Supporting Information

Water Dispersible Upconverting Nanoparticles: Effects of Surface Modification on Luminescence and Colloidal Stability


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1. Large Scale Synthesis of Oleate-coated $\beta$-NaYF$_4$(Yb$^{3+}$/Er$^{3+}$) UCNPs

The salts YCl$_3$·6H$_2$O (15.6 mmol), YbCl$_3$·6H$_2$O (4.0 mmol), and ErCl$_3$·6H$_2$O (0.4 mmol) were dissolved in ~40 mL of methanol by sonication. This solution was transferred into a 1 L three-necked flask, mixed with 160 mL of oleic acid and 300 mL of 1-octadecene under an atmosphere of nitrogen and heated to 160 °C. A homogeneous, clear solution was formed after 30 minutes at 160 °C under vacuum. The reaction mixture was then cooled to room temperature and 200 mL of methanol containing NaOH (0.25 M) and NH$_4$F (0.4 M) were added at once. The resulting colloidal suspension was stirred for 30 minutes at 120 °C under a gentle flow of nitrogen and then heated to reflux at ~320 °C for ~22 minutes. The visible green upconversion luminescence occurring at this point can easily be seen by the bare eye. Subsequently, the mixture was cooled to 200 °C. An additional heating step (>300 °C for ~5 minutes) was applied. Afterwards, the mixture was cooled to room temperature. The formed oleate-coated hexagonal-phase UCNPs were precipitated by addition of ~400 mL of ethanol after cooling to room temperature and were isolated via centrifugation at a relative centrifugal force (RCF) of 1000 g for 5 minutes. The white pellet was washed three times by dispersion of the UCNPs in ~10 mL of chloroform and cyclohexane, and then precipitated by the addition of ~150 mL of ethanol and acetone. Finally, the purified oleate-coated (OA) UCNPs were dispersed in 160 mL of cyclohexane.
Figure S1: Setup of a large scale synthesis of oleate-coated $\beta$-NaYF$_4$(Yb$^{3+}$,Er$^{3+}$) UCNPs. Once the reaction mixture had reached 300 °C a timer was started. The images were taken at 0 (1), 22 (2), 28 (3), 50 (4), and 58 (5) minutes, respectively. The diagram shows the course of the reaction mixture’s temperature as a function of time. Green upconversion luminescence (see inset) could be observed by the bare eye after ~ 22 minutes (2).
2. Surface Modifications

2.1. Modification with DSPE-mPEG2000

A method reported by Li et al. was used [1]. In detail, 2 mL of UCNPs (4·10^{15} UCNPs mL^{-1}, concentration determined by ICP-OES, see Section 3) dispersed in cyclohexane were transferred into a 50 mL round bottom flask. Subsequently, cyclohexane was evaporated under reduced pressure and the UCNPs redispersed in 4 mL of chloroform. DSPE-mPEG(2000) (10 µmol) dissolved in 4 mL of chloroform was added, and the resulting mixture was sonicated for 3 minutes at room temperature. Then, the chloroform was evaporated under reduced pressure, which resulted in the formation of a colorless, transparent film on the bottom of the flask. Hereby, driven by hydrophobic interaction, the hydrophobic tails of DSPE-mPEG(2000) intercalated the OA coating of the UCNPs, leading to an additional layer of DSPE-mPEG(2000) around the UCNPs. Afterwards, the film was hydrated with 10 mL of double distilled (dd) water. As a result, a clear dispersion of UCNPs coated with DSPE-mPEG(2000) acting as an amphiphilic layer around the original hydrophobic surfactant layer was obtained. The dispersion was purified by three centrifugation steps (RCF: 17000 g; 15 minutes) and subsequent redispersion in dd water. Finally, the DSPE-mPEG(2000)-coated UCNPs were dispersed in 10 mL of dd water.

2.2. Modification with a Silica-shell

We used a modified reverse-microemulsion technique for the silica-shell modification of oleate-coated UCNPs [2]. First, 2 mL of UCNPs (4·10^{15} UCNPs mL^{-1}) dispersed in cyclohexane were transferred into a 50 mL round bottom flask and diluted with 8 mL cyclohexane. Then, 500 µL of IGEPAL® CO-520 were added, forming inverted micelles with embedded UCNPs. The flask was sealed with a ground-in glass stopper and the clear dispersion was sonicated for 10 minutes. Afterwards, 80 µL of an aqueous ammonia solution (32%) were added and again sonicated for 10 minutes. Finally, 80 µL (0.27 mmol) of TEOS were added, forming a silica shell around the micelles, and the dispersion was magnetically stirred (~ 600 rpm) for 24 hours at room temperature. The silica-coated UCNPs were precipitated by adding 40 mL of acetone and collected via centrifugation (RCF: 1000 g; 5 minutes). The pellet was redispersed in 1 mL of chloroform, again precipitated with 10 mL of acetone, and collected via centrifugation. This step was repeated one more time. Then, the
pellet was redispersed in 2 mL of sodium borate buffer (SBB12, 50 mM, pH 12), precipitated again with 20 mL of acetone, and collected via centrifugation. The silica-coated UCNPs were redispersed in 2 mL dd water, precipitated with 20 mL of acetone, and collected via centrifugation. This step was repeated twice. Finally, the silica-modified UCNPs were dispersed in 10 mL of dd water.

2.3. Modification with PMA

The synthesis of the amphiphilic polymer, comprising hydrophobic side chains and a hydrophilic backbone, has been reported previously [3,4]. In more detail, 2.70 g (15 mmol) of dodecylamine (which acts as a hydrophobic side chain) were dissolved in 100 mL of THF in a 250 mL round bottom flask. Once, dodecylamine dissolved completely, all of the clear solution was poured into another 250 mL round bottom flask containing 3.084 g of poly(isobutylene-alt-maleic anhydride), (PMA; 20 mmol monomer units; there are ~39 monomer units per polymer chain). PMA acts as a hydrophilic backbone. The ratio of dodecylamine to the anhydride rings of PMA was chosen in a way that ~75 % of anhydride rings of the amphiphilic polymer backbone react with the amino groups of hydrophobic side chains, leaving ~25 % of anhydride rings intact for further modification with other functionalities. In the following also the whole polymer will be termed as PMA. The cloudy mixture was sonicated for a few seconds (~20 s) and then refluxed at 55-60 °C for about three hours under magnetic stirring. Afterwards, the solution was concentrated to 30-40 mL by evaporation of THF to enhance the reaction between maleic anhydride rings of the PMA backbone and the amino groups of the dodecylamine side chains. Then, the solution was refluxed overnight under continuous stirring. The next day, the solvent was completely evaporated under reduced pressure and the product was re-dissolved in 400 mL anhydrous chloroform to give a final molar concentration of 0.05 M of monomer units. This solution was used as a polymer stock solution.

Approximately 100 monomer units per nm² ($R_P$) of UCNP surface were applied [5,8]. The average particle diameter of a single UCNP core is ~22.7 nm ($d_c$), which was determined by the TEM analysis. The thickness of the organic shell, which consists of OA ions, around the inorganic $\beta$-NaYF$_4$(Yb$^{3+}$/Er$^{3+}$) core, is estimated as ~1.1 nm ($l_{OA}$)[6,7]. The effective diameter ($d_{eff}$) is calculated to be ~24.9 nm ($d_{eff} = d_c + 2l_{OA} = 22.7 \text{ nm} + 2\cdot1.1 \text{ nm}$).
Hence, the volume of the polymer stock solution \( V_{\text{PMA}} \) needed for the amphiphilic polymer coating can be calculated according to Equation (1).

\[
V_{\text{PMA}} = R_p \cdot \pi \cdot c_{\text{UCNP}} \cdot V_{\text{UCNP}} \cdot d_{\text{eff}} \cdot c_{\text{PMA}}^{-1}
\]  

(1)

With:

- \( R_p = 100 \), is the number of monomer units per \( \text{nm}^2 \) of UCNP surface area;
- \( c_{\text{UCNP}} \sim 6.8 \cdot 10^{-7} \text{ M} \), is the UCNP concentration in the particle stock solution;
- \( V_{\text{UCNP}} \sim 2 \cdot 10^{-3} \text{ L} \), is the used volume of the particle stock solution;
- \( d_{\text{eff}} \sim 24.9 \text{ nm} \)
- \( c_{\text{PMA}} 0.05 \text{ M} \), is the concentration of monomer in the polymer stock solution.

A volume \( V_{\text{PMA}} \) of \( \sim 5 \text{ mL} \) of PSS is needed in order to coat 2 mL of UCNPs \( \sim 6.8 \cdot 10^{-7} \text{ M} \) with PMA, resulting in \( \sim 100 \) monomer units of PMA per \( \text{nm}^2 \) of nanoparticle surface area.

The PMA polymer solution (5 mL, 0.05 M monomer units in chloroform, 75% of the anhydride rings occupied by dodecylamine) was mixed with 2 mL of OA-coated UCNPs \( \sim 6.8 \cdot 10^{-7} \text{ M} \) dispersed in cyclohexane, resulting in a molar ratio of 100 monomer units of polymer per \( \text{nm}^2 \) of effective nanoparticle surface area (the thickness of the OA capping \( l_{\text{OA}} \) was assumed to be \( \sim 1.1 \text{ nm} \)). The PMA hereby is wrapped around the surface of the OA-capped UCNPs, driven by hydrophobic interaction. Immediately after mixing, sonication for 1 minute was applied. Afterwards, the solvent was slowly evaporated under reduced pressure until the sample got completely dry. The remaining solid film was redissolved in SBB12 under vigorous stirring until the solution turned clear. The 25% of the anhydride, which are not occupied by dodecylamine, hydrolyze in contact with water forming negatively charged carboxyl groups and providing colloidal stability in aqueous solutions. The resulting polymer-coated UCNPs were purified, removing unreacted ligand molecules with centrifuge filters (membrane: 100 kDa \( M_w \) cut off, polyethersulfone (PES)). For this purpose the dispersion of PMA-coated UCNPs was further pre-concentrated using a centrifuge filter (RCF: 870 g; 15 minutes) in order to give a volume of less than 250 \( \mu \text{L} \). At this step the reservoir of the
filter was filled again with milli-Q water to a volume of 10 mL and the pre-concentration of the diluted solution was repeated by centrifugation. The dilution-concentration sequence was repeated for three times. Between each step the remaining UCNP dispersion was vigorously shaken in order to avoid agglomeration of the UCNPs inside the filter. In the final step, the sample solution was concentrated to a volume of 300 μL. Then, this concentrated sample was diluted with milli-Q water up to 4 mL for further characterization.

2.4. Modification with Py-PMA

The synthesis of the amphiphilic polymer was modified in order to introduce pyridine ligands together with the dodecylamine in the hydrophilic backbone of PMA. For the synthesis of the Py-PMA polymer the procedure reported previously [3,4] was modified by addition of 2.7 g (15 mmol) of dodecylamine and 0.306 mL (3 mmol) of 4-(2-aminoethyl)pyridine in the reaction mixture which consists of 3.084 g (20 mmol expressed as monomer) of poly(isobutylene-alt-maleic anhydride) dissolved in 100 mL of tetrahydrofuran. In this way 75% and 15% of the anhydride groups were occupied by dodecylamine and 4-(2-aminoethyl)pyridine, respectively. Coating of the UCNPs with this AP was carried out in the same way as described in the Section 2.3.

2.5. Modification with PEG-PMA

First, the polymer coating procedure using PMA was carried out following the same procedure as described in the Section 2.3. Then, amino-modified PEG was attached to the carboxylic groups, which were present on the surface of the PMA-coated UCNPs, by EDC chemistry [8]. For linkage of PEG, a stock solution of 3 mM of amino-modified PEG with a molecular weight of ~ 1.2 kDa was prepared by dissolving 9 mg of amino-modified PEG in 2.5 mL of sodium borate buffer (SBB9; 50 mM, pH 9). Afterwards, 227 μL of the amino-modified PEG stock solution (3 mM) was added to 227 μL of the PMA-coated UCNP solution (7 μM UCNP concentration), resulting in a ratio of 500 PEG molecules per UCNP. Then, 227 μL of a freshly prepared EDC solution (384 mM in SBB9) was added, yielding a ratio of 64000 EDC molecules per UCNP within the reaction mixture. The samples were allowed to react for 3 hours before they were diafiltrated once with SBB9 using a centrifuge
filter (membrane: 100 kDa M\textsubscript{w} cut off, PES). Finally, the sample solution was changed to milli-Q water and concentrated to a volume of 300 \(\mu\text{L}\), which was then diluted with milli-Q water to a volume of 4 mL.

2.6. Modification with BF\textsubscript{4}⁻

We used a modified ligand-exchange strategy as reported by Dong \textit{et al.} [9]. In detail, 8 mL of dimethylformamide (DMF) were transferred into a 50 mL round bottom flask. Then, 10 mL of UCNPs (\(4 \cdot 10^{15}\) UCNPs mL\textsuperscript{-1}) dispersed in cyclohexane were added. This resulted in a two phase system consisting of an upper layer of cyclohexane (containing the OA-coated UCNPs) and a subjacent layer of DMF. Subsequently, 120 mg (1.0 mmol) of NOBF\textsubscript{4} were added at once under vigorous stirring. The mixture was further stirred for 10 minutes, whereby the original hydrophobic OA-coating of the UCNPs was replaced by a layer of hydrophilic BF\textsubscript{4}⁻. This resulted in the phase transfer of the UCNPs from the cyclohexane phase to the DMF phase. The UCNPs within the slightly turbid DMF phase were precipitated by adding 20 mL of chloroform and were collected \textit{via} centrifugation (RCF: 1000 g; 5 minutes). The transparent pellet was redispersed in ~ 1 mL of DMF, precipitated again by addition of an excess of chloroform, and collected \textit{via} centrifugation. This step was repeated two times. Afterwards, the pellet was redispersed in 10 mL of DMF and centrifuged (RCF: 1000 g; 3 minutes) in order to get rid of larger agglomerates. This dispersion (concentration of UCNPs ~ 0.6 \(\mu\text{M}\)) was used as stock for all further ligand-exchange strategies.

2.7. Modification with citrate

First, 2.5 g (8.5 mmol) of trisodium citrate dihydrate were dissolved in 4 mL of dd water. Then, 5 mL of the stock dispersion of UCNPs in DMF were slowly added under vigorous stirring. The turbid mixture was further stirred for 15 minutes. Hereby the BF\textsubscript{4}⁻ capping was replaced by a citrate capping. Afterwards, the dispersion was centrifuged for 5 minutes at 1000 g and the supernatant was discarded. The pellet was redispersed in 500 \(\mu\text{L}\) of dd water and centrifuged for 15 minutes at 14000 g. This step was repeated two times. Finally, the pellet was redispersed in 5 mL of dd water and centrifuged for 3 minutes at 1000 g in order to get rid of larger agglomerates.
2.8. Modification with PEG-PA

We used 5 mL of the stock dispersion of UCNPs in DMF and added 80 mg (0.14 mmol) of PEG-PA dissolved in 1 mL of dd water under vigorous stirring. The turbid mixture was further stirred for 20 minutes. Hereby the BF$_4^-$ ligand shell was replaced by PEG-PA, which is then attached to the surface of the UCNPs via the phosphonic acid terminal. Afterwards, the dispersion was centrifuged for 15 minutes at 14000 g. The pellet was redispersed in 500 µL of dd water and again centrifuged for 15 minutes at 14000 g. This step was repeated two times. Finally, the pellet was redispersed in 5 mL of dd water and centrifuged for 3 minutes at 1000 g in order to get rid of larger agglomerates.

2.9. Modification with PAA

We used 5 mL of the stock dispersion of UCNPs in DMF and added 80 mg (0.04 mmol) of PAA dissolved in 1 mL of dd water under vigorous stirring, which displace the original BF$_4^-$ shell. The following steps were the same as described in Section 2.8.

2.10. Modification with Layer-by-Layer (LbL) coating

The polyelectrolytes used for multilayer deposition were PAH and PSS [10,11]. They were dissolved in milli-Q water for preparing stock solutions of 20 mg mL$^{-1}$. An amount of 1 mL of citrate-capped UCNPs (0.68 µM) in dd water was added drop by drop and under vigorous stirring (1000 rpm) to a total volume of 1 mL of a stock solution of 20 mg mL$^{-1}$ of PAH (around 100 polyelectrolyte chains added per UCNP). After mixing, the dispersion was stirred for 1 hour and then centrifuged for 1 hour at 8000 rpm in 2 mL Eppendorf tubes. The supernatant containing unbound PAH was then carefully removed and replaced by ultrapure water. The centrifugation procedure was repeated one more time. Then, the precipitated sample was redispersed in 1 mL milli-Q water. This sample was then used to form the second layer with PSS. For this purpose the sample was added drop by drop and under vigorous stirring (1000 rpm) to a total volume of 1 mL of a stock solution of 20 mg mL$^{-1}$ of PSS and left stirring for 20 minutes. Again, the sample was centrifuged for 1 hour at 8000 rpm. Then, the supernatant was replaced by ultrapure water and the centrifugation procedure was repeated. The third (PAH) and fourth (PSS) layers were prepared following the sample
procedure as used for the second layer. Finally, the sample was redispersed in 1 mL of milli-Q water. Note that this protocol first involves ligand exchange to transfer the UCNPs to aqueous solution by replacing the hydrophobic OA-coating by citrate, followed by further overcoating with four polyelectrolyte layers.
3. Quantification of UCNP Composition

The quantitative content of rare-earth ions of UCNPs was determined using inductively coupled plasma optical emission spectrometry (ICP-OES). The OA-coated β-UCNPs obtained after synthesis (see Section 1) were dispersed in a total volume of 160 mL of cyclohexane. From this dispersion 16 µL of OA-coated β-UCNPs were taken and dried until a constant weight was reached. The resulting 0.3 mg of OA-coated UCNPs were then solubilized in 417 µL of sulfuric acid (95-97%). Afterwards, 7.083 mL dd water and 7.5 mL of HNO₃ (1 M) were added. Finally, a clear solution with a total volume of 15 mL was obtained and analyzed using ICP-OES. The results are summarized in Table S1.

Table S1 | Content of rare-earth ions of NaYF₄(Yb³⁺,Er³⁺) UCNPs

<table>
<thead>
<tr>
<th>Element</th>
<th>Concentration [µM]</th>
<th>Content [mol%]</th>
<th>Theoretical Content(*) [mol%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yttrium</td>
<td>319.5 ± 0.3</td>
<td>78.4 ± 0.1</td>
<td>78.0</td>
</tr>
<tr>
<td>Ytterbium</td>
<td>78.8 ± 0.4</td>
<td>19.3 ± 0.1</td>
<td>20.0</td>
</tr>
<tr>
<td>Erbium</td>
<td>9.2 ± 0.1</td>
<td>2.3 ± 0.1</td>
<td>2.0</td>
</tr>
</tbody>
</table>

(*)Theoretical content as revealed from the original sample weight.
4. Quantification of UCNPs Concentration

The OA-coated β-UCNPs obtained after synthesis (see Section 1) were dispersed in a total volume of 160 mL of cyclohexane. The UCNPs concentration \( \frac{\text{UCNPs}}{\text{mL}} \) was calculated as follows:

The volume of one UCNP core (\( V_{\text{UCNP}} \); i.e. without the organic capping) was calculated using Equation (2), assuming a spherical shape of the UCNP core:

\[
V_{\text{UCNP}} = \frac{4}{3} \left( \frac{d_c}{2} \right)^3 \pi
\]

With the core diameter, \( d_c = 22.7 \text{ nm} \) (from TEM).

\[
V_{\text{UCNP}} = 6.1 \cdot 10^3 \text{ nm}^3
\]

The volume of one UCNP core is \( 6.1 \cdot 10^3 \text{ nm}^3 \).

The volume of the unit cell (\( V_{\text{unit\_cell}} \)) of \( \beta\)-NaYF\(_4\)(Yb\(^{3+}\),Er\(^{3+}\)) was taken from literature [12].

\[
V_{\text{unit\_cell}} = 107.44 \text{ Å}^3
\]

The volume of the unit cell is \( 107.44 \text{ Å}^3 \).

The number of unit cells (\( N_{\text{unit\_cells}} \)) per one UCNP core is calculated using Equation (3).

\[
N_{\text{unit\_cells}} = \frac{V_{\text{UCNP}}}{V_{\text{unit\_cell}}}
\]

Therefore, one spherical core UCNP contains \(~57 \cdot 10^3\) unit cells.
As each unit cell contains one rare-earth ion (either \( \text{Y}^{3+} \), or \( \text{Yb}^{3+} \), or \( \text{Er}^{3+} \)) the number of rare-earth ions (\( N_{\text{RE}^{3+}} = V_{\text{UCNP}} / V_{\text{Unit cell}} \)) per one UCNP can be derived from this result, being also \( \sim 57 \cdot 10^3 \). Again, each UCNP comprises \( N_{\text{unit cells}} \) unit cells, each of which contains one RE\(^{3+}\) ion, which is either \( \text{Y}^{3+} \), or \( \text{Yb}^{3+} \), or \( \text{Er}^{3+} \). The total number of RE\(^{3+}\) ions in once UCNP is the sum of all \( \text{Y}^{3+} \), \( \text{Yb}^{3+} \), and \( \text{Er}^{3+} \) ions in one UCNP. The ratios of \( \text{Y}^{3+} \), \( \text{Yb}^{3+} \), and \( \text{Er}^{3+} \) ions in one UCNP are given in Table S1. The total amount of rare-earth ions (\( n_{\text{RE}^{3+}} \)) per one spherical core UCNP is calculated using Equation (4):

\[
n_{\text{RE}^{3+}} = \frac{N_{\text{RE}^{3+}}}{N_A} \tag{4}
\]

With:

\[
N_{\text{RE}^{3+}} = 57 \cdot 10^3; \\
\text{and the Avogadro constant; } N_A = 6.022 \cdot 10^{23} \text{ mol}^{-1}
\]

\[
n_{\text{RE}^{3+}} = 9.5 \cdot 10^{-20} \text{ mol}
\]

The total amount of rare-earth ions (\( n_{\text{RE}^{3+}} \)) per one spherical core UCNP is \( 9.5 \cdot 10^{-20} \) mol.

For ICP-OES measurements (see Section 3), 0.3 g of OA-coated UCNPs were solubilized in a total volume of 15 mL. The results of the ICP-OES measurements can be found in Table S1. The total amount of RE\(^{3+}\) (\( n_{\text{RE}^{3+}_{15 \text{ mL}}} \)) in the solution used for ICP-OES (volume of 15 mL) is calculated using Equation (5). Here, the concentrations of \( \text{Y} \), \( \text{Yb} \), and \( \text{Er} \) as given in Table S1 are summed up.

\[
n_{\text{RE}^{3+}_{15 \text{ mL}}} = (c_Y + c_{\text{Yb}} + c_{\text{Er}}) V_{\text{ICP-OES}} \tag{5}
\]

With:

\[
c_Y = 319.5 \, \mu\text{mol} \, \text{L}^{-1} \\
c_{\text{Yb}} = 78.8 \, \mu\text{mol} \, \text{L}^{-1} \\
c_{\text{Er}} = 9.2 \, \mu\text{mol} \, \text{L}^{-1} \\
V_{\text{ICP-OES}} = 15 \, \text{mL}
\]
And thus:

\[ n_{RE^{3+}_{15 \text{ mL}}} = 6.1 \cdot 10^{-6} \text{ mol} \]

The total amount of RE$^{3+}$ ($n_{RE^{3+}_{15 \text{ mL}}}$) in the solution used for ICP-OES (volume of 15 mL) is 6.1 µmol.

The total amount of rare-earth ions ($n_{RE^{3+}}$) per one spherical core UCNP is $9.5 \cdot 10^{-20}$ mol as calculated above. Thus, the number of UCNPs ($N_{UCNPs_{15mL}}$) in the solution used for ICP-OES (volume of 15 mL) can be calculated using Equation (6).

\[
N_{UCNPs_{15mL}} = \frac{n_{RE^{3+}_{15 \text{ mL}}}}{n_{RE^{3+}}} \tag{6}
\]

With:

\[
n_{RE^{3+}_{15 \text{ mL}}} = 6.1 \cdot 10^{-6} \text{ mol}
\]
\[
n_{RE^{3+}} = 9.5 \cdot 10^{-20} \text{ mol}
\]

\[
N_{UCNPs_{15mL}} = 6.4 \cdot 10^{13}
\]

The number of UCNPs ($N_{UCNPs_{15mL}}$) in the solution used for ICP-OES (volume of 15 mL) is $6.4 \cdot 10^{13}$.

As described in Section 3, the volume of OA-coated UCNPs (dispersed in cyclohexane) used for ICP-OES measurements was 16 µL ($V_{ICP-OES \_cyclohexane}$). Accordingly, the number of UCNPs in 16 µL of the original cyclohexane dispersion is the same as in the solution (volume of 15 mL) used for ICP-OES measurements, viz. $6.4 \cdot 10^{13}$.

From these results one can calculate the UCNPs concentration ($c_{UCNPs_{\text{Stock}}}$) in 160 mL of the original cyclohexane dispersion using Equation (7). This dispersion was used as a stock for all further surface modifications.
The UCNPs concentration of the stock dispersion is $4 \cdot 10^{15}$ UCNPs mL$^{-1}$ in cyclohexane. This dispersion was used as a stock for all further surface modifications.
5. Quantification of OA Surface Ligands

Thermal gravimetric analysis (TGA) experiments were performed under a nitrogen atmosphere with a heating rate of 10 °C·min⁻¹ in the temperature range of 35 to 600 °C (see Figure 3; main article). A relative mass loss of ~9.1% can be observed for the initially OA-coated UCNPs. One can calculate the number of OA ions per single UCNP from the absolute mass loss and the density of pure NaYF₄ (4.21·10⁻²¹ g nm⁻³) [13].

The volume of one UCNP core \( V_{UCNP} \); i.e. without the OA capping was calculated using Equation (2), assuming a spherical shape of the UCNP core (for calculation see Section 4).

\[
V_{UCNP} = 6.1 \cdot 10^3 \text{ nm}^3
\]

The average mass of one single UCNP core \( m_{UCNP} \) without the OA capping can be calculated using Equation (8).

\[
m_{UCNP} = \frac{\rho_{NaYF_4}}{V_{UCNP}} \tag{8}
\]

With:

\[
\rho_{NaYF_4} = 4.21 \cdot 10^{-21} \text{ g nm}^{-3}
\]

\[
V_{UCNP} = 6.1 \cdot 10^3 \text{ nm}^3
\]

\[
m_{UCNP} = 2.6 \cdot 10^{-17} \text{ g}
\]

The average mass of one single UCNP core \( m_{UCNP} \) without the OA capping is \( 2.6 \cdot 10^{-17} \text{ g} \).

The mass of the OA-sample did not change above ~500 °C as can be seen from the thermogram (see Figure 3; main article). We take the absolute mass (15.162 mg; \( m_{\text{absolute, 500°C}} \)) at 500 °C and assumed that this is to be the mass of plain UCNPs (plUCNPs) without any surface ligands. The number of plUCNPs \( N_{plUCNPs} \) in the sample thus is calculated using Equation (9):

\[
N_{plUCNPs} = \frac{m_{\text{absolute, 500°C}}}{m_{UCNP}} \tag{9}
\]

With:
\[ m_{\text{absolute, } 500^\circ\text{C}} = 15.162 \text{ mg} \]
\[ m_{\text{UCNP}} = 2.6 \cdot 10^{-17} \text{ g} \]

\[ N_{\text{plUCNP}s} = 5.8 \cdot 10^{14} \]

The number of plUCNPs \( (N_{\text{plUCNP}s}) \) in the sample is \( 5.8 \cdot 10^{14} \).

The total number of OA ions \( (N_{\text{OA}}) \) is calculated using Equation (10) from the absolute mass loss of the TGA experiment \( (1.53 \text{ mg}; m_{\text{absolute_mass_loss}}) \). It is assumed that the absolute mass loss is only due to the loss of OA ions. The molar mass of OA ions \( (M_{\text{OA}}) \) is 281.45 g mol\(^{-1}\).

\[ N_{\text{OA}} = \left( \frac{m_{\text{absolute_mass_loss}}}{M_{\text{OA}}} \right) \cdot N_A \tag{10} \]

With:
\[ m_{\text{absolute_mass_loss}} = 1.53 \text{ mg} \]
\[ M_{\text{OA}} = 281.45 \text{ g mol}^{-1} \]
\[ N_A = 6.022 \cdot 10^{23} \text{ mol}^{-1} \]

\[ N_{\text{OA}} = 3.3 \cdot 10^{18} \]

The total number of OA ions \( (N_{\text{OA}}) \) is \( 3.3 \cdot 10^{18} \).

The number of OA ions per one single UCNP \( (N_{\text{OA \text{UCNP}}} / \text{UCNP}) \) is calculated using Equation (11).

\[ N_{\text{OA \text{UCNP}}} / \text{UCNP} = \frac{N_{\text{OA}}}{N_{\text{plUCNP}}} \tag{11} \]

With:
\[ N_{\text{OA}} = 3.3 \cdot 10^{18} \]
\[ N_{\text{plUCNP}} = 5.8 \cdot 10^{14} \]

\[ N_{\text{OA \text{UCNP}}} / \text{UCNP} = 5.7 \cdot 10^3 \]

The number of OA ions per one single UCNP \( (N_{\text{OA \text{UCNP}}} / \text{UCNP}) \) is \( 5.7 \cdot 10^3 \).
The surface area of one single UCNP ($A_{UCNP}$) core without OA capping can be calculated using Equation (12).

$$A_{UCNP} = 4 \cdot \pi \left( \frac{d_c}{2} \right)^2 \quad (12)$$

With the core diameter, $d_c = 22.7$ nm (from TEM).

$$A_{UCNP} = 1.6 \cdot 10^{-3} \, \mu m^2$$

The surface area of one single UCNP ($A_{UCNP}$) core without OA capping is $1.6 \cdot 10^{-3} \, \mu m^2$.

The number of OA ions per $\mu m^2$ of the surface area of one single UCNP ($N_{\text{oleate}} \frac{A_{OA}}{A_{UCNP}}$) can be calculated using Equation (13).

$$N_{\text{OA}} \frac{A_{OA}}{A_{UCNP}} = \frac{N_{OA}}{A_{UCNP}} \quad (13)$$

With:

$$N_{OA} \frac{A_{OA}}{A_{UCNP}} = 5.7 \cdot 10^3$$

$$A_{UCNP} = 1.6 \cdot 10^{-3} \, \mu m^2$$

$$N_{OA} \frac{A_{OA}}{A_{UCNP}} = 3.6 \cdot 10^6 \, \mu m^2$$

The number of OA ions per $\mu m^2$ of the surface area of one single UCNP ($N_{\text{oleate}} \frac{A_{OA}}{A_{UCNP}}$) is $3.6 \cdot 10^6 \, \mu m^2$.

Assuming a diameter of an OA ion of 0.5 nm, its area is $\pi \cdot (0.5 \, \text{nm}/2)^2 \approx 0.2 \, \text{nm}^2$ [14]. Therefore, $\sim 70\%$ of the UCNP’s surface area is covered by OA ions.
6. References