thickness) were purchased form the clean room facilities at University of Zaragoza.

2. Characterisation of Fe_3O_4 nanoparticles and surfaces

Transmission electron microscopy (TEM) images were obtained on a Tecnai T20 microscope (FEI) operating at 200 kV (Laboratorio de Microscopias Avanzadas LMA, University of Zaragoza). A single drop (10 μL) of a solution (0.1 mg $Fe \cdot mL^{-1}$) of the different nanoparticles was placed onto a copper grid coated with a carbon film (Electron Microscopy Sciences). The grid was left to dry in air for several hours at room temperature. Particle size distribution was evaluated from several micrographs using an automatic image analyzer (ImageJ). 200 particles were selected for analysis, which resulted in a stable size distribution statistic.

Scanning electron microscopy (SEM) images were obtained on a CSEM-FEG INSPECT F50 (FEI, LMA, University of Zaragoza).

A Malvern Instruments Zetasizer ZS-NANO was used for the determination of the hydrodynamic diameter (by dynamic light scattering, DLS) and for the measurement of the zeta potential of the nanoparticles. All samples were irradiated with a 633 nm He-Ne laser. Samples were prepared in Milli-Q water (0.05 mg mL^{-1}), final volume of 1.5 mL. Each sample was measured five times combining 100 runs per measurement at 25 ^{o}C. Results were treated using the Malvern software Zetasizer Nano 7.03.

The iron concentrations of the nanoparticle suspensions were determined using a spectrophotometric method. Samples were prepared by diluting 5 or 10 μL of nanoparticle suspension in hexane or water to a final volume of 50 μL. Samples were incubated with 100 μL

of aqua regia solution (HCl/HNO_3 3/1) at 60 ºC for 15 minutes in order to eliminate the organic coating. Subsequently, 350 µL of Milli-Q water were added and 50 µL of the final total volume of each solution were transferred to a 96 well plate. 100 µL of 0.2 M Na_3PO_4 (pH = 9.7) and 60 µL of a solution consisting of 50 µL of 4 N KOH and 10 µL of 0.25 M Tiron (1,2-dihidroxybenzen-3,5-disulfonic acid,) were added. The samples were maintained at room temperature for 15-20 minutes. Finally, sample absorbance was measured at 480 nm on a Multiskan Go spectrophotometer (Thermo Scientific). A similar protocol was followed using six standard solutions of known iron(III) concentrations (0, 100, 200, 400, 600 and 800 µg/mL) in order to obtain a calibration curve. Each sample was analysed by triplicate. Standard solutions were fitted to a calibration curve and the sample absorption value was interpolated in order to obtain the iron concentration.

1% (W/v) agarose gels for electrophoresis were prepared by dissolving agarose in a 0.5X tris-borate-EDTA (TBE) buffer. Nanoparticle samples were loaded in the wells by mixing 3 µL of glycerol (20% in 0.5X TBE) and 4 µL of nanoparticle suspension. Gels were run for 30 minutes at 80 V and 3 A. Visible light was used to detect nanoparticles while UV light under a blue filter was used to detect the blue emitting excess of unbounded polymer (in the case of PMAO-coated NPs).

Thermogravimetric analysis (TGA) was performed using a TA STD 2960 simultaneous DTA-DTGA instrument in air at a heating rate of 10 $^{o}C\ min^{-1}$.

Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectra were recorded on a Bruker VERTEX 70/70v spectrometer using 128 scans over the 650−4000 cm^{-1} spectral range.

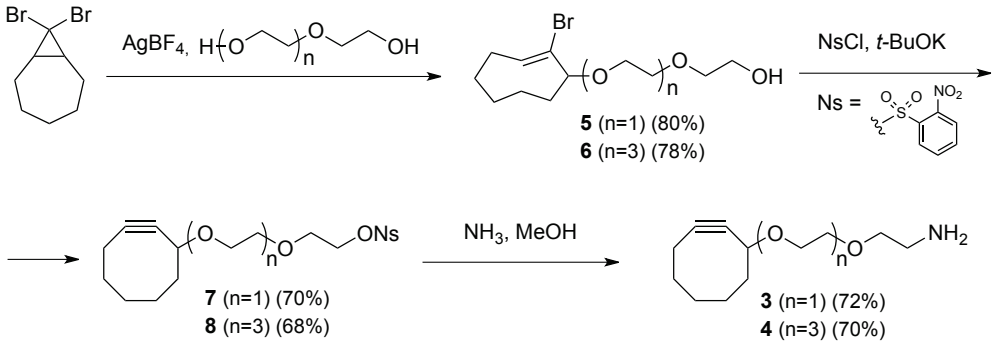
X-ray photoelectron spectroscopy (XPS) spectra were recorded on an AXIS SUPRA^{TM} XPS spectrometer (Kratos Analytical, LMA, Universidad de Zaragoza). XPS spectra were analysed using the CASAXPS software.

Contact angle measurements were performed using a Krüss DSA 10 MK2 drop shape analyser (Krüss GmbH, Germany). The instrument was equipped with a Hamilton microliter syringe whose Z position was controlled by the software. The sessile drop method was used. A droplet of water (5 µL) was formed on the end of the syringe and lowered onto the surface; the syringe was then retracted until the drop detached itself. Three measurements per sample were made at different points on the sample. All contact angles are given as mean values with standard deviation.

^{1}H NMR spectra were recorded on a Bruker 500 MHz; and ^{13}C NMR spectra at 125 MHz. The chemical shifts are reported as δ values (ppm) relative to residual deuterated solvent as internal standards: for $CDCl_3$ δ H (7.26 ppm) and δ C (77.16 ppm), respectively; for DMSO-d_6 δ H (2.50 ppm) and δ C (39.52 ppm), respectively. Infrared spectra of compounds **3** and **4** were recorded on a *Bruker Alpha P* spectrometer.

3. Synthesis of cyclooctynylamines 3 and 4

The synthesis of aminocyclooctynes **3** and **4** was achieved following the parallel three-step reactions shown in the following scheme:



(*Z*)-2-[2-(2-Bromocyclooct-2-en-1-yloxy)ethoxy]ethanol (5): To a flame-dried flask containing $AgBF_4$ (3.3 mmol, 642 mg) under nitrogen atmosphere were added successively anhydrous diethylene glycol (60 mmol, 5.7 mL) and 8,8-dibromobicyclo-[5.1.0]-octane (3.0 mmol, 800 mg). The reaction mixture was stirred at 90 ºC for 100 min while a precipitate of silver bromide was formed. Then, it was cooled down to room temperature and aq sat ammonia (30% NH_3, 4.0 mL) was added. After stirring the mixture for 2 min, the suspension was filtered through a pad of celite, which was washed successively with aq sat NH_4Cl (4.0 mL) and EtOAc (10.0 mL). The organic layer was separated from the filtrate, washed with brine (2 x 6 mL), dried (Na_2SO_4) and evaporated under reduced pressure to provide the crude product, which was used without further purification in the next transformation.
Yield: 696 mg (80%). Oil. IR (cm^{-1}): 3415, 1630. ^1H NMR (400 MHz, $CDCl_3$): δ 6.19 (dd, *J* = 11.7, 4.3 Hz, 1H), 3.92 (dd, *J* = 10.3, 5.1 Hz, 1H); 3.44–3.80 (m, 8H); 2.74 (td, *J* = 11.9, 5.5 Hz, 1H); 2.28 (dt, *J* = 10.7, 4.7 Hz, 1H); 1.82–2.07 (m, 4H); 1.69 (m, 1H); 1.50 (m, 1H); 1.30 (m, 1H); 0,83 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 134.8; 131.7; 85.7; 72.5; 70.6; 68.0; 62.0; 39.5; 36.5; 33.5; 28.2; 26.4.

(*Z*)-11-(2-Bromocyclooct-2-en-1-yloxy)-3,6,9-trioxaundecanol (6): The same procedure as before was followed starting from $AgBF_4$ (3.3 mmol, 643 mg), 8,8-dibromobicyclo-[5.1.0]-octane (3.0 mmol, 800 mg) and tetraethylene glycol (29.84 mmol, 5.2 mL).
Yield: 886 mg (78%). Oil. IR (cm^{-1}): 3457. ^1H NMR (400 MHz, $CDCl_3$): δ 6.18 (dd, *J* = 11.7, 3.6 Hz, 1H), 3.92 (dd, *J* = 10.6, 4.9 Hz, 1H), 3.79-3.42 (m, 16H); 2.73 (dd, *J* = 11.7, 5.8, 3.0 Hz, 1H); 2.32-2.25 (m, 1H); 1.80–2.06 (m, 4H); 1.70 (m, 1H); 1.56–1.41 (m, 1H); 1.37–1.23 (m, 1H); 0,79 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 133.0, 131.4, 85.1, 72.4, 70.5, 70.5, 70.4, 70.3, 70.2, 67.9, 61.6, 39.5, 36.4, 33.2, 28.1, 26.4.

2-[2-(Cyclooct-2-yn-1-yloxy)ethoxy]ethyl 2-nitrobenzenesulfonate (7): A solution of alcohol **5** (0.34 mmol, 100 mg) in anhydrous THF (4.0 mL) was added at 0 ºC into a flame-dried flask containing *t*-BuOK (0.75 mmol, 84 mg) under nitrogen atmosphere and the reaction mixture was stirred for 10 minutes at the same temperature. Then, the solution was cooled to –12 ºC and 2-nitrobenzenesulfonyl chloride (0.41 mmol, 91 mg) was added. After stirring the mixture at the same temperature for 30 minutes, it was quenched with aq sat NH_4Cl (2.0 mL) and the organic solvent was evaporated keeping the temperature below 5-10 ºC. The aqueous residue was taken up in EtOAc (10 mL) the organic layer was washed with aq sat NH_4Cl (2 x 3 mL), dried

(Na_2SO_4) and evaporated under reduced pressure to provide the crude product, which was used without further purification in the next transformation.
Yield: 95 mg (70 %). Oil. IR (cm^{-1}): 2205, 1448, 1335. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19–7.74 (m, 4H), 4.40 (t, *J* = 4.6 Hz, 2H), 4.18 (t, *J* = 6.3 Hz, 1H), 3.83–3.74 (m, 6H), 2.30–2.11 (m, 3H), 1.91 (m, 3H), 1.82 (dd, *J* = 15.3, 7.3 Hz, 2H), 1.74–1.56 (m, 1H), 1.41 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 148.4, 137.0, 135.0, 132.6, 131.3, 124.9, 100.3, 92.8, 72.9, 71.5, 70.6, 68.6, 68.5, 42.4, 34.4, 29.8, 26.4, 20.8.

11-(Cyclooct-2-yn-1-yloxy)-3,6,9-trioxaundecyl 2-nitrobenzenesulfonate (8): The same procedure as before was followed starting from alcohol **6** (0.26 mmol, 100 mg), *t*-BuOK (0.58 mmol, 65 mg) and 2-nitrobenzenesulfonyl chloride (0.31 mmol, 69 mg).
Yield: 85 mg (68 %). Oil. IR (cm^{-1}): 2205, 1525, 1448, 1336, 729. ^{1}H NMR (400 MHz, $CDCl_3$): 8.26–7.74 (m, 4H), 4.42 (t, *J* = 4.6 Hz, 2H), 4.22 (t, *J* = 6.2 Hz, 1H), 3.78 (t, *J* = 4.6 Hz, 2H), 3.76–3.44 (m, 12H), 2.34–2.11 (m, 3H), 1.95 (dd, *J* = 13.9, 7.3 Hz, 2H), 1.83 (dt, *J* = 17.4, 9.1 Hz, 2H), 1.76–1.54 (m, 2H), 1.43 (dd, *J* = 17.2, 9.0 Hz, 1H).^{13}C NMR (101 MHz, $CDCl_3$): δ 148.6, 136.8, 134.9, 132.6, 131.5, 125.0, 100.3, 93.0, 72.9, 71.5, 71.0, 70.8, 70.7, 70.6, 68.8, 68.7, 42.5, 34.5, 30.0, 26.6, 20.9.

2-[2-(Cyclooct-2-yn-1-yloxy)ethoxy]ethylamine (3): A solution of nosylate **7** (0.13 mmol, 50 mg) and 7M NH_3 in MeOH (8 mmol, 1.2 mL) was stirred at room temperature for 5h under anhydrous conditions. The reaction mixture was evaporated in vacuo to give a residue which was dissolved in EtOAc (3 mL) and washed successively with aq 5M NaOH (2 x 1.5 mL) and brine (2 mL). Drying (Na_2SO_4) and evaporation under vacuo of the organic phase gave the without further purification.
Yield: 20 mg (72%). Oil. IR (cm^{-1}): 2207, 1449, 1100. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.26–4.20 (t, *J* = 6.2 Hz, 1H), 3.76–3.48 (m, 6H), 2.86 (t, *J* = 5.1 Hz, 2H), 2.28–2.08 (m, 2H), 2.03–1.92 (m, 3H), 1.83 (td, *J* = 15.0, 13.3, 5.8 Hz, 2H), 1.66 (m, 2H), 1.46–1.40 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 100.0, 92.7, 73.1, 72.7, 70.0, 68.4, 42.2, 41.6, 34.2, 29.6, 26.3, 20.6.

11-(Cyclooct-2-yn-1-yloxy)-3,6,9-trioxaundecylamine (4): The same procedure as before was followed starting from nosylate **8** (0.26 mmol, 100 mg) and 7M NH_3 in MeOH (16 mmol, 2.5 mL).
Yield: 55 mg (70 %). Oil. IR (cm^{-1}): 2243, 1449, 1094. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.27–4.19 (m, 1H), 3.77–3.59 (m, 14H), 2.87 (t, *J* = 5.2 Hz, 2H), 2.30–2.13 (m, 3H), 2.02–1.90 (m, 2H), 1.85–1.77 (m, 2H), 1.66 (m, 2H), 1.41 (m, 1H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 100.0, 92.8, 73.2, 72.7, 70.5, 70.4, 70.3, 68.4, 42.2, 41.7, 34.2, 29.7, 26.3, 20.7.

4. Determination of the rate constant for the reaction of cyclooctynylamine 4 and benzylazide

The rate constant of the [3+2] cycloaddition reaction of cyclooctynamine **4** (A) with benzylazide (B) was determined in DMSO-*d6* solvent by monitoring the reaction conversion by ^{1}H NMR (500 MHz) at 5 min intervals in an NMR tube thermostatized at 40 ºC. Two cycloadducts ($C_{1,4}$) and ($C_{1,5}$) were formed in 57:43 proportion, respectively. The initial concentrations were set at $[A]_0$ = 30 mM for cyclooctynylamine **4** and $[B]_0$ = 90 mM for benzylazide. Figure ESI-1 shows the compilation of ^{1}H NMR spectra indicating a virtually total conversion of the starting cyclooctynamine after 1h.

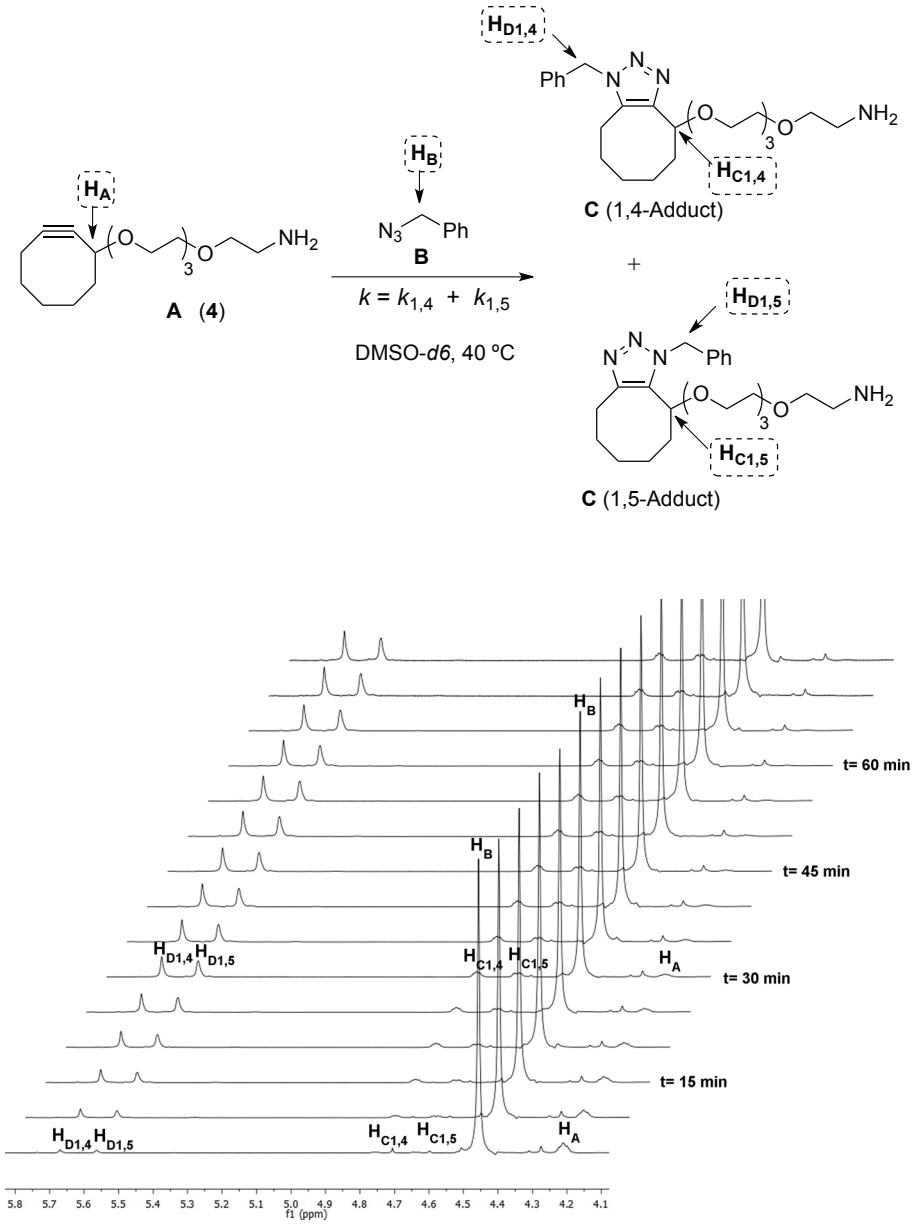
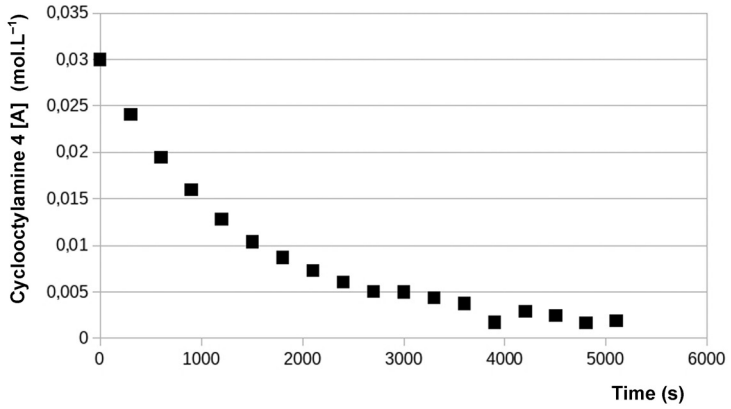


Figure S1. *^{1}H NMR (500 MHz, DMSO-d_6) spectra of the cycloaddition reaction of cyclooctylamine **4** with benzyl azide **3a**. Two cycloadducts ($C_{1,4}$) and ($C_{1,5}$) were formed in a 57:43 proportion, respectively.*



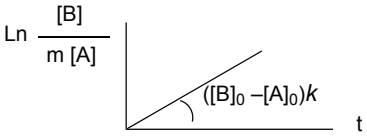
$$A + B \xrightarrow{k_{1,4}} C_{1,4}$$

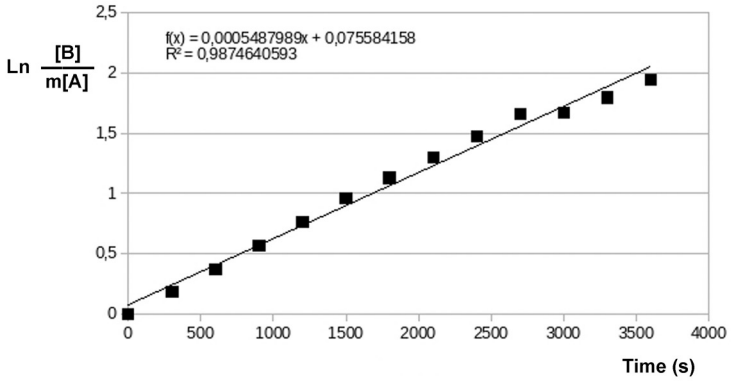
$$A + B \xrightarrow{k_{1,5}} C_{1,5}$$

$[A]_0 = 30 \times 10^{-3}$ M $m = \frac{[B]_0}{[A]_0} = 3$

$[B]_0 = 90 \times 10^{-3}$ M $[B]_0 - [A]_0 = 60 \times 10^{-3}$ M

$$\boxed{\text{Ln}\frac{[B]}{m\,[A]} = ([B]_0 - [A]_0)\,k\,t}$$





$k_{(40\,^{\circ}C,\ DMSO\text{-}d6)} = 9.1 \times 10^{-3}$ M

Figure S2. *Conversion plot (Top) and second order reaction rate plot measured at 40 ºC for the reaction of cyclooctynylamine* ***4*** *with benzyl azide in DMSO-d_6 (Bottom).*

5. Synthesis of 12 nm hydrophobic iron oxide NPs

Monodisperse iron oxide nanoparticles of 12 nm mean diameter were synthesized following a seed-mediated growth method. [1, 2]

Synthesis of 6 nm iron oxide nanoparticle seeds:

In a three-neck flask equipped with mechanical stirring, temperature probe and water condenser, $Fe(acac)_3$ (0.71 g, 2.01 mmol), 1,2-hexadecanediol (2.58 g, 9.98 mmol), oleic acid (2 mL, 5.65 mmol) and oleylamine (2 mL, 4.22 mmol) were mixed in solubilized in benzyl ether (40 mL). The mixture was subjected to three degassing and nitrogen flushing cycles and then was heated to 200 °C (3 °C/min) and maintained at this temperature for 2 h to promote nucleation. The temperature was further increased to 305 ^{o}C at a heating rate of 10 ^{o}C/min and maintained for 2 h. The mixture was then allowed to cool down to room temperature. To remove excess reagents, the nanoparticles were precipitated with ethanol, collected with a magnet and redispersed in hexane; this procedure was repeated three times and then the nanoparticles were redispersed in a mixture of hexane, oleic acid and oleylamine.

Synthesis of 12 nm iron oxide nanoparticle seeds:

In order to obtain 12 nm nanoparticles, 40 mg[1] of the 6 nm Fe_3O_4 seeds in hexane were added to a mixture containing $Fe(acac)_3$ (1.42 g, 4.02 mmol), 1,2-hexadecanediol (5.16 g, 19.97 mmol), oleic acid (1 mL, 3.12 mmol), oleylamine (1 mL, 3.06 mmol), and 40 mL of benzyl ether. The mixture was heated to 100 ^{o}C (3 °C/min) and maintained for 30 minutes to remove the hexane, then heated to 200 ^{o}C (3 ^{o}C/min) and kept at this temperature for 1 h. The temperature was further increased to 305 ^{o}C at a heating rate of 10 ^{o}C/min and maintained for 1h. The mixture was then allowed to cool down to room temperature, the nanoparticles were washed as indicated above for the 6 nm seeds and redispersed mixture of hexane, oleic acid and oleylamine.

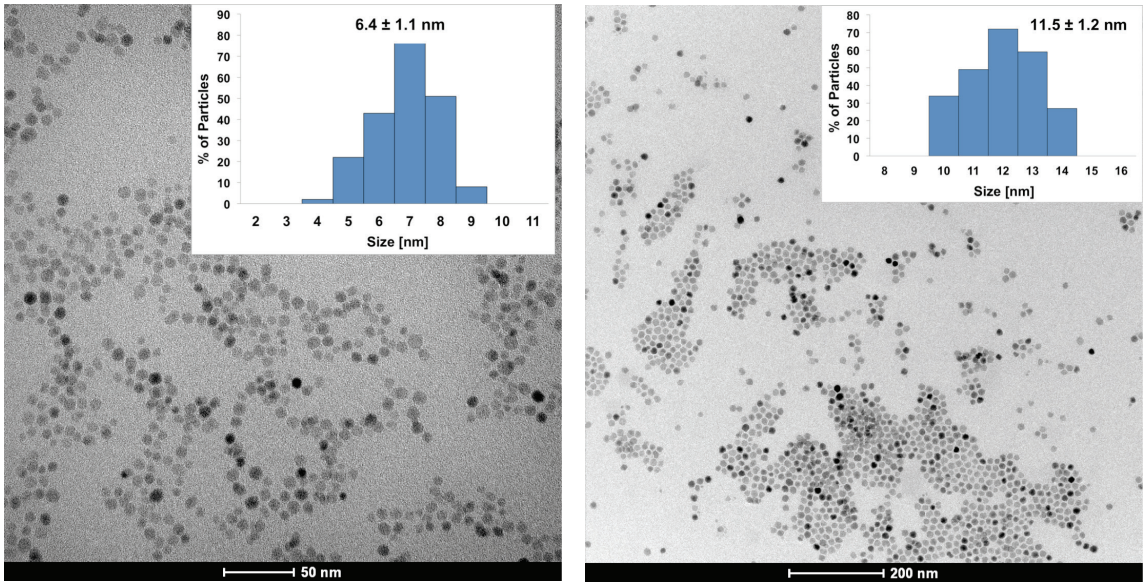


Figure S3: *TEM micrographs and size distribution histograms of 6 (left) and 12 (right) nm hydrophobic iron oxide nanoparticles.*

[1] This quantity corresponds to 40 mg of Fe, taking into account the Fe concentration of the NP solution calculated as described in Section 2. The same applies to the rest of the experimental procedures in this ESI.

6. Water transfer of hydrophobic iron oxide NPs

140 mg (0.4 mmol monomers) of poly(maleic anhydride-alt-1-octadecene) (PMAO, MW: 30000-50000 Da) were added to a 500 mL flask containing 15 mL of chloroform. The polymer was dissolved under magnetic stirring and 2 mg (3.77 µmol) of TAMRA-cadaverine in 2-3 mL of ethanol were added and the reaction was stirred overnight at room temperature in the absence of light. Afterwards, 80 mL of chloroform were added (until a final total volume of 98 mL) and then 10 mg of the hydrophobic nanoparticles (previously washed three times with ethanol and resuspended in 2 mL of chloroform) were added drop-wise in an ultrasonic bath and the mixture was kept in the bath for 15 minutes at room temperature. Then the most part of the chloroform was removed under vacuum leaving a volume of 5-10 mL solution. 10 mL of Milli-Q water and 10 mL of 0.1 M NaOH were added at once (foaming occurs due to the hydrolysis of the maleic anhydride groups) and the solvent was removed at 70 ^{o}C increasing the vacuum progressively until the complete disappearance of foam. The solution was then filtered using a 0.2 µm syringe filter. Finally, four ultracentrifugation steps were carried out during 2 hours at 24000 rpm and 20 ^{o}C (first two) and 10 ºC (last two) to eliminate unbound polymer.

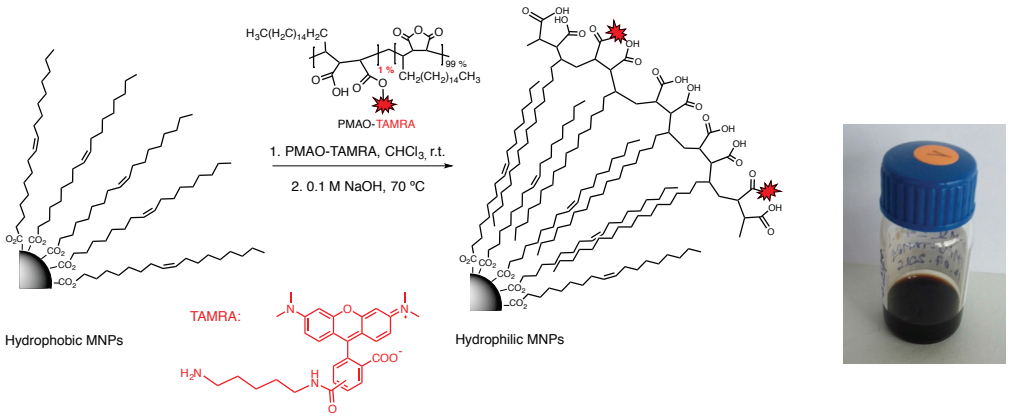


Figure S4: *Water transfer of hydrophobic MNPs with PMAO-TAMRA.*

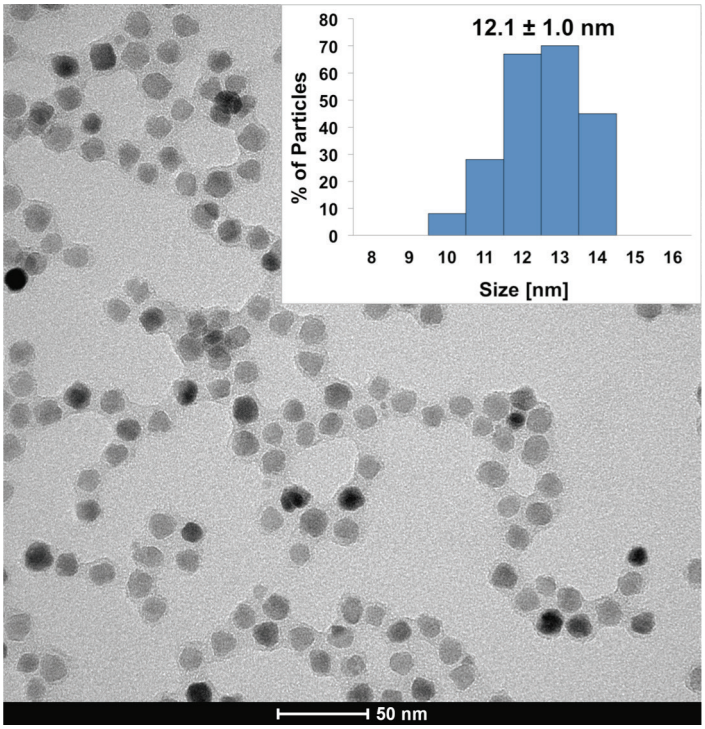


Figure S5: *TEM micrograph and size distribution histogram of 12 nm hydrophilic iron oxide nanoparticles.*

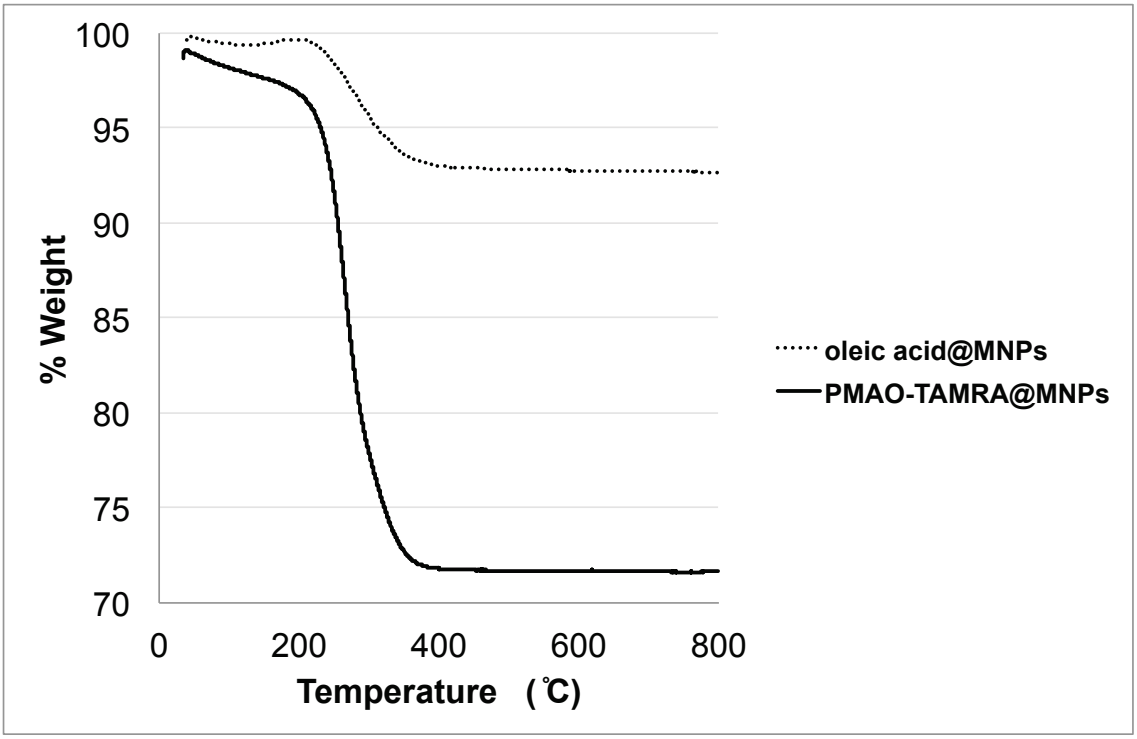


Figure S6: *Thermogravimetric analysis curves of oleic acid MNPs and PMAO coated MNPs.*

7. Functionalization of PMAO-TAMRA-coated MNPs (I) with polyetheleneglycol/glucose and cyclooctynylamines

7.1. Functionalization of PMAO-TAMRA-coated MNPs I with polyethylene glycol (PEG, 1) or glucose (Glc, 2)

The same protocol was followed for both PEG and Glc derivatives, using the amounts in Table S1.

Table S1.

NPs **I** (mg Fe)	PEG (mg, µmol)	Glc (mg, µmol)	EDC (mg, µmol)
1	16.7 mg (22.26 µmol)	-	6.25 mg (32.6 µmol) x 2
1	-	4.17 mg (15.37 µmol)	7.5 mg (39 µmol) x 2

1 mg of PMAO-TAMRA functionalised MNPs were incubated with the corresponding amounts (Table S1) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and α-methoxy-ω-amino polyethylene glycol (PEG, **1**, MW 750 Da) or 4-aminophenyl β-D-glucopyranoside (Glc, **2**) in SSB buffer (50 mM of boric acid and 50 mM of sodium borate, pH = 9) for 30 minutes at room temperature and protected from light. Then, a second portion of EDC was added to the solution and the mixture was stirred for three hours at room temperature and protected from light. In order to eliminate the excess of reagents several washing steps with Milli-Q water (centrifugation at 5000 rpm for 15 min, 10 times for PEG and 4 times for Glc) were carried out using 4 mL cellulose membrane centrifugal filters (Amicon, MilliPore, 100 kDa). The PEG- and Glc-coated nanoparticles **II** and **V** were recovered from the filters using Milli-Q water and stored at 4 ºC and protected from light. In the case of Glc-NPs an additional filtration using a 0.2 µm syringe filter was carried out before storage to eliminate larger aggregates that are typically observed with this kind of NPs.

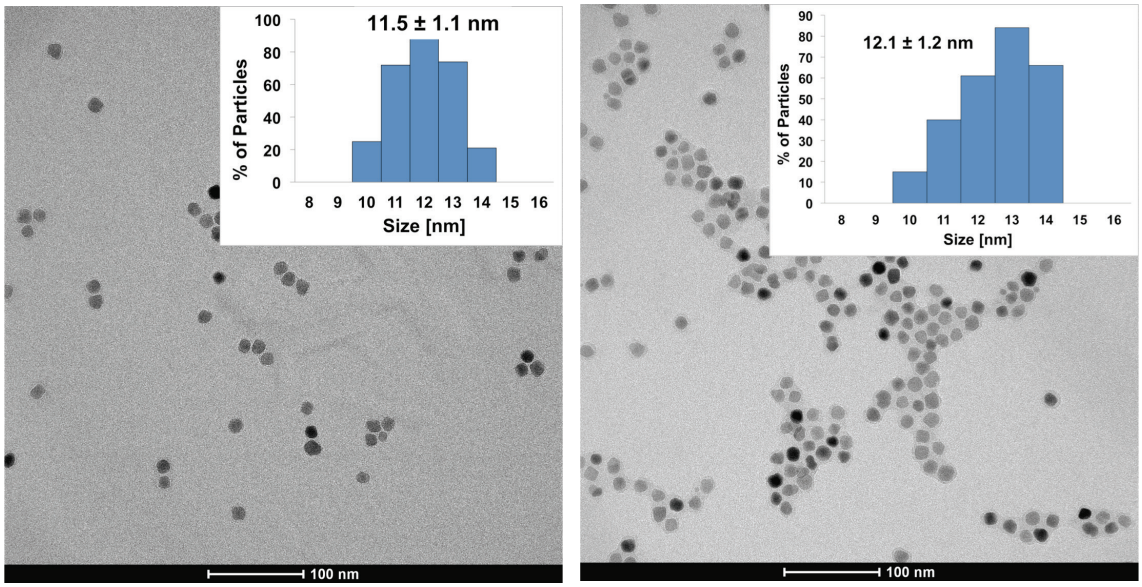


Figure S7: *TEM micrographs and size distribution histograms of PEG (left) and Glc-coated (right) nanoparticles.*

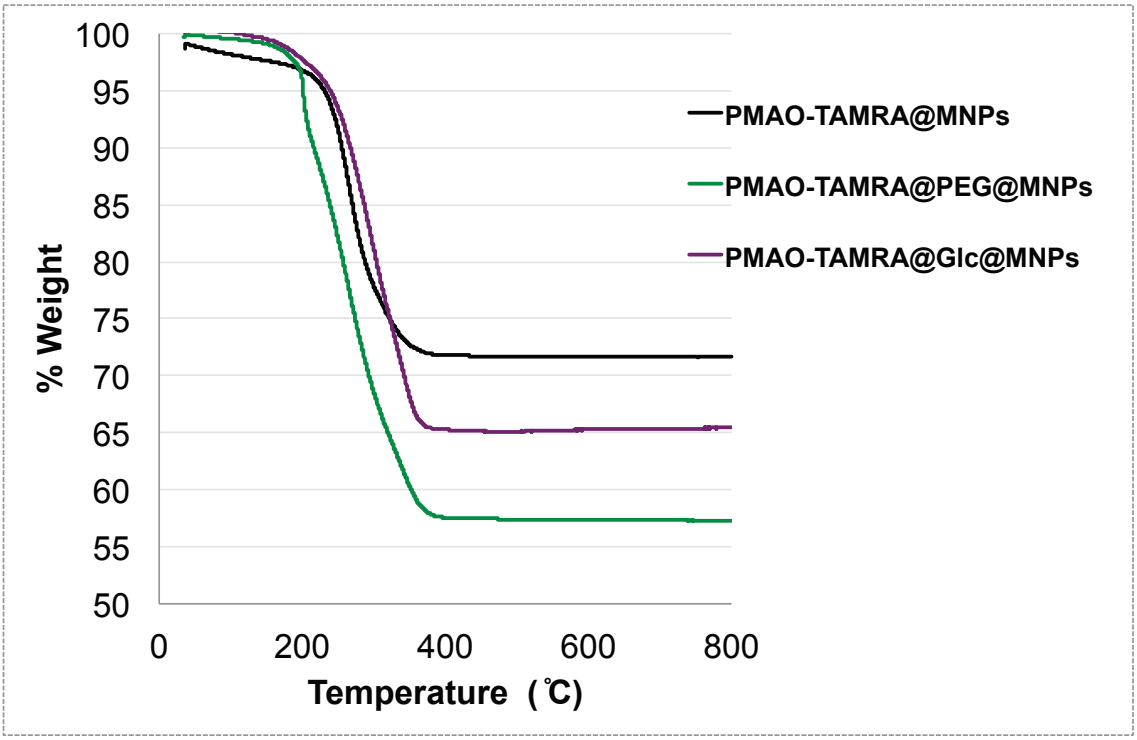


Figure S8: *Thermogravimetric analysis curves of PMAO-TAMRA (**I**), PMAO-TAMRA@PEG (**II**) and PMAO-TAMRA@Glc (**V**) MNPs.*

7.2 Functionalization of PMAO-TAMRA-PEG/Glc-coated MNPs with cyclooctynylamine derivatives 3 and 4

The same protocol was followed for both cyclooctynylamine derivatives.
0.5 mg of PMAO-TAMRA-PEG-coated MNPs were incubated with 3.125 mg (16.1 µmol) of EDC and 27.3 µmol of cyclooctynylamine **3** or **4** in SSB buffer (final reaction volume of 1.2 mL) for 30 minutes at room temperature and protected from light. Then, 3.125 mg (16.1 µmol) of EDC were added to the solution and the mixture was stirred for three hours at room temperature and protected from light. In order to eliminate the excess reagents, 4 washing steps with Milli-Q water (centrifugation at 5000 rpm for 15 min) were carried out using 4 mL cellulose membrane centrifugal filters (Amicon, MilliPore, 100 kDa).

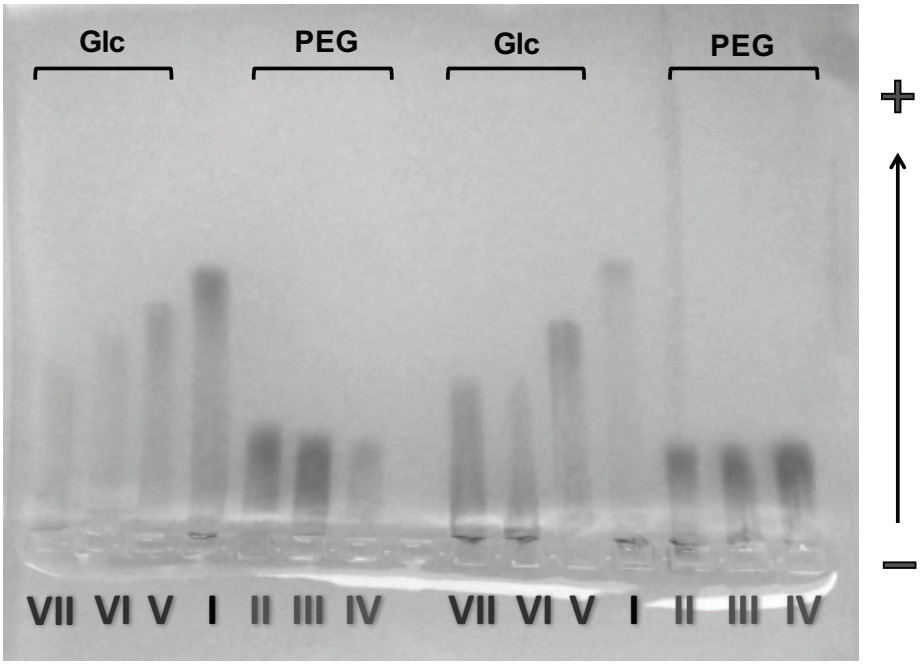


Figure S9: *Agarose gel electrophoresis showing the different electrophoretic mobilities of MNPs* ***I-VII*** *depending on their surface functionalization. Two different MNP batches starting from the same PMAO-TAMRA@MNPs* ***I*** *but prepared at different times are shown to demonstrate the robustness and reproducibility of the functionalization protocol.*

Table S2. Hydrodynamic diameter (d, nm) of MNPs **I-VII** in water and PBS (25 ºC, pH = 7.4) expressed in number, intensity and volume including standard deviation.

MNP	Number		Intensity		Volume	
	Water	**PBS**	**Water**	**PBS**	**Water**	**PBS**
PMAO (**I**)	26 ± 1	26 ± 2	94 ± 4	91 ± 2	260 ± 80	140 ± 82
PMAO@PEG (**II**)	23 ± 3	32 ± 3	88 ± 5	89 ± 2	39 ± 2	50 ± 2
PMAO@PEG@A1 (**III**)	25 ± 2	31 ± 5	79 ± 6	78 ± 5	39 ± 3	44 ± 6
PMAO @PEG@A2 (**IV**)	27 ± 6	22 ± 5	75 ± 3	74 ± 4	40 ± 6	37 ± 5
PMAO @Glc (**V**)	23 ± 3	24 ± 4	55 ± 2	56 ± 2	32 ± 3	33 ± 4
PMAO@Glc@A1 (**VI**)	30 ± 5	29 ± 4	84 ± 1	69 ± 1	50 ± 4	41 ± 3
PMAO@Glc@A2 (**VII**)	33 ± 2	31 ± 2	75 ± 1	79 ± 1	50 ± 2	45 ± 3

8. Calculation of the number of ligands per nanoparticle

The numbers of COOH, PEG and Glc groups per NP were estimated from TGA data as detailed below.

Example: PMAO-TAMRA-coated MNPs (I)

For simplicity, we consider the polymer as being only PMAO and neglect the 1 % of TAMRA.

TGA analysis results:

- sample weight: 3.8×10^{-4} g
- inorganic residue: 71.7 %
- organic residue (oleic acid + PMAO): 28.3 %

From TGA data of the hydrophobic NPs a percentage of oleic acid of 11.13 % was determined. Therefore, taking into account that no oleic acid is lost in the water transfer step, the percentage of PMAO coating the NPs is:

28.3 - 11.13 = 17.17 %

Taking into account the molecular weight of a monomer of PMAO (350 g/mol) we can calculate the number of PMAO monomers present in a 3.8×10^{-4} g sample:

$\mathbf{n_{PMAO\ mon}} = 0.1717 \times 3.8 \times 10^{-4}$ g $\times 6.023 \times 10^{23}$ molecules $\times$ mol^{-1} / 350 g $\times$ mol^{-1} = $\mathbf{1.123 \times 10^{17}}$

Considering that the inorganic residue is composed only of iron oxide, we can calculate the number of NPs present in a sample of 3.8×10^{-4} g:

- The **volume of one NP** of 12 nm diameter is:

$R = 6 \times 10^{-9}$ m

$\mathbf{V_{NP}} = 4/3\pi R^3 = 9.048 \times 10^{-19}$ cm^3

-The **mass of one NP** is then (density of iron oxide is d = 5.24 g/cm^3):

$\mathbf{m_{NP}} = d \times V_{NP} = 4.74 \times 10^{-18}$ g

- The **number of NPs** in a sample of 3.8×10^{-4} g of NPs is:

$\mathbf{n_{NPs}} = 0.717 \times 3.8 \times 10^{-4}$ g/ 4.74×10^{-18} g $\times$ NP^{-1}= $\mathbf{5.747 \times 10^{13}}$ **NPs**

The **number of PMAO molecules per NP** is then:

$1.123 \times 10^{17} / 5.747 \times 10^{13}$ = **1954**

Considering that the hydrolysis of each maleic anhydride unit yields two carboxyl groups, we can estimate the **number of COOH groups per NP**:

$\mathbf{n_{COOH}} = 2 \times 1954$ = **3908 COOH groups / NP**

Similar calculations were carried out for the other functionalised NPs, yielding the following results:

PMAO-PEG (II): 958 PEG ligands / NP, 24.5 % of COOH functionalization)

PMAO-Glc (V): ~ 1000 Glc ligands / NP, 25.6 % of COOH functionalization)

9. Stability tests

9.1. Stability tests *in vitro*

HTC 116 human colon carcinoma cells were seeded at a density of 8000 cells/well in a 96-well plate and grown for 24 h in complete Dulbecco´s Modified Eagle Medium (CDMEM) supplemented with 10% foetal bovine serum, penicillin (100 U/mL), streptomycin (100 μg/mL) and L-glutamine (2 mM) at 37 ºC in a humidified atmosphere with 5% CO_2. After 24 h, the medium was aspirated gently and replaced with 200 μL of cyclooctynyl-functionalised (**III**, **IV**, **VI** and **VII**) and PMAO-coated MNP (**I**) suspensions at a final concentration of 100 μg Fe/mL. Cells were imaged at three different time points: 5 min, 1 h and 24 h using a Nikon Eclipse Ti-E inverted microscope.

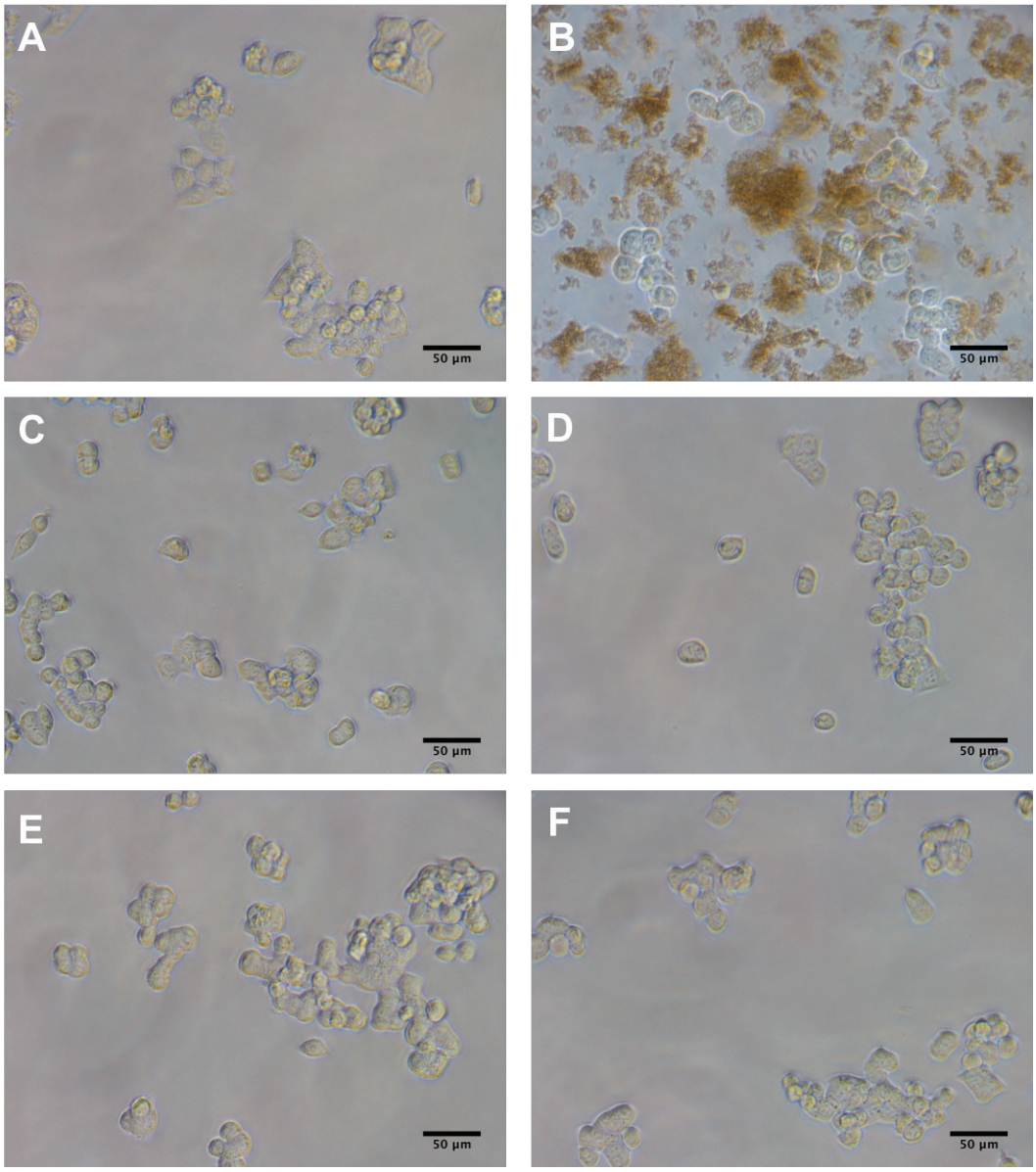


Figure S10: *Bright field images of HTC 116 cells after 5 min of incubation. A: control experiment (cells not incubated with MNPs). B: cells incubated with PMAO-coated MNPs* ***I****. C: cells incubated with PEG@CO1 MNPs* ***III****. D: cells incubated with PEG@CO2 MNPs* ***IV****. E: cells incubated with Glc@CO1 MNPs* ***VI****. F: cells incubated with Glc@CO2 MNPs* ***VII****. Scale bar is 50 μm in all images.*

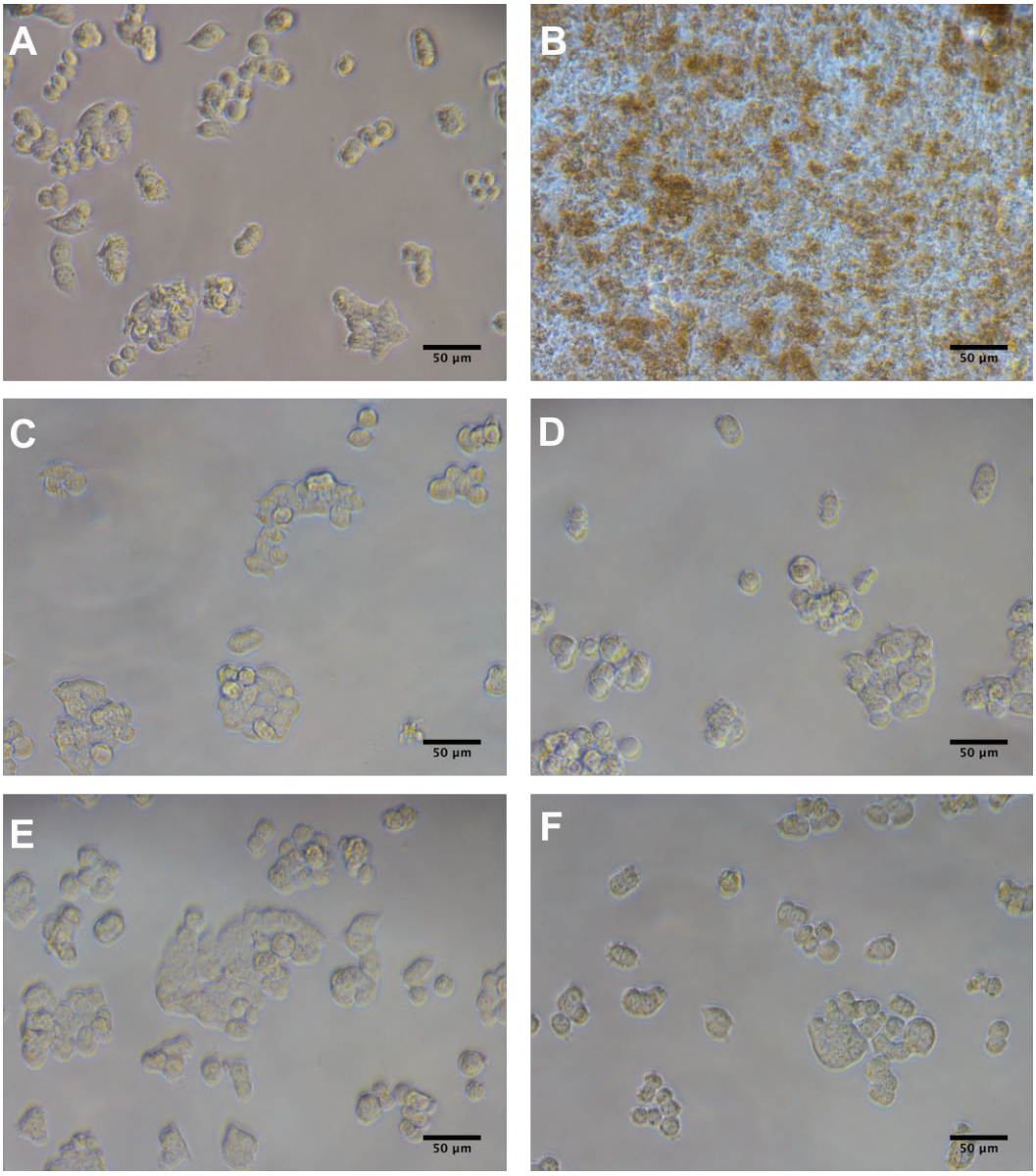


Figure S11: *Bright field images of HTC 116 cells after 1 h of incubation. A: control experiment (cells not incubated with MNPs). B: cells incubated with PMAO-coated MNPs* ***I****. C: cells incubated with PEG@CO1 MNPs* ***III****. D: cells incubated with PEG@CO2 MNPs* ***IV****. E: cells incubated with Glc@CO1 MNPs* ***VI****. F: cells incubated with Glc@CO2 MNPs* ***VII****. Scale bar is 50 µm in all images.*

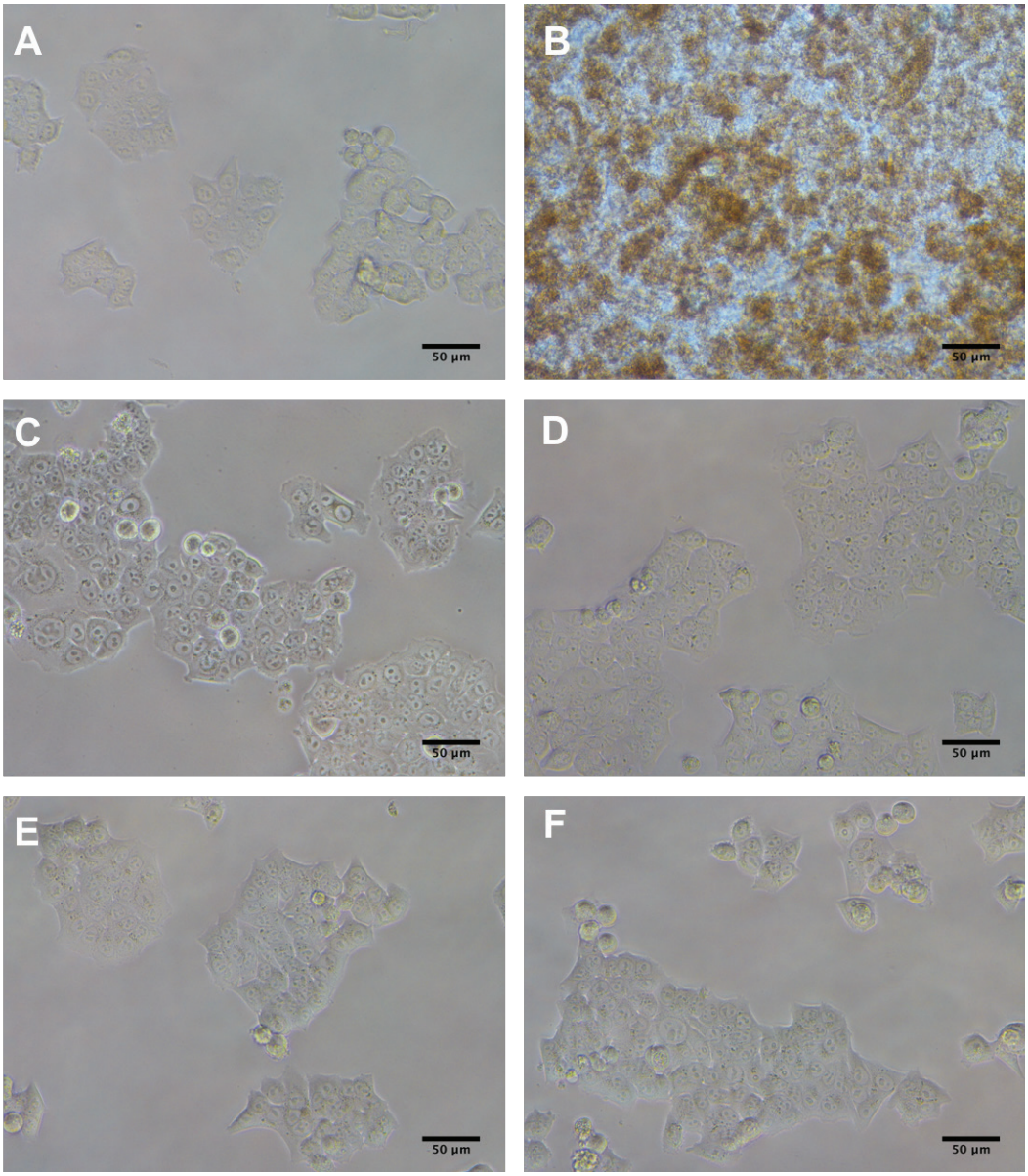


Figure S12: *Bright field images of HTC 116 cells after 24 h of incubation. A: control experiment (cells not incubated with MNPs). B: cells incubated with PMAO-coated MNPs* ***I****. C: cells incubated with PEG@CO1 MNPs* ***III****. D: cells incubated with PEG@CO2 MNPs* ***IV****. E: cells incubated with Glc@CO1 MNPs* ***VI****. F: cells incubated with Glc@CO2 MNPs* ***VII****. Scale bar is 50 µm in all images.*

9.2 Stability tests in suspension

Suspensions of MNPs **I**, **III**, **IV**, **VI** and **VII** were prepared in phosphate buffered saline (PBS) and complete Dulbecco´s Modified Eagle Medium (CDMEM) supplemented with 10% foetal bovine serum at a final concentration of 100 µg Fe/mL. Figure S11 shows images of the suspension five minutes after their preparation for the PMAO-coated MNPs **I** (Figure S11 A) and 24 h later for MNPs **III**, **IV**, **VI** and **VII** in PBS (Figure S11 B) and CDMEM (Figure S11 C).

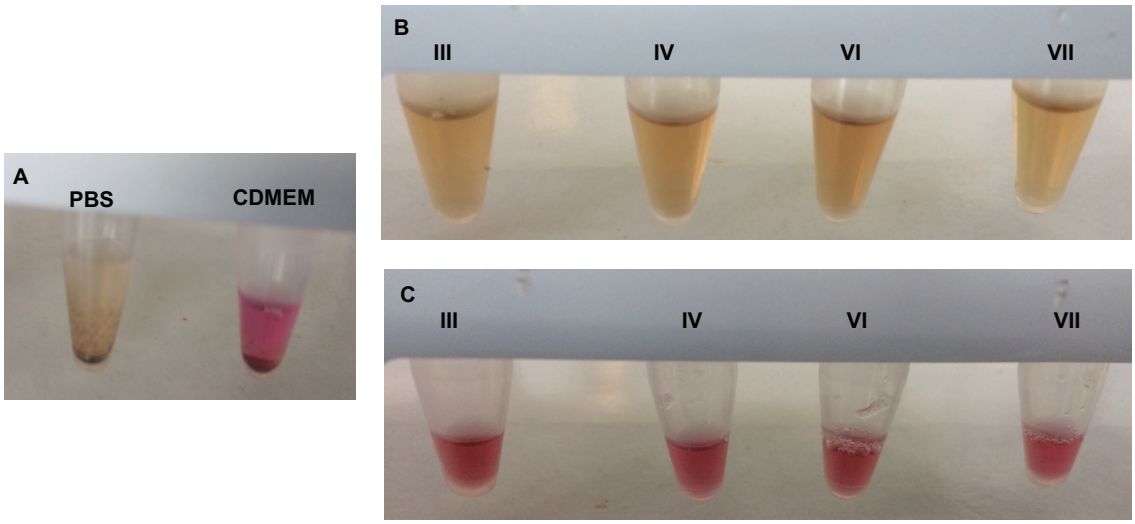


Figure S13: *Photographs of PMAO-coated MNPs (**I**) in PBS and CDMEM (left) and MNPs **III**, **IV**, **VI** and **VII** in PBS (top right) and CDMEM (bottom right).*

10. Bioorthogonal click chemistry on surfaces

10.1 Activation of Si surfaces

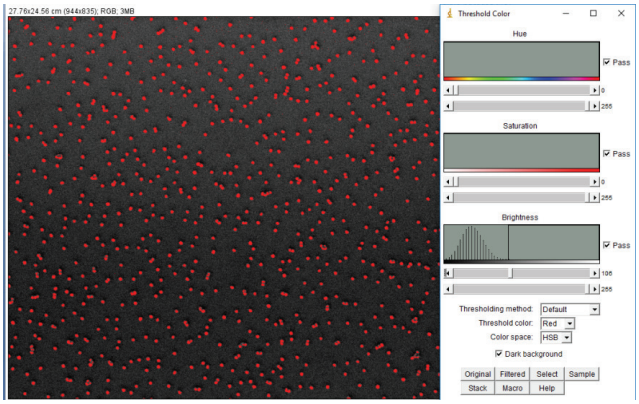
Single side polished p-type Si(100) wafers were diced into pieces of approximately 1 cm^2 and cleaned before use with ethanol and acetone (15 min in an ultrasonic bath), rinsed copiously with Milli-Q water and dried under a flow of nitrogen. Then, the substrates were immersed in Piranha solution (3:1 v:v concentrated H_2SO_4 : 30 %H_2O_2) for 30 min (***Warning!*** *Piranha solution should be handled with extreme caution. It has been reported to detonate unexpectedly)* to install silanol groups. The substrates were then rinsed several times with Milli-Q water and dried under a flow of nitrogen. Dry substrates were immersed in a solution of 11-bromoundecyltrichlorosilane in dry toluene (1% vol.) for 45 minutes, sonicated in toluene for 15 minutes, rinsed with toluene, acetone and ethanol, sonicated in ethanol during 15 minutes and finally dried under a flow of nitrogen. Then, dry substrates were immersed in a saturated solution of sodium azide in *N*,*N*-dimethylformamide (DMF, 99.9% HPLC grade) for 48 h, under nitrogen and gentle shaking at 37 ^oC. After 48 h the substrates were sonicated in acetone and ethanol for 5 minutes and finally rinsed with Milli-Q water and dried under a flow of nitrogen. Functionalised Si surfaces were typically used immediately after their preparation, as azide groups can degrade over time.

10.2 Bioorthogonal click reaction on surfaces

Freshly functionalised Si surfaces were incubated for 1h at room temperature with 125 mM aqueous solutions of MNPs **V**-**VIII** and DBCO-PEG_4-sulforhodamine B. After that time, the substrate were extensively rinsed with Milli-Q water, acetone and ethanol and dried under a flow of nitrogen.

10.3 Surface coverage calculation

The quantification of the surface area percentage occupied by MNPs was carried out using the ImageJ software.
Due to the poor contrast of the SEM images, a 0.5% contrast enhancement was applied prior to the analysis using the "Process" and "Enhance contrast" commands available in ImageJ. (Note: it is necessary to remove all text parameters embedded in the figure which have black or white colours since they prevent contrast enhancement). Next, by using the commands "Image" -> "Adjust"-> "Threshold", an automatic colour histogram is enabled, which allows the modification of the brightness threshold fitting the automatic detection to the real image.



Finally, the **total area occupied by nanoparticles** (A_{NPs}) was measured using the commands "Analyze" -> "Analyze Particles" The **total area of the image** (A_{total}) was considered as the 100% of coating. The percentage of nanoparticles surface coating was calculated using the formula % = 100*(A_{NPs}/A_{total}).

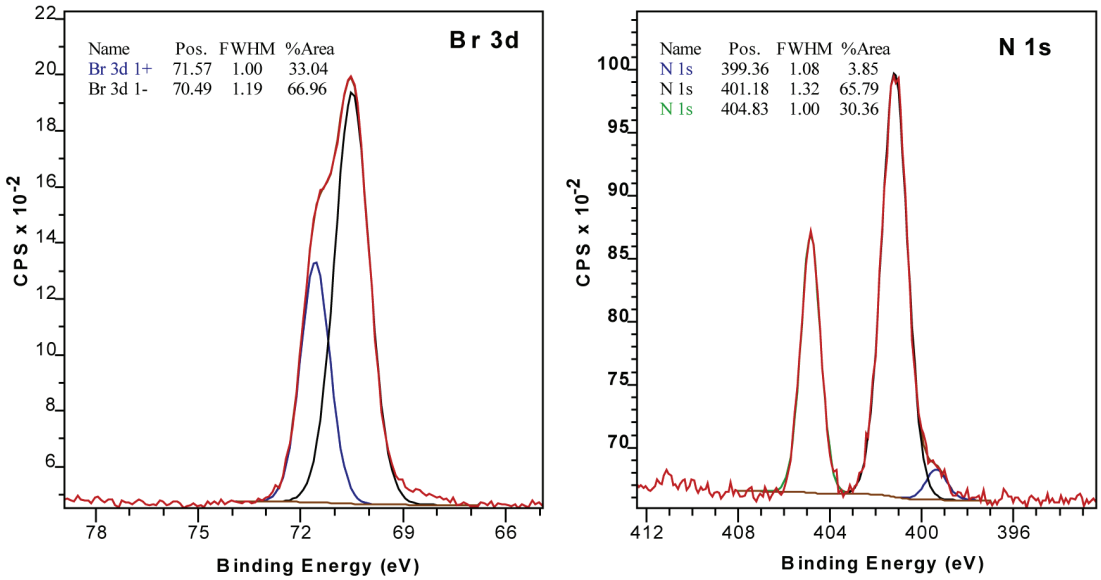


Figure S14: *High-resolution XPS spectra of Br3d and N1s peaks corresponding to the 11-bromoundecyltrichlorosilane and azide-functionalised silicon substrates used for bioorthogonal "click" chemistry.*

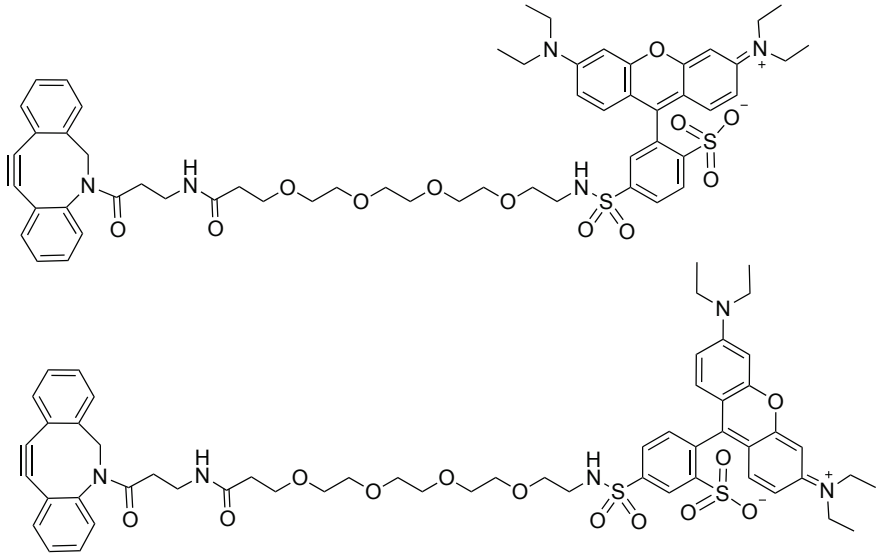


Figure S15: *Chemical structure of dibenzylcyclooctyne-PEG4-5/6-sulforhodamine B.*

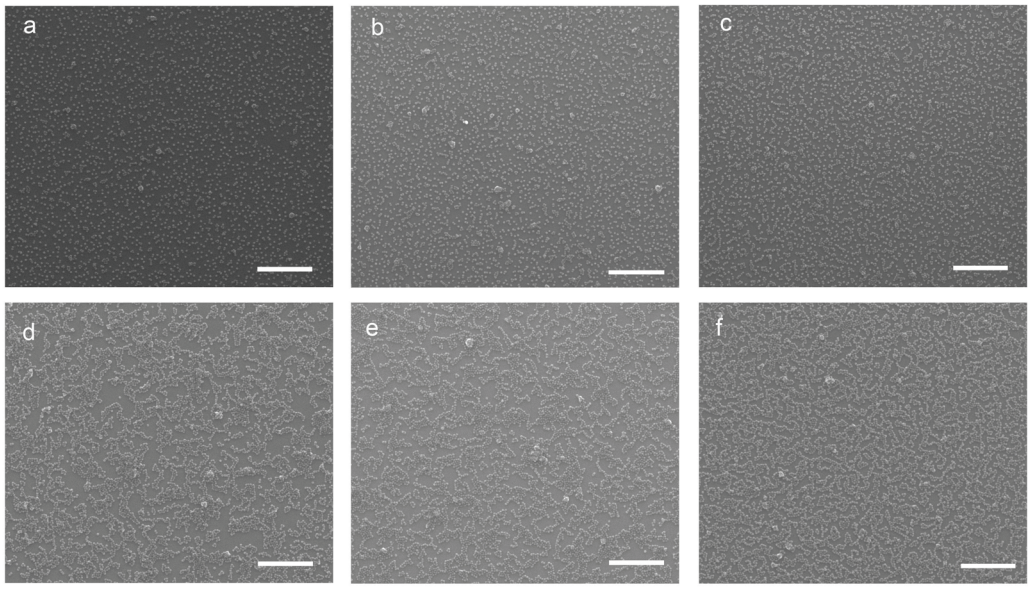


Figure S16: *SEM micrographs of the piranha-activated surfaces reacted with different NPs: a) PEG (**II**); b) PEG@CO1 (**III**); c) PEG@CO2 (**IV**); d) Glc (**V**); e) Glc@CO1 (**VI**); d) Glc@CO2 (**VII**). Scale bar is 500 nm.*

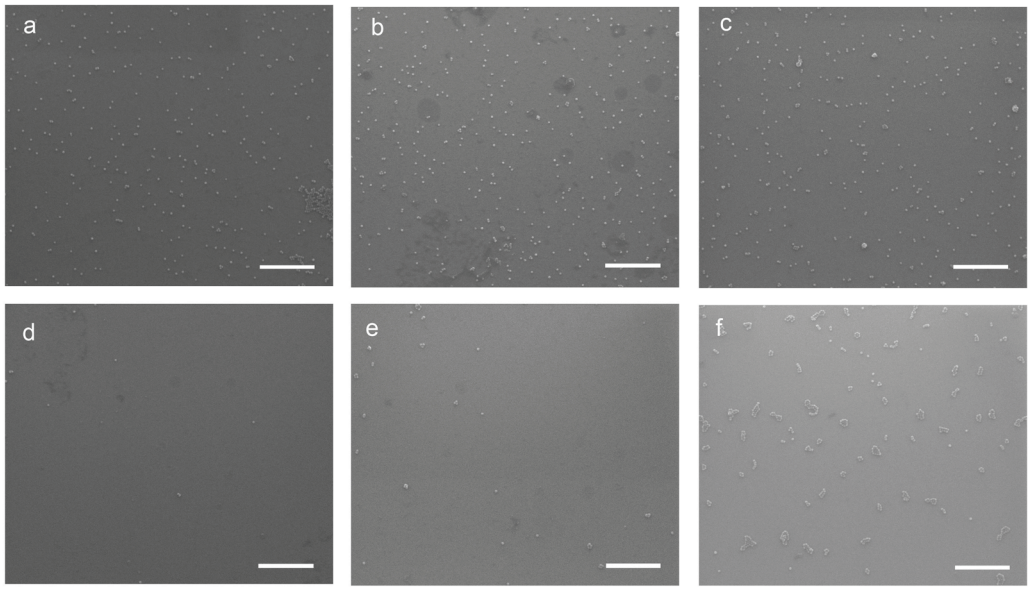


Figure S17: *SEM micrographs of the Br-functionalised surfaces reacted with different NPs: a) PEG (**II**); b) PEG@CO1 (**III**); c) PEG@CO2 (**IV**); d) Glc (**V**); e) Glc@CO1 (**VI**); d) Glc@CO2 (**VII**). Scale bar is 500 nm.*

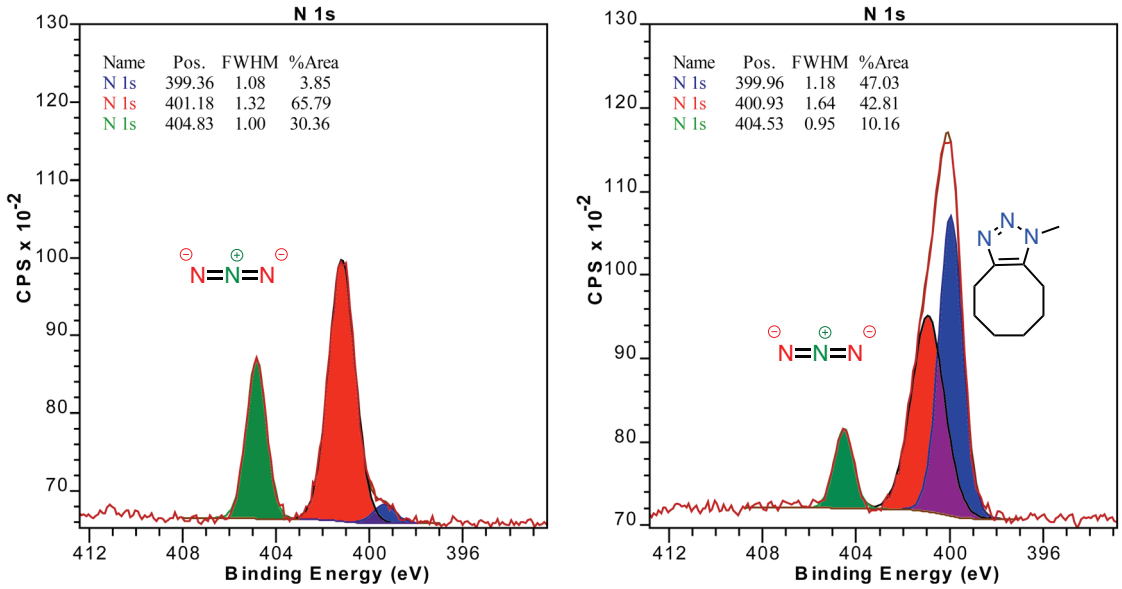


Figure S18: *High-resolution XPS spectra of N1s peaks corresponding to the azide-functionalised silicon substrate before (left) and after bioorthogonal "click" reaction with DBCO.*

References

1. M. Moros, B. Pelaz, P. López-Larrubia, M. L. García-Martin, V. Grazú and J. M. de la Fuente, *Nanoscale*, 2010, **2**, 1746–55.

2. M. Moros, B. Hernáez, E. Garet, J. T. Dias, B. Sáez, V. Grazú, Á. González-Fernández, C. Alonso and J. M. de la Fuente, *ACS Nano*, 2012, **6**, 1565–1577.