

Lanthanide-Chelate Silica Nanospheres as Robust Multicolor Vis-NIR Tags

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Experimental section

¹⁰ General Details

Solvents and starting materials were obtained from Aldrich, Fluka, Acros and Alfa and used without further purification unless otherwise stated. Solvents were dried over the appropriate drying agents when required. Water and H₂O refer to high purity water with resistivity value of 18 MΩ.cm, obtained from the “Millipore/MilliQ” purification system.

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Luminescence measurements

Low-resolution luminescence measurements (spectra and lifetimes) were recorded on a Fluorolog FL 3-22 spectrometer from Spex-Jobin-Yvon-Horiba with double grating emission and excitation monochromators, and a R928P photomultiplier. For measurements in the NIR spectral range, the ²⁰ spectrometer was fitted with a second measuring channel equipped with a FL-1004 single grating monochromator and light intensity was measured by two Jobin-Yvon solid state InGaAs detectors (i) DSS-IGA020L, cooled to 77 K (range 800-1600 nm) and (ii) DSS-IGA020A (range 800-1700 nm) working at room temperature and inserted into a LN2 housing including an elliptical mirror (90° beam path) and coupled to a Jobin Yvon SpectrAcq2 data acquisition system. The equipment ²⁵ and experimental procedures for luminescence measurements in the visible and NIR range have been published previously by Comby et al.¹ All spectra were corrected for the instrumental functions. Lifetimes were measured in time-resolved mode and are averages of three independent measurements, which were made by monitoring the decay at the maxima of the emission spectra. The mono-exponential decays were analyzed with Origin 7.0[®]. Quantum yields of the complexes ³⁰ in solution at pH 7.4 and in solid state were determined using a home-modified integrating sphere from Oriel and the previously described procedure.² Spectra were corrected for the instrumental function with an absolute method with an integrations sphere.

Supplementary Information 1: Synthesis of the nanoparticles

A microemulsion is created by the consecutive addition of the oil Cyclohexane (Sigma-Aldrich), the co-surfactant 1-Hexanol (anhydrous, >99%, Sigma-Aldrich), the non ionic surfactant Triton X-100 (Sigma-Aldrich) and a certain amount of an aqueous solution of the complexes. The silica precursor TEOS (Tetraethyl Orthosilicate, >99%, Sigma-Aldrich) is then added when the microemulsion has been stabilized. The hydrolysis-condensation reactions are catalyzed by an aqueous ammonium hydroxide solution (Puriss, 30-33% in H₂O, Sigma-Aldrich).

The following preparation of NP-Yb describes the general procedure for the synthesis of the nanoparticles:

To a mixture of Triton X-100 (1,65 mL, 2.82 mmol), hexanol (1,6 mL), cyclohexane (7,5 mL), 80 μ L of a 0.01 M aqueous solution of the lanthanide complex Yb-thqtcnSO₃ (pH 7.4) is added followed by ammonium hydroxide (100 μ L) and water (400 μ L) up to pH 9. The mixture is stirred 30 min at room temperature and then 100 μ L of tetraethyl orthosilicate (TEOS, 0.678 mmol) are added. After 24 h under magnetic stirring, a large excess of ethanol is added. The nanoparticles are then washed under centrifugation three times by ethanol and one more time by water. This process is followed by dialysis (Roth, MWCO: 10,000 Dalton) one week in water in a 10L volume buffer (changed every day) under magnetic stirring. Then, the resulting solution is washed under centrifugation by water to give the NP-Yb nanoparticles. The water was tested by different methods (see below) to verify the absence of lanthanide ion free or complexed.

The NP-tpatcn are prepared by an analogous synthetic procedure by using 80 μ L of a 0.01 M solution of the ligand tpatcn. The NP-NdYb nanoparticles are prepared by using 40 μ L of a 0.01 M solution of Yb-thqtcnSO₃ and 40 μ L of a 0.01 M solution of Nd-tpatcn. The NP-EuTb nanoparticles are prepared by using 40 μ L of a 0.01 M solution of Eu-tpatcn and 40 μ L of a 0.01 M solution of Tb-tpatcn. The NP-NdYbtpatcn nanoparticles are prepared by using 40 μ L of a 0.01 M solution of Ndtpatcn and 40 μ L of a 0.01 M solution of Yb-tpatcn.

Leaking and photobleaching of the nanoparticles were tested by luminescence spectroscopy, absorption spectroscopy and physico-chemical methods (colorimetric titration in acetate buffer at pH 4.5 using standardized H₂Na₂edta solutions from Merck and a Xylenol orange indicator). The luminescence spectra of the water used to wash the nanoparticles after dialysis did not show the characteristic emission of the lanthanide ions or of the singlet states of the ligands and did not show UV-vis absorption peaks. After immersion of the nanoparticles for one month and after

removal of the nanoparticles by centrifugation, the resulting water solution did not show detectable luminescence or UV-vis absorption peaks indicating the absence of complexes leakage; Moreover, colorimetric titrations in acetate buffer have confirmed the absence of free lanthanide ions released from the nanoparticles. Finally, the quantum yields measured and the photophysical properties (intensity, luminescence decays) of the nanoparticles solutions remain unchanged after one month.

Supplementary Information 2: ICPMS Method

The centrifuged NP-NdYb and NP-NdYb-tpatcn nanoparticles and the resulting ethanolic mother liquor were analyzed before washing of the nanoparticles by ICPMS to determine the percentage of encapsulation of the different chelate.

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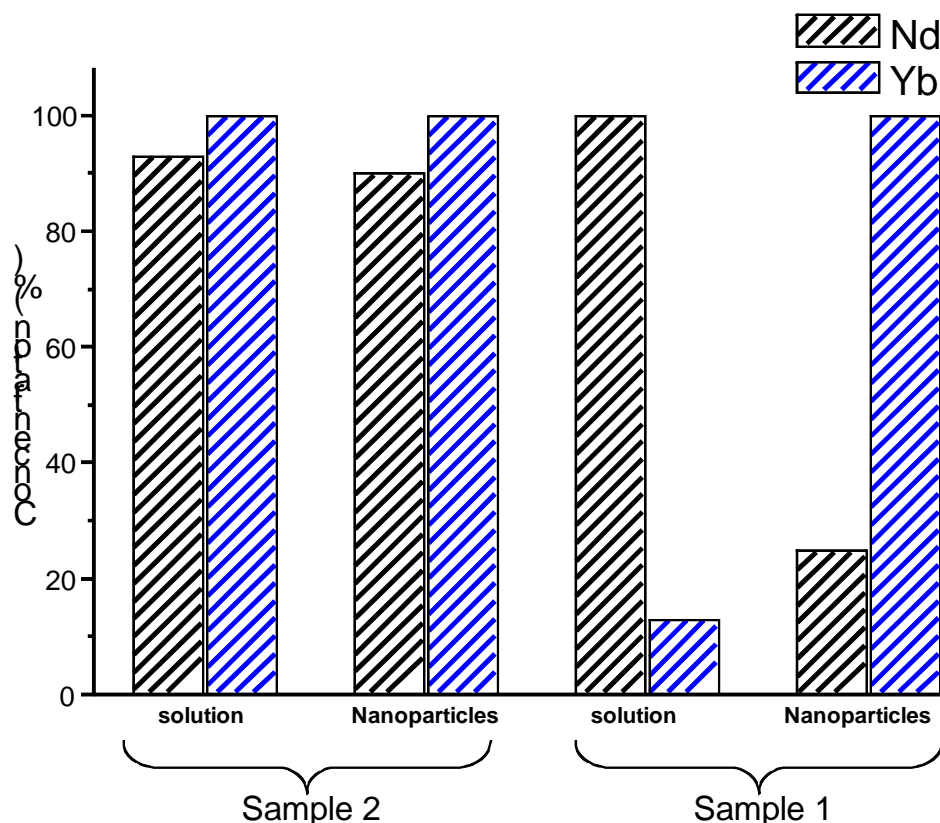


Figure S2: Percentage of encapsulation measured by ICPMS (Sample 1 : Centrifuged NP-NdYb nanoparticles and mother liquor; Sample 2 Centrifuged NP-NdYbtpatcn nanoparticles and mother liquor)

10 The titrations of the lanthanide ions by ICPMS method indicate the encapsulation of the complexes in the nanoparticles (blue column). For sample 1 where two different ligands are present, tpatcn-Nd and thqtcnSO₃-Yb, the concentration of thqtcnSO₃-Yb in the nanoparticles is three times that of tpatcn-Nd (500 compare to 110 ppb). This could be due to the localisation of the Yb complex in the center of the nanoparticle. For sample 2 the concentrations of the two ions are identical (70
15 ppb) indicating an equimolar insertion of the two lanthanide complexes in perfect agreement with the starting concentration of both ions. These results suggest a better insertion of the hydrophilic complexes of the nanoparticles compared to the hydrophobic complexes.

Supplementary Information 3: 2D TEM images of nanoparticles

These images were recorded on two different nanoparticles, the first one synthesised with the tpatch ligand alone and the second one with its lanthanide (Eu and Tb) complexes. They clearly demonstrate the absence of aggregation, the presence of spherical nanoparticles uniform in size and the porosity of the nanoparticles.

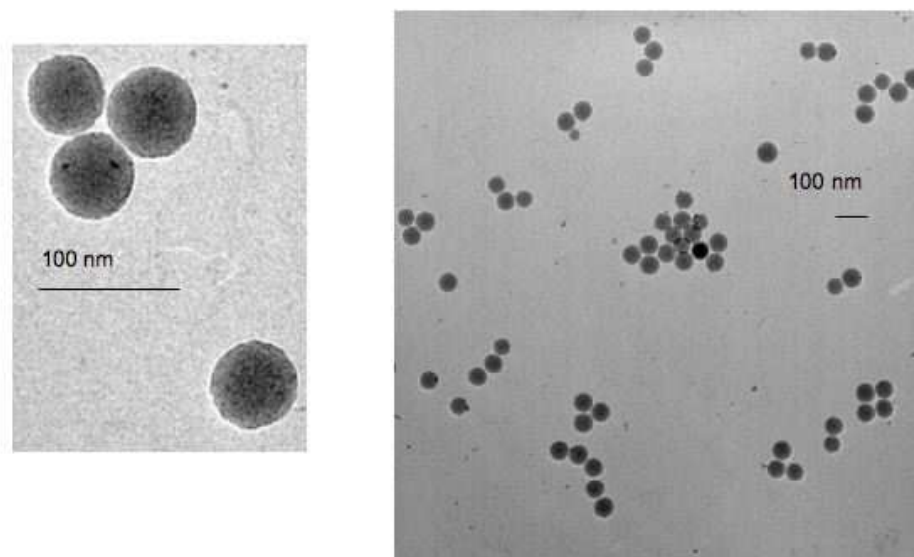


Figure S3. 2D TEM images of silica nanospheres NP-tpatch containing the tpatch ligand alone

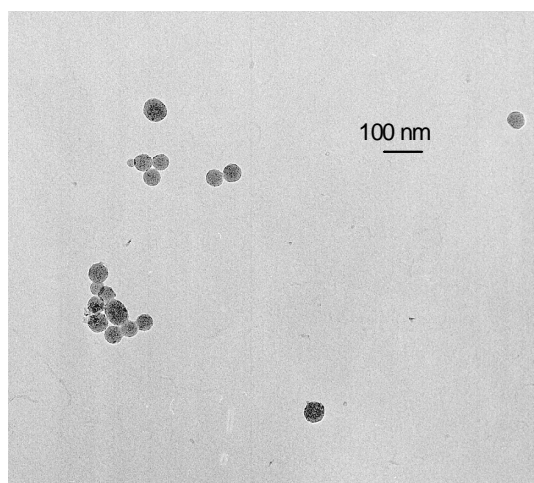
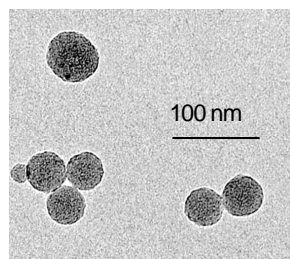
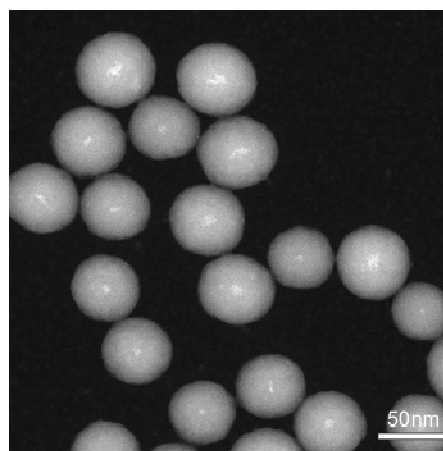
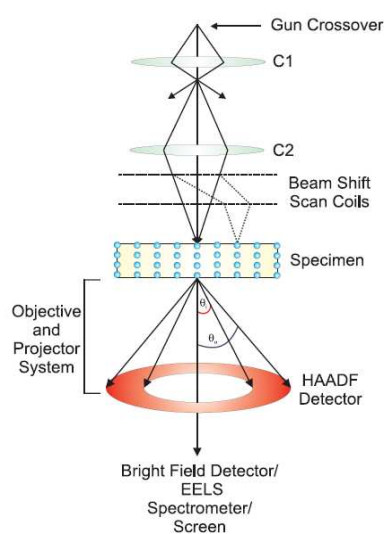


Figure S4. 2D TEM images of silica nanospheres NP-EuTb with a mixture of tpatcn-Eu and tpatcn-Tb

Supplementary Information 4: 3D structural characterization by electron tomography

Electron tomography is employed, with experiments carried out on an FEI Titan S/TEM, operating at 300kV. A series of 141 high angle annular dark field (HAADF) images were acquired with a 1° spacing over a range of $\pm 70^\circ$. This imaging mode gives images with Z contrast. A tomographic reconstruction is obtained using the simultaneous iterative reconstruction technique (SIRT).³ The resultant tomograms allow the study of the 3D distribution of lanthanide complexes within the silica substrate.

1) High angle annular dark field (HAADF) images

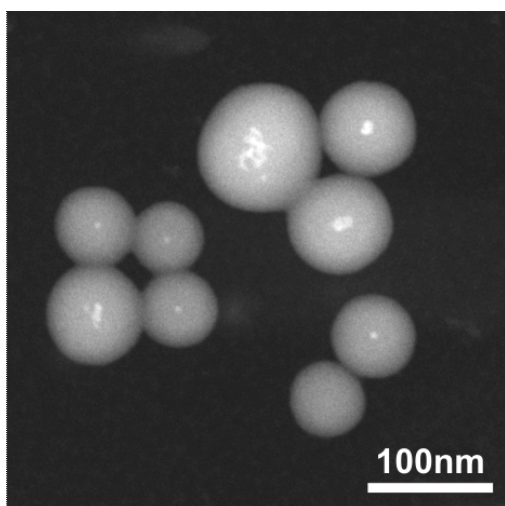


Images are acquired in scanning TEM mode on the FEI Titan at 300kV with a 0.1-0.5nm probe

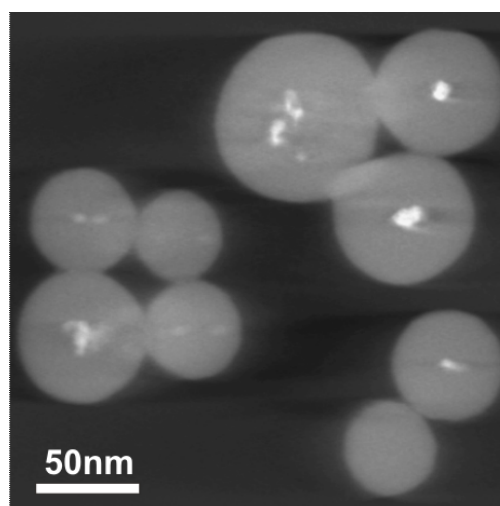
The intensity is proportional to Z^2 . The fact that the central region of each sphere appears brighter than the surrounding suggests that the lanthanide, with the higher Z, is concentrated in the center of the NPs.

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2) Resulting 3D images



HAADF Image – a single projection from the tilt series. In total there are 141 images like this taken at different angles.



Slice through tomogram, after 3D reconstruction. This is not a projection, but a single plane of intensities in the tomogram.

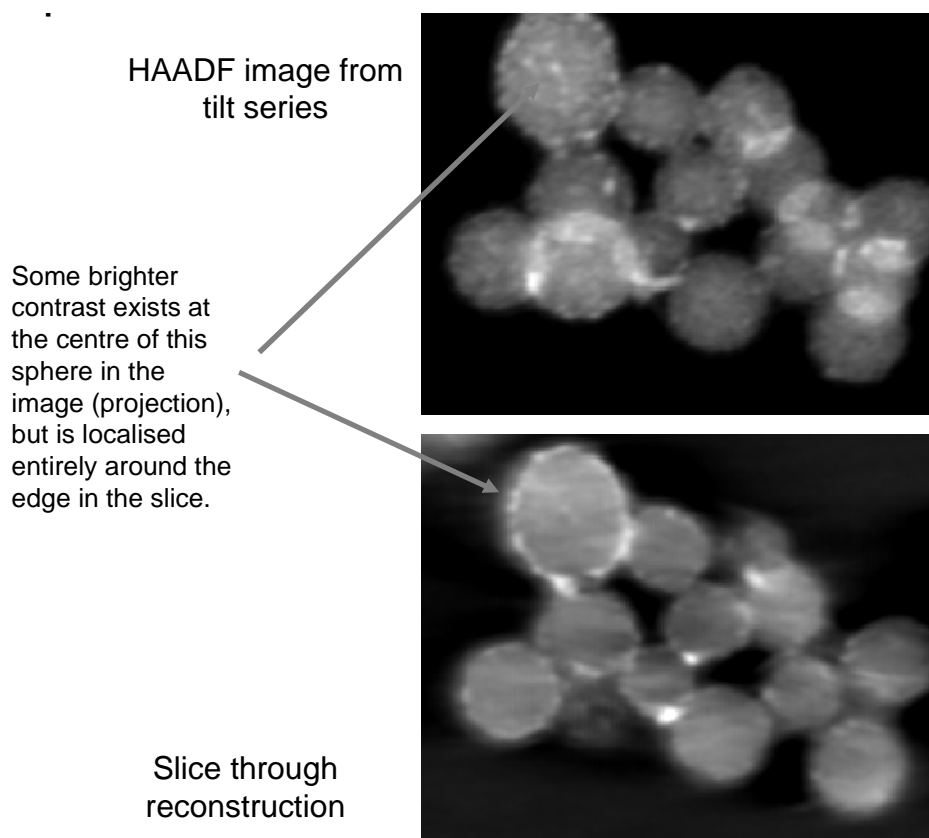


Figure S5. HAADF STEM images and slice through reconstruction for NP-NdYb and NP-EuTb

Supplementary Information 5: Excitation and emission spectra of the NP-EuTb nanoparticles doped with the Eu-tpatcn and Tb-tpatcn complexes.

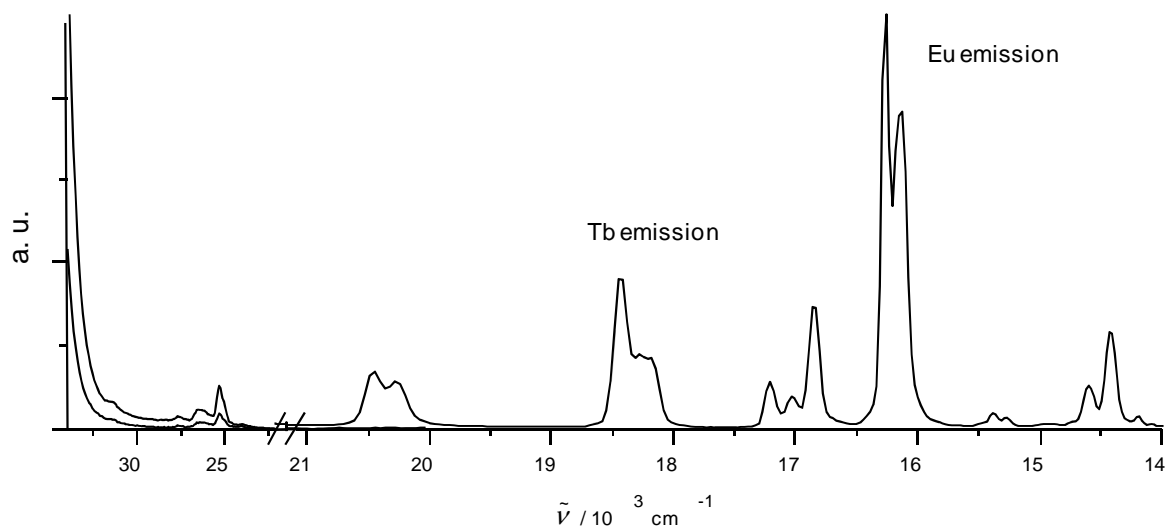


Figure S6. Excitation spectra (left) with analysis at 545 ($18\,348\text{ cm}^{-1}$, Tb) and 615 nm ($16\,260\text{ cm}^{-1}$, Eu) and emission spectrum (Right) of the NP-EuTb nanoparticles with excitation at 279 nm ($35\,843\text{ cm}^{-1}$).

1. Comby, S.; Imbert, D.; Chauvin, A.-S.; Bünzli, J.-C. *Inorg. Chem.* 2006, 45, 732..
- 10 2. De Mello, J. C.; Wittmann, H. F.; Friend, R. H. *Adv. Mater.* 1997, 9, 230.
3. Midgley, P. A.; Ward, E. P. W.; Hungria, A. B.; Thomas, J. M. *Chem. Soc. Rev* 2007, 36, 1477..

Supplementary Information 5: Metal Ion Centered Lifetimes for Nd($^4F_{3/2}$), Yb($^2F_{5/2}$), Eu(5D_0) and Tb(5D_4) in the Ln-complexes in water solution and in the nanoparticles.

Compound	τ (ms)
Eu-tpatcn	1.08(1)
NP-Eu	1.23(2)
Tb-tpatcn	2.00(1)
NP-Tb	2.06(2)
Nd-tpatcn	$0.045(3) \cdot 10^{-3}$
NP-NdYb	$0.058(4) \cdot 10^{-3}$
Yb-thqtcnSO ₃	$2.05(1) \cdot 10^{-3}$
NP-Yb	$4.17(1) \cdot 10^{-3}$
NP-NdYb	$4.01(5) \cdot 10^{-3}$

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