Gold Nanoparticles protected by fluorinated ligands for ^{19}F MRI

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ELECTRONIC SUPPORTING INFORMATION

Materials and Instruments

All commercially available reagents were from Aldrich, Fluka and Fluorochem and used without purification unless otherwise mentioned. Solvents are purchased from Aldrich, VWR, Fluka and Riedel, and deuterated solvents from Cambridge Isotope Laboratories and Aldrich. Dry solvents were obtained from Aldrich. Chlorinated solvents were stirred over K_2CO_3 for at least 24 h prior to use. All other solvents were reagent grade and used as received. Reactions were monitored by TLC on Merck silica gel plates (0.25 mm) and visualized by UV light, I_2, or by $KMnO_4$–H_2SO_4. Chromatography was performed on Merck silica gel 60F-254 (230–400 mesh) and the solvents employed were of analytical grade. Size exclusion chromatography was carried out using Sephadex^{TM} LH-20 (Amersham Biosciences). Spectra/Por Dialysis membrane with MWCO: 3500 was used for dialysis purifications. Bodipy 650/665-X succinimidyl ester was purchase from Invitrogen, Oregon, USA.

NMR spectra were recorded on a Varian 500 spectrometer (operating at 500 MHz for proton and at 125 MHz for carbon), on a Jeol GX-400 MHz (operating at 400 for proton and at 100.5 MHz for carbon), on a Jeol GX-270 spectrometer (operating at 270 MHz for proton and at 67.8 for carbon) or on a Bruker Avance 300 MHz (operating at 282 MHz for ^{19}F). ^{1}H NMR spectra were referenced to the residual protons in the deuterated solvent. ^{13}C NMR spectra were referenced to the solvent chemical shift. ^{19}F NMR spectra were referenced to trifluorotoluene as external standard. Chemical shifts (δ) are reported in ppm and the multiplicity of each signal is designated by the conventional abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad; dd, doublet of doublets. Coupling constants (J) are quoted in Hz. Mass spectra were obtained by electrospray ionization (ESI) with a Perkin Elmer APII at 5600 eV and recorded by Dr Fabio Hollan, Department of Chemical and Pharmaceutical Sciences, University of Trieste, Italy. MALDI-TOF spectra were obtained using a 4800 Plus MALDI TOF spectrometer equipped with a TOF-analyzer. Infrared spectra (FT-IR) were recorded on a Perkin-Elmer FT-IR/Raman 2000 instrument in the transmission mode on NaCl disks. UV-Visible spectra were recorded on a Shimadzu UV-1800 spectrophotometer. Fluorescence spectra were recorded on a Varian Cary Eclipse Fluorescence spectrophotometer. TGA analysis was performed on TGA Q-500 V6.3 Build 189 or on TGA Q5000 V3.15 Build 263 using a heating rate of 10 °C/min up to 1000 °C. TEM images were obtained with a Jeol 3010 high resolution electron microscope (1.7 nm point-to-point) operating at 300 keV using a Gatan slow-scan CCD camera (mod. 794). TEM samples of protected gold nanoparticles were prepared by placing a single drop of 0.5 mg/mL isopropanol dispersion onto a 200-mesh copper grid coated with an amorphous carbon film. The grid was then dried in air for 24 h. Images were

obtained with a JEOL 3010 high resolution electron microscope (1.7 nm point-to-point) operating at 300 keV using a Gatan slow-scan CCD camera.

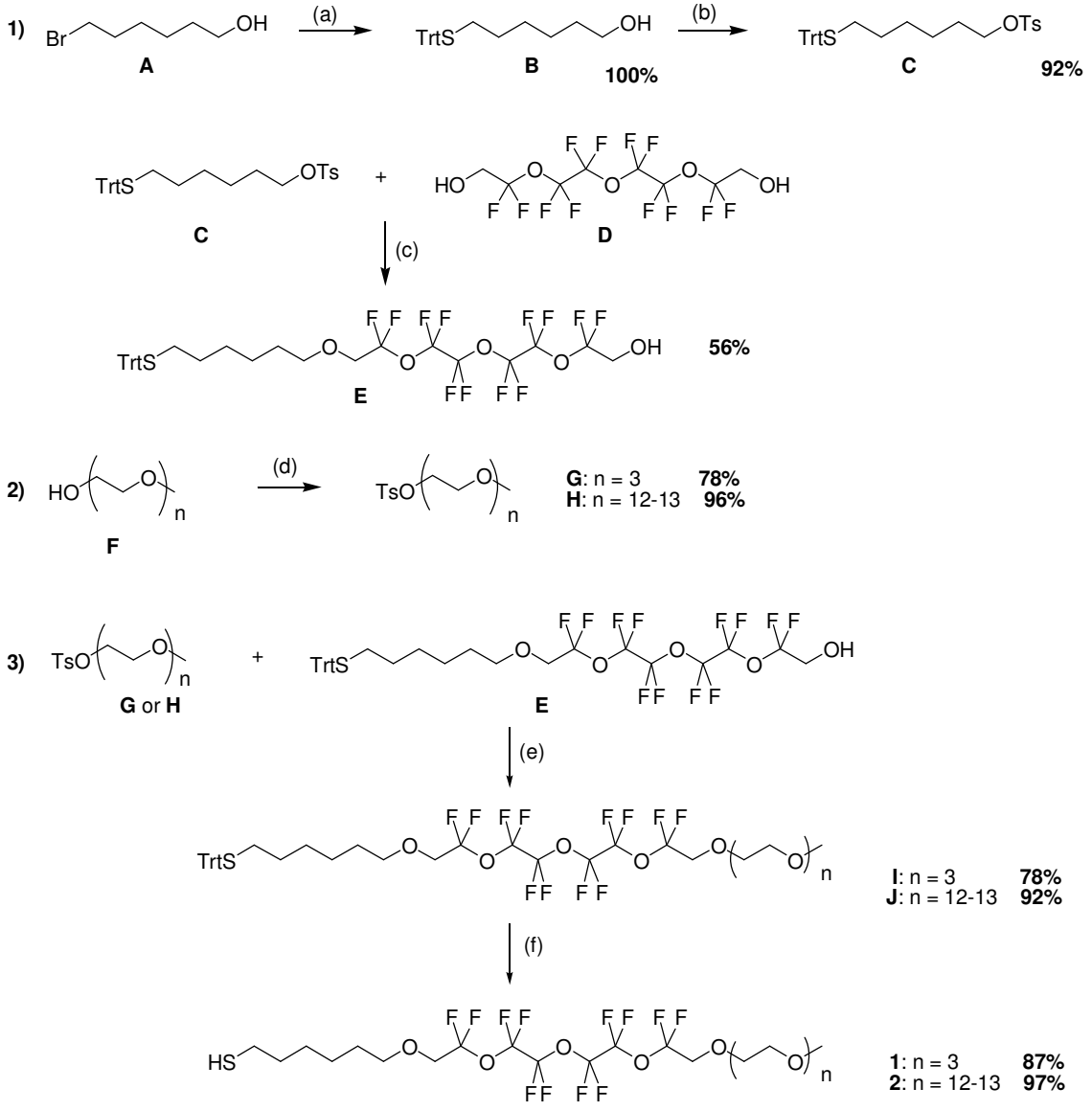
(mod. 794). Depending on the Au core size, magnifications between 250000 and 600000 were used for counting purposes. Diameters were measured manually using Gatan software Digital Micrograph (ver. 3.4.1) on at least 250 particles. HAADF-STEM images were recorded on a JEOL2010-F microscope with 0.19 nm spatial resolution at Scherzer defocus conditions and they were obtained by using an electron probe of 0.5 nm of diameter at a diffraction camera length of 10 cm.

DLS and ζ potentials were measured with a Malvern Instruments, Zetasizer, Nano Series, Nano-ZS instrument on 1 mg/mL solutions of F-MPCs.

Radical (*para*-*n*-pentyl-benzyl)-1-hydro-2-methyl-2-propylnitroxide (**6**) was generated by mixing 0.5 μL of a methanol solution containing the corresponding amine (0.1 M) and 0.5 μL of a water solution containing Oxone (0.1 M) with 100 μL of a water solution containing variable amounts of F-MPCs **4**. Samples were transferred in capillary tubes (diameter 1 mm) and then placed inside the thermostatted cavity of the EPR spectrometer. EPR spectra were collected using a Bruker ESP300 spectrometer equipped with an NMR gaussmeter for field calibration and a Hewlett-Packard 5350B microwave frequency counter for the determination of the *g*-factors, which were referenced to that of the perylene radical cation in concentrated H_2SO_4 (g = 2.00258). The sample temperature was controlled with a standard variable temperature accessory and monitored before and after each run using a copper-constantan thermocouple. The instrument settings were as follows: microwave power 5.0 mW, modulation amplitude 0.05 mT, modulation frequency 100 kHz, scan time 180 s. Digitized EPR spectra were transferred to a personal computer for analysis using digital simulations carried out with a program developed in our laboratory and based on a Monte Carlo procedure. The input data for the program are the number of nonequivalent nuclei, the hyperfine splitting constants of the free and included radical, the intrinsic line width in the absence of exchange, and the rate constants for the exchange process.

SUPPORTING INFORMATION

Synthesis of fluorinated thiols



Scheme 1. Scheme for the synthesis of fluorinated thiols **1** and **2**. (a) TrtSH, K_2CO_3, EtOH/H_2O 1:1, 90°C, 18h (b) TsCl, Et_3N, DCM, Ar, 2h, RT (c) KOH, 1,4-dioxane, Ar, reflux, overnight (d) TsCl, DCM, Ar, 18h, RT (e) NaH, 1,4-dioxane, Ar, 50°C, overnight (f) *i*Pr_3Si, TFA, DCM, Ar, 4h, RT.

Synthesis of 6-tritylthio-1-hexanol (B)

1-bromo-hexanol (2.5 mL, 19.1 mmol), tritylthiol (5.28 g, 19.1 mmol) and potassium carbonate (5.28 g, 38.2 mmol) were dissolved in a mixture of distilled water and ethanol 1:1 (200 mL). The reaction mixture was refluxed at 90° C for 18 h. After the completeness of the reaction the residue was neutralized with HCl 1M and extracted with dichloromethane (3 x 100 mL). The organic layers were collected and washed with distilled water (1 x 150 mL) and brine (1 x 150 mL), then dried

over anhydrous Na_2SO_4. After filtration the solvent was removed in vacuo to afford compound **B** as a yellow oil (yield 7.17 g, 100%).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.42-7.40 (m, 6H, Trt),7.30 (m, 6H, Trt), 7.22-7.18 (m, 3H, Trt), 3.57 (t, 2H, J = 6.6 Hz, CH_2OH); 2.14 (t, 2H, J = 7.3 Hz, CH_2STrt), 1.55-1.40 (m, 4H, CH_2), 1.27-1.22 (m, 4H, CH_2) ppm. ^{13}C-NMR (67.8 MHz, $CDCl_3$): δ = 145.15, 129.68, 127.88, 126.59, 66.36, 62.80, 32.39, 31.78, 28.60, 28.41, 25.15 ppm. MS-ESI (m/z): 377.2 [M+H$^+$]. IR (NaCl, film): $\tilde{\nu}$ = 3346, 2930-2856, 742 cm^{-1}.

Synthesis of 6-tritylthio-1-*p*-toluenesulfonylhexane C

A solution of *p*-toluenesulfonyl chloride (789 mg, 4.14 mmol) in dry dichloromethane (3 mL) was cooled at 0°C under Ar. A mixture of compound **B** (1.5 g, 3.98 mmol), triethylamine (1.11 mL, 7.96 mmol) in dry dichloromethane (1 mL) was dropwise added. The reaction mixture was stirred at room temperature for two hours, then it was poured into a mixture of dichloromethane (30 mL) and distilled water (30 mL). The aqueous layer was extracted with dichloromethane (3 x 20 mL). The organic layers were collected and washed with HCl 6N (1 x 50 mL), with a solution of $NaHCO_3$ 5% (1 x 50 mL), and distilled water (1 x 50 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crud product was purified by column chromatography over silica gel using chloroform-petroleum ether (6:4 v/v) as an eluent. Solvent was removed in vacuo to afford compound **C** as a white solid (yield 2.02 g, 92%).

^{1}H-NMR (270 MHz, $CDCl_3$): δ = 7.79 (m, 2H, Ts), 7.42-7.21 (m, 17H, Trt+Ts), 3.98 (m, 2H, CH_2OTs); 2.44 (s, 3H, CH_3), 2.11 (t, 2H, J = 7.3 Hz, CH_2STrt), 1.53 (m, 2H, CH_2), 1.28 (m, 2H, CH_2), 1.18 (m, 2H, CH_2) ppm. ^{13}C-NMR (67.8 MHz, $CDCl_3$): δ = 145.15, 144.77, 133.30, 129.9, 127.9, 126.64, 70.41, 66.42, 31.63, 28.47, 24.78, 22.54, 21.51, 13.99 ppm. MS-ESI (m/z): 553.2 [M+Na$^+$], 569.2 [M+K$^+$]. IR (NaCl, nujol): $\tilde{\nu}$ = 3346, 2930-2856, 742 cm^{-1}.

Coupling of C with oxy-fluorinated tetraethylene glycol D

To a solution of fluorinated tetraethylene glycol **D** (1.383 g, 3.382 mmol) in dry dioxane (2 mL) a solution of tosylate **C** (0.816 g, 1.537 mmol) in dry of dioxane (2 mL) was added dropwise in 10 minutes. KOH pellets (0.138 g, 2.460 mmol) was added to the mixture and the reaction was stirred overnight at 100°C. The reaction mixture was poured into distilled water (30 mL) and the residue was extracted with ethyl acetate (5 x 20 mL). The organic layers were washed with distilled water (1 x 50 mL) and brine (1 x 50 mL) and dried over anhydrous Na_2SO_4, filtered and concentrated at reduced pressure. The crud product was purified by column chromatography over silica gel using hexane-ethyl acetate (9:1 v/v) as eluent to afford compound **E** as colorless oil (yield 661 mg, 56%).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.43 (m, 6H, Trt), 7.28 (m, 6H, Trt), 7.26 (m, 3H, Trt), 3.89 (t, J = 9.6 Hz, 2H, $HOCH_2CF_2$); 3.78 (t, J = 9.7 Hz, 2H, OCH_2CF_2); 3.55 (t, J = 6.5 Hz, 2H, OCH_2CH_2); 2.15 (t, 2H, J = 7.3 Hz, CH_2STrt), 1.50 (m, 2H, CH_2), 1.28 (m, 2H, CH_2), 1.18 (m, 4H, CH_2) ppm. ^{13}C-NMR (67.8 MHz, $CDCl_3$): δ = 145.01, 129.58, 127.91, 126.52, 72.99, 69.64 (t, J = 30.7 Hz), 66.41, 62.61 (t, J = 32.9 Hz), 60.45, 31.88, 29.20, 28.64, 28.42, 25.28, 21.01. MS-ESI (m/z): 791.3 [M+Na^+].

Synthesis of TsOEG3, G

A solution of *p*-toluenesulfonyl chloride (5.98 g, 31.3 mmol) in dry dichloromethane (20 mL) was cooled at 0°C under Ar. A mixture of **F** (4.77 mL, 30.4 mmol), triethylamine (8.46 mL, 60.8 mmol) in dry DCM (10 mL) was added drop wise to the mixture and stirred under Ar for 18 h. The reaction mixture was poured into distilled water (30 mL) and the aqueous layer was extracted with dichloromethane (3 x 50 mL). The organic layers were collected and were washed with HCl 6N (1 x 100 mL), 5% $NaHCO_3$ (1 x 100 mL) and distilled water (1 x 100 mL), dried over anhydrous Na_2SO_4 and filtered. The solvent was removed in vacuo to afford compound **G** as a colorless oil (yield 7.805 g, 78%).

^{1}H-NMR (270 MHz, $CDCl_3$): δ = 7.80-7.31 (m, 4H, Ts), 4.14 (t, J = 4.75 Hz, 2H, CH_2OTs); 3.67 (t, J = 4.75 Hz, 2H), 3.61-3.19 (m, 10H, OCH_2), 3.35 (s, 3H, OCH_3), 2.43 (s, 3H, CH_3) ppm. ^{13}C-NMR (67.8 MHz, $CDCl_3$): δ = 144.10, 132.16, 129.14, 127.08, 71.02, 69.61, 68.70, 67.73, 58.01, 20.71 ppm. MS-ESI (m/z): 341.1 [M+Na^+], 357.1 [M+K^+]. IR (NaCl, film): $\tilde{\nu}$ = 2880, 1453, 1356, 1292, 1247, 1176, 1099, 922, 817, 775, 663 cm^{-1}.

Synthesis of TsOPEGOMe, H

A solution of *p*-toluenesulfonyl chloride (785 mg, 4.12 mmol) in dry dichloromethane (5 mL) was cooled at 0°C under Ar. A mixture of PEG550 (2.00 mL, 3.96 mmol), triethylamine (1.1 mL, 7.92 mmol) in dry DCM (5 mL) was added drop wise at the mixture and stirred under Ar for 18 h. The reaction mixture was poured into distilled water (30 mL) and the aqueous layer was extracted with dichloromethane (3 x 50 mL). The organic layers were collected and were washed with HCl 6N (1 x 100 mL), 5% $NaHCO_3$ (1 x 100 mL), and distilled water (1 x 100 mL), dried over anhydrous Na_2SO_4, filtered and concentrated. The crud product was purified by column chromatography over silica gel using chloroform as eluent. Solvent was removed in vacuo to afford compound **H** as a colorless oil (yield 2.32 g, 92%).

^{1}H-NMR (270 MHz, $CDCl_3$): δ = 7.81 (d, J = 8.1 Hz, 2H, Ts), 7.34 (d, J = 8.1 Hz, 2H, Ts), 4.15 (t, J = 4.7, 2H, CH_2OTs); 3.67- 3.52 (m, 42H, OCH_2), 3.38 (s, 3H, OCH_3), 2.45 (s, 3H, CH_3) ppm. ^{13}C-

NMR (67.8 MHz, $CDCl_3$): δ = 144.40, 132.54, 129.44, 127.49, 71.42, 70.21, 70.19, 70.03, 69.95, 68.90, 68.18, 58.54, 21.18 ppm. MS-ESI (m/z): 517.3, 561.3, 605.3, 649.3, 693.4, 737.4, 781.4, 825.4, 869.5, 913.5, 957.6. IR (NaCl, film): $\tilde{\nu}$ = 2872, 1454, 1354, 1176, 1103, 923, 818, 775, 662 cm^{-1}.

Coupling of E with G

Fluorinated alcohol **E** (147 mg, 0.19 mmol) was dissolved in dry dioxane (3 mL) under Ar atmosphere. To the mixture NaH (7.7 mg of NaH 60%, 0.19 mmol, washed 5 times with petroleum ether) was added and then it was stirred for 20 minutes. To the mixture TsOEG3 **G** (61 mg, 0.19 mmol) dissolved in dried dioxane (2 mL) was added dropwise in 10 minutes. The reaction mixture was heated under stirring for 18 h at 50 °C then was cooled at room temperature, diluted with chloroform (20 mL). The organic layer was washed with brine (1 x 15 mL), dried over Na_2SO_4, filtered and the solvent was removed at reduced pressure. The product was purified by column chromatography over silica gel using chloroform-ethyl acetate (9:1 v/v) as an eluent. Solvent was removed in vacuo to afford compound **I** as a colorless oil (yield 135 mg, 78%).

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.39 (m, 6H, Trt), 7.25 (m, 6H, Trt), 7.24 (m, 3H, Trt), 3.95 (t, J = 9.7 Hz, 2H, OCF_2CH_2), 3.90 (t, J = 9.9 Hz, 2H, OCH_2CF_2); 3.6-3.5 (m, 14 H, CH_2O); 3.36 (s, 3H, OCH_3); 2.13 (t, 2H, J = 7.3 Hz, CH_2STrt), 1.49 (m, 2H, CH_2), 1.38 (m, 2H, CH_2), 1.21 (m, 4H, CH_2) ppm. ^{13}C-NMR (67.8 MHz, CDCl3): δ = 144.99, 129.74, 127.86, 126.44, 72.86, 72.0, 71.83, 70.66, 70.60, 70.59, 70.49, 70.43, 70.08, 70.01, 69.63, 69.57, 69.16, 69.12, 68.92, 66.35, 62.30, 58.88, 31.82, 29.19, 28.58, 28.41, 25.30. ^{19}F NMR (300 MHz, CD_3OD): δ = -83.0-84.0(m, 4H, CH_2CF_2O), -93-94 (m, 4H, $CF_2C\underline{F}_2O$), -94.4-95.2 (m, 4H, $CF_2C\underline{F}_2O$). MS-ESI (m/z): 937.5 [M+Na^+], 953.5 [M+K^+]. IR (NaCl, film): $\tilde{\nu}$ = 3433, 2950, 1489, 1444, 1290, 1202, 1112, 969, 744, 702 cm^{-1}.

Coupling of E with H

Fluorinated alcohol **E** (0.29 g, 0.377 mmol) was dissolved in dry dioxane (3 mL) under Ar atmosphere. To the mixture NaH (36.2 mg of NaH 60%, 1.508 mmol, washed 5 times with petroleum ether) was added and then it was stirred for 20 minutes. To the mixture TsOPEG **H** (0.266 g, 0.377 mmol) dissolved in dried dioxane (2 mL) was added dropwise in 10 minutes. The reaction mixture was heated under stirring for 18 h at 50 °C then was cooled at room temperature, diluted with chloroform (20 mL). The organic layer was washed with brine (1 x 15 mL), dried over Na_2SO_4, filtered and the solvent was removed at reduced pressure. The product was purified by

column chromatography over silica gel using chloroform-ethyl acetate (9:1 v/v) as an eluent. Solvent was removed in vacuo to afford compound **J** as a colorless oil (Yield 451mg, 92%).
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.44 (m, 6H, Trt), 7.25 (m, 9H, Trt), 3.96 (t, J = 9.9 Hz, 2H, OCH_2CF_2), 3.92 (t, J = 9.9 Hz, 2H, OCH_2CF_2); 3.76 (m, 4 H, OCH_2CF_2 + CH_2O); 3.67 (m, 44H, CH_2O)); 3.39 (s, 3H, OCH_3); 2.15 (t, 2H, J = 7.3 Hz, CH_2STrt), 1.51 (m, 2H, CH_2), 1.38 (m, 2H, CH_2), 1.21 (m, 4H, CH_2) ppm. ^{13}C-NMR (67.8 MHz, CDCl3): δ = 145.07, 129.38, 127.43, 126.28, 72.35, 72.32, 71.65, 70.25, 70.18, 69.97, 69.69, 69.45, 69.27, 69.13, 69.03, 68.78, 67.83, 57.73, 33.71, 29.14, 28.33, 27.69, 25.03, 23.49. ^{19}F NMR (300 MHz, CD_3OD): δ = -83.7-84.0 (m, 4H, CH_2CF_2O), -94.6-94.7 (m, 4H, $CF_2C\underline{F}_2O$), -95.0-95.12 (m, 4H, $CF_2C\underline{F}_2O$). MS-ESI (m/z): 783.2, 827.3, 871.3, 915.3, 959.3, 1003.3, 1047.4, 1091.4, 1157.3, 1201.4, 1245.4, 1289.5, 1333.5, 1377.4, 1421.5, 1465.6, 1509.6. IR (NaCl, film): $\tilde{\nu}$ = 2871, 1446, 1350, 1291, 1203, 1110, 959, 745, 701 cm^{-1}.

Synthesis of thiol 1 (HS-C6ossifluoroEG3)

Compound **I** (398 mg, 0.48 mmol) was dissolved in deoxygenated dichloromethane (5 mL) under Ar. At the mixture, trifluoroacetic acid (0.71 mL, 9.6 mmol) was added and then triisopropylsilane (0.20 mL, 0.96 mmol) was added. The reaction mixture was stirred at room temperature for 4h then the solvent was removed in vacuo and the residue co-evaporated with methanol. The crud product was purified by column chromatography over silica gel using chloroform-ethyl acetate (9:1 v/v), the solvent was removed in vacuo afford compound **1** as a colorless oil (Yield 280 mg, 87%).
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 3.90 (t, J = 9.9 Hz, 2H, OCH_2CF_2); 3.76 (m, 4 H, OCH_2CF_2 + CH_2O); 3.66 (m, 12H, CH_2O), 3.36 (s, 3H, OCH_3); 2.54 (q, 2H, J = 7.5 Hz, CH_2SH), 1.62 (m, 4H, CH_2), 1.37 (m, 4H, CH_2) ppm. ^{13}C-NMR (67.8 MHz, CDCl3): δ = 72.95, 72.10, 71.91, 70.68, 70.65, 70.57, 70.52, 70.17, 69.70, 58.99, 33.85, 29.43, 29.02, 28.16, 25.41, 24.49. MS-ESI (m/z): 695.2 [M+Na^+]. IR (NaCl, film): $\tilde{\nu}$ = 2960, 1469, 1351, 1291, 1204, 1110, 962 cm^{-1}.

Synthesis of thiol 2 (HS-C6ossifluoroPEG)

Compound **J** (518 mg, 0.399 mmol) was dissolved in deoxygenated dichloromethane (5 mL) under Ar. At the mixture, trifluoroacetic acid (0.59 mL, 7.98 mmol) was added and then triisopropyl silane (0.16 mL, 0.798 mmol) was added. The reaction mixture was stirred at room temperature for 4h then the solvent was removed in vacuo and the residue co-evaporated with methanol. The crud product was purified by column chromatography over silica gel using chloroform-ethyl acetate (9:1 v/v), the solvent was removed in vacuo afford compound **2** as a colorless oil (Yield 409 mg, 97%).

^{1}H-NMR (400 MHz, CD_3OD): δ = 4.02 (t, J = 9.9 Hz, 2H, $HOC\underline{H_2}CF_2$); 3.89 (t, J = 9.8 Hz, 2H, $CH_2OC\underline{H_2}CF_2$); 3.67 (m, 44H, CH_2O), 3.30 (s, 3H, OCH_3); 2.50 (t, 2H, J = 7.1 Hz, CH_2SH), 1.62 (m, 4H, CH_2), 1.42 (m, 4H, CH_2) ppm. ^{13}C-NMR (67.8 MHz, CDCl3): δ = 72.35, 72.32, 71.65, 71.65, 70.25, 70.18, 69.979, 69.69, 69.458, 69.27, 69.21, 69.03, 57.73, 33.71, 29.14, 28.33, 27.69, 25.03, 23.49. MS-ESI (m/z): 783.2, 827.3, 871.3, 915.3, 959.3, 1003.3, 1047.4, 1091.4, 1135.4, 1201.3, 1246.3 IR (NaCl, film): $\tilde{\nu}$ = 2872, 1456, 1351, 1292, 1205, 1113, 960 cm^{-1}.

Synthesis of HS-C6ossifluoroEG3 (F-MPCs 3) and HS-C6ossifluoroPEG (F-MPCs 4) monolayer-protected gold clusters

Gold nanoparticles protected by a homoligand monolayer of thiol **1** or thiol **2** were prepared following the homogeneous-phase synthesis reported by some of us for the synthesis of small water soluble nanoparticles[1]

Synthesis of F-MPCs 3a

$HAuCl_4 \cdot 3H_2O$ (81.5 mg, 0.207 mmol) and 50 ml of milliQ water were introduced into a 250 ml round-bottom flask. To the pale yellow solution, thiol **1** (242 mg, 0.41 mmol) dissolved in 50 ml of deoxygenated methanol was added. The solution turned immediately reddish-brown; the mixture was left to stir for 30 minutes, while its color faded on standing. The solution was cooled to 0 °C and kept at that temperature for 30 minutes, further fading was observed. A solution of $NaBH_4$ 0.1M (23.6 mL, 2.4 mmol) in milliQ water was added in 10 seconds, the reaction mixture turned immediately dark-brown, the mixture was stirred at 0 °C for 30 minutes and then at room temperature for 2 hours. The solvent was removed under reduced pressure without heating above 35 °C. The residue was concentrated to a small volume and treated with ether in order to precipitate the nanoparticles. The black solid was washed copiously with ether and purified by gel permeation chromatography (Sephadex LH-20, methanol) and by dialysis giving 115 mg of nanoparticles.

^{1}H-NMR (500 MHz, CD_3OD): δ = 4.02 (br,); 3.89 (br); 3.77 (br), 3.66 (br), 3.30 (s, 3H, OCH_3); 1.59 (br), 1.40 (br) ppm. ^{19}F NMR (300 MHz, CD_3OD): δ = -83.0-84.0 (br), -93-95.2 (br). IR (NaCl, film): $\tilde{\nu}$ = 2916, 1453, 1280, 1192, 1107, 957, 822. UV-Vis (methanol): λmax = 385 nm.

Synthesis of F-MPCs 4a

$HAuCl_4 \cdot 3H_2O$ (78.6 mg, 0.199 mmol) and 50 ml of milliQ water were introduced into a 250 ml round-bottom flask. To the pale yellow solution, thiol **2** (422 mg, 0.399 mmol) dissolved in 50 ml of deoxygenated methanol was added. The solution turned immediately reddish-brown; the mixture was left to stir for 30 minutes, while its color faded on standing. The solution was cooled to 0 °C

and kept at that temperature for 30 minutes, further fading was observed. A solution of $NaBH_4$ 0.1M (22 mL, 2.19 mmol) in milliQ water was added in 10 seconds, the reaction mixture turned immediately dark-brown, the mixture was stirred at 0°C for 30 minutes and then at room temperature for 2 hours. The solvent was removed under reduced pressure without heating above 35 °C. The residue was concentrated to a small volume and treated with ether in order to precipitate the nanoparticles. The black solid was washed copiously with ether and purified by gel permeation chromatography (Sephadex LH-20, methanol) and by dialysis giving 165 mg of nanoparticles.
^{1}H-NMR (500 MHz, CD_3OD): δ = 4.04 (br,); 3.87 (br); 3.76 (br), 3.64 (br), 3.30 (s, 3H, OCH_3); 1.60 (br), 1.40 (br) ppm. ^{19}F NMR (300 MHz, CD_3OD): δ = -83.0-84.0 (br), -93-95.5 (br). IR (NaCl, film): $\tilde{\nu}$ = 2872, 1457, 1350, 1291, 1203, 1112, 959, 851. UV-Vis (methanol): λmax = 383 nm.
Average composition from TEM and TGA measurements: $Au_{100}SR_{60}$
ζ potential: -24.6 mV in water and -10.1 mV in PBS 1% solution.

Synthesis of fluorescently labelled nanoparticles F-MPCs 3b and F-MPCs 4b by place exchange reactions

The synthesis of fluorescent dye **5** followed the procedure reported by Stellacci and coworkers.[2] 2.3 mg of BODIPY-SH dye **5** was dissolved in 1.6 mL of a deionized water/dimethylformamide mixture (2:1 vol:vol). This solution was used as a stock solution for all of the place exchange reactions.

Synthesis of F-MPCs 3b

10 mg of F-MPCs **3a** (~1.7 x 10^{-7} mol) were dissolved in 5 mL of deoxygenated methanol. Then, 430 µL (~8.3 x 10^{-7} mol) of the dye stock solution was added to this nanoparticle solution and left in a thermostatic bath at 28°C for 4 days in a sealed and dark environment. The residue was concentrated to a small volume and purified by gel permeation chromatography (Sephadex LH-20, methanol) giving 8.2 mg of dye functionalized nanoparticles (F-MPCs **3b**) free of unbound ligands. The number of fluorescent dyes per nanoparticle was determined from UV-Vis absorption at 650 nm after decomposition of a small quantity of nanoparticle with KCN solution and using Lambert-Beer low; this value is 2.3 dye/nanoparticle.

Synthesis of MPCs 4b

10 mg of F-MPCs **4a** (~1.3 x 10^{-7} mol) were dissolved in 5 mL of deoxygenated methanol. Then, 400 µL (~7.8 x 10^{-7} mol) of the dye stock solution was added to this nanoparticle solution and left

in a thermostatic bath at 28°C for 4 days in a sealed and dark environment. The residue was concentrated to a small volume and purified by gel permeation chromatography (Sephadex LH-20, methanol) giving 9.1 mg of dye functionalized nanoparticles (F-MPCs **4b**) free of unbound ligands. The number of fluorescent dyes per nanoparticle was determined from UV-Vis absorption at 665 nm after decomposition of a small quantity of nanoparticle with KCN solution and using Lambert-Beer low; this value is 2 dye/nanoparticle.

F-MPCs 3a (OEG3)

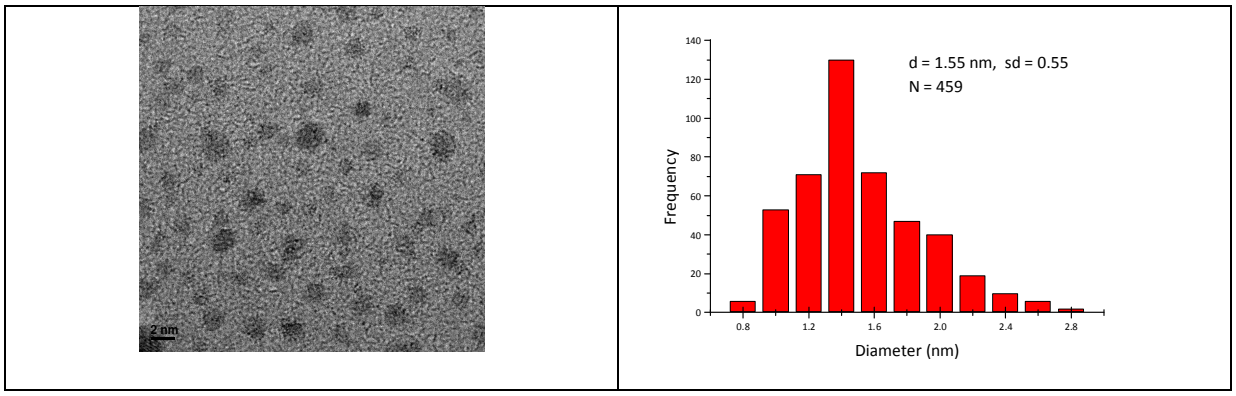


Figure S1. A representative TEM image and histogram of F-MPCs **3**.

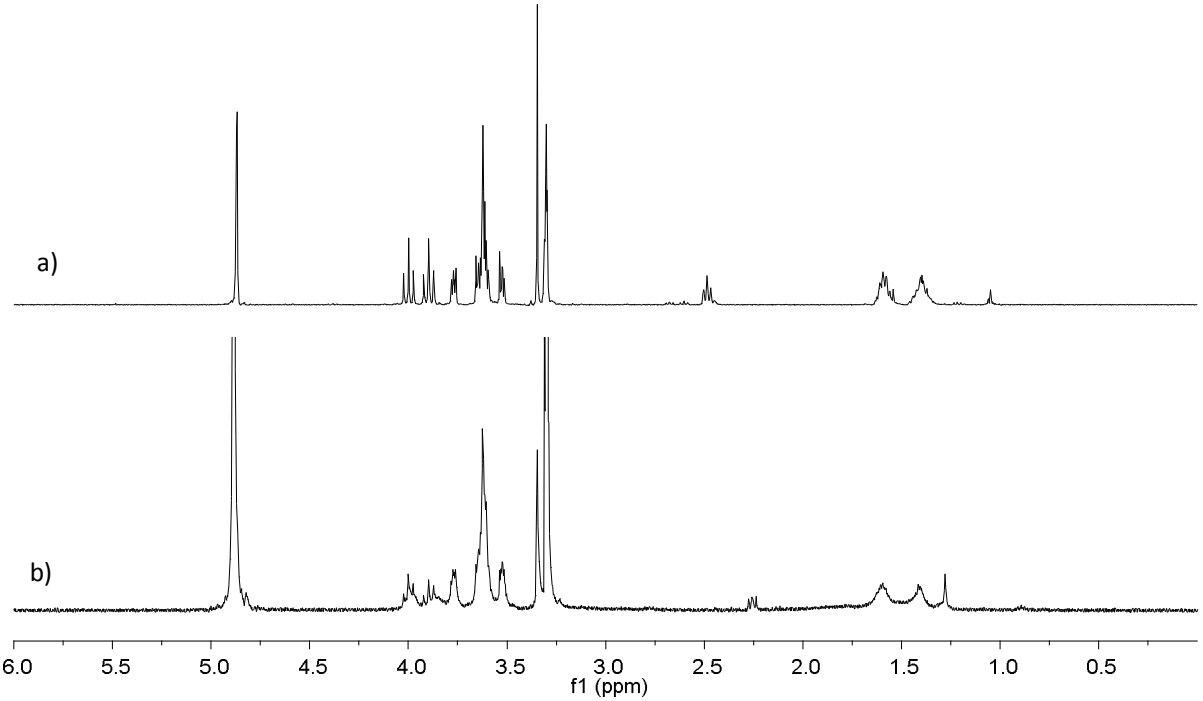


Figure S2. ^{1}H-NMR (500 MHz, CD_3OD) spectra of: a) thiol **1** and b) F-MPCs **3a**.

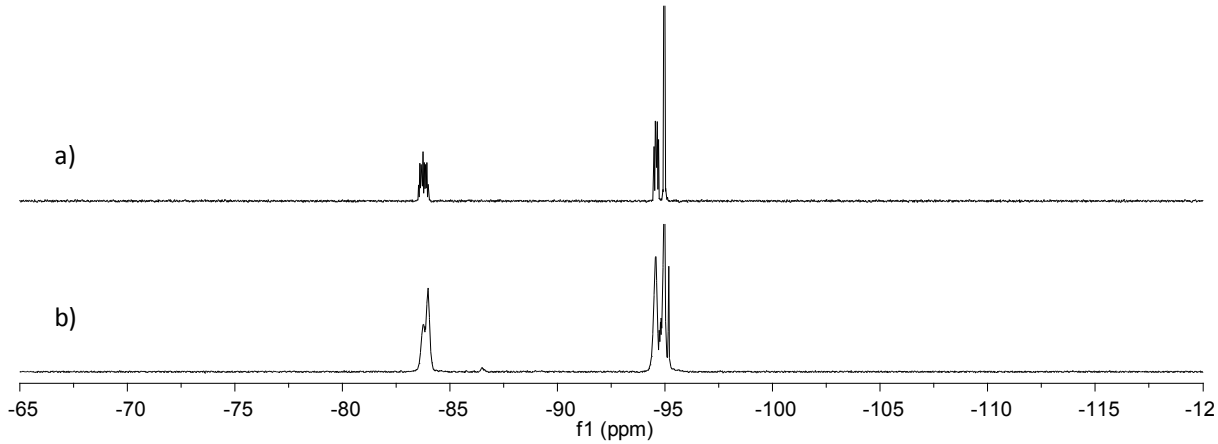


Figure S3. ^{19}F NMR (300 MHz, CD_3OD) spectra of: a) protected thiol **I** and b) F-MPCs **3a**.

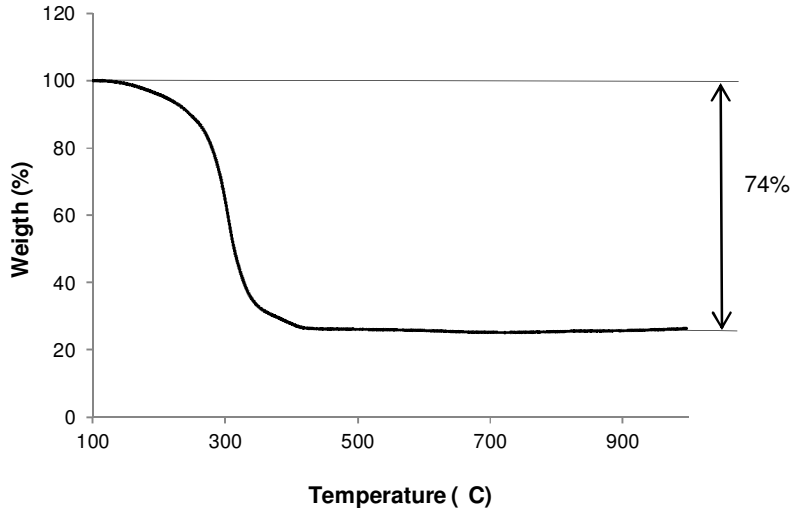


Figure S4. TGA of F-MPCs **3a**.

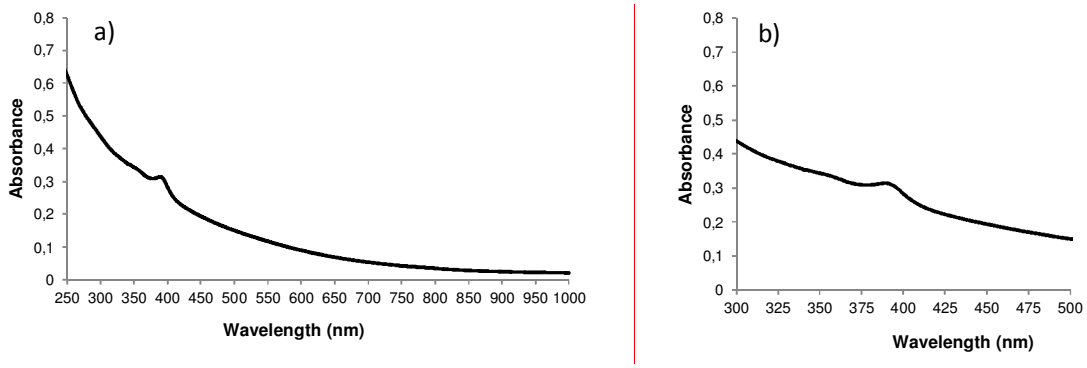


Figure S5. a) UV-Vis spectrum of F-MPCs **3a** in methanol (c = 0.5 mg/mL) and b) enlargement from 300 to 500 nm.

SUPPORTING INFORMATION

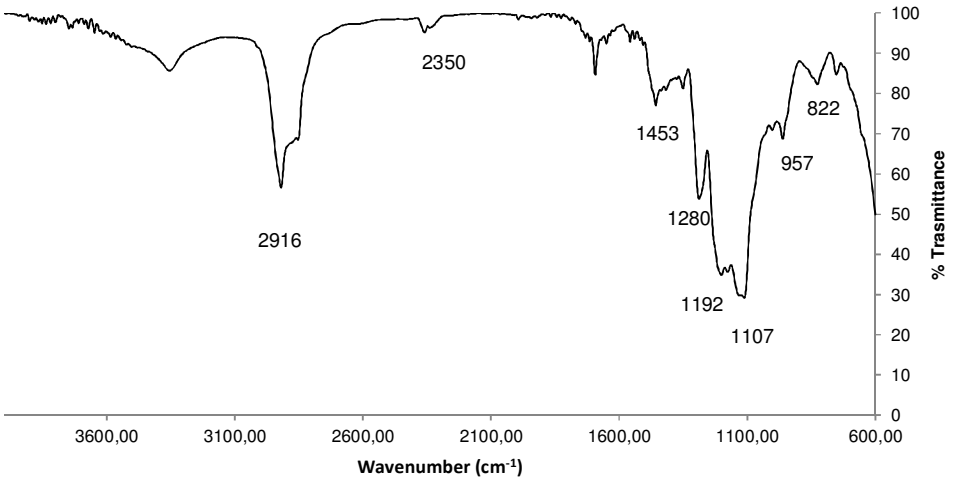


Figure **S6**. IR of F-MPCs **3a**.

F-MPCs 4a (PEG550)

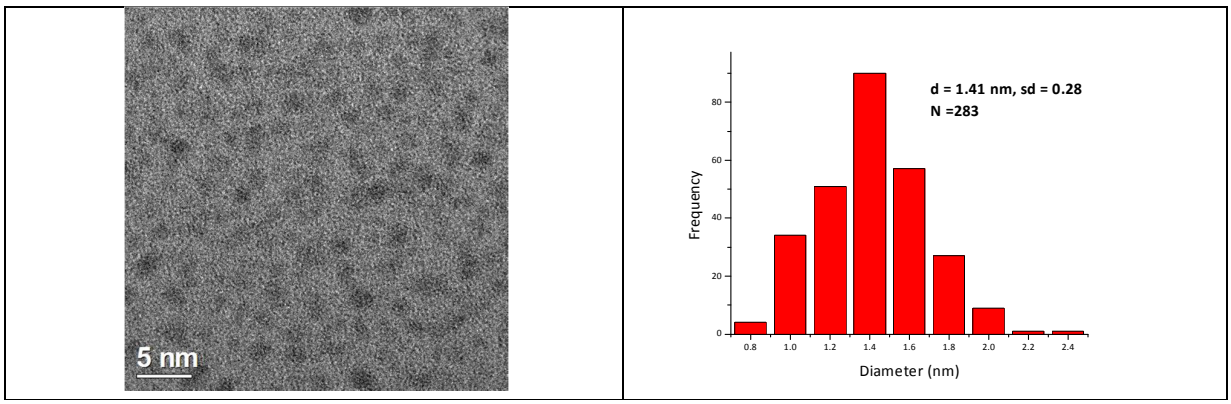


Figure S7. A representative TEM image and histogram of F-MPCs **4a**

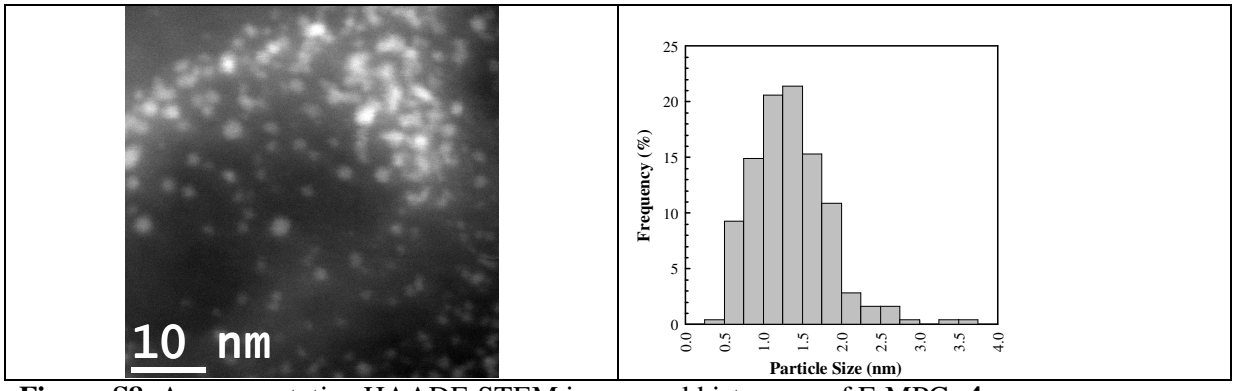


Figure S8. A representative HAADF-STEM image and histogram of F-MPCs **4a**.

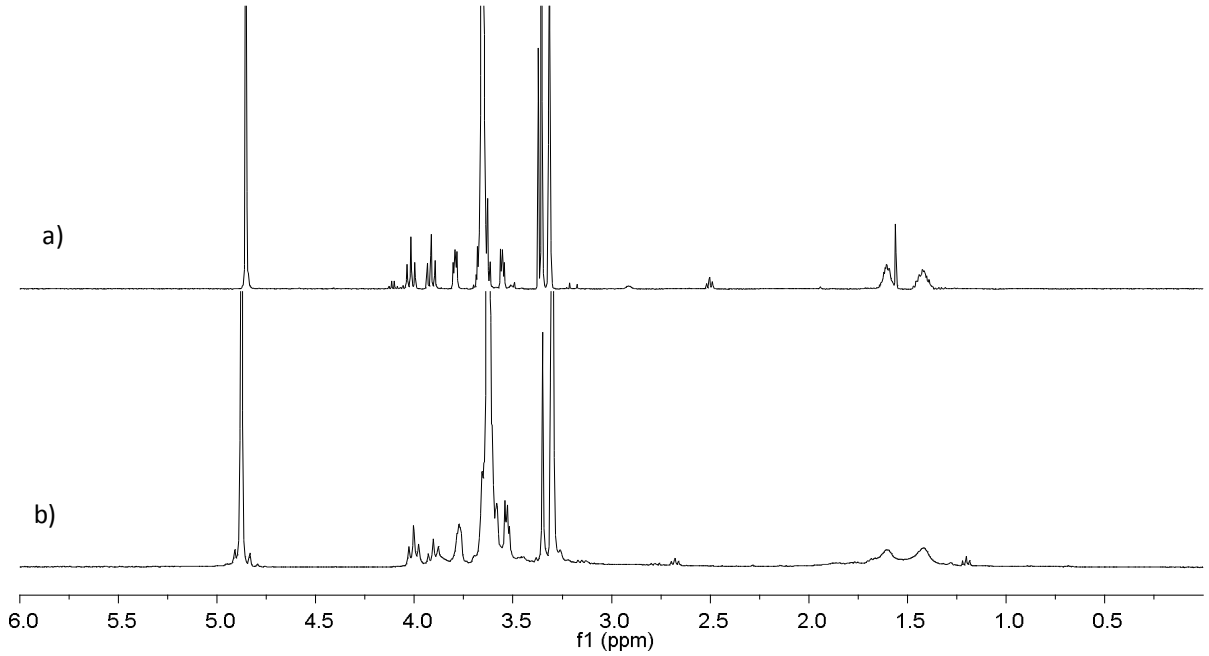


Figure S9. ^{1}H-NMR (500 MHz, CD_3OD) spectra of: a) thiol **2** and b) F-MPCs **4a**.

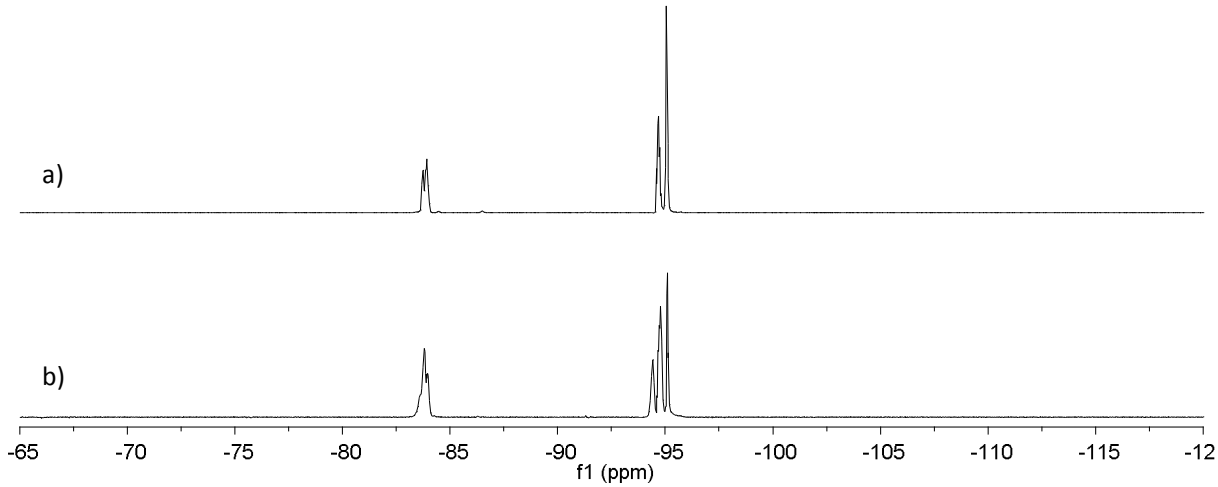


Figure S10. ^{19}F NMR spectra of: a) protected thiol **J** and b) F-MPCs **4a**.

SUPPORTING INFORMATION

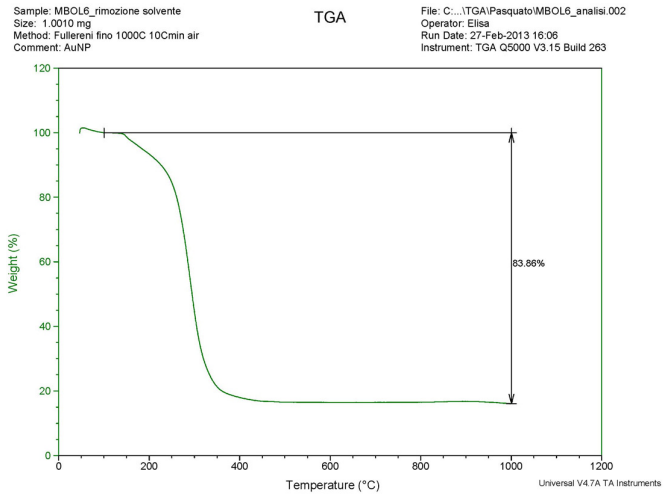


Figure S11. TGA of F-MPCs **4a**.

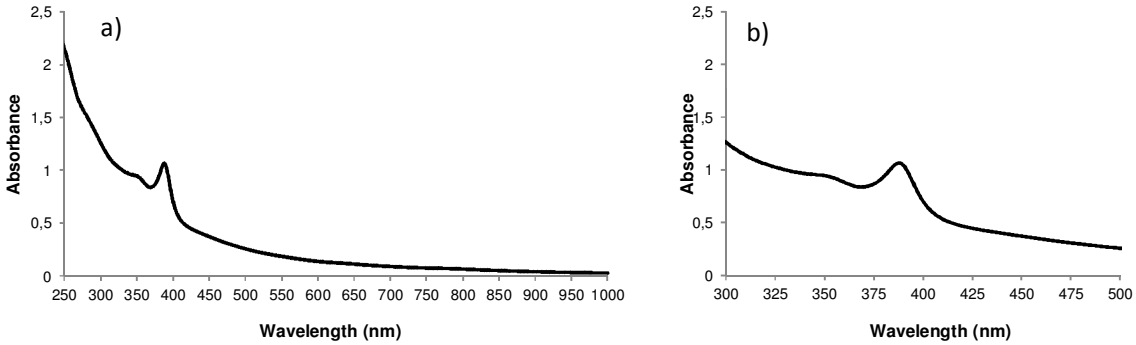


Figure S12. a) UV-Vis spectrum in methanol of F-MPCs **4a** in methanol (c = 0.8 mg/mL) and b) enlargment from 300 to 500 nm.

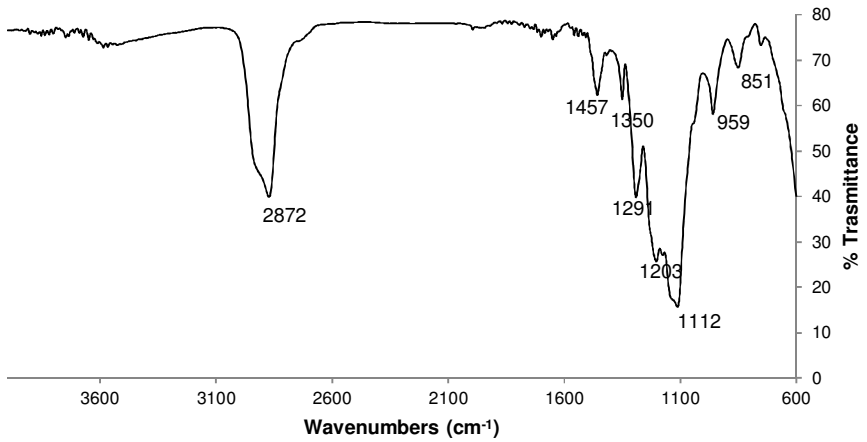


Figure S13. IR of F-MPCs **4a**.

SUPPORTING INFORMATION

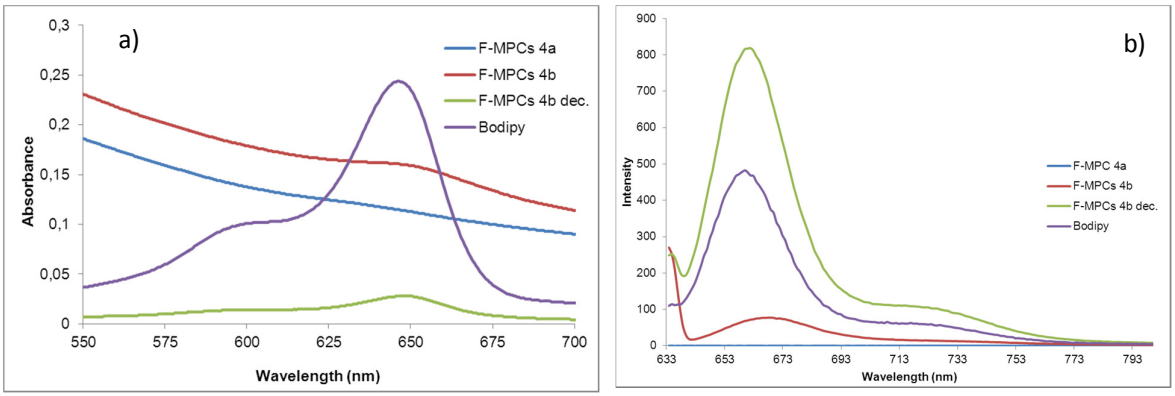


Figure S14. a) UV-Vis spectra of F-MPCs 4a (0.8 mg/mL), F-MPCs 4b (0.9 mg/mL) , F-MPCs 4b after addition of KCN (0.83 mg/mL of F-MPCs 4b) and Bodipy ($7x10^{-5}$M) in methanol and b) emission spectra (λ_{exc} = 650 nm) of F-MPCs 4a (0.8 mg/mL), F-MPCs 4b (0.9 mg/mL) , F-MPCs 4b after addition of KCN (0.83 mg/mL of F-MPCs 4b) and Bodipy ($1.6x10^{-5}$M) in methanol.

MRI experiments. MRI experiments were performed on a BioSpec 70/30 USR (Bruker, Ettlingen, Germany) preclinical MRI scanner. The system has a magnetic field strength of 7T and a 30 cm bore diameter. The scanner is equipped with an actively shielded gradient system with integrated shims set up to 2nd order. The maximum gradient amplitude is 400 mT/m. Studies of ^{1}H MRI, ^{19}F MRI and ^{19}F MRS were carried out using a transceiver double tunable ^{1}H/^{19}F linear birdcage RF coil having a diameter of 72 mm. This coil is able to perform MR experiments both on ^{1}H and ^{19}F nuclei in the same session, maintaining the same set-up. All measurements were done at room temperature. The test tube containing a 2 ml solution of free ligands or F-MPCs was placed at the gradient isocenter and the magnetic field homogeneity within the field of view (FOV) was guaranteed by a 2nd order shimming procedure. Then the sequences protocol was optimized to obtain the best sensitivity from the ^{19}F signal. First, from the spectral data acquired by single pulse excitation sequences, the correct resonance frequency of the compound and pulse gain in the 7T field was determined. From the spectrum analysis, the peaks number and position were also determined for the receiver bandwidth selection. T_1, T_2 and T_2* relaxation times were then assessed by means of the following sequences: rapid acquisition with relaxation enhancement (RARE) sequences with varying repetition times (TR: 3000, 1000, 800, 600, 400, 200, 100 ms) allowed the creation of T_1 maps; multislice multiecho sequences with 40 echoes and 10.5 ms echo spacing was used for T_2 mapping and multi gradient echo sequences with 40 echoes 2 ms echo spacing for T_2* mapping. In order to optimize the imaging protocol, MRI of the sample was carried out by means of RARE sequences (Rare Factor RF = 8; Slice thickness ST = 5 mm; Field of View FOV = 5,50 x 5,50 mm^2; Acquisition Time AT = 1h5min; number of averages NA = 3000) with varying TR, excitation bandwidth (EBW) and matrix size (MTX). The resulting optimal setting was used in the

subsequent acquisitions. In order to evaluate the minimal concentration detectable in an acquisition time compatible with future *in vivo* applications, the same protocol (TR = 1303 ms; MTX = 32 px; Res = 1,719 mm/px; EBW = 2700 Hz) were applied to F-MPC mother solution (40 mg/ml for MPCs **3a** and 18.75 mg/ml for F-MPCs **4a** and to the diluted samples.

Cell culture and fluorescence imaging. Human cervical carcinoma cells (HeLa) were grown in a standard culture media at 37 °C and in 95% air, 5% CO_2 atmosphere. Cells were seeded in a µ-Slide 8-well ibidi plate (Martinsried, Germany) at a density of 5 x 10^4 cells per well (1.0 cm^2) and were allowed to adhere overnight. Before cell incubation with nanoparticles, the medium containing fetal bovine serum was replaced with serum-free medium to avoid unspecific binding of the NPs to serum proteins. Cells were then incubated with 2.5 µM of F-MPCs labeled with BODIPY (650/665 nm) for 4h at 37 °C. After incubation the cells were washed 3 times with PBS. Nuclei were counterstained with Hoechst 33342 (Invitrogen, Oregon, USA), according to the manufacturer's instructions. Cellular internalization of the fluorescently labeled F-MPCs was visualized with an inverted confocal laser scanning microscope (CLSM, Carl Zeiss LSM 510) equipped with a 63×/1.3 oil DIC objective, using excitation lines at 405 (Hoechst 33342) and 633 nm (BODIPY (650/665 nm)). ImageJ software was used for image analysis.

Cell Viability – FACS analysis. HeLa cell incubation with BODIPY F-MPCs in a concentration range of 0.6-10 µM mg/mL was performed as described above. After incubation, 5 x10^5cell were collected by trypsination and washed with PBS containing 1% BSA. The staining of nonviable cells was performed with propidium iodide (50 µg/mL in PBS) for 5 minutes at room temperature. The samples were analyzed immediately on a flow cytometer (FACS Canto II, BD Biosciences) with excitation at 488 nm for PI and an excitation at 633 nm for BODIPY.

SUPPORTING INFORMATION

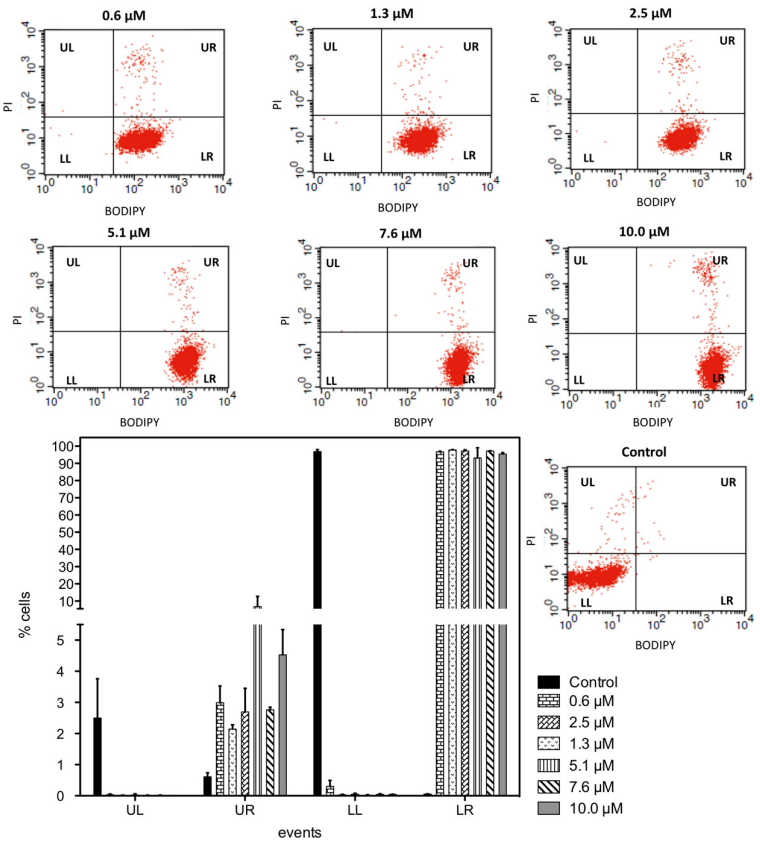


Figure S15. FACS analysis of HeLa cells incubated with F-MPCs **4b** for 4h at different concentration. The events represent: UL is the percentage dead cells without F-MPCs; UR is the percentage dead cells with F-MPCs; LL is the percentage viable cells without F-MPCs; LR is the percentage viable cells with F-MPCs.

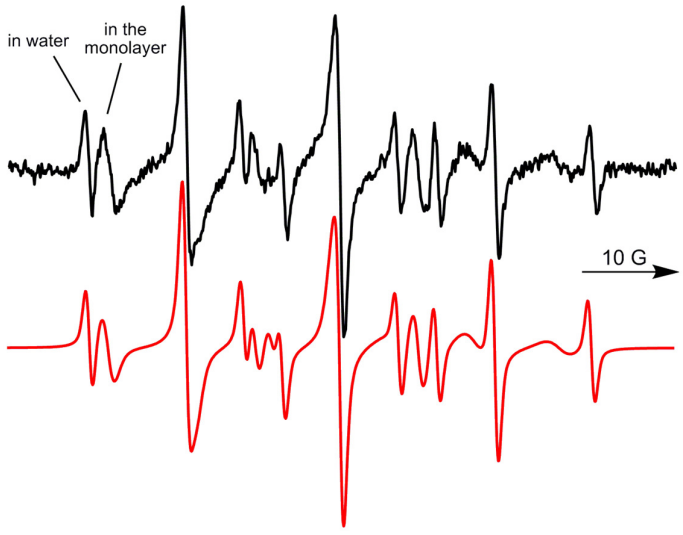


Figure S16. ESR spectrum (in black) recorded at 298 K (H_2O/MeOH, 90/10) in the presence of F-MPC **4a**, 0.039 mM ([RS] = 2.34 mM) and the corresponding computer simulation (in red). The signals of radicals both in water and in the monolayer are present.

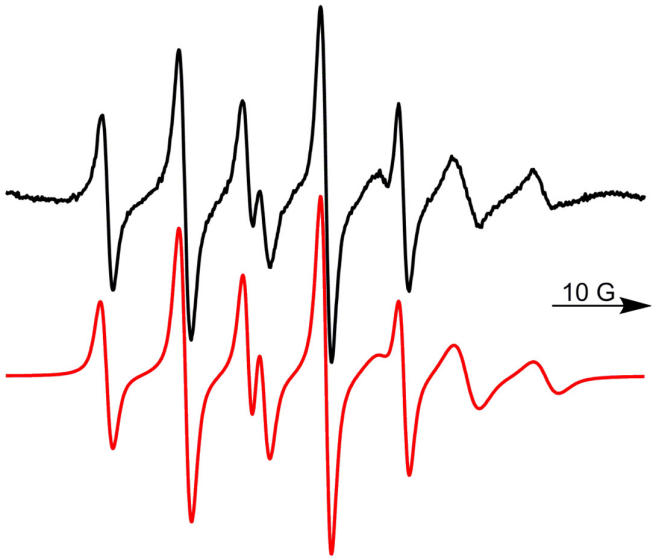


Figure S17. ESR spectrum (in black) recorded at 298 K (H_2O/MeOH, 90/10) in the presence of F-MPC 1.97 mM ([RS] = 118.2 mM) and the corresponding computer simulation (in red). Only the radical partitioned in the monolayer is present.

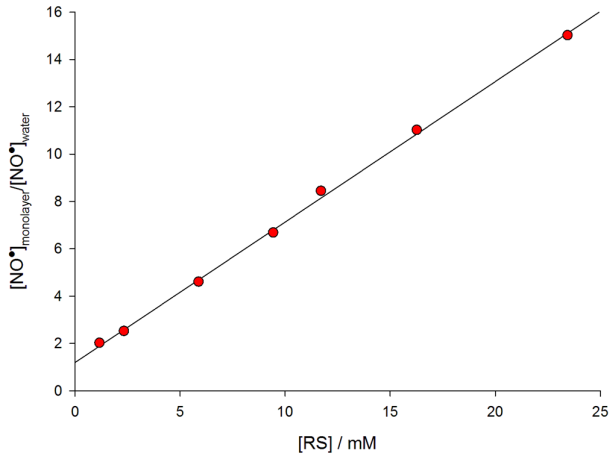


Figure S18. Plot of the ratio between the concentration of the radical probe **6** partitioned in the monolayer and that of the free species as a function of thiol **2** concentrations bound to the gold.

References

1. Pengo, P.; Polizzi, S.; Battagliarin, M.; Pasquato, L.; Scrimin, P. *J. Mater. Chem.* **2003**, *13*, 2471–2478.

2. Verma, A.; Uzun, O.; Hu, Y.; Hu, Y.; Han, H.-S.; Watson, N.; Chen, S.; Irvine, D. J.; Stellacci, F. *Nature Materials* **2008**, *7*, 588-595.