Supporting Information

Materials

All reagents and solvents were obtained from Acros Organics (Geel, Belgium), ABCR (Karlsruhe, Germany), BDH prolabo (Leuven, Belgium), Sigma-Aldrich (Bornem, Belgium), Alfa Aesar (Ward Hill, USA), Chemlab (Zedelgem, Belgium) and all the lanthanide oxides were purchased from Heraeus (Hanau, Germany) and were used without further purification.

Instrumentation

Elemental analysis was performed with a CE Instruments EA-1110 elemental analyzer. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker Avance 300 MHz for ^{1}H and 75 MHz for ^{13}C. Mass spectra were obtained with a Thermo Finnigan LCQ Advantage mass spectrometer. IR spectra were measured using a Bruker Vertex 70 FT-IR spectrometer (Bruker, Ettlingen, Germany) equipped with a Platinum ATR accessory and data were processed with OPUS 6.5 software. Preparative HPLC was performed by using a Waters Delta 600 system equipped with a Waters 996 Photo Diode Array detector. A Phenomenex Luna C18 column (150 mm × 21.20 mm, particle size 5 µm) was employed. Lanthanide concentrations were obtained using a TXRF Bruker S2 Picofox with a Bruker AXS sample carrier.[1, 2] An internal standard a Chem-Lab gallium(III) standard solution (1000 µg mL^{-1}, 2-5% HNO_3) was further diluted to 20 ppm and added to an equivolume of the sample. The resulting solution was dropcasted on a hydrophobic Quartz plate and dried in an oven to remove all solvent. Three Quartz plates were made in the same manner and as such used to determine the concentrations. The collected data were processed with excel to retrieve the average value with their standard deviation. Vacuum dried nanoparticles suitable for powder X-ray diffraction were mounted on a nylon loop attached to a copper pin and placed on an Agilent SuperNova diffractometer at room temperature (293 K) using Mo Kα radiation (l= 0.71073 Å) and the absorption corrections were applied using CrysAlisPro.[3] Transmission electron microscopy measurements were performed using a 80 kV Zeiss EM-900 using 300 mesh Formvar/carbon coated copper grids. Distribution data were calculated by ImageJ. Oleic acid-coated nanoparticles were dispersed in toluene while the citrate-coated nanoparticles and the nanoconstruct were dispersed in ethanol and subsequently deposited onto the grid. Nanoparticles were dispersed in a 180 W Bandelin Sonorex RK 510 H sonicator. Photon correlation spectroscopy was performed at room temperature with a BIC multiangle laser light scattering system with a 90º scattering angle (Brookhaven Instruments Corporation, Holtsville, USA). Analysis was done using Igor Pro 6.20 software. Photophysical data were recorded with an Edinburgh Instruments FS920 steady-state spectrofluorimeter. This instrument was equipped with a 230 mW diode laser (980 nm, Power Technology), a 450 W Xenon arc lamp and a high energy microsecond flashlamp µF900H and an extended re-sensitive photomultiplier (185-1010 nm, Hamamatsu R 2658P). All spectra were analyzed with Edinburgh software and corrected for the instrumental functions. The exponential fitting of the lifetimes measurements were done using Igor Pro 6.20 software. Proton Nuclear Magnetic Relaxation Dispersion (NMRD) profiles were measured with a Stelar Spinmaster FFC, fast-field cycling NMR relaxometer Stelar, Mede PV, Italy) over a magnetic field strength range that extended from 0.24 mT to 0.7 T. Measurements were performed on samples (0.6 mL) contained in 10 mm (outside diameter) Pyrex tubes. Additional relaxation rates at 20, 60 and 300 MHz were obtained with a Minispec mq20, a Minispec mq60 and a Bruker Avance 300 spectrometer, respectively (Bruker, Karlsruhe, Germany). The proton NMRD curves were fitted using data-processing software,[4, 5] including different theoretical models describing the nuclear relaxation phenomena (Minuit, CERN Library).[6-8]

Synthesis

tert-butyl 2,2',2''-(1,4,7,10-tetraazacyclododecane-1,4,7-triyl)tri-acetate (1):

The synthesis was based on an existing procedure.[9, 10] To a solution of 210 mg (1.22 mmol) tetraazacyclodedecaan, 453 mg (3.5 eq) sodium carbonate in 150 mL ACN under an argon atmosphere a mixture of 616 µL (3.5 eq) tert-butyl bromoacetate in 50 mL ACN was added dropwise. The solution was refluxed for 17h. After removing the salts via filtration the compound was purified using column chromatography (Al_2O_3, DCM/MeOH 98:2) and 514 mg (0.939mmol, 77%) tert-butyl 2,2',2''-(1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate **1** as an off-white powder was retrieved after removal of the solvents under reduced pressure. ESI-MS in DCM: m/z calcd 516 $[M+H]^+$, 538 $[M+Na]^+$, found 516.0 $[M+H]^+$, 537.5 $[M+Na]^+$. ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 1.46 (s, 27H, ^{t}Bu C**H$_3$**), 2.88 (t, 12H, cyclenring C**H$_2$**), 3.08 (t, 4H, cyclenring C**H$_2$**), 3.30 (s, 2H, N-C**H$_2$**-COO^{t}Bu), 3.38 (s, 4H, N-C**H$_2$**-COO^{t}Bu). Elemental analysis: calcd. (%) for $C_{26}H_{50}N_4O_6(H_2O)_4$: C:53.22, H:9.96, N: 9.55, O: 27.27 , found C:52.62, H:9.04, N: 9.34.

tert-butyl 2,2',2''-(10-(2-(tert-butoxycarbonylamino)ethyl)-1,4,7,10-tetraazacyclo-dodecane-1,4,7-triyl)triacetate (2):

A solution of 500 mg (0.81 mmol) of product **1**, 217.8 mg (1.2 eq) 2-(Boc-amino)ethylbromide, 134.35 mg (1.2 eq) potassium carbonate in 25 mL ACN was stirred at 353 K for 17h. The salts were removed via filtration and 335.5 mg (0.51 mmol, 63%) of product **2** was obtained after column chromatography (Al_2O_3, DCM/MeOH 98:2). ESI-MS in DCM: m/z calcd 658.9 $[M+H]^+$, 680.9 $[M+Na]^+$, found 659.7 $[M+H]^+$, 680.7 $[M+Na]^+$. ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 1.46 (s, 36H, ^{t}Bu C**H$_3$**), 2.46 (t, 2H, -CH$_2$-C**H$_2$**-NH-), 2.53 (t, 4H, cyclenring C**H$_2$** 1^e gen ethylamine), 2.78 (t, 4H, cyclenring C**H$_2$** 2^e gen ethylamine), 2.85 (t, 8H, cyclenring C**H$_2$** rest), 3.15 (t, 2H, -C**H$_2$**-CH$_2$-NH-), 3.28 (s, 4H, N-C**H$_2$**-COO^{t}Bu), 3.32 (s, 2H, N-C**H$_2$**-

COO^{t}Bu). ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 27.4, 28.0, 28.4 & 28.7 (4C, ^{t}Bu **C**H_3), 38.9 (1C, -CH_2-**C**H_2-NH-), 51.6 (4C, cyclenring **C**H_2), 52.7 (2C, cyclenring **C**H_2), 53.1 (1C, -**C**H_2-CH_2-NH-), 55.6 (2C, cyclenring **C**H_2), 56.6 (3C, N-**C**H_2-COO^{t}Bu), 78.1 (1C, -NH-CH_2-COO^{t}Bu), 80.6 (3C, N-**C**H_2-COO^{t}Bu), 156.2 (1C, -NH- **C**OO^{t}Bu), 170.7 (3C, N-CH_2-**C**OO^{t}Bu). Elemental analysis: calcd. (%) for $C_{33}H_{63}N_5O_8$: C:60.25, H:10.65, N: 10.65, O: 18.46 , found C:59.89, H:10.80, N: 10.67.

2,2',2''-(10-(2-aminoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl) triacetic acid TFA salt(3):

300 mg (0.456 mmol) of product **2** was stirred at room temperature for 12h in 20 mL DCM/TFA (1:3). The raw material was obtained after removal of the solvents under reduced pressure and dissolved in 5 mL of water. 143.6 mg product **3** was obtained as an off-white solid after precipitation with Et_2O and centrifugation. ESI-MS in DCM: m/z calcd 504.5 [M], found 504.2 [M]. ^{1}H NMR (D_2O, 300 MHz): δ (ppm) 2.85 (t, 4H, cyclenring C**H₂**), 2.96 (t, 4H, cyclenring C**H₂**), 3.22 (t, 4H, cyclenring C**H₂**), 3.29 (t, 4H, cyclenring C**H₂**), 3.34 (t, 2H, -CH_2-C**H₂**-NH_2), 3.45 (t, 2H, -C**H₂**-CH_2-NH_2), 3.59 (s, 2H, N-C**H₂**-COOH), 3.82 (s, 4H, N-C**H₂**-COOH). ^{13}C NMR (D_2O, 75 MHz): δ (ppm) 36.10 (1C, -CH_2-**C**H_2-NH_2), 47.90, 48.13, 48.87, 50.07, 50.40, & 51.97 (8C, cyclenring **C**H_2), 52.43 (1C, -**C**H_2-CH_2-NH_2), 56.97 (3C, N-**C**H_2-COOH), 110.51, 114.35, 118.29 & 122.08 (1C, **C**F_3COO^-), 162.07, 162.48, 163.09 & 163.49 (1C, CF_3**C**OO^-), 170.26 (2C, N-CH_2-**C**OOH), 175.30 (1C, N-CH_2-**C**OOH). Elemental analysis: calcd. (%) for $C_{16}H_{35}N_5O_6(CF_3COOH)_2$: C:35.92, H:5.21, N: 9.52, O: 26.10, F: 23.25 , found C:36.56, H:5.63, N: 10.04

Lanthanide(III) 2,2',2''-(10-(2-aminoethyl)-1,4,7,10-tetraazacyclo-dodecane-1,4,7-triyl)triacetate (4):

A solution of 140 mg (0.36 mmol) **3**, 10 mL water and 1.05eq $LnCl_3.6H_2O$ was brought to a pH of 5.5 with 0.2 M KOH. The solution was stirred at 323 K until the pH remained constant, where after the complex **4** was purified with HPLC (H_2O/ACN*0-20-20-100*10-15-10min) and the absence of free lanthanide(III)-ions was ascertained using Arsenazo(III)[11]. Product **4** was retrieved as a white solid.

Lanthanum: 409.1 mg (74%), ^1H NMR (D_2O, 300 MHz): δ (ppm) 2.81-3.35 (m, 16H, cyclenring C**H₂**), 3.54 (t, 2H, $-CH_2-C\mathbf{H_2}-NH_2$), 3.59 (s, 2H, $N-C\mathbf{H_2}-COOH$), 3.82 (s, 4H, $N-C\mathbf{H_2}-COOH$), 4.39 (t, 2H, $-C\mathbf{H_2}-CH_2-NH_2$). ^{13}C NMR (D_2O, 75 MHz): δ (ppm) 37.13 (1C, $-CH_2-\mathbf{C}H_2-NH_2$), 40.40, 41.65, 44.64, 48.06, 50.02, 50.32, 52.16 & 53.73 (8C, cyclenring $\mathbf{C}H_2$), 57.09 (1C, $-\mathbf{C}H_2-CH_2-NH_2$), 60.33, 63.35 & 66.05 (3C, $N-\mathbf{C}H_2-COOH$), 170.71 (2C, $N-CH_2-\mathbf{C}OOH$), 179.55 (1C, $N-CH_2-\mathbf{C}OOH$). FT-IR (ῦ, cm^{-1}): 3422 (NH_2 stretch), 3008-2908-2878 (aliphatic asymmetric and symmetric stretches), 1672-1585 (asymmetric CO stretch), 1438-1400 (symmetric CO stretch). Elemental analysis: calcd. (%) for C16H27N5O6La: C: 36.65, H: 5.19, N: 13.36, O: 18.31, found C: 35.93, H: 5.24, N: 13.02.

Europium: 265.7 mg (77%), FT-IR (ῦ, cm^{-1}): 3402 (NH_2 stretch), 2987-2933-2879 (aliphatic asymmetric and symmetric stretches), 1691-1608 (asymmetric CO stretch), 1456-1437 (symmetric CO stretch)

Gadolinium: 155.4 mg (79%), FT-IR (ῦ, cm-1): 3413 (NH_2 stretch), 2995-2925-2883 (aliphatic asymmetric and symmetric stretches), 1685-1606 (asymmetric CO stretch), 1458-1427 (symmetric CO stretch)

Oleate-capped NaGdF4:Yb3+,Tm3+ Nanoparticles:[12-14]

Using a two-step process starting with the preparation of the lanthanide trifluoroacetate precursors, followed by thermal decomposition reaction, the oleate-capped nanoparticles were synthesised. The lanthanide precursors were generated via the addition of 10 mL of a water/TFA (1:1) mixture to 337.6 mg (9.31×10^{-4} mol, 74.5 mol%) Gd_2O_3, 123.2 mg (3.13×10^{-3} mol, 25 mol%) Yb_2O_3 and 2.4 mg (6.25×10^{-6} mol, 0.5 mol%) Tm_2O_3 and the solution was left to reflux until the solution was clear, where after it was dried under atmospheric pressure at 333 K. In the second step 12.5 mL of oleic acid and 12.5 mL of 1-octadecene were added to a 3-neck round bottom flask. The solution was degassed at 423 K for 30 minutes under vacuum (A). To the dried lanthanide precursors 7.5 mL oleic acid, 7.5 mL 1-octadecene and 340.0 mg (2.50×10^{-3} mol) CF_3COONa were added (B). B was successively degassed at 323 K, 348 K and 403 K respectively for 10 minutes under vacuum. Subsequently, A was placed under a gentle flow of argon and heated to 583 K. Using a syringe and pump system B was added at a rate of 1.5 $mLmin^{-1}$ to A. After addition the mixture was left stirring for 60 minutes at 583 K, where after the solution was cooled down to room temperature and the nanoparticles were precipitated with ethanol and recovered via centrifugation. The nanoparticles were dispersed in hexane and precipitated and purified using ethanol. A general production under these conditions deliver 1.0204 g nanoparticles and were stored as a wet solid under ethanol at room temperature. FT-IR (ʋ̃, cm-1): 1469 (CO symmetric stretch), 1560 (CO asymmetric stretch), 2976 (CH stretch), 2924 (asymmetric CH_2 stretch), 2854 (symmetric CH_2 stretch).

Citrate-capped NaGdF4:Yb3+,Tm3+ Nanoparticles:

200 mg of oleate-capped $NaGdF_4:Yb^{3+},Tm^{3+}$ nanoparticles were dispersed in 20 mL of a 0.2 M citric acid buffer (pH=3) and subsequently shaken for 3h at 800 rpm. The solution was extracted with cyclohexane (3 x) and diethylether (3 x). The particles were then precipitated with acetone and recovered via centrifugation. After recovery the particles were dispersed in 20 mL of a 0.2

M citric acid buffer (pH=6) and subsequently shaken for 12h at 800 rpm. The solution was again extracted with cyclohexane (3 x) and diethylether (3 x) and 175.26 mg citrate-capped nanoparticles were obtained via precipitation with acetone and centrifugation and kept at room temperature under ethanol. FT-IR (ῦ, cm^{-1}): 1388 (CO symmetric stretch), 1583 (CO asymmetric stretch), 3352 (OH stretch), 1263 (OH bending).

Nanoconstruct:

Synthesis was based on literature procedure.[15] Under dry conditions 100 mg (0.18 mmol) of product **(4)** was dissolved in 5 mL of DMF and 48 µL (1.5 eq) of DIPEA. This mixture was stirred at room temperature for 15 min under an argon atmosphere. In another flask 100 mg of citrate-capped $NaGdF_4:Yb^{3+},Tm^{3+}$ nanoparticles were sonicated at room temperature for 15 min under argon atmosphere in 5 mL of dry DMF, 48 µL of dry DIPEA and 102.6 mg (1.5 eq) HATU. The first solution was then added slowly to the latter solution and the complete batch was stirred overnight at room temperature under argon atmosphere. The product was purified via successive extraction with diethylether. The diethylether phases were extracted with water and the water and DMF phases were collected, centrifuged and stored under ethanol at room temperature.

Europium: 54.6 mg, FT-IR (ῦ, cm-1): 1664-1597 (asymmetric CO stretch), 1425-1400 (symmetric CO stretch)

Gadolinium: 138.7 mg, FT-IR (ῦ, cm^{-1}): 3456 (NH and OH stretch), 1658-1599 (asymmetric CO stretch), 1409-1396 (symmetric CO stretch)

Lanthanum: Synthesis was slightly adjusted, 25 mg (1.05eq) 2-ethyl-2-hydroxybutyric acid was used instead of 100mg citrate-capped $NaGdF_4:Yb^{3+},Tm^{3+}$ nanoparticles. This experiment was performed to check the purity of the product with the NMR technique. ^1H NMR (D_2O, 300 MHz): δ (ppm) 0.72 (t, 6H, CH_3 butyric acid), 1.59 (m, 4H, CH_2 butyric acid), 2.82-3.27 (m, 16H, cyclenring C**H$_2$**), 3.62 (t, 2H, -CH$_2$-C**H$_2$**-NHCOO), 3.50 (s, 2H, N-C**H$_2$**-COOH), 3.62 (t, 2H, -CH$_2$-C**H$_2$**-NHCOO), 3.84 (s, 4H, N-C**H$_2$**-COOH), 4.42 (t, 2H, -C**H$_2$**-CH$_2$-NHCOO). FT-IR (ṽ, cm-1): 1662-1581 (asymmetric CO stretch), 1425-1394 (symmetric CO stretch). Elemental analysis: calcd. (%) for $C_{22}H_{38}N_5O_8La$: C: 41.32, H: 5.99, N: 10.95, O: 20.02, found C: 40.88, H: 6.21, N: 11.06.

Theoretical calculation of amount ligands per nanoparticle

Starting from the crystallographic data of the JPDS card 27-699 the following parameters were obtained for the hexagonal structure:

$$a = 0.6020\, nm$$
$$c = 0.3601\, nm$$

The volume of a hexagonal unit cell ($Vol_{\text{unit cell}}$) is calculated using the following equation:

$$\begin{aligned} Vol_{unit\ cell} &= a^2c \times \sin 60° \\ &= 0.113\, nm^3 \end{aligned}$$

However the unit cell exists of $Na_2Ln_2F_8$[16, 17] and further calculations should take this into account.

The volume of one nanoparticle can easily be calculated from the average diameter size (20.28 nm), measured by TEM and treating a nanoparticle as a sphere.

$$Vol_{NP} = \frac{4}{3}\pi r^3$$

$$= 4367\, nm^3$$

The total amount of lanthanides in the nanoparticle is the sum off the concentration of the gadolinium, ytterbium and thulium acquired from TXRF measurements, and reported in the paper (Table 1).

$c(Ln) = 33.549 \times 10^{-3} M$

In the next step the amount of lanthanide ions in one nanoparticle is found by simply dividing the volume of one nanoparticle by the volume of the unit cell, taking into account that two lanthanide ions are in one unit cell.

$$\#\frac{Ln}{NP} = \frac{4367}{0.113} * 2$$
$$= 77295$$

From this result the amount of NPs per volume unit (L) is obtained, using the total concentration of lanthanide ions in the nanoparticles and Avogadro's number.

$$\#\frac{Ln}{L} = 33.549 \times 10^{-3} * N_A$$

Dividing the last two equations by each other gives the amount of nanoparticles per liter.

$$\#\frac{NPs}{L} = \frac{\#\frac{Ln}{L}}{\#\frac{Ln}{NP}} = 2.61 \times 10^{17}$$

In the final step of these theoretical calculations the amount of ligands at the surface of one nanoparticle is obtained as well as the concentration (moles) per nanoparticle.

$$\#\frac{Gd-DO3A}{NP} = \frac{0.9 \times 10^{-3} * N_A}{3.406 \times 10^{16}} = 2074$$

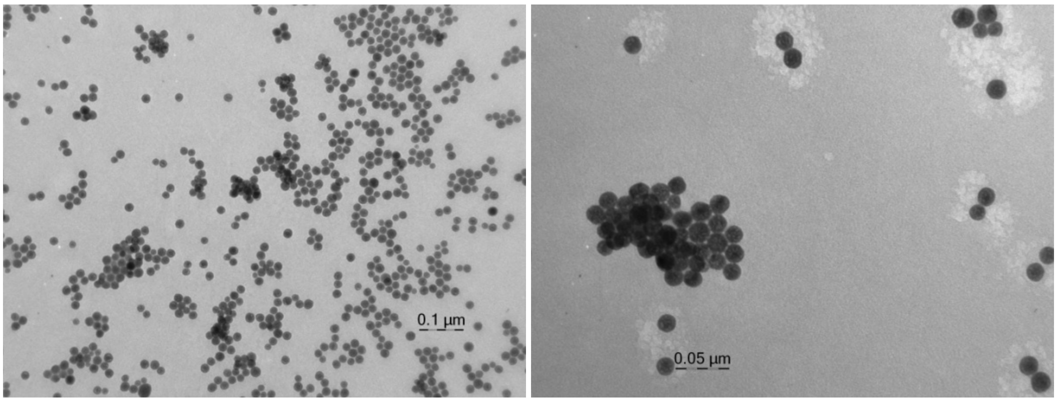
$$moles\frac{Gd-DO3A}{NP} = \frac{0.9 \times 10^{-3}}{3.406 \times 10^{16}} = 3.44 \times 10^{-21}$$

Experimental data

All nanoparticles were redispersed through sonication in the required solvent. For IR and XRD the samples were dried under vacuum.

Transmission Electron Microscopy (TEM)

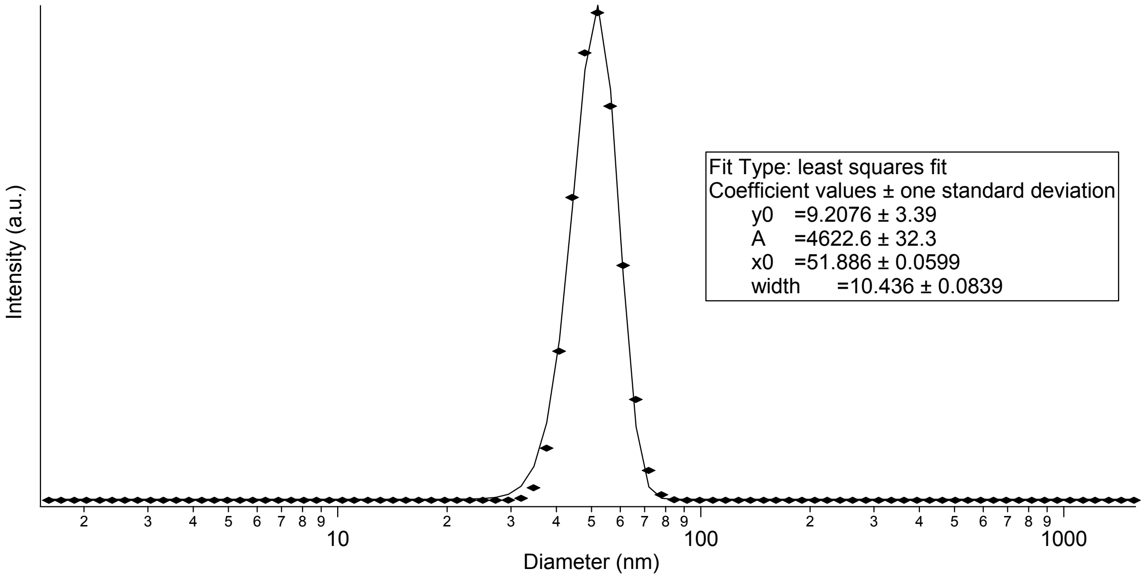
UCNP-oleate



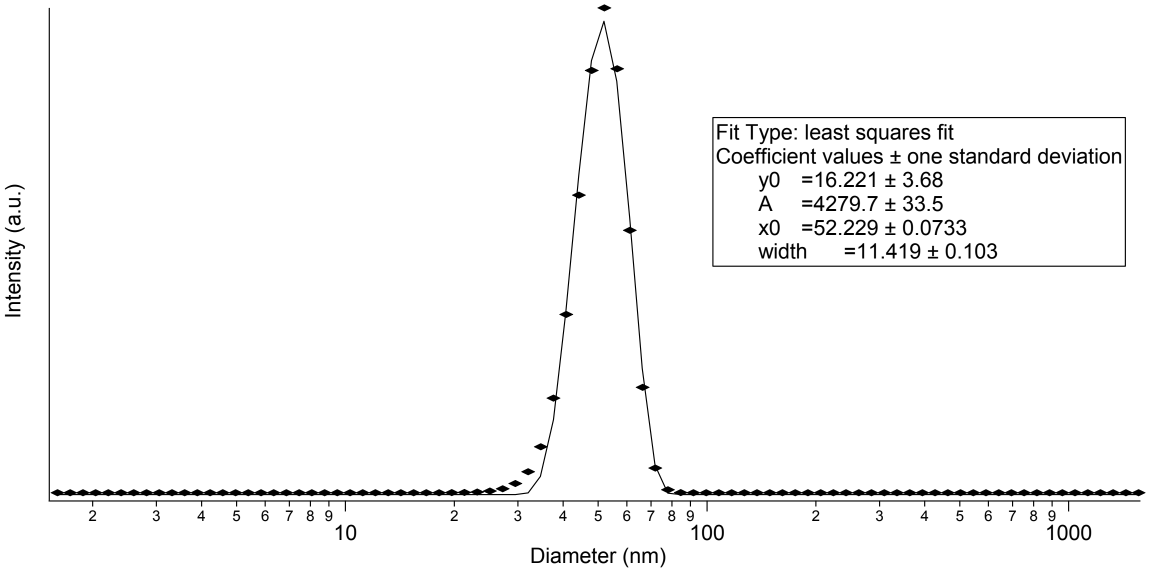
SI 1: TEM image of a 0.1 wt% solution of the oleate capped upconverting nanoparticles in toluene.

Dynamic Light Scattering (DLS)

Citrate-capped Nanoparticles

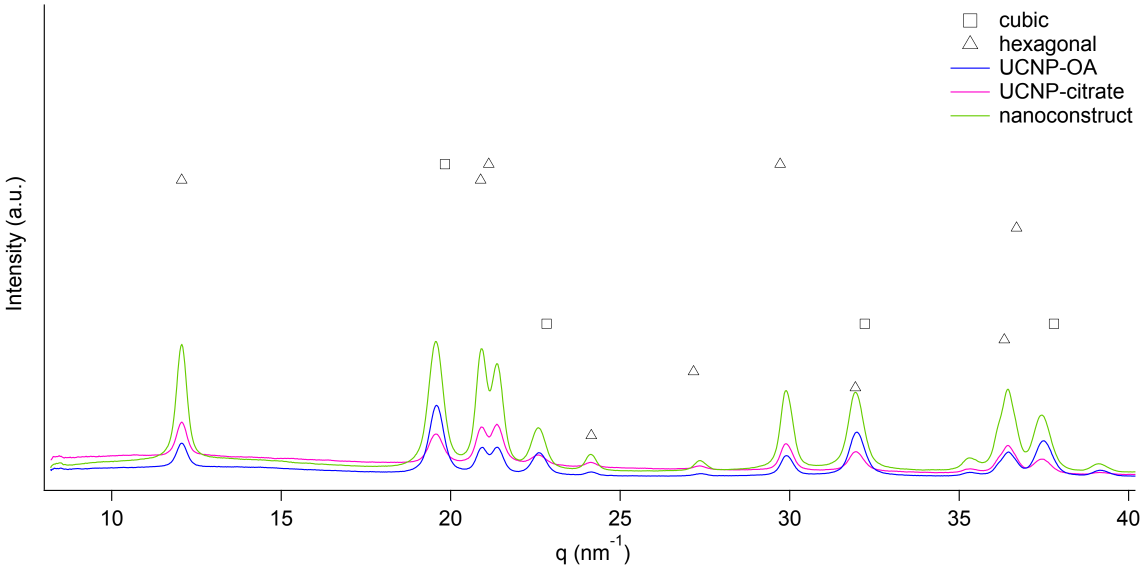


Nanoconstruct



SI 2: DLS measurement of the nanoconstruct. In the box the results of fitted data are shown.

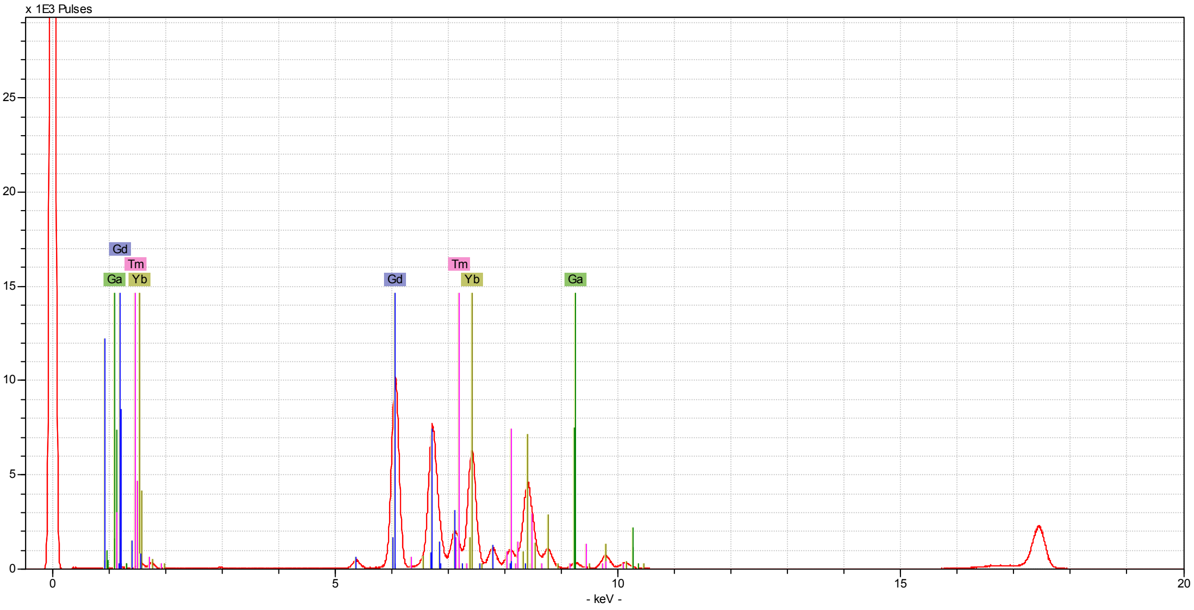
XRD



SI 3: XRD image of the oleate capped upconverting nanoparticles (blue), the citrate capped nanoparticles (pink) and the nanoconstruct (green). The theoretical XRD peaks for a cubic (square) and hexagonal (triangle) phase is added to the figure and originates from JPDS 27-0697 (cubic) and JPDS 27-0699 (hexagonal).

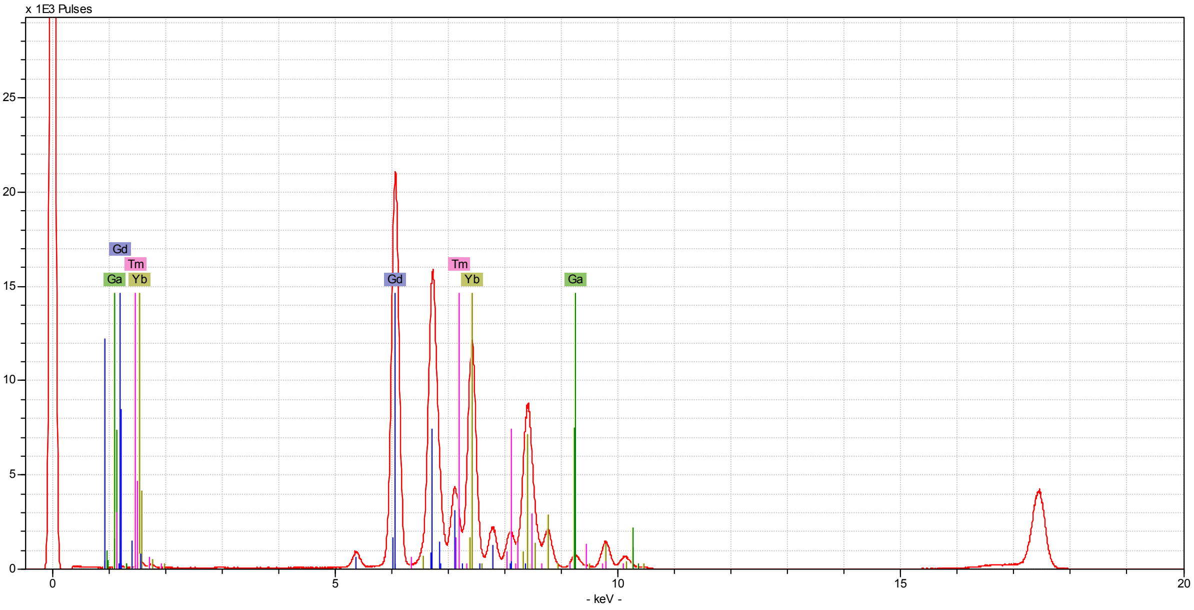
TXRF

Oleate-capped UCNPs



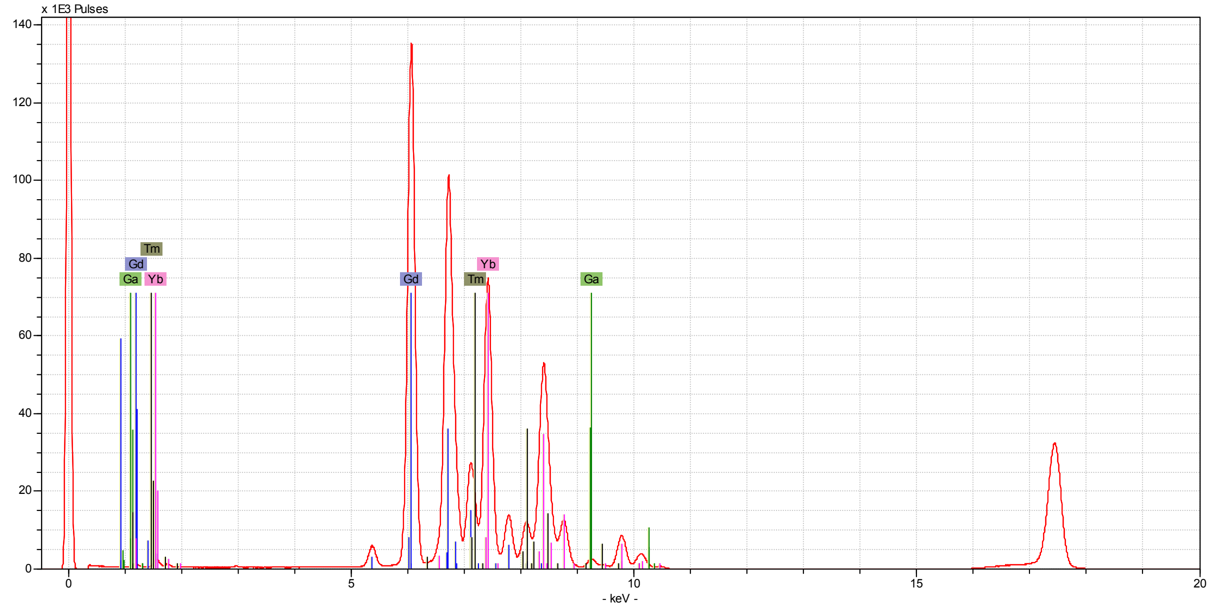
SI 4: TXRF measurement of the oleate-capped upconverting nanoparticles.

Citrate-capped UCNPs



SI 5: TXRF measurement of the citrate-capped upconverting nanoparticles.

Nanoconstruct

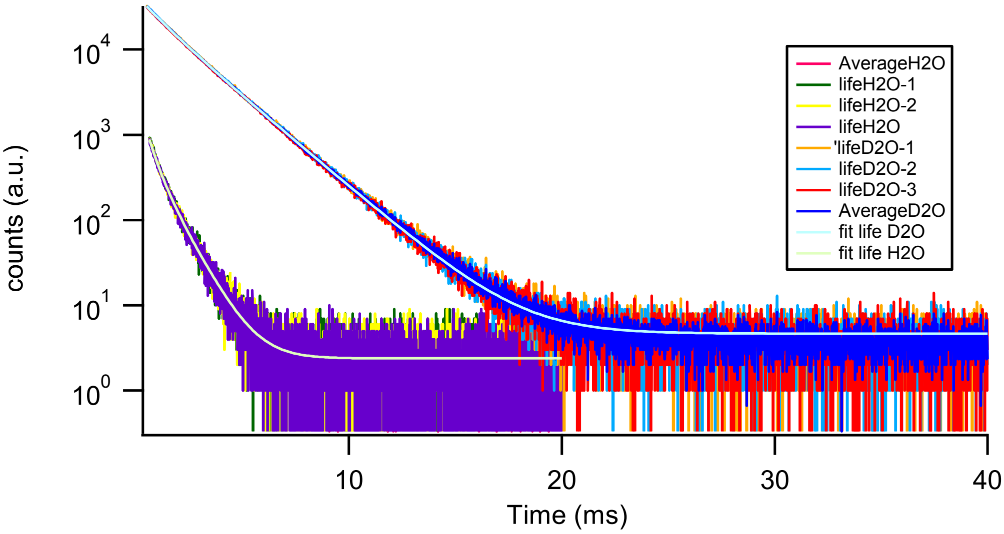


SI 6: TXRF measurement of the nanoconstruct.

Lifetime measurements

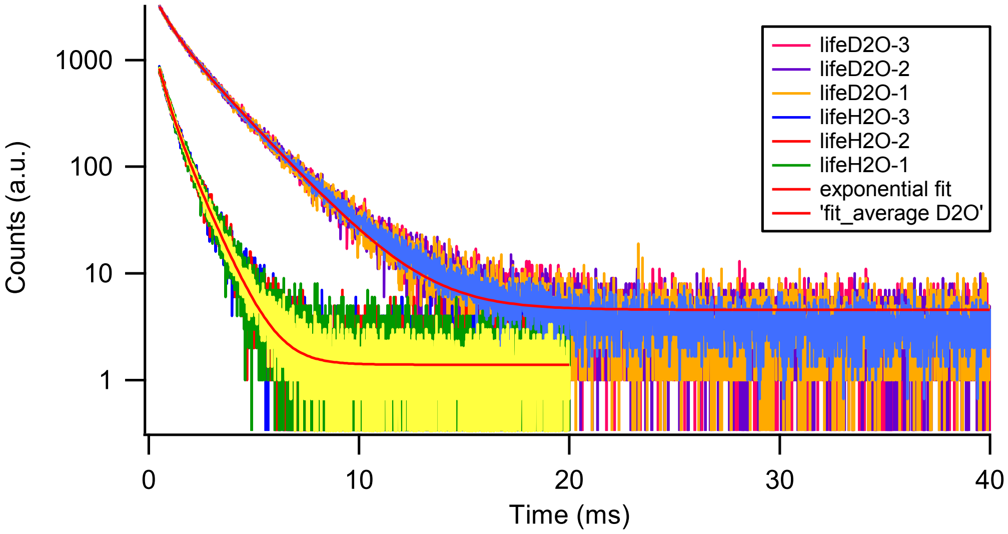
The luminescence decay curves were obtained for the Eu-DO3A **(4)** and the Eu-nanoconstruct in water and deuterium oxide at pH 7.4 and at 293 K. The luminescence decay was observed after irradiation at 319.2 nm and monitoring the emission at 613.5 nm. Three data sets were acquired and were averaged to fit for exponential decay parameters.

Eu-DO3A-EA (4)



SI 7: Luminescence decay with the exponential fit for Eu-DO3A-EA (4) in water and deuterium oxide at 293K and pH=7.4.

Eu-nanoconstruct



SI 8: Luminescence decay with the exponential fit for Eu-nanoconstruct in water and deuterium oxide at 293K and pH=7.4.

References

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