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Upconversion Nanoparticles: From Hydrophobic to Hydrophilic Surfaces

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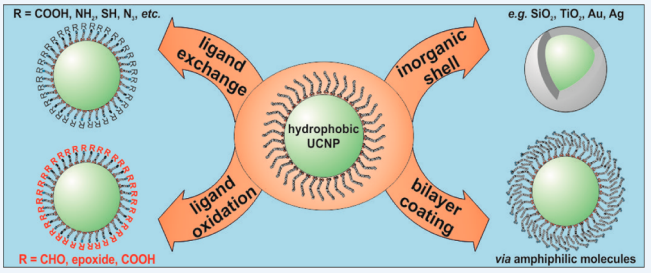
CONSPECTUS: Photon upconversion nanoparticles (UCNPs) have emerged as a promising new class of nanomaterials due to their ability to convert near-IR light into visible luminescence. Unfortunately, most efficient methods for preparing UCNPs yield hydrophobic materials, but water-dispersibility is needed in the major fields of applications of UCNPs, that is, in bioimaging, labeling, and bioassays. Numerous methods therefore have been reported in the past years to convert the hydrophobic surface of UCNPs to a more hydrophilic one so to render them dispersible in aqueous systems.



We present a classification respective for these strategies and assess the main methods. These include (A) chemical modification of the hydrophobic (typically oleate) ligand on the surface, (B) addition of an extra layer, (C) addition of a thin shell on top of the UCNP, and (D) complete replacement of the original ligand by another one. Chemical modification (A) involves oxidation of the oleate or oleylamine and leads to particles with terminal oxygen functions. This method is less often used because solutions of the resulting UCNPs in water have limited colloidal stability, protocols are time-consuming and often give low yields, and only a limited number of functional groups can be introduced. Methods B and C involve coating of UCNPs with amphiphiles or with shells made from silica oxide, titanium oxide, or metallic gold or silver. These methods are quite versatile in terms of further modifications, for example, by further cross-linking or by applying thiol−gold chemistry. Growing an extra shell is, however, often accompanied by a higher polydispersity. Method D can be divided into subgroups based on either (i) the direct (single-step) replacement of the native ligand by a new ligand or (ii) two-step protocols using nitrosyltetrafluoroborate ($NOBF_4$) or strong acids as reagents to produce ligand-free UCNPs prior to the attachment of a new ligand. These methods are simple and versatile, and the distance between the new ligand and the luminescent particle can be well controlled. However, the particles often have limited stability in buffer systems. The methods described also are of wider interest because they are likely to be applicable to other kinds of nanomaterials.

We additionally address the need for (a) a better control of particle size and homogeneity during synthesis, (b) more reproducible methods for surface loading and modification, (c) synthetic methods giving higher yields of UCNPs, (d) materials displaying higher quantum yields in water solution without the need for tedious surface modifications, (e) improved methods for workup (including the suppression of aggregation), (f) new methods for surface characterization, and (g) more affordable reagents for use in surface modification. It is noted that most synthetic research in the area is of the trial-and-error kind, presumably due to the lack of understanding of the mechanisms causing current limitations. Finally, all particles are discussed in terms of their biocompatibility (as far as data are available), which is quintessential in terms of imaging, the largest field of application.

INTRODUCTION

Upconverting (or upconversion) luminescent materials, first described in the 1960s, are capable of converting light of long wavelength (typically 800−1000 nm) into shorter-wave (mostly visible) luminescence.[1] Unlike in two-photon excitation (where two photons are absorbed at the same time), the effect is based on the sequential absorption of multiple lower energy photons, not the least because the first excited states have long lifetimes where the probability of absorbing a second photon to form a higher excited state therefore is quite high. Relaxation from such an excited state results in the emission of light at higher energy, with typical wavelengths that lie between 350 and 800 nm.

Upconversion nanoparticles (UCNPs), like the corresponding bulk phases, also display upconverted luminescence, albeit with lower brightness.[2] The most efficient upconverting luminescent nanomaterials known to date consist of hexagonal-phase lanthanide-doped $NaYF_4$ nanocrystals. The excitation wavelengths required for upconversion to occur lie in the optical window of most biological matter so that absorption of light in this range is comparably weak. This leads to a longer penetration depth of the excitation light and makes strong laser light sources (that can damage tissue) dispensable. The fact that upconverted luminescence is anti-Stokes-shifted also facilitates

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the separation of luminescence from Raman bands and other scattered light. If luminescence of biomatter is induced at all, it will occur far in the NIR. In other words, the intensity of background visible fluorescence (i.e., in the region where upconverted luminescence occurs) is virtually zero.

In contrast to quantum dots, the color of the emission of UCNPs does not depend on the size of the particles. UCNPs are not known to be cytotoxic, are chemically stable, and neither blink nor bleach. On the other hand, their quantum yields depend on their size, on the kind of surface coating, and on the power density of the laser used for photoexcitation.[3] These features have been discussed in numerous reviews that can be easily found. Their outstanding features make UCNPs highly interesting materials for purposes including photodynamic therapy,[4] photoinduced drug delivery,[5] (targeted) cell imaging,[6] sensing of fundamental parameters such as pH values,[7] oxygen,[8] ammonia,[9] heavy metal ions,[10] or CO_2,[11] screening,[12] and immunoassays.[13]

Initially, UCNPs were produced by top-down strategies. Such methods yield stable colloidal solutions, but the particles were rather polydisperse and fairly large, leading to slow or no cellular uptake. A comparative study on upconversion luminescence and cell bioimaging based on single-step synthesized hydrophilic UCNPs capped with various functional groups has been presented by Tsang et al.[14] To overcome the limitations of high polydispersity and poor flexibility in terms of surface modification (by either small molecules or thin additional layers), bottom-up strategies have been developed with the aim to synthesize small and monodisperse nanoparticles possessing bright upconversion luminescence. The most common strategies are coprecipitation, thermal decomposition, and solvothermal syntheses.[15] Depending on the kind of host lattice and the protocol used for synthesis, UCNPs can be prepared in shapes such as symmetrical spheres, rods, and even plates. The reviews by Chen et al.[15] and DaCosta et al.[16] provide an overview of synthetic strategies and material properties. DeCosta et al. also have introduced a system for the classification of methods of making apolar surfaces of UCNPs more polar (based on ligand exchange, ligand oxidation, ligand absorption, layer-by-layer assembly, ligand-free modifications, and silanizations). Other reviews are available on lanthanide-doped luminescent nanoprobes (with a small section on UCNPs)[17] and on UCNPs for use in small-animal imaging (with a short section on surface modification).[18] The groups of Selvin[19] and Li[20] have briefly reviewed methods for surface engineering of UCNPs, while other reviews are ignoring the need for converting hydrophobic to hydrophilic surfaces.[21]

Hexagonal phase $NaYF_4$ nanoparticles doped with trivalent lanthanides (Ln^{3+}) are by far most often used. In 2008, Li and Zhang[22] presented an efficient protocol for preparation of *hydrophobic* (oleate-capped) UCNPs. It involves heating of rare earth chlorides in a mixture of octadecene and oleic acid, first to generate the respective oleate salts, which act as in situ precursors. The addition of ammonium fluoride and sodium hydroxide and an increase in the reaction temperature to 300 °C leads to the formation of highly monodisperse, oleate-capped, hexagonal UCNPs that can be dispersed in nonpolar solvents. Oleylamine (OlAm) may be added to, or even used in place of oleic acid.[23] This bottom-up method in high-boiling solvents is said to be superior to others with respect to the monodispersity, shape uniformity, and phase purity of the resulting UCNPs. On the other hand, it suffers from the disadvantage of giving UCNPs that are dispersible in hydrophobic solvents only and not in aqueous solutions including buffers. If intended for use in biosciences, water dispersibility and colloidal stability in buffers is, however, mandatory. In addition, these particles are highly inert in being devoid of any useful functional group on their surface. In order to exploit the large potential of UCNPs, appropriate functional groups have to be introduced.

This Account throws a critical look at the methods for surface modification and functionalization that lead to UCNPs for use in aqueous media. In terms of bioimaging (which is the most widespread application of such particles at present), features such as small size, brightness, and tunable emission and excitation spectra also are paramount.[24] We summarize general principles, discuss advantages and disadvantages of the strategies, and give selected examples for respective applications.

SURFACE MODIFICATION OF HYDROPHOBIC UPCONVERSION NANOPARTICLES

A large variety of methods for surface modification have been developed to convert hydrophobic UCNPs into more hydrophilic particles. They often are not limited to UCNPs but also may be applied to other types of nanoparticles.[25] The respective strategies can be categorized into four groups: (1) chemical modification of the hydrophobic (usually oleate or oleylamine) ligand on the surface; (2) bilayer coating with amphiphilic molecules or polymers, (3) addition of an extra layer or shell on top of the UCNP; and (4) complete replacement of the original ligand by another one. These will be discussed in the following.

Modification of the Original Ligand

The direct modification of the hydrophobic ligand on a particle's surface to generate hydrophilic UCNPs is simple but not common. It is based on the oxidation of the carbon−carbon double bond of the oleate or oleylamine. Depending on the reagents employed, it can lead to the formation of carboxy groups or epoxy groups. Oxidizing agents include the Lemieux−von Rudloff reagent,[26] ozone,[27] and 3-chloroperoxy-benzoic acid.[28] In addition to the formation of reactive groups, the dispersibility of the resulting particles in water is strongly enhanced. Such particles then may been covalently coupled to other species, for example, to the cancer drug doxorubicin in order to enable controlled drug delivery[29] or to poly(ethylene glycol) in order to impart biocompatibility.[28] Notwithstanding this, oxidative surface modification is rarely used because dispersions in water have poor colloidal stability and only a limited number of ligands (aldehydes, epoxides, or carboxylic acids) can be introduced.

Amphiphilic Coatings

This technique involves coating of the UCNPs with molecules containing long alkyl chains to form a bilayer that is stabilized via van-der-Waals interactions between the hydrophobic oleate and the new coating. Amphiphilic molecules are preferred in this context because they (a) undergo strong van-der-Waals interaction, (b) enable the surface charge to be easily altered, and (c) may even be deposited as a so-called layer-by-layer coating, that is, in the form of multiple layers of alternating charge. If oleate-capped UCNPs are treated with long-chain amphiphiles, their hydrophobic tails intercalate between the oleate chains, while their hydrophilic head groups are directed outward. This results in the formation of a bilayer around the

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UCNP as shown in Figure 1. The hydrophilic head groups render the particles well dispersible in water.

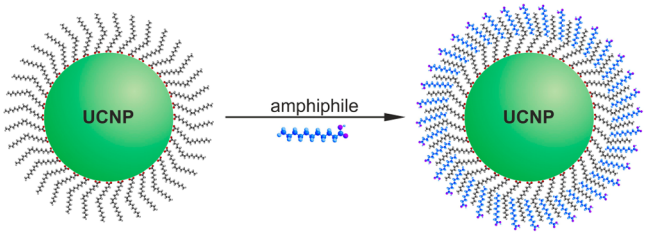

Figure 1. Principle of bilayer formation by coating the oleate-capped UCNP with an amphiphile possessing a hydrophilic or ionic end group, thus converting the hydrophobic particles to hydrophilic particles.

Phospholipids (PLs) have often been used to modify surfaces. Resulting particles (not only UCNPs) are readily internalized by cells, are nonimmunogenic, and possess a long functional lifetime even *in vivo*. PLs have been widely used in drug delivery. Quite a variety of PLs are commercially available with various kinds of head groups such as maleimide (for binding the particle to protein thiol groups), biotin (with its high affinity for streptavidin), and several others. Phospholipids also are known with highly different chain lengths, and variation in length is often accomplished by incorporating poly(ethylene glycol) (PEG) units, which has the beneficial effect of imparting biocompatibility.[30,31] In a typical example, maleimide and folate head groups were used to conjugate UCNPs to gold nanoparticles and image HeLa cells (see Figure 2).[19]

While phospholipids with polar head groups and PEG spacers are easy to use, they are difficult to make and purify and expensive if commercially available. The following calculation may reflect the costs to be expected in a typical experiment: The surface area of one single nanocrystal with a diameter of 20 nm is 1250 nm^2. If 1 μmol of such particles is to be covered with, say, a PEGylated distearoyl phospholipid (with a size of ~80 $Å^2$), the total surface to be coated is as large as 760 m^2. This requires, roughly, 4.5 g of the phospholipid, which will actually cost *more than US $30,000*. This number may be even higher if phospholipids are applied in excess to warrant complete coverage of the surface. Obviously, less expensive methods are desirable to create bilayers. Zhao et al.[32] have coated UCNPs with the detergent Tween 80 to obtain particles for use as a carrier for doxorubicin that was trapped in the hydrophobic bilayer. Other long-chain alkylammonium derived surfactants were tested by the Yang group,[33] but the colloidal stability of the particles in water was poor. Subsequent surface modification with silica was required. This will be discussed in the next section.

In another approach, amphiphilic polymers were used in place of the relatively small surfactants as shown by the Parak group[34] in order to modify the surface of gold nanoparticles, quantum dots, or iron oxide particles. Poly(maleic anhydride-*alt*-1-octadecene) (PMAO) is a widely used polymeric amphiphile[35,36] because it contains multiple alkyl chains per molecule and has a weak chelating effect, which stabilizes the surface coating against ligand detachment. Particles coated with PMAO display good temporal stability in aqueous media, which can be further increased by reacting the anhydride groups with bis(hexamethylene)triamine (BHMT).[37] This method enabled

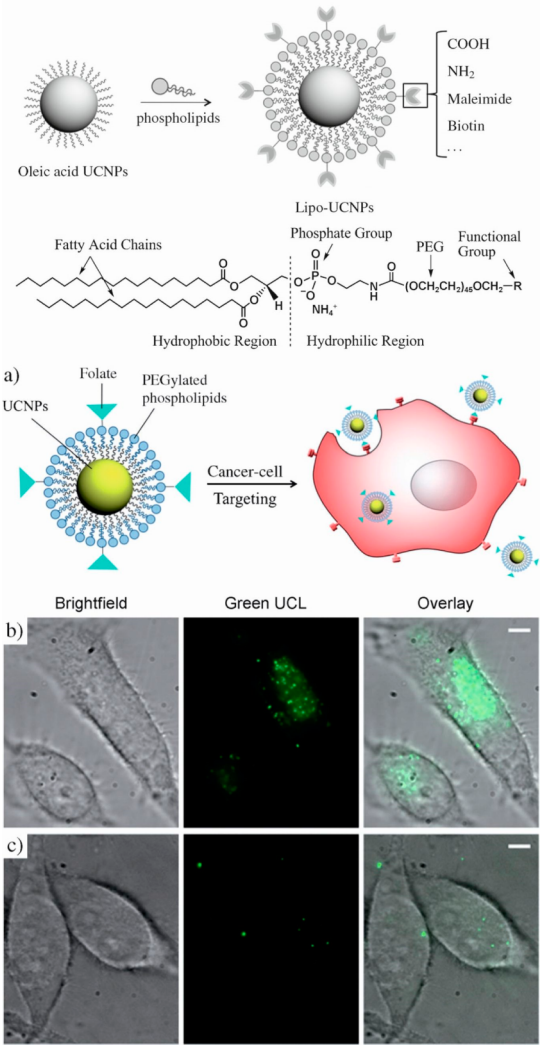

Figure 2. Illustration of the assembly of the water-dispersible UCNPs by adding a monolayer of phospholipids. (a) Illustration of the targeted imaging of cancer cells with Lipo-UCNPs carrying folic acid (FA). (b, c) Transmission and luminescence microscopy images of HeLa cells treated with Lipo-UCNPs-FA (b) and with Lipo-UCNPs without folate ligand (c). Scale bar is 5 μm. UCL, upconversion luminescence. Reprinted with permission from ref 19. Copyright 2012 by Wiley & Sons, Inc.

UCNPs to be stabilized over the pH 3−13 range and in cell cultures for even several weeks (see Figure 3).

Poly(acrylic acid) modified with long alkyl chains also binds to the surface of oleate-capped UCNPs.[38] This results in the introduction of carboxy groups that are negatively charged at near-neutral pH values and will render the particles water-soluble. The carboxy groups can further serve as functional groups to couple the particles to proteins.[39] There have also been reports on UCNPs coated (a) with amphiphilic chitosan for use in photodynamic therapy[40] and (b) with an amphiphilic silane for use in optical probing of temperature using a Eu(III) chelate as an indicator[41] as schematically shown in Figure 4. UCNPs that are highly stable under physiological conditions were obtained[42] by coating them with methoxy-poly(ethylene glycol-*block*-caprolactone). Table 1 gives a selection of amphiphilic molecules applied to surface modification of UCNPs.

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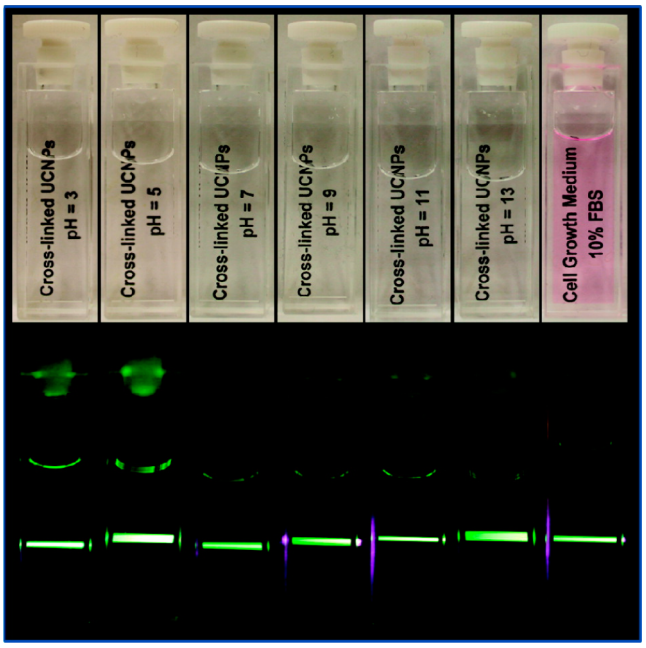

Figure 3. Core/shell nanoparticles ($NaYF_4$:20% Yb^{3+},2% Er^{3+}/$NaYF_4$−PMAO−BHMT) dispersed in water at different pH from 3 to 13 and serum-supplemented cell growth medium and respective images under 980 nm excitation (bottom). Reprinted with permission from ref 37. Copyright 2012 American Chemical Society.

Encapsulation with Inorganic Materials or Noble Metals Forming a Shell

Water dispersibility was accomplished by deposition of an additional shell on top of hydrophobic UCNPs. Typical shell materials include oxides like SiO_2 and TiO_2 (to impart solubility and, sometimes, catalytic activity) or noble metals like gold or silver, which pave the way to plasmonic modulation of upconverted luminescence (Figure 5). Table 2 gives a selection of respective materials.

The deposition of a silica shell on a UCNP is a useful technique to generate functionalized and water dispersible NPs. Both hydrophilic and hydrophobic UCNPs can be coated by using either the Stöber method or the reverse microemulsion method. The latter is better suited for oleate or oleylamine-capped UCNPs and gives core−shell particles coated with a uniform layer of silica (SiO_2).[50] A product having the trade name Igepal CO-520 is widely used because it forms adequately stable reverse microemulsions for polymerization of precursors such as tetraethyl orthosilicate. Ammonia is added as a catalyst. It causes the formation of silicic acid at a concentration above the nucleation concentration, thus warranting a steady growth of the silica shell as schematically shown in Figure 6. The resulting silica-coated particles (usually referred to as UCNPs@SiO_2) readily disperse in water. They have a negative ζ potential that depends on solvent, salinity, and surface charges and display low cytotoxicity.[50]

The silica coating strategy suffers from the classical drawback of almost all coating methods of that kind in that polydispersity increases compared to untreated particles. In case of thin silica shells, it is difficult to prove whether the formation of the shell is complete. In addition, UCNPs@SiO_2 possess poor temporal stability in aqueous solution in that they tend to aggregate and precipitate within a couple of hours.[51,52] On the positive side, coatings with comparably thick layers of silica do not strongly compromise the "brightness" of particles, which is in contrast to coatings with small molecules or thin films (see below).

If UCNPs@SiO_2 are separated by centrifugation, they often cannot be redispersed in water and no longer form a clear dispersion. This problem may be overcome by introducing a

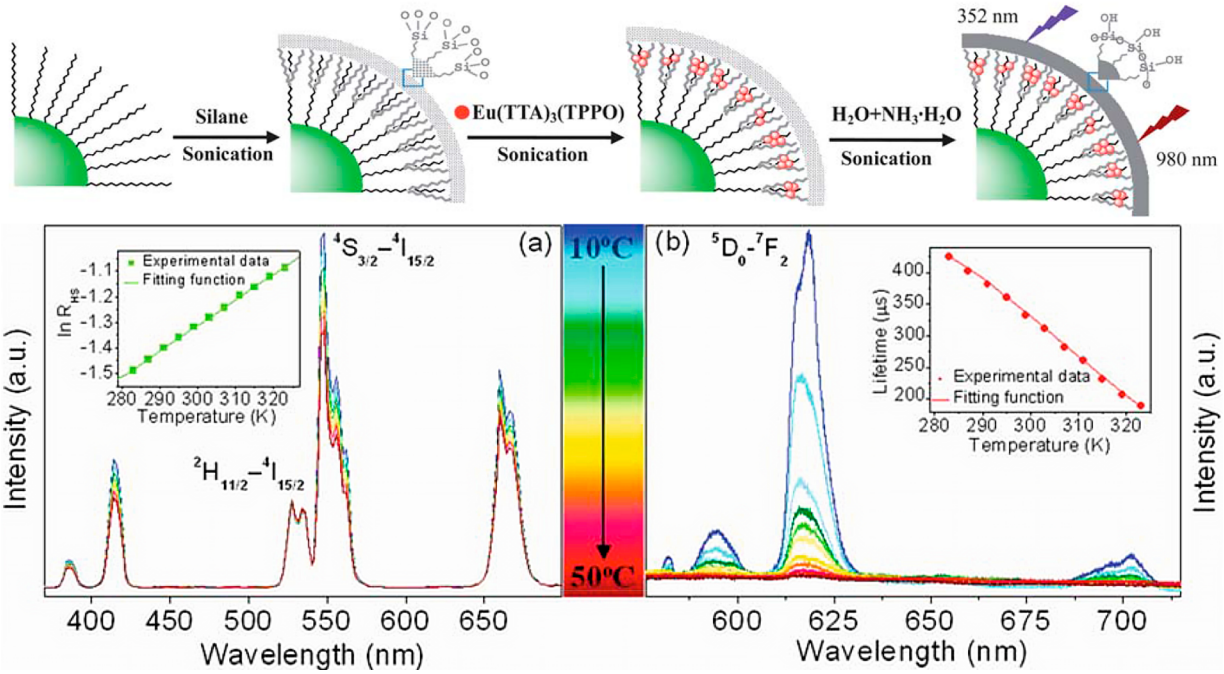

Figure 4. Schematic illustration of the synthesis of silane-modified $NaYF_4$:Yb^{3+},Er^{3+} loaded with the probe $Eu(TTA)_3(TPPO)_2$ (top), and temperature-dependent spectra of C_{18} silane-modified NPs under 980 nm (a) and 352 nm (b) excitation (bottom). The inset in panel a shows the temperature-dependent intensity ratio value of the two upconversion luminescence emissions at 525 and 544 nm. The inset in panel b shows the temperature-dependent lifetime of the upconversion luminescence. Reproduced from ref 41 with permission of The Royal Society of Chemistry.

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Table 1. Examples for Amphiphilic Molecules Used for Coating of UCNPs and Selected Applications of the Resulting Water-Dispersible Nanoparticles

UCNP and native ligand	amphiphilic molecule	application	refs
$NaYF_4$:Yb,Er and $NaYF_4$:Yb,Tm@oleate	phospholipids with various head groups	optical and magnetic resonance imaging	19, 30, 31
$NaYF_4$:Yb,Er@oleate	Tween 80	bioimaging and drug delivery	32
$NaYF_4$:Yb,Er@oleate	surfactants	water dispersibility	33
$NaYF_4$:Yb,Er and $NaYF_4$:Yb,Tm@oleate	poly(maleic anhydride-*alt*-1-octadecene)	photodynamic therapy, detection of Hg^{2+} ions in water	35, 36
$NaYF_4$:Yb,Er and $NaYF_4$:Yb,Tm@oleate	amphiphilic poly(acrylic acid)	bioimaging, cell tracking	38, 39
$NaYF_4$:Yb,Er@oleate	amphiphilic chitosan	photodynamic therapy	40
$NaYF_4$:Yb,Er@oleate	amphiphilic silane	temperature sensing, cell imaging	41

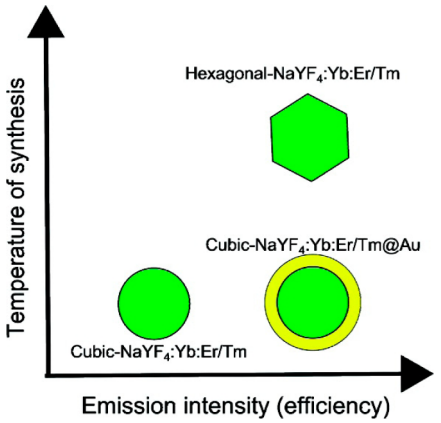

Figure 5. Illustration of the luminescence enhancement of cubic $NaYF_4$:Yb,Er/Tm UCNPs achieved by the growth of a thin Au-shell compared with the luminescence intensity of conventional hexagonal $NaYF_4$:Yb,Er/Tm UCNPs. Reprinted with permission from ref 43. Copyright 2011 American Chemical Society.

Table 2. Examples for $NaYF_4$ Nanoparticles Encapsulated by Various Inorganic Materials and Corresponding Applications

UCNP and its native ligand	shell	application	refs
$NaYF_4$:Yb,Tm, $NaYF_4$:Yb,Er@oleate	SiO_2	imaging, drug delivery	44, 45
$NaYF_4$:Yb,Tm@oleate, $NaYF_4$:Yb,Er@SiO_2	TiO_2	dye sensitized solar cells, photocatalysis	46, 47
$NaYF_4$:Yb,Tm@oleate	Au	plasmonic modulation of upconversion emission	43, 48
$NaYF_4$:Yb,Er@oleylamine	Ag	imaging, photothermal therapy	49

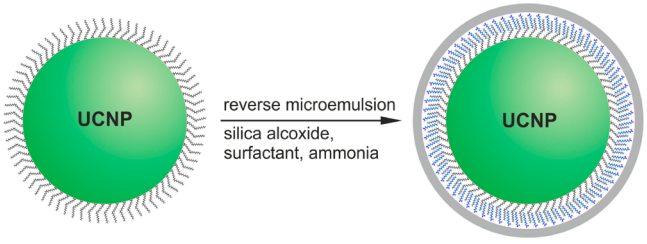

Figure 6. Schematic representation of the silica shell formed on oleate-capped UCNPs. The initially hydrophobic particles are converted to hydrophilic particles. This process is accompanied by large changes in the ζ potential.

high density of surface charges, which will reduce the tendency toward aggregation.[53] It was shown that agarose gel electrophoresis (AGE) is well suited for the purification of silica-coated UCNPs.[54] The silica shell of a fraction of the particles was doped with a fluorescent dye for direct detection. The shell was prepared by reverse microemulsion and resulted in individual nanoparticles but also in aggregates that were separated and isolated. The preparation of an ultrathin carboxylated silica shell, in contrast, yielded nonaggregated UCNPs that can be directly used for protein conjugation.

Functional groups can be created on the surface of the UCNPs in two ways. In one, the preformed UCNPs@SiO_2 particles are modified with organically modified silanizing agents. In the other, functional organosilanes are added during the polymerization process, which leads to the formation of the shell so that postsynthetic modification is not needed. Organosilanes that have been used in either method are summarized in Table 3.

Amino-functionalized UCNPs@SiO_2 may be prepared by adding aminopropyltriethoxysilane to the microemulsion.[44] The water-dispersible UCNPs can then be conjugated to folic acid to enable targeting of tumor cells. Similarly, silica-coated $NaYF_4$:Yb,Er UCNPs were further endowed with folic acid and anti-Her2 antibody to label the folate receptors and Her2 receptors of certain cells. Our group has reported the preparation of protein-reactive hydrophilic particles (Figure 7) by modifying the surface of UCNPs@SiO_2 with a silane-modified poly(ethylene glycol) with a terminal *N*-hydroxysuccinimide group.[58] The nanoparticles were then conjugated to proteins as verified by surface plasmon resonance spectroscopy.

UCNPs coated with mesoporous silica were modified with azo groups via silanization and then loaded with the cancer drug doxorubicin.[5] The azo groups acted as motors to trigger the controlled release of the drug under photoexcitation at 980 nm. Mesoporous silica shells are characterized by a large specific surface area and a pore size that can be fine-tuned (see Figure 8). Other UCNPs were coated with mesoporous silica and loaded with photosensitizers such as zinc(II) phthalocyanine, which causes the formation of singlet oxygen upon NIR excitation.[4] Li et al.[60] have incorporated doxorubicin into particles coated with mesoporous silica, which then were studied with respect to cellular uptake and cytotoxicity. Their potential for imaging of nasopharyngeal epidermal carcinoma cells was demonstrated.[61] More recently, core−shell−shell particles of the type β-$NaYF_4$:Yb,Er@SiO_2@mSiO_2 have been reported,[45] again for use in imaging and drug storage and delivery. So-called yolk−shell UCNPs were obtained by forming a hollow mesoporous silica shell around $NaLuF_4$:Yb,Er,Tm nanoparticles.[62] Their large cavities were loaded with a chromophore to construct nanoprobes for cysteine, homocysteine, and cyanide.

Replacement of the Native Ligand

Ligand exchange is a versatile strategy to modify the surface of UCNPs. Two major methods are known. One is based on

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Table 3. Examples of Silane Reagents Used for Coating or Modification of UCNPs To Render Them Water-Dispersible (and Sometimes Also Reactive) and Corresponding Applications of the Hydrophilic Particles

UCNP and native ligand	silane reagent	application	refs
$NaYF_4$:Yb,Er@oleate	aminopropyltrimethoxysilane	targeting and imaging of tumor cells	55
$NaYF_4$:Yb,Er@oleate	aminopropyltriethoxysilane	targeting and imaging of tumor cells	44
$NaYF_4$:Yb,Er@oleate	*N*-[3-(trimethoxysilyl)propyl]-ethylenediamine	assay for the detection and characterization of a caspase-3 inhibitor	
$NaYF_4$:Yb,Er@oleate	carboxyethylsilanetriol	biolabeling, energy transfer	56
$NaYF_4$:Yb,Er and $NaYF_4$:Yb,Tm@oleate	mesoporous silica loaded with doxrubicin	imaging and drug delivery	5, 57
$NaYF_4$:Yb,Er and $NaYF_4$:Yb,Tm@oleate	silane-modified PEG-NHS	protein conjugation	58
$NaYF_4$:Yb,Er@oleate	silane modified IR-783	NIR imaging and photothermal therapy	59
$NaYF_4$:Yb,Er@oleate	mesoporous silica loaded with zinc(II) phthalocyanine	photodynamic therapy	4
$NaYF_4$:Yb,Er@oleate	silica@mesoporous silica loaded with ibuprofen	imaging and drug delivery	45

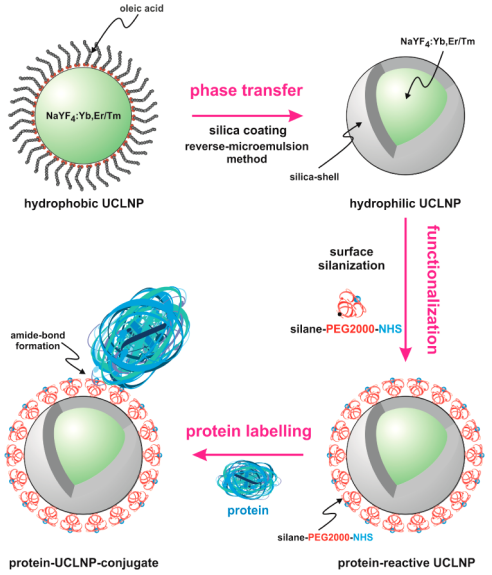

Figure 7. Surface engineering of UCNPs toward protein-reactive, multicolor upconverting labels by coating them with a reagent of the type silane-PEG-NHS.[58]

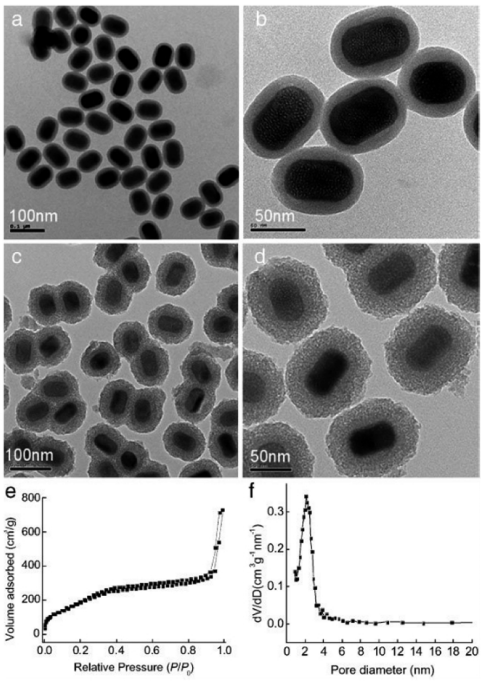

Figure 8. Transmission electron microscopy images of $NaYF_4$:Yb,Er@silica nanoparticles (a, b) and mesoporous-silica-coated $NaYF_4$:Yb,Er@silica nanoparticles (c, d). N_2 adsorption/desorption isotherm (e) and pore-size distribution (f) of mesoporous-silica-coated $NaYF_4$:Yb,Er@silica nanoparticles. Reprinted with permission from ref 4. Copyright 2009 by John Wiley Sons, Inc.

direct exchange of the first ligand by the new one; the other is based on two-step strategies using $NOBF_4$ or acid treatment with HCl to strip off the oleate or oleylamine and subsequent attachment of a new coating. Unfortunately, practically all coatings with small molecules (for example via oleate replacement or the $NOBF_4$ technique) for phase transfer to aqueous solvents drastically reduce the "brightness" of UCNPs. Coating the particles with $NaYF_4$, in contrast, does not cause such an effect. However, a systematic study on the effect of small-molecule coatings on quantum yields and luminescence decay times has not been presented so far.

Direct (Single Step) Replacement of the Native Ligand by a New Ligand

In this case, the native ligand on the UCNP is (almost) completely displaced by another ligand that is supposed to be more polar to confer water solubility. Ideally, it contains a functional group that coordinates to the surface of the UCNP so that it can easily replace the native ligand. The strength of interaction is likely to increase in the order $-SH$, $-NH_2$, $-COOH$, $-PO_3H$, but no comparative study covering the different binding strengths is available. Respective methods are fairly simple, at least in principle, but work up is tedious and more challenging than the chemical reaction itself. In a typical procedure, oleate-capped UCNPs and the new ligand are stirred for 4 h to several days, usually at elevated temperature.[63,49] The protocols have to be optimized for each single ligand because each ligand requires specific reaction conditions in terms of concentrations, stirring time, temperature, and need for an inert atmosphere. Furthermore, the particles tend to aggregate during ligand exchange.[64] The group of Pérez-Prieto used heterobifunctional PEG with a thiol group at one end and an amine or carboxylic group at the other.[65] In this protocol, the PEG ligands are used as both the capping ligand and the water-stabilizing agent. PEG moieties can function as polydentate ligands and bind to lanthanide ions. Representative reagents and cappings and the properties and application of the resulting hydrophilic UCNPs are summarized in Table 4. Ligands usually have to be added in excess in order to displace

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Table 4. Examples for Direct Replacement of Hydrophobic Surface Ligands by Hydrophilic Ligands and Properties and Uses of the Resulting Water-Dispersible Products

UCNP and native ligand	reagent or new ligand	application	refs
$NaYF_4$:Yb,Er@oleate	citrate	bioimaging, conjugation	66
$NaYF_4$:Yb,Er and $NaYF_4$:Yb,Tm@ oleylamine	hexanedioic acid	making particles water-soluble, conjugation	67
$NaYF_4$:Yb,Er and $NaYF_4$:Yb,Tm@ oleylamine	PEGylated carboxylate	making particles water-soluble, biocompatibility	68
$NaYF_4$:Yb,Er and $NaYF_4$:Yb,Tm@ oleate	PEGylated phosphate	making particles water-soluble, biocompatibility	69
$NaGdF_4$:Yb,Er@oleate	poly(amido amine) (PAMAM)	conjugation to carbohydrates and recognition of lectins	70
$NaYF_4$:Yb,Er@oleate	poly(allyl amine)	conjugation to zinc(II)-phthalocyanine as photosensitizer for photodynamic therapy	64
$NaYF_4$:Yb,Tm@oleate	mercaptopropionic acid	imaging and photothermal therapy	71
$NaYF_4$:Yb,Er@oleylamine	thioglycolic acid	growth of Ag-shell for photothermal therapy	49
$NaYF_4$:Yb,Tm and $NaGdF_4$:Yb,Ho@ oleate	poly(acrylic acid)	studies on the distribution and toxicity of polyacrylate-coated UCNPs	72, 73
$NaYF_4$:Yb, Er@oleate	poly(vinylpyrrolidone)	making particles water-soluble	74
$NaYF_4$:Yb,Er@oleate	monothiolated heterobifunctional PEGs	bioimaging, conjugation	65, 75, 76

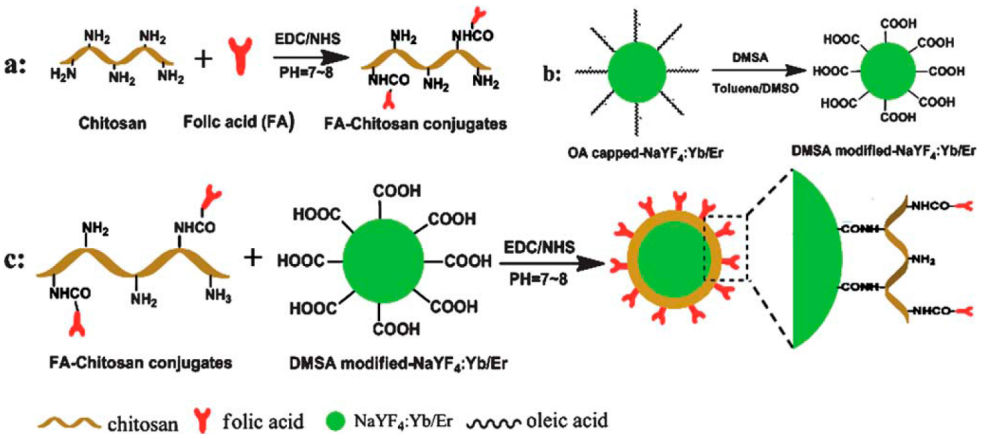

Figure 9. Illustration of the formation of $NaYF_4$:Yb,Er nanoparticles coated with folic acid (FA) and chitosan. From ref 79 with permission of The Royal Society of Chemistry.

the former ligand. Even organic polymers may be used in this replacement strategy as can be seen in Table 4.

The introduction of PEG chains not only imparts hydrophilicity but also results in improved biocompatibility when used in imaging or cell targeting. Ultrasmall core−shell UCNPs of the type $NaYF_4$:Yb,Tm@SiO_2 were further modified with PEG and found to be bound by MCF-7 tumors,[77] while others were coated with similarly hydrophilic multihydroxy dendritic molecules to provide water dispersibility and hydrophilicity.[78] The introduction of carboxy groups, in turn, allows UCNPs to be conjugated to biomolecules containing amino groups (see Figure 9),[79] and maleimides can be conjugated to thiols.[80] If the oleate ligand is exchanged by 2-bromo-2-methylpropionic acid and polymerized with the hydrophilic polymer oligo(ethylene glycol) methacrylate, a dispersion is obtained that is stable in phosphate buffer.[81] The UCNPs obtained were conjugated to lectins and applied to imaging of cancer cells. Strong interaction of UCNPs with the phosphate groups of DNA also has been claimed,[82] but questions remain such as the lack of cross-linking between particles (via DNA chains) and how hybridization can occur such that one end of the oligomer remains bound to the UCNP.

Modification of oleylamine-capped magnetic UCNPs via ligand exchange with a mixture of aminocaproic acid, oleic acid, and folic acid and simultaneous cation exchange with Gd^{3+} ions gives mixed hydrophobic surfaces.[83] Positively or negatively charged layers consisting of small molecules or even of polymers may be deposited alternatively, a technique known as layer-by-layer coating. This strategy allows for a precise control of surface charge and thickness of the particles. Various kinds of molecules may be placed between (or in) the layers to result in materials for controlled drug delivery and photodynamic therapy,[84] for example, as shown in Figure 10. Dispersions of polymer-modified UCNPs generally display better colloidal stability in aqueous media than their small molecule-modified counterparts. Nonetheless, their tendency to aggregate if placed in buffers or cell culture media remains a problem.[69,85]

Two-Step Replacement of the Native Ligand Using the $NOBF_4$ Reagent

The group of Murray[86] have introduced a widely applicable strategy for modification of surfaces of nanoparticles by using the reagent nitrosyl tetrafluoroborate ($NOBF_4$). If added to dispersions of nanoparticles capped with oleate or oleylamine, the ligand is stripped off and the BF_4^- ions are said to take their place. Other tetrafluoroborates work much less well, or even not at all, so the involvement of the NO^+ cation in the process also should be taken into consideration. Aggregation during the

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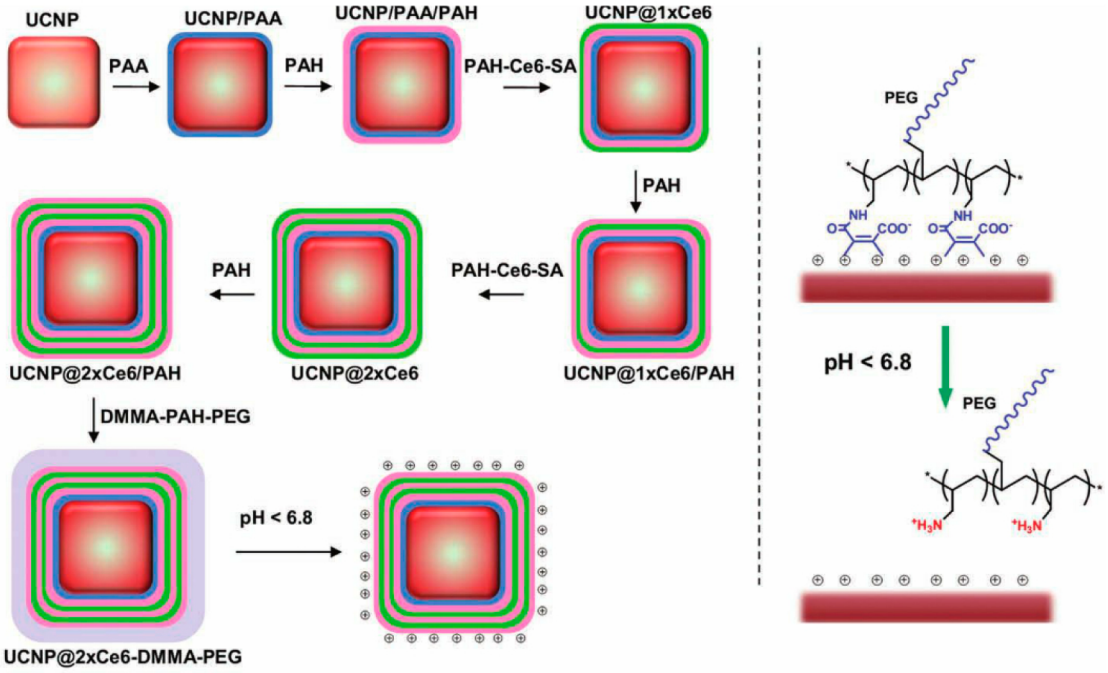

Figure 10. Illustration of pH-responsive smart theranostic UCNPs. (left) A schematic showing the fabrication process of pH-sensitive charge-reversible UCNPs with multilayers of Ce6 loading. (right) Detachment of the PEGylated polymer layer from the surface of a positively charged nanoparticle by adjusting the pH value to below 6.8. Reprinted with permission from ref 84. Copyright 2013 by John Wiley Sons, Inc.

exchange process is strongly reduced compared with other protocols, and dispersions of the resulting quasi-ligand-free particles in DMF are stable for months. New ligands (such as oleylamine, tetradecylphosphonic acid, or poly(vinylpyrrolidone)) can then be attached to the surface in a second step as schematically shown in Figure 11.

This method is quite important because it is independent of the core of the particles and thus has a wide scope. It also works with iron oxide nanoparticles, titanium oxide nanorods, or

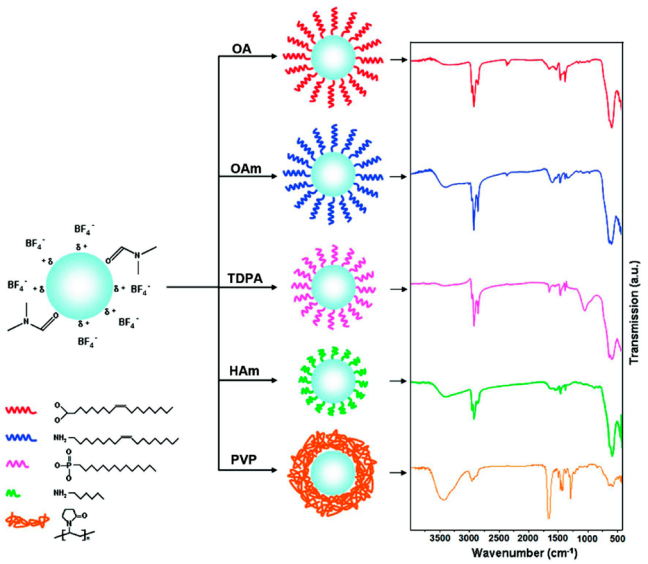

Figure 11. Illustration of the ligand exchange process at the surface of Fe_3O_4 nanocrystals modified with the BF_4^- by various capping molecules. The right column shows the corresponding FTIR spectra. Reprinted with permission from ref 86. Copyright 2011 American Chemical Society.

$NaYF_4$ nanoplates. Additional advantages include the direct attachment of the new ligand to the surface of the UCNPs. Linkers, like those needed in surface modifications with amphiphilic molecules or silica shells, are not required. Examples are given in Table 5. The so-called Meerwein salt (Et_3OBF_4) was also applied to ligand stripping of oleate-passivated nanocrystals.[87]

Two-Step Replacement of Native Ligands via Strong Acids

A strategy developed by Capobianco et al.[92] involves treatment of hydrophobic UCNPs with hydrochloric acid that can strip the native ligands off the surface to generate ligand-free and water-dispersible particles. These can then be coated with new ligands as demonstrated by the attachment of heparin[93] as schematically shown in Figure 12. The strategy was transferred to other ligands (see Table 6) and represents a quick and easy way to make UCNPs water dispersible. However, further studies on the stability and aggregation tendency of the uncoated particles may be needed.

CONCLUSIONS

In short and somewhat simplified terms, one can make the following statements: Ligand modification (mainly by oxidation) gives low yields and is time-consuming, and the particles tend to aggregate. It is the least often applied method. The formation of an additional layer on the surface of an UCNP using amphiphilic reagents gives particles of good stability in water solution and enables a large variety of head groups to be deposited but requires expensive reagents and increases the thickness of the outer layer and thus the distance of the functional group to the particle core. Coatings with SiO_2, TiO_2, silver, or gold result in water-stable particles that have low cytotoxicity, but those coated with SiO_2 or TiO_2 tend to aggregate during workup, and the size of the particle is enlarged. Two-step methods based on complete ligand

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Table 5. Typical Examples for Indirect Replacement of Oleate Ligands by Using the $NOBF_4$ Reagent, New Ligands, and Properties and Uses of the Resulting Water-Dispersible Nanoparticles (NPs)

UCNP and native ligand	new surface capping	properties and applications	ref
$NaYF_4$:Nd,Yb,Er and $NaYF_4$:Nd,Yb,Tm@ oleate	poly(acrylic acid)	results in water-soluble NPs; method for tuning the excitation wavelength	88
$NaYF_4$:Yb,Er@oleate	poly(acrylic acid)	multiphoton microscopy with low-power continuous wave light sources	89
$NaGdF_4$:Nd,Yb,Er@oleate	poly(acrylic acid)	*in vivo* bioimaging with minimized heating effect	90
$NaYF_4$:Yb,Tm@oleate	poly(vinylpyrrolidone)	photoinduced release of biomacromolecules from hydrogels loaded with upconversion NPs	91

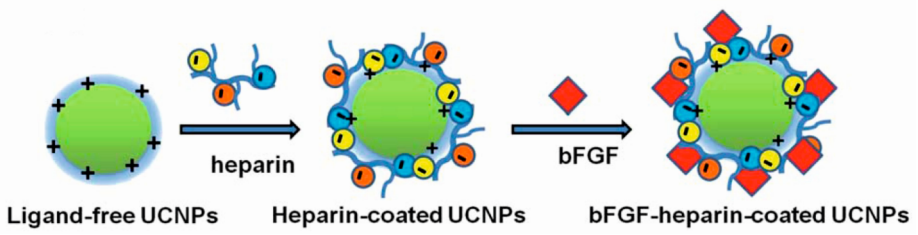

Figure 12. Illustration of the procedure for preparing UCNPs coated with heparin and basic fibroblast growth factor (bFGF). Reproduced from ref 93 with permission of The Royal Society of Chemistry.

Table 6. Examples for Indirect Replacement of Native Ligands by First Stripping off the Ligand with Hydrochloric Acid and Then Adding the New Capping Ligand and Properties and Applications of the Resulting Water-Dispersible Particles

UCNP and native ligand	new surface capping	properties and applications	ref
$NaYF_4$:Yb,Tm@oleate	azobenzene-modified mesoporous silica	drug delivery	5
$NaYF_4$:Yb,Er,Tm@ oleate	polyallylamine	conjugation to PEGylated graphene oxide for combined imaging and photothermal and photodynamic cancer therapy	94
$NaYF_4$:Yb,Er@oleate	lysine	conjugation to AuNPs for fluorescence resonance energy transfer assay to detect Cr(III) Ions	95
$NaYF_4$:Yb,Tm,Gd@ oleate	HS-PEG-NH_2	bimodal magnetic resonance and fluorescence imaging of intracranial glioblastoma	96
$NaYF_4$:Yb,Er@oleate	citrate	conjugation to streptavidin for fluorescence hybridization assay on paper	97

exchange (using HCl or $NOBF_4$) are simple and affordable, and the new ligands are directly attached to the surface without substantially enlarging the particle size. While aggregation can occur after stripping off the oleate, the method results in particles of superior quality and homogeneity but of limited stability in buffer solution.

In terms of biocompatibility, there is no systematic study available so far that would allow comparisons to be made. We note that the term biocompatibility is often used in the wrong way. We remind the reader that biocompatibility is defined by IUPAC as the "ability to be in contact with a living(!) system without producing an adverse effect." This definition applies to adverse effects on both the NP and the living system. Biocompatibility and potential toxicity of NPs usually are being tested via (commercial) test kits using normal rat kidney cells. Biocompatibility is not an issue when studying blood or urine samples. UCNPs coated with PEG or phospholipids generally are likely to possess excellent biocompatibility, while chemical coatings such as silica are adequate but not excellent in this respect. Even surfaces are preferred over uneven surfaces, and surface defects (which may cause the release of lanthanide ions, compromise biocompatibility, or cause an immune response) are disadvantageous even though the trivalent ions released by conventional UCNPs are less toxic than those released by Cd-based quantum dots. No studies are available on the biocompatibility of the less often used UCNPs based on heavy metal ions, though.

In terms of cytotoxicity, it appears that UCNPs are less toxic than other particles, but as was stated by Gu et al.,[98] data from in vivo cytotoxicity studies may not reflect chronic toxicity. Few data are available on dose–effect relationships and less data on quantitative correlations between their toxicological properties and their nanocharacteristics including size, surface chemistry, surface charge, shape and morphology. While not compromising current uses in imaging of cells and tissue, the lack of systematic fundamental research on the toxicity of UCNPs may obstruct medical applications at present.

In terms of applications, the following comments may be useful. If UCNPs are intended for use in (cellular) imaging, coatings with SiO_2 and the like result in particles of good stability in culture media. UCNPs for use in bioconjugation may be better coated with amphiphilic molecules where a variety of functional head groups is available or with gold, which can be further modified, for example, via gold–thiol interactions. FRET studies are best performed with UCNPs modified by ligand exchange (using $NOBF_4$ as a reagent, for example) because this results in a small, constant, and well controllable distance between the core and any fluorophore on the surface. UCNPs for use in electrophoresis, in turn, can be well modified using amphiphilic polymers, which provide long-term stability because ligand detachment hardly occurs.

Current challenges include better control of particle size and homogeneity, more reproducible methods for surface loading or modification, the search for synthetic methods providing higher yields of UCNPs, the need for materials displaying

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higher quantum yields in water solution (ideally without tedious surface modification), improved methods for workup (including the suppression of aggregation), new methods for surface characterization, and the design of more affordable reagents for surface modifications. Unfortunately, much synthetic research in the area is of the trial-and-error kind due to the lack of understanding of the mechanisms causing the above limitations. Better control of the reproducibility of particle size and composition requires experimental skill, chemicals of high purity, nonleaching labware (glass!), and the careful exclusion of oxygen. Surface loading can be tested best via thermogravimetric analysis (TGA), which presently is the method of choice but requires 10−15 mg of particles. Interestingly, inductively coupled plasma mass spectrometry, which is a powerful technique, is not often applied, possibly because of costs. The fight against aggregation is never-ending. No single good method can be recommended because aggregation tendency strongly depends on the kind of surface and its charge. A simple rule of thumb tells that particles with negatively charged surfaces tend to aggregate in the presence of divalent ions, while positively charged do (less) so in the presence of bivalent anions. One also notes the lack of a fast method for the determination of the degree of aggregation and sedimentation. Despite these challenges, UCNPs are considered to represent very promising new materials as evidenced by the almost exponential increase in the number of articles covering the subject.

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REFERENCES

(1) Auzel, F. Upconversion and Anti-Stokes Processes with f and d Ions in Solids. *Chem. Rev.* **2004**, *104*, 139−173.
(2) Boyer, J.-C.; van Veggel, F. C. J. M. Absolute Quantum Yield Measurements of Colloidal $NaYF_4$:Er^{3+},Yb^{3+} Upconverting Nanoparticles. *Nanoscale* **2010**, *2*, 1417−1419.
(3) van Veggel, F. C. J. M.; Dong, C.; Johnson, N. J. J.; Pichaandi, J. Ln^{3+}-Doped Nanoparticles for Upconversion and Magnetic Resonance Imaging: Some Critical Notes on Recent Progress and Some Aspects to Be Considered. *Nanoscale* **2012**, *4*, 7309−7321.
(4) Qian, H. S.; Guo, H. C.; Ho, P. C.-L.; Mahendran, R.; Zhang, Y. Mesoporous-Silica-Coated Up-Conversion Fluorescent Nanoparticles for Photodynamic Therapy. *Small* **2009**, *5*, 2285−2290.
(5) Liu, J.; Bu, W.; Pan, L.; Shi, J. NIR-Triggered Anticancer Drug Delivery by Upconverting Nanoparticles with Integrated Azobenzene-Modified Mesoporous Silica. *Angew. Chem., Int. Ed.* **2013**, *52*, 4375−4379.
(6) Mader, H. S.; Kele, P.; Saleh, S. M.; Wolfbeis, O. S. Upconverting Luminescent Nanoparticles for Use in Bioconjugation and Bioimaging. *Curr. Opin. Chem. Biol.* **2010**, *14*, 582−596.
(7) Arppe, R.; Nareoja, T.; Nylund, S.; Mattsson, L.; Koho, S.; Rosenholm, J. M.; Soukka, T.; Schäferling, M. Photon Upconversion Sensitized Nanoprobes for Sensing and Imaging of pH. *Nanoscale* **2014**, *6*, 6837−6843.
(8) Achatz, D. E.; Meier, R. J.; Fischer, L. H.; Wolfbeis, O. S. Luminescent Sensing of Oxygen Using a Quenchable Probe and Upconverting Nanoparticles. *Angew. Chem., Int. Ed.* **2011**, *50*, 260−263.
(9) Mader, H. S.; Wolfbeis, O. S. Optical Ammonia Sensor Based on Upconverting Luminescent Nanoparticles. *Anal. Chem.* **2010**, *82*, 5002−5004.
(10) Saleh, S. M.; Ali, R.; Wolfbeis, O. S. Quenching of the Luminescence of Upconverting Luminescent Nanoparticles by Heavy Metal Ions. *Chem.—Eur. J.* **2011**, *17*, 14611−14617.
(11) Ali, R.; Saleh, S. M.; Meier, R. J.; Azab, H. A.; Abdelgawad, I. I.; Wolfbeis, O. S. Upconverting Nanoparticle Based Optical Sensor for Carbon Dioxide. *Sens. Actuators, B* **2010**, *150*, 126−131.
(12) Achatz, D. E.; Ali, R.; Wolfbeis, O. S. Fluorescent Sensing, Biosensing, and Screening Using Upconverting Nanoparticles. *Top. Curr. Chem.* **2011**, *300*, 29−50.
(13) Wang, M.; Hou, W.; Mi, C.-C.; Wang, W.-X.; Xu, Z.-R.; Teng, H.-H.; Mao, C.-B.; Xu, S.-K. Immunoassay of Goat Antihuman Immunoglobulin G Antibody Based on Luminescence Resonance Energy Transfer between Near-Infrared Responsive $NaYF_4$:Yb, Er Upconversion Fluorescent Nanoparticles and Gold Nanoparticles. *Anal. Chem.* **2009**, *81*, 8783−8789.
(14) Tsang, M.-K.; Chan, C.-F.; Wong, K.-L.; Hao, J. Comparative Studies of Upconversion Luminescence Characteristics and Cell Bioimaging Based on One-step Synthesized Upconversion Nanoparticles Capped with Different Functional Groups. *J. Lumin.* **2015**, *157*, 172−178.
(15) Chen, G.; Qiu, H.; Prasad, P. N.; Chen, X. Upconversion Nanoparticles: Design, Nanochemistry, and Applications in Theranostics. *Chem. Rev.* **2014**, *114*, 5161−5214.
(16) DaCosta, M. V.; Doughan, S.; Han, Y.; Krull, U. J. Lanthanide Upconversion Nanoparticles and Applications in Bioassays and Bioimaging: A Review. *Anal. Chim. Acta* **2014**, *832*, 1−33.
(17) Liu, Y.; Tu, D.; Zhu, H.; Chen, X. Lanthanide-Doped Luminescent Nanoprobes: Controlled Synthesis, Optical Spectroscopy, and Bioapplications. *Chem. Soc. Rev.* **2013**, *42*, 6924−6958.
(18) Zhou, J.; Liu, Z.; Li, F. Upconversion Nanophosphors for Small-Animal Imaging. *Chem. Soc. Rev.* **2012**, *41*, 1323−1349.

(19) Li, L.-L.; Zhang, R.; Yin, L.; Zheng, K.; Qin, W.; Selvin, P. R.; Lu, Y. Biomimetic Surface Engineering of Lanthanide-Doped Upconversion Nanoparticles as Versatile Bioprobes. *Angew. Chem.* **2012**, *124*, 6225−6229.
(20) Feng, W.; Zhu, X.; Li, F. Recent Advances in the Optimization and Functionalization of Upconversion Nanomaterials for in vivo Bioapplications. *NPG Asia Mater.* **2013**, *5*, No. e75.
(21) Rao, L.; Lu, W.; Ren, G.; Wang, H.; Yi, Z.; Liu, H.; Zeng, S. Monodispersed LaF_3 Nanocrystals: Shape-Controllable Synthesis, Excitation-Power-Dependent Multi-color Tuning and Intense Near-Infrared Upconversion Emission. *Nanotechnology* **2014**, *25*, No. 065703.
(22) Li, Z.; Zhang, Y.; Jiang, S. Multicolor Core/Shell-Structured Upconversion Fluorescent Nanoparticles. *Adv. Mater.* **2008**, *20*, 4765−4769.
(23) Chan, E. M.; Han, G.; Goldberg, J. D.; Gargas, D. J.; Ostrowski, A. D.; Schuck, P. J.; Cohen, B. C.; Milliron, D. J. Combinatorial Discovery of Lanthanide-Doped Nanocrystals with Spectrally Pure Upconverted Emission. *Nano Lett.* **2012**, *12*, 3839−3845.
(24) Sun, L.-D.; Wang, Y.-F.; Yan, C.-H. Paradigms and Challenges for Bioapplication of Rare Earth Upconversion Luminescent Nanoparticles: Small Size and Tunable Emission/Excitation Spectra. *Acc. Chem. Res.* **2014**, *47*, 1001−1009.
(25) Erathodiyil, N.; Ying, J. Y. Functionalization of Inorganic Nanoparticles for Bioimaging Applications. *Acc. Chem. Res.* **2011**, *44*, 925−935.
(26) Chen, Z.; Chen, H.; Hu, H.; Yu, M.; Li, F.; Zhang, Q.; Zhou, Z.; Yi, T.; Huang, C. Versatile Synthesis Strategy for Carboxylic Acid-Functionalized Upconverting Nanophosphors as Biological Labels. *J. Am. Chem. Soc.* **2008**, *130*, 3023−3029.
(27) Zhou, H.-P.; Xu, C.-H.; Sun, W.; Yan, C.-H. Clean and Flexible Modification Strategy for Carboxyl/Aldehyde-Functionalized Upconversion Nanoparticles and their Optical Applications. *Adv. Funct. Mater.* **2009**, *19*, 3892−3900.
(28) Hu, H.; Yu, M.; Li, F.; Chen, Z.; Gao, X.; Xiong, L.; Huang, C. Facile Epoxidation Strategy for Producing Amphiphilic Up-Converting Rare-Earth Nanophosphors as Biological Labels. *Chem. Mater.* **2008**, *20*, 7003−7009.
(29) Dai, Y.; Yang, D.; Ma, P.; Kang, X.; Zhang, X.; Li, C.; Hou, Z.; Cheng, Z.; Lin, J. Doxorubicin Conjugated $NaYF_4$:Yb^{3+}/Tm^{3+} Nanoparticles for Therapy and Sensing of Drug Delivery by Luminescence Resonance Energy Transfer. *Biomaterials* **2012**, *33*, 8704−8713.
(30) Park, Y. I.; Kim, J. H.; Lee, K. T.; Jeon, K.-S.; Na, H. B.; Yu, J. H.; Kim, H. M.; Lee, N.; Choi, S. H.; Baik, S.-I.; Kim, H.; Park, S. P.; Park, B.-J.; Kim, Y. W.; Lee, S. H.; Yoon, S.-Y.; Song, I. C.; Moon, W. K.; Suh, Y. D.; Hyeon, T. Nonblinking and Nonbleaching Upconverting Nanoparticles as an Optical Imaging Nanoprobe and T1 Magnetic Resonance Imaging Contrast Agent. *Adv. Mater.* **2009**, *21*, 4467−4471.
(31) Nam, S. H.; Bae, Y. M.; Park, Y. I.; Kim, J. H.; Kim, H. M.; Choi, J. S.; Lee, K. T.; Hyeon, T.; Suh, Y. D. Long-Term Real-Time Tracking of Lanthanide Ion Doped Upconverting Nanoparticles in Living Cells. *Angew. Chem.* **2011**, *123*, 6217−6221.
(32) Ren, W.; Tian, G.; Jian, S.; Gu, Z.; Zhou, L.; Yan, L.; Jin, S.; Yin, W.; Zhao, Y. Tween-Coated $NaYF_4$:Yb,Er/$NaYF_4$ Core/Shell Upconversion Nanoparticles for Bioimaging and Drug Delivery. *RSC Adv.* **2012**, *2*, 7037−7041.
(33) Liang, S.; Zhang, X.; Wu, Z.; Liu, Y.; Zhang, H.; Sun, H.; Sun, H.; Yang, B. Decoration of Up-Converting $NaYF_4$:Yb,Er(Tm) Nanoparticles with Surfactant Bilayer. A Versatile Strategy to Perform Oil-to-Water Phase Transfer and Subsequently Surface Silication. *CrystEngComm* **2012**, *14*, 3484−3489.
(34) Pellegrino, T.; Manna, L.; Kudera, S.; Liedl, T.; Koktysh, D.; Rogach, A. L.; Keller, S.; Rädler, J.; Natile, G.; Parak, W. J. Hydrophobic Nanocrystals Coated with an Amphiphilic Polymer Shell: A General Route to Water Soluble Nanocrystals. *Nano Lett.* **2004**, *4*, 703−707.
(35) Wang, C.; Tao, H.; Cheng, L.; Liu, Z. Near-Infrared Light Induced *in vivo* Photodynamic Therapy of Cancer Based on Upconversion Nanoparticles. *Biomaterials* **2011**, *32*, 6145−6154.
(36) Li, X.; Wu, Y.; Liu, Y.; Zou, X.; Yao, L.; Li, F.; Feng, W. Cyclometallated Ruthenium Complex-Modified Upconversion Nanophosphors for Selective Detection of Hg^{2+} Ions in Water. *Nanoscale* **2014**, *6*, 1020−1028.
(37) Jiang, G.; Pichaandi, J.; Johnson, N. J. J.; Burke, R. D.; van Veggel, F. C. J. M. An Effective Polymer Cross-Linking Strategy To Obtain Stable Dispersions of Upconverting $NaYF_4$ Nanoparticles in Buffers and Biological Growth Media for Biolabeling Applications. *Langmuir* **2012**, *28*, 3239−3247.
(38) Yi, G.-S.; Chow, G.-M. Water-Soluble $NaYF_4$:Yb,Er(Tm)/$NaYF_4$/Polymer Core/Shell/Shell Nanoparticles with Significant Enhancement of Upconversion Fluorescence. *Chem. Mater.* **2007**, *19*, 341−343.
(39) Cheng, L.; Yang, K.; Zhang, S.; Shao, M.; Lee, S.; Liu, Z. Highly-Sensitive Multiplexed *in vivo* Imaging Using PEG-ylated Upconversion Nanoparticles. *Nano Res.* **2010**, *3*, 722−732.
(40) Cui, S.; Chen, H.; Zhu, H.; Tian, J.; Chi, X.; Qian, Z.; Achilefu, S.; Gu, Y. Amphiphilic Chitosan Modified Upconversion Nanoparticles for *in vivo* Photodynamic Therapy Induced by Near-Infrared Light. *J. Mater. Chem.* **2012**, *22*, 4861−4873.
(41) Chen, B.; Dong, B.; Wang, J.; Zhang, S.; Xu, L.; Yu, W.; Song, H. Amphiphilic Silane Modified $NaYF_4$:Yb,Er Loaded with Eu-$(TTA)_3(TPPO)_2$ Nanoparticles and their Multi-Functions: Dual Mode Temperature Sensing and Cell Imaging. *Nanoscale* **2013**, *5*, 8541−8549.
(42) Budijono, S. J.; Shan, J.; Yao, N.; Miura, Y.; Hoye, T.; Austin, R. H.; Ju, Y.; Prud'homme, R. K. Synthesis of Stable Block-Copolymer-Protected $NaYF_4$:Yb^{3+}, Er^{3+} Up-Converting Phosphor Nanoparticles. *Chem. Mater.* **2010**, *22*, 311−318.
(43) Sudheendra, L.; Ortalan, V.; Dey, S.; Browning, N. D.; Kennedy, I. M. Plasmonic Enhanced Emissions from Cubic $NaYF_4$:Yb:Er/Tm Nanophosphors. *Chem. Mater.* **2011**, *23*, 2987−2993.
(44) Hu, H.; Xiong, L.; Zhou, J.; Li, F.; Cao, T.; Huang, C. Multimodal-Luminescence Core−Shell Nanocomposites for Targeted Imaging of Tumor Cells. *Chem.—Eur. J.* **2009**, *15*, 3577−3584.
(45) Liu, B.; Li, C.; Yang, D.; Hou, Z.; Ma, P.; Cheng, Z.; Lian, H.; Huang, S.; Lin, J. Upconversion-Luminescent Core/Mesoporous-Silica-Shell-Structured β-$NaYF_4$:Yb^{3+},Er^{3+}@SiO_2@$mSiO_2$ Composite Nanospheres: Fabrication and Drug-Storage/Release Properties. *Eur. J. Inorg. Chem.* **2014**, *2014*, 1906−1913.
(46) Liang, L.; Liu, Y.; Zhao, X.-Z. Double-Shell β-$NaYF_4$:Yb^{3+}, Er^{3+}/SiO_2/TiO_2 Submicroplates as a Scattering and Upconverting Layer for Efficient Dye-Sensitized Solar Cells. *Chem. Commun.* **2013**, *49*, 3958−3960.
(47) Zhang, Y.; Hong, Z. Synthesis of Lanthanide-Doped $NaYF_4$@TiO_2 Core-Shell Composites with Highly Crystalline and Tunable TiO_2 Shells Under Mild Conditions and Their Upconversion-Based Photocatalysis. *Nanoscale* **2013**, *5*, 8930−8933.
(48) Zhang, H.; Li, Y.; Ivanov, I. A.; Qu, Y.; Huang, Y.; Duan, X. Plasmonic Modulation of the Upconversion Fluorescence in $NaYF_4$:Yb/Tm Hexaplate Nanocrystals Using Gold Nanoparticles or Nanoshells. *Angew. Chem., Int. Ed.* **2010**, *49*, 2865−2868.
(49) Dong, B.; Xu, S.; Sun, J.; Bi, S.; Li, D.; Bai, X.; Wang, Y.; Wang, L.; Song, H. Multifunctional $NaYF_4$:Yb^{3+},Er^{3+}@Ag Core/Shell Nanocomposites: Integration of Upconversion Imaging and Photothermal Therapy. *J. Mater. Chem.* **2011**, *21*, 6193−6200.
(50) Jalil, R. A.; Zhang, Y. Biocompatibility of Silica Coated $NaYF_4$ Upconversion Fluorescent Nanocrystals. *Biomaterials* **2008**, *29*, 4122−4128.
(51) Wang, M.; Mi, C.; Zhang, Y.; Liu, J.; Li, F.; Mao, C.; Xu, S. NIR-Responsive Silica-Coated $NaYbF_4$:Er/Tm/Ho Upconversion Fluorescent Nanoparticles with Tunable Emission Colors and Their Applications in Immunolabeling and Fluorescent Imaging of Cancer Cells. *J. Phys. Chem. C* **2009**, *113*, 19021−19027.
(52) Idris, N. M.; Gnanasammandhan, M. K.; Zhang, J.; Ho, P. C.; Mahendran, R.; Zhang, Y. In Vivo Photodynamic Therapy Using

Upconversion Nanoparticles as Remote-Controlled Nanotransducers. *Nat. Med.* **2012**, *18*, 1580−1585.
(53) Bagwe, R. P.; Hilliard, L. R.; Tan, W. Surface Modification of Silica Nanoparticles to Reduce Aggregation and Nonspecific Binding. *Langmuir* **2006**, *22*, 4357−4362.
(54) Hlavacek, A.; Sedlmeier, A.; Skladal, P.; Gorris, H. H. Electrophoretic Characterization and Purification of Silica-Coated Photon-Upconverting Nanoparticles and Their Bioconjugates. *ACS Appl. Mater. Interfaces* **2014**, *6*, 6930−6935.
(55) Wang, M.; Mi, C.-C.; Wang, W.-X.; Liu, C.-H.; Wu, Y.-F.; Xu, Z.-R.; Mao, C.-B.; Xu, S.-K. Immunolabeling and NIR-Excited Fluorescent Imaging of HeLa Cells by Using $NaYF_4$:Yb,Er Upconversion Nanoparticles. *ACS Nano* **2009**, *3*, 1580−1586.
(56) Liu, F.; Zhao, Q.; You, H.; Wang, Z. Synthesis of Stable Carboxy-Terminated $NaYF_4$: Yb^{3+}, Er^{3+}@SiO_2 Nanoparticles with Ultrathin Shell for Biolabeling Applications. *Nanoscale* **2013**, *5*, 1047−1053.
(57) Li, C.; Hou, Z.; Dai, Y.; Yang, D.; Cheng, Z.; Ma, P.; Lin, J. A. Facile Fabrication of Upconversion Luminescent and Mesoporous Core-Shell Structured β-$NaYF_4$:Yb^{3+}, Er^{3+}@$mSiO_2$ Nanocomposite Spheres for Anti-Cancer Drug Delivery and Cell Imaging. *Biomater. Sci.* **2013**, *1*, 213−223.
(58) Wilhelm, S.; Hirsch, T.; Patterson, W. M.; Scheucher, E.; Mayr, T.; Wolfbeis, O. S. Multicolor Upconversion Nanoparticles for Protein Conjugation. *Theranostics* **2013**, *3*, 239−248.
(59) Shan, G.; Weissleder, R.; Hilderbrand, S. A. Upconverting Organic Dye Doped Core-Shell Nano-Composites for Dual-Modality NIR Imaging and Photo-Thermal Therapy. *Theranostics* **2013**, *3*, 267−274.
(60) Li, C.; Hou, Z.; Dai, Y.; Yang, D.; Cheng, Z.; Ma, P.; Lin, J. A Facile Fabrication of Upconversion Luminescent and Mesoporous Core-Shell Structured b-$NaYF_4$:Yb^{3+}, Er^{3+}@$mSiO_2$ Nanocomposite Spheres for Anti-Cancer Drug Delivery and Cell Imaging. *Biomater. Sci.* **2013**, *1*, 213−223.
(61) Sun, L.; Liu, T.; Qiu, Y.; Liu, J.; Shi, L.; Wolfbeis, O. Direct Formation of Mesoporous Upconverting Core-Shell Nanoparticles for Bioimaging of Living Cells. *Microchim. Acta* **2013**, *180*, 1−7.
(62) Zhao, L.; Peng, J.; Chen, M.; Liu, Y.; Yao, L.; Feng, W.; Li, F. Yolk-Shell Upconversion Nanocomposites for LRET Sensing of Cysteine/Homocysteine. *ACS Appl. Mater. Interfaces* **2014**, *6*, 11190−11197.
(63) Schäfer, H.; Ptacek, P.; Kömpe, K.; Haase, M. Lanthanide-Doped $NaYF_4$ Nanocrystals in Aqueous Solution Displaying Strong Up-Conversion Emission. *Chem. Mater.* **2007**, *19*, 1396−1400.
(64) Xia, L.; Kong, X.; Liu, X.; Tu, L.; Zhang, Y.; Chang, Y.; Liu, K.; Shen, D.; Zhao, H.; Zhang, H. An Upconversion Nanoparticle - Zinc Phthalocyanine Based Nanophotosensitizer for Photodynamic Therapy. *Biomaterials* **2014**, *35*, 4146−4156.
(65) Voliani, V.; González-Béjar, M.; Herranz-Pérez, V.; Duran-Moreno, M.; Signore, G.; Garcia-Verdugo, J. M.; Pérez-Prieto, J. Orthogonal Functionalization of Upconverting $NaYF_4$ Nanocrystals. *Chem.—Eur. J.* **2013**, *19*, 13538−13546.
(66) Cao, T.; Yang, T.; Gao, Y.; Yang, Y.; Hu, H.; Li, F. Water-Soluble $NaYF_4$:Yb/Er Upconversion Nanophosphors: Synthesis, Characteristics and Application in Bioimaging. *Inorg. Chem. Commun.* **2010**, *13*, 392−394.
(67) Zhang, Q.; Song, K.; Zhao, J.; Kong, X.; Sun, Y.; Liu, X.; Zhang, Y.; Zeng, Q.; Zhang, H. Hexanedioic Acid Mediated Surface-Ligand-Exchange Process for Transferring $NaYF_4$:Yb/Er (or Yb/Tm) Up-Converting Nanoparticles from Hydrophobic to Hydrophilic. *J. Colloid Interface Sci.* **2009**, *336*, 171−175.
(68) Yi, G. S.; Chow, G. M. Synthesis of Hexagonal-Phase $NaYF_4$:Yb,Er and $NaYF_4$:Yb,Tm Nanocrystals with Efficient Up-Conversion Fluorescence. *Adv. Funct. Mater.* **2006**, *16*, 2324−2329.
(69) Boyer, J.-C.; Manseau, M.-P.; Murray, J. I.; van Veggel, F. C. J. M. Surface Modification of Upconverting $NaYF_4$ Nanoparticles with PEG-Phosphate Ligands for NIR (800 nm) Biolabeling within the Biological Window. *Langmuir* **2010**, *26*, 1157−1164.
(70) Bogdan, N.; Vetrone, F.; Roy, R.; Capobianco, J. A. Carbohydrate-Coated Lanthanide-Doped Upconverting Nanoparticles for Lectin Recognition. *J. Mater. Chem.* **2010**, *20*, 7543−7550.
(71) Nyk, M.; Kumar, R.; Ohulchanskyy, T. Y.; Bergey, E. J.; Prasad, P. N. High Contrast *in Vitro* and *in Vivo* Photoluminescence Bioimaging Using Near Infrared to Near Infrared Up-Conversion in Tm^{3+} and Yb^{3+} Doped Fluoride Nanophosphors. *Nano Lett.* **2008**, *8*, 3834−3838.
(72) Xiong, L.; Yang, T.; Yang, Y.; Xu, C.; Li, F. Long-Term *in Vivo* Biodistribution Imaging and Toxicity of Polyacrylic Acid-Coated Upconversion Nanophosphors. *Biomaterials* **2010**, *31*, 7078−7085.
(73) Naccache, R.; Vetrone, F.; Mahalingam, V.; Cuccia, L. A.; Capobianco, J. A. Controlled Synthesis and Water Dispersibility of Hexagonal Phase $NaGdF_4$:Ho^{3+}/Yb^{3+} Nanoparticles. *Chem. Mater.* **2009**, *21*, 717−723.
(74) Johnson, N. J. J.; Sangeetha, N. M.; Boyer, J.-C.; van Veggel, F. C J. M. Facile Ligand-Exchange with Polyvinylpyrrolidone and Subsequent Silica Coating of Hydrophobic Upconverting β-$NaYF_4$:Yb^{3+}/Er^{3+} Nanoparticles. *Nanoscale* **2010**, *2*, 771−777.
(75) González-Béjar, M.; Liras, M.; Francés-Soriano, L.; Voliani, V.; Herranz-Pérez, V.; Duran-Morena, M.; Garcia-Verdugo, J. M.; Alarcon, E. I.; Scaiano, J. C.; Pérez-Prieto, J. NIR Excitation of Upconversion Nanohybrids Containing a Surface Grafted Bodipy Induces Oxygen-Mediated Cancer Cell Death. *J. Mater. Chem. B* **2014**, *2*, 4554−4563.
(76) Liras, M.; Gónzales-Béjar, M.; Peinado, El; Francés-Soriano, L.; Pérez-Prieto, J.; Quijada-Garrido, I.; García, O. Thin Amphiphilic Polymer-Capped Upconversion Nanoparticles: Enhanced Emission and Thermoresponsive Properties. *Chem. Mater.* **2014**, *26*, 4014−4022.
(77) Zhu, X.; Da Silva, B.; Zou, X.; Shen, B.; Sun, Y.; Feng, W.; Li, F. Intra-Arterial Infusion of PEGylated Upconversion Nanophosphors to Improve the Initial Uptake by Tumors in Vivo. *RSC Adv.* **2014**, *4*, 23580−23584.
(78) Zhou, L.; He, B.; Huang, J.; Cheng, Z.; Xu, X.; Wei, C. Multihydroxy Dendritic Upconversion Nanoparticles with Enhanced Water Dispersibility and Surface Functionality for Bioimaging. *ACS Appl. Mater. Interfaces* **2014**, *6*, 7719−7727.
(79) Chen, Q.; Wang, X.; Chen, F.; Zhang, Q.; Dong, B.; Yang, H.; Liu, G.; Zhu, Y. Functionalization of Upconverted Luminescent $NaYF_4$:Yb/Er Nanocrystals by Folic Acid-Chitosan Conjugates for Targeted Lung Cancer Cell Imaging. *J. Mater. Chem.* **2011**, *21*, 7661−7667.
(80) Liebherr, R. B.; Soukka, T.; Wolfbeis, O. S.; Gorris, H. H. Maleimide Activation of Photon Upconverting Nanoparticles for Bioconjugation. *Nanotechnology* **2012**, *23*, No. 485103.
(81) Zhang, W.; Peng, B.; Tian, F.; Qin, W.; Qian, X. Facile Preparation of Well-Defined Hydrophilic Core-Shell Upconversion Nanoparticles for Selective Cell Membrane Glycan Labeling and Cancer Cell Imaging. *Anal. Chem.* **2014**, *86*, 482−489.
(82) Li, L.-L.; Wu, P.; Hwang, K.; Lu, Y. An Exceptionally Simple Strategy for DNA-Functionalized Up-Conversion Nanoparticles as Biocompatible Agents for Nanoassembly, DNA Delivery, and Imaging. *J. Am. Chem. Soc.* **2013**, *135*, 2411−2414.
(83) Liu, Q.; Sun, Y.; Li, C.; Zhou, J.; Li, C.; Yang, T.; Zhang, X.; Yi, T.; Wu, D.; Li, F. ^{18}F-Labeled Magnetic-Upconversion Nanophosphors via Rare-Earth Cation-Assisted Ligand Assembly. *ACS Nano* **2011**, *5*, 3146−3157.
(84) Wang, C.; Cheng, L.; Liu, Y.; Wang, X.; Ma, X.; Deng, Z.; Li, Y.; Liu, Z. Imaging-Guided pH-Sensitive Photodynamic Therapy Using Charge Reversible Upconversion Nanoparticles under Near-Infrared Light. *Adv. Funct. Mater.* **2013**, *23*, 3077−3086.
(85) Budijono, S. J.; Shan, J.; Yao, N.; Miura, Y.; Hoye, T.; Austin, R. H.; Ju, Y.; Prud'homme, R. K. Synthesis of Stable Block-Copolymer-Protected $NaYF_4$:Yb^{3+}, Er^{3+} Up-Converting Phosphor Nanoparticles. *Chem. Mater.* **2010**, *22*, 311−318.
(86) Dong, A.; Ye, X.; Chen, J.; Kang, Y.; Gordon, T.; Kikkawa, J. M.; Murray, C. B. A Generalized Ligand-Exchange Strategy Enabling Sequential Surface Functionalization of Colloidal Nanocrystals. *J. Am. Chem. Soc.* **2011**, *133*, 998−1006.

(87) Rosen, E. L.; Buonsanti, R.; Llordes, A.; Sawvel, A. M.; Milliron, D. J.; Helm, B. A. Exceptionally Mild Reactive Stripping of Native Ligands from Nanocrystal Surfaces by Using Meerwein's Salt. *Angew. Chem.* **2012**, *51*, 684−689.
(88) Shen, J.; Chen, G.; Vu, A.-M.; Fan, W.; Bilsel, O. S.; Chang, C.-C.; Han, G. Engineering the Upconversion Nanoparticle Excitation Wavelength: Cascade Sensitization of Tri-Doped Upconversion Colloidal Nanoparticles at 800 nm. *Adv. Opt. Mater.* **2013**, *1*, 644−650.
(89) Esipova, T. V.; Ye, X.; Collins, J. E.; Sakadžić, S.; Mandeville, E. T.; Murray, C. B.; Vinogradov, S. A. Dendritic Upconverting Nanoparticles Enable *in vivo* Multiphoton Microscopy with Low-Power Continuous Wave Sources. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 20826−20831.
(90) Wang, Y.-F.; Liu, G.-Y.; Sun, L.-D.; Xiao, J.-W.; Zhou, J.-C.; Yan, C.-H. Nd^{3+}-Sensitized Upconversion Nanophosphors: Efficient *in Vivo* Bioimaging Probes with Minimized Heating Effect. *ACS Nano* **2013**, *7*, 7200−7206.
(91) Yan, B.; Boyer, J.-C.; Habault, D.; Branda, N. R.; Zhao, Y. Near Infrared Light Triggered Release of Biomacromolecules from Hydrogels Loaded with Upconversion Nanoparticles. *J. Am. Chem. Soc.* **2012**, *134*, 16558−16561.
(92) Bogdan, N.; Vetrone, F.; Ozin, G. A.; Capobianco, J. A. Synthesis of Ligand-Free Colloidally Stable Water Dispersible Brightly Luminescent Lanthanide-Doped Upconverting Nanoparticles. *Nano Lett.* **2011**, *11*, 835−840.
(93) Bogdan, N.; Rodríguez, E. M.; Sanz-Rodríguez, F.; de la Cruz, M. C. I.; Juarranz, Á.; Jaque, D.; Solé, J. G.; Capobianco, J. A. Bio-Functionalization of Ligand-Free Upconverting Lanthanide Doped Nanoparticles for Bio-Imaging and Cell Targeting. *Nanoscale* **2012**, *4*, 3647−3650.
(94) Wang, Y.; Wang, H.; Liu, D.; Song, S.; Wang, X.; Zhang, H. Graphene Oxide Covalently Grafted Upconversion Nanoparticles for Combined NIR Mediated Imaging and Photothermal/Photodynamic Cancer Therapy. *Biomaterials* **2013**, *34*, 7715−7724.
(95) Liu, B.; Tan, H.; Chen, Y. Upconversion Nanoparticle-Based Fluorescence Resonance Energy Transfer Assay for Cr(III) Ions in Urine. *Anal. Chim. Acta* **2013**, *761*, 178−185.
(96) Ni, D.; Zhang, J.; Bu, W.; Xing, H.; Han, F.; Xiao, Q.; Yao, Z.; Chen, F.; He, Q.; Liu, J.; Zhang, S.; Fan, W.; Zhou, L.; Peng, W.; Shi, J. Dual-Targeting Upconversion Nanoprobes across the Blood−Brain Barrier for Magnetic Resonance/Fluorescence Imaging of Intracranial Glioblastoma. *ACS Nano* **2014**, *8*, 1231−1242.
(97) Zhou, F.; Noor, M. O.; Krull, U. J. Luminescence Resonance Energy Transfer-Based Nucleic Acid Hybridization Assay on Cellulose Paper with Upconverting Phosphor as Donors. *Anal. Chem.* **2014**, *86*, 2719−2726.
(98) Gu, Z.; Yan, L.; Tian, G.; Li, S.; Chai, Z.; Zhao, Y. Recent Advances in Design and Fabrication of Upconversion Nanoparticles and Their Safe Theranostic Applications. *Adv. Mater.* **2013**, *25*, 3758−3779.