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### Structured doping of upconversion nanosystems for biological applications

Wang, Y.

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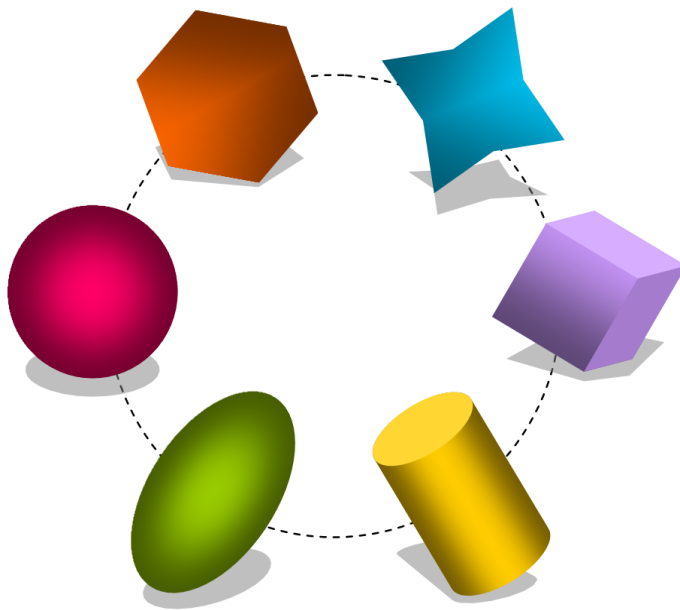
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# CHAPTER 1

## Introduction



Chapter 1

## 1.1 Nanotechnology

“There’s Plenty of Room at the Bottom”, a lecture given by Richard Feynman at an American Physical Society meeting at Caltech in 1959, founded the new era of “nanotechnology”. In the present thesis we will be concerned with nanoparticles, amorphous or crystalline structures of which one or more of its dimensions is below about 100 nm. At the nanoscale two important considerations need to be taken into account. Firstly, due to reduction in size quantum confinement effects start to play an important role as is beautifully demonstrated in the dependence of optical spectra of, for example, quantum dots. Secondly, for nanoscale materials the ratio of surface area and volume is markedly different from that of bulk materials. As a consequence, physical and chemical properties of nanomaterials become increasingly determined by the properties of the surface instead of the properties of the bulk. This subtle interplay of influencing material properties by addressing their quantum behavior and/or making explicitly use of the difference in properties of surface and bulk material has become one of the important targets of scientists in their quest for producing novel materials and devices. In recent years it has led to broad applications of nanomaterials, for instance, opaque substances become transparent (copper); inert materials attain catalytic properties (platinum); stable materials turn combustible (aluminum); insulators become conductors (silicon). Materials, such as gold which are regarded as chemically inert, can serve as a potent chemical catalyst at nanoscales.

## 1.2 Upconversion and rare earth ions doped upconversion nanoparticles

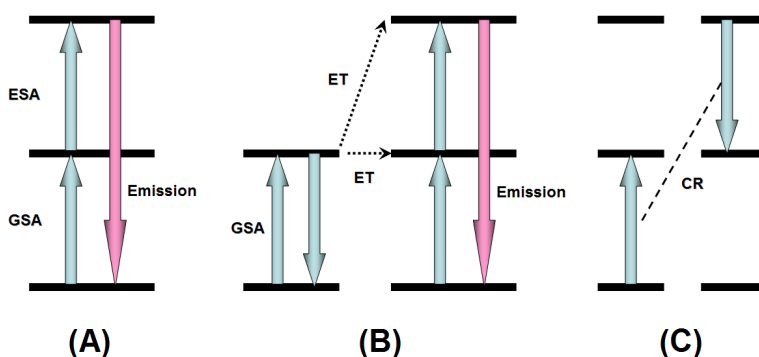
### 1.2.1 Upconversion process

One of the spectroscopic processes this thesis revolves around is upconversion. In an upconversion process two or more photons are combined to produce photons with a higher energy than each of the individual photons. Glasses and crystals doped with rare earth ions have in recent years attracted particular attention as upconverting materials since they are able to convert light in the near-infrared part of the spectrum into wavelengths in the UV-Vis region. Moreover, since in these materials upconversion occurs as a non-coherent process it can be done by an economic continuous wave (CW) diode laser as opposed to the high peak power lasers that are needed to drive coherent upconversion processes.

Various upconversion mechanisms have been proposed (**Figure 1.1**) and confirmed experimentally. In most cases, the upconversion mechanism involves absorption and non-radiative energy transfer (ET). Ground state absorption (GSA) results in promotion of an ion from its ground state to an electronically excited state. Excited-state absorption (ESA)

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involves absorption of a photon by an excited ion, and results in promotion of that ion to a higher level excited state.



**Figure 1.1.** Schematic illustration of different upconversion processes: (A) Two-photon absorption; (B) Energy transfer upconversion; (C) Cross-relaxation

Energy transfer may take a variety of forms. ET is the basis for the common phenomenon of energy migration. A similar process between different ions may lead to energy trapping or sensitization effects. ET among different energy levels in different ions may result in cross relaxation (CR), by which an ion in a high electronic level is deactivated to a lower level, which promotes the ion in the vicinity to a higher level. The reverse of this process can also happen. In that case a lower-lying neighbor donates its excitation energy to a neighboring excited ion, which is then promoted to an even higher excited state. This process is referred to as energy transfer upconversion (ETU). ETU is inherently a pairwise or multicenter effect, and is therefore very strongly dependent on ion concentration. Whereas GSA/ESA mechanisms often dominate upconversion processes in low-doped samples, upconversion in highly-doped samples is very often dominated by ET processes, in particular GSA/ETU. The dependence of ETU on concentration results directly from the strong dependence of the ET rate constant on the distance between the donor and acceptor centers.

### ***1.2.2 Rare earth ions doped upconversion nanoparticles/nanocrystals***

Upconversion nanoparticles (UCNPs), sometimes called upconverting nanoparticles or photo-upconversion nanoparticles and abbreviated as UCPs, can be defined as particles with any of its dimensions in the nano scale (0-100 nm) that generate higher-energy light from lower-energy radiation (usually NIR or IR radiation) through the doping of a transition metal, lanthanide, or actinide ions into a solid state host lattice. The research in the field of rare earth (RE)-doped nanoparticles for infrared to visible upconversion emission has been recently recognized to hold tremendous potential in the areas of photonic and biophotonic applications. Intensive research efforts have therefore been

devoted to designing and tuning the upconversion properties of such materials. In rare earth nanoparticles or compounds, the 4f or 5f electrons are efficiently shielded and thus not strongly involved in the metal-to-ligand bonding. As a consequence, electron-phonon coupling to f-f transitions is reduced, and multiphonon relaxation processes are less competitive. The phenomenon of upconversion is therefore most common and best studied in materials containing lanthanide ions. However, not only the doping ions, but also the matrix plays an important role in the luminescence, RE<sup>3+</sup> (Ho<sup>3+</sup>, Tm<sup>3+</sup>, Er<sup>3+</sup> etc.) doped oxide (Gd<sub>2</sub>O<sub>3</sub>, Y<sub>2</sub>O<sub>3</sub>, ZrO<sub>2</sub> etc.) and fluoride (NaYF<sub>4</sub>, NaGdF<sub>4</sub>, YF<sub>3</sub> etc.) nanocrystals have attracted particular interest because those compounds are chemically and photochemically stable. Fluorides demonstrate more efficient upconversion processes because they have lower phonon energy, although the chemical stability is not as good as that of oxides. Güdel *et al.* have identified micro-sized Er<sup>3+</sup>/Yb<sup>3+</sup>- or Tm<sup>3+</sup>/Yb<sup>3+</sup> co-doped hexagonal NaYF<sub>4</sub> as the materials with highest upconversion efficiency.<sup>[1]</sup> The most popular combination is the Er<sup>3+</sup>/Yb<sup>3+</sup>- or Tm<sup>3+</sup>/Yb<sup>3+</sup>-co-doped NaYF<sub>4</sub> crystals, shown as the materials with the highest upconversion efficiency.<sup>[2]</sup> The crystal structure of NaREF<sub>4</sub> exhibits two polymorphic forms named cubic  $\alpha$ -phase (metastable high-temperature phase) and hexagonal  $\beta$ -phase (thermodynamically stable low-temperature phase).<sup>[3]</sup> For biological applications, the required nanoparticles should have a suitable size and suitable surface for conjugation with biological molecules, and exhibit high-intensity emission as well.<sup>[4]</sup>

Until now, few publications report the absolute quantum yields of upconversion nanomaterials.<sup>[5]</sup> The external radiative quantum yield,  $\eta$ , is defined as:<sup>[6]</sup>

$$\eta = (\text{number of photons emitted}) / (\text{number of photons absorbed})$$

Since upconversion is not a linear process, quantum yield becomes a function of radiation density. The efficiency of the upconversion process in RE ion-doped nanoparticles is extremely low compared with quantum dots and organic dyes. In a recently published paper,<sup>[7]</sup> a quantum yield of 3% was measured for hexagonal phase NaYF<sub>4</sub>:20%Yb<sup>3+</sup>, 2%Er<sup>3+</sup> bulk samples at a radiation power density of 20 W/cm<sup>2</sup>. For 30 nm and 10 nm hexagonal phase NaYF<sub>4</sub>:20%Yb<sup>3+</sup>, 2% Er<sup>3+</sup> nanoparticles, the quantum yield are about only 0.1% and 0.005%, respectively, under radiation conditions of 150 W/cm<sup>2</sup>. The drop in quantum yield with decreasing particle size is attributed to the increase in surface area of the smaller particles. Growing a homogeneous shell outside UCNPs is considered an efficient route to enhance the quantum yield.<sup>[8]</sup> Optimization of the synthetic procedures to obtain upconversion nanoparticles with the highest possible quantum yields is a very critical step in realizing the potential of these nanoparticles.

### 1.3 Synthesis of upconversion nanoparticles

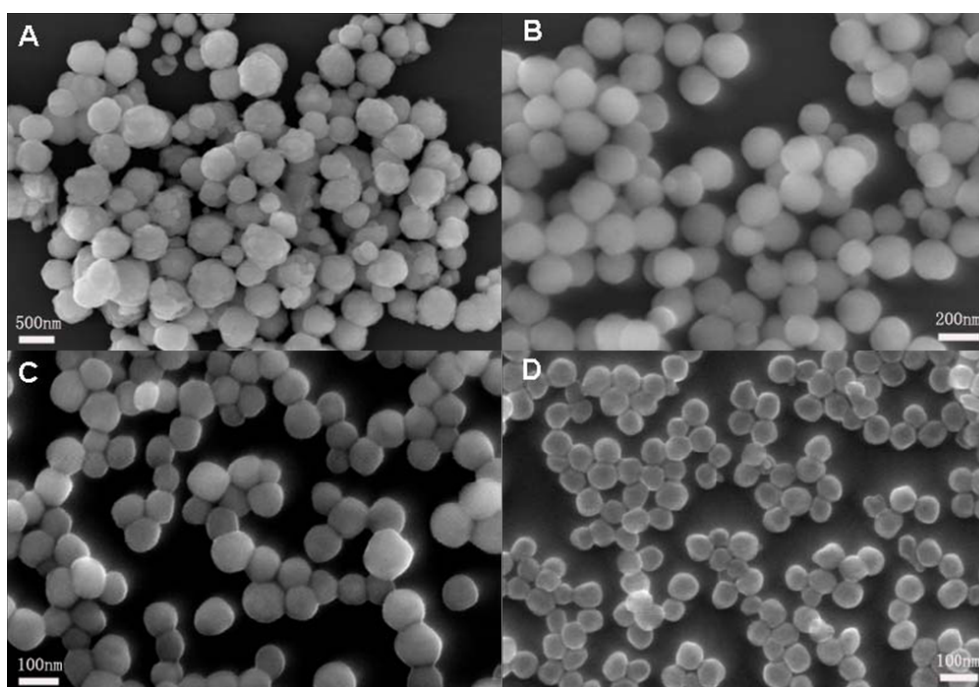
In the past decades, many different methods were invented for synthesizing

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nanomaterials: physics grinding, high-energy ball mill, sputtering, explosion, spraying, freeze drying, chemical vapor deposition, precipitation, hydrothermal synthesis, solvothermal synthesis, sol-gel synthesis, radiation chemical synthesis, micro emulsion, self-assembly, ionic liquid etc. To obtain upconversion nanocrystals, hydrothermal/solvothermal, sol-gel, thermal decomposition and ionic thermal methods are mostly used.

For biological applications, the desired upconversion nanoparticles should have a suitable size, a suitable size distribution, a suitable surface for conjugation with biological molecules, and exhibit high intensity emission. To obtain high-quality nanoparticles, the growth dynamics of the nanoparticles needs to be optimized by changing various reaction parameters such as the concentration of the reactants, reaction time, temperature and capping ligand used.

### **1.3.1 Hydrothermal/solvothermal method**



**Figure 1.2.** FESEM photograph of cubic  $\text{NaYF}_4:\text{Yb}^{3+},\text{Er}^{3+}$  nanoparticles synthesized by hydrothermal method with different precursor concentrations: (A) 0.004 mol/L; (B) 0.01 mol/L; (C) 0.02 mol/L, and (D) 0.04 mol/L.<sup>[11a]</sup>

Hydrothermal/solvothermal synthesis can be defined as a method of synthesis of single crystals that depends on the solubility of minerals in hot water or hot solvent under

high vapor pressures. The crystal growth is performed in an apparatus consisting of a steel pressure vessel called autoclave, in which a nutrient is supplied along with aqueous solution or solvent. Compared with other synthetic routes in nanocrystal preparation, the hydrothermal method has the following advantages: (1) synthesis occurs at relatively low reaction temperature (in general < 250 °C); (2) size, structure, and morphology of the products are subject to hydrothermal conditions and easy to be controlled (**Figure 1.2**); (3) the purity of product is high owing to recrystallization in hydrothermal solution; and (4) the required equipment and processes are simple. In order to obtain small and uniform particles, organic additives are often used to stabilize the particles in solution and control particle growth, e.g., cetyl-trimethylammonium bromide (CTAB),<sup>[9]</sup> ethylenediamine tetraacetic acid (EDTA),<sup>[10]</sup> and trisodium citrate (TSC)<sup>[11]</sup> etc..

### **1.3.2 Sol-gel method**

The sol-gel approach is a wet-chemical technique widely used in the fields of materials science and ceramic engineering. This technique is used primarily for the fabrication of materials starting from a chemical solution, which acts as the precursor for an integrated network of either discrete particles or network polymers.<sup>[12]</sup>

### **1.3.3 Thermal decomposition**

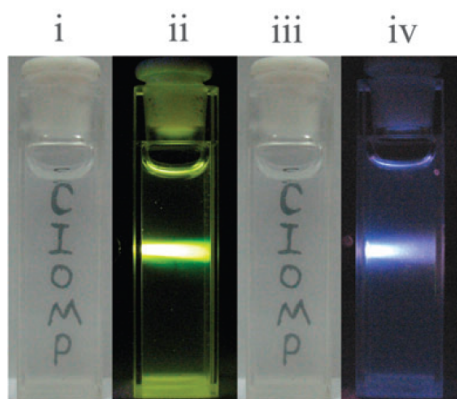
High-quality rare earth fluoride nanocrystals with smaller sizes can be synthesized efficiently with control over size and shape in non-aqueous media by a thermal decomposition process. Rare earth precursors are first dissolved in high-boiling organic solvents with the assistance of surfactants, subsequently those precursors are decomposed at elevated temperature. The first commonly used rare earth precursors are rare earth organic acid salts (e.g. trifluoroacetate, acetonacetate, oleate, acetate, etc.), and the surfactants typically contain polar capping groups and long hydrocarbon chains such as oleic acid (OA),<sup>[13]</sup> oleylamine (OM),<sup>[14]</sup> trioctylphosphine oxide (TOPO),<sup>[15]</sup> liquid paraffin<sup>[16]</sup> etc. Sometimes microwave assistance is introduced to the synthesis.<sup>[17]</sup> Due to the high temperature (> 250 °C) at which the reaction is carried out and the use of organic ligands to control the growth, the nanocrystals synthesized by this method often have small size (< 20 nm or even smaller than 5 nm) and a high degree of crystallization. However, further complex surface modification steps are required before the particles can be used for biological applications.

### **1.3.4 Ionothermal synthesis**

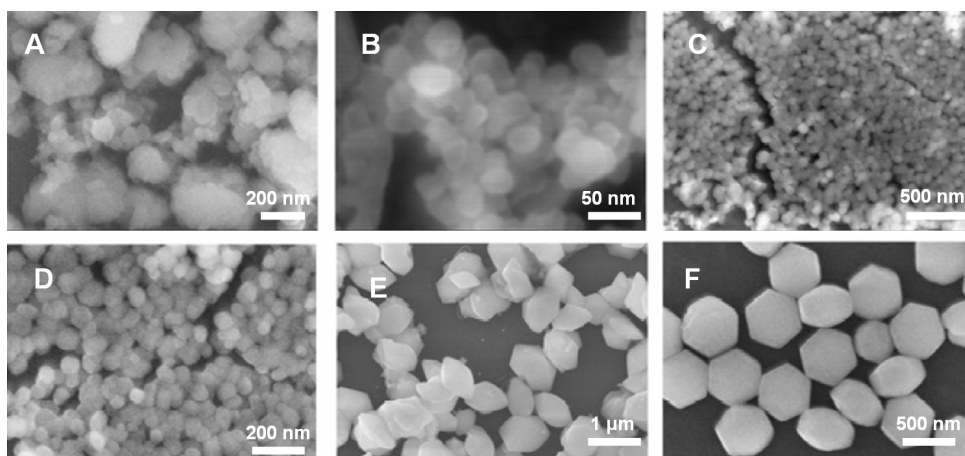
Ionic liquids (ILs) are non-volatile, non-flammable and thermally stable liquid state organic salts, which have been recently suggested as a ‘green’ alternative to the conventional organic solvents for the synthesis of inorganic compounds. Small, water-soluble and pure hexagonal phase NaYF<sub>4</sub>:Yb<sup>3+</sup>,Er<sup>3+</sup>/Tm<sup>3+</sup> upconversion nanoparticles were prepared for the first time in our group by an ionothermal method (**Figure 1.3** and **Figure 1.4**).<sup>[18]</sup> The key to this approach is the use of an ionic liquid, [BMIM] [BF<sub>4</sub>],

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which acts as solvent and template as well as fluoride source. Due to the overlay of ILs on the surface, the nanoparticles are strongly positively charged, and can be directly dispersed in water.



**Figure 1.3.** 1 wt% colloidal water solutions of  $\text{NaYF}_4:20\%\text{Yb}^{3+},2\%\text{Er}^{3+}$  (i and ii) and  $\text{NaYF}_4:20\%\text{Yb}^{3+},2\%\text{Tm}^{3+}$  (iii and iv) with (ii and iv) and without (i and iii) 980 excitation.<sup>[18]</sup>



**Figure 1.4.** SEM images of the  $\text{NaYF}_4:20\%\text{Yb}^{3+},2\%\text{Er}^{3+}$  obtained at 120 °C (A: amorphism); 140 °C (B: aggregate sphere with bad crystallization); 160 °C (C: 30-50 nm particle); 180 °C (D: 60-80 nm particle); 220 °C (E: spindle) and 240 °C (F: hexagonal flake).<sup>[18]</sup>

Up to date, thermal decomposition and hydro (solvo)-thermal reactions are the most widely used and the best methods for the precise control of shape and size of rare earth

fluoride nanoparticles. The hydrothermal route is effective to yield one-dimensional (1D) rare earth fluoride structures.<sup>[19]</sup> In contrast to these three methods, ionic liquid-based synthesis is a relatively green route, but the drawback is that particles synthesized following this approach are of less quality. For example, they are of lower monodispersity, less uniformity and have a broader size distribution relative to those obtained from the thermal decomposition processes.

## **1.4 Strategies to enhance upconversion luminescence**

Compared to the well-developed tags such as organic dyes and quantum dots, the application of upconversion nanomaterials leads to less photodamage to living organisms, weak background fluorescence due to excitation in NIR spectral range, and deeper detection and/or treatment range. However, these upconversion nanomaterials usually have low emission efficiency, partly because of their structure defects and large surface area with a variety of quenchers. At the same time, the small absorption cross section of excitation light also limits the efficiency. Therefore, how to enhance the upconversion emission is one of the most important issues towards the application of these nanomaterials. Doping concentration optimization, Yb<sup>3+</sup>, Er<sup>3+</sup>/Tm<sup>3+</sup> co-doping and high-temperature annealing are well-known traditional methods to improve the upconversion intensity, which have not yet led, however, to satisfactory result for (bio-)medical applications, especially in in-vivo applications. In recent years new approaches have come into sight.

### ***1.4.1 Inert shell coating***

Coating with a homogeneous layer outside the core has become a common method to improve the optical properties of nanoparticles. NaYF<sub>4</sub>:Yb<sup>3+</sup>,Er<sup>3+</sup>@NaYF<sub>4</sub> core/shell structured nanoparticles were designed and studied.<sup>[8]</sup> The shell has essentially two functions: it protects the emission centers (luminescent lanthanide ions: Tm<sup>3+</sup>, Er<sup>3+</sup> etc.) in the core, especially those near the surface, from non-radiative energy transfer to surface defects, which are more prevalent in nano-sized materials. In addition, the shell protects the ions from non-radiative relaxation processes assisted by the large vibrational energies of the solvent and surface-associated ligands. Core/shell nanoparticles offer clear advantages over similar uncoated materials in terms of protection of photoluminescence. Amongst the many contributions to this approach, it is worthwhile to mention the work of the van Veggel's group which proved the core/shell structure<sup>[20]</sup> and investigated the structure and chemical composition of the NaYF<sub>4</sub>/NaGdF<sub>4</sub> nanocrystals in detail.<sup>[21]</sup>

### ***1.4.2 Active shell coating***

In 2009, it was reported that active doping of both core and shell with ytterbium ions (active shell) as a sensitizer brings further improvement in upconversion emission intensity.<sup>[22]</sup> The upconversion luminescence intensity of NaGdF<sub>4</sub>:Yb<sup>3+</sup>,Er<sup>3+</sup>@

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$\text{NaGdF}_4:\text{Yb}^{3+}$  is stronger than that of  $\text{NaGdF}_4:\text{Yb}^{3+},\text{Er}^{3+}@\text{NaGdF}_4$  nanoparticles. It has been proposed that the reason for the increase is the role of the active shell which can transfer NIR absorbed radiation to  $\text{Er}^{3+}$ .

### **1.4.3 Au/Ag enhancement**

Another well-known method to enhance fluorescence is to couple emitters to noble metal surfaces or particles. Au and Ag nanomaterials are commonly chosen noble metals due to their well-studied surface plasmonic resonance (SPR) behavior. The luminescence of UCNPs has been improved in various structures, such as Ag nanowires decorated with UCNPs,<sup>[23]</sup> Au shell-coated on UCNPs,<sup>[24]</sup> and  $\text{Y}_2\text{O}_3:\text{Er}$  hollow nanoparticles with an Ag core inside.<sup>[25]</sup> In 2010, Schietinger *et al.* coupled single  $\text{NaYF}_4:\text{Yb}^{3+},\text{Er}^{3+}$  nanoparticles to a single Au sphere to obtain enhanced upconversion emission as demonstrated with a combined confocal and atomic force microscope setup.<sup>[26]</sup> However, two different interactions may occur simultaneously when a noble metal approaches a phosphor: fluorescence enhancement and non-radiative quenching. In some other experiments,<sup>[27]</sup> instead of upconversion enhancement the emitter-quencher (UCNPs-Au/Ag) nano-complex was obtained for tuning the emission of UCNPs or biosensor constructions etc. where the noble metallic nanomaterials were used only for quenching the emission of UCNPs.

### **1.4.4 $\text{Li}^+/\text{Sc}^{3+}$ doping**

In 2008 Zhang *et al.*<sup>[28]</sup> found that tri-doping with  $\text{Li}^+$  ions could enhance the visible green and red emissions in  $\text{Y}_2\text{O}_3:\text{Yb}^{3+},\text{Er}^{3+}$  nanocrystals by 25 times. In 2010, Yu *et al.*<sup>[29]</sup> doped trivalent  $\text{Sc}^{3+}$  ions in  $\text{NaYF}_4:\text{Yb}^{3+},\text{Er}^{3+}$  nanocrystals. The local symmetry of rare earth ions was changed and the blue, green, red, and UV upconversion luminescence was clearly enhanced. This enhancement was explained by the fact that  $\text{Li}^+/\text{Sc}^{3+}$  ions, which have a smaller cationic radius than  $\text{Y}^{3+}/\text{Yb}^{3+}/\text{Er}^{3+}$  ions, cause a change of local symmetry of rare earth ions. Because  $\text{Ln}^{3+}$  ions are sensitive to the surrounding environment, tailored modification of the local crystal field was ascribed to be responsible to the enhancement.

## **1.5 Hydrophobic to hydrophilic phase transfer of nanoparticles**

Amongst known chemical routes, high-temperature thermolysis of organometallic species in nonpolar solvents with a high boiling point has been widely shown to be a general approach capable of making colloidal nanostructure materials with a narrow size distribution, low crystalline defects, tunable shape and a good dispersibility in organic solvents. The problem of this approach is that it typically produces nanocrystals with hydrophobic surfaces. As is well known, the ideal luminescence nanoparticles used for biology/biomedicine should meet several requirements, such as being homogeneously dispersed and colloidally stable in aqueous solvents, maintaining a high quantum yield,

and showing high specific binding to biological components. The insolubility of such nanocrystals in aqueous solution thus greatly limits their bio-applications. Surface modification of nanocrystals has become one of the research foci in the fields of materials science. The surface coating of nanoparticles is crucial as it determines their properties. Several strategies have been developed to phase-transfer nanocrystals from hydrophobic into hydrophilic, not only for UCNPs but also for other hydrophobic nanoparticles such as quantum dots, magnetic nanoparticles and others.

### ***1.5.1 Ligand exchange (ligand substitution)***

The dispersibility of inorganic nanoparticles in water or organic solvent is achieved by ligand-stabilization. The ligand is normally a molecule that binds to a central metal atom to form a coordination complex. The bonding between metal and ligand is in general realized by formal donation of one or more of the ligand's electron pairs. One popular method to make a nanoparticle hydrophilic is based on ligand exchange, where the hydrophobic surfactants on the particle surfaces are replaced by molecules containing polar groups on both ends. The advantage of this method is that it generates water-soluble nanoparticles with the lowest possible hydrodynamic diameter.

In 2007, Yin *et al.*<sup>[30]</sup> reported a general approach for transferring hydrophobic nanocrystals into water through a ligand exchange procedure. Polyelectrolytes such as poly(acrylic acid) and poly(allylamine) are used to replace the original hydrophobic ligands on the surface of nanocrystals at an elevated temperature in a glycol solvent and eventually render the nanocrystals highly water soluble. The physical properties of the nanocrystals, such as superparamagnetism, photocatalytic activity, and photoluminescence, are maintained or improved after ligand exchange.

In 2009, Yang *et al.*<sup>[31]</sup> utilized poly(ethylene glycol) grafted hyperbranched poly(amino amine) (*h*-PAMAM-*g*-PEG) to modify different types of hydrophobic nanoparticles, and transferred them into water with better stability. In the case of CdSe nanoparticles, the *h*-PAMAM-*g*-PEG layer leads to a lower cytotoxicity when compared with bare CdSe particles, and the applications in biology are extended.

### ***1.5.2 Polymer encapsulation***

Polymer encapsulation is another method for phase transfer. In this method the original hydrophobic ligands are retained on the nanoparticles surface. Because single polymer chains can contain multiple hydrophobic units and their interactions with the native organic coatings on nanocrystals can be numerous, they can be bound more strongly than conventional surfactants. One advantage of this method is that the functionality of the nanocrystal-polymer assembly can be widely varied through introduction of different amphiphilic moieties.

In 2004, a general strategy was reported by Parak *et al.*<sup>[32]</sup> for phase transfer by wrapping an amphiphilic polymer around the particles. Here, the hydrophobic alkyl chains

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of the polymer intercalate with the surfactant coating. The surface of the polymer shell becomes negatively charged, stabilizing the particles in water by electrostatic repulsion. In particular, high-quality CoPt<sub>3</sub>, Au, CdSe/ZnS, and Fe<sub>2</sub>O<sub>3</sub> nanocrystals were water-solubilized in this way.

In 2007, Colvin *et al.*<sup>[33]</sup> used amphiphilic copolymers containing poly(ethylene glycol) (PEG). Those copolymers are generated using a maleic anhydride coupling scheme that permits the coupling of a wide variety of PEG polymers, both unfunctionalized and functionalized, to hydrophobic tails. The benefit of this approach is that after encapsulating the hydrophobic nanocrystals the amphiphilic polymers offer not only PEG chains available for biocompatibility but also free carboxylic acid groups for further reaction. Nonspecific binding can thus be reduced.<sup>[34]</sup>

Yi *et al.*<sup>[35]</sup> synthesized upconversion nanoparticles with an amphiphilic layer, which was 25 % octylamine and 40 % isopropylamine modified poly(acrylic acid) (PAA). The upconversion luminescence decreased 30 % due to the interaction of PAA with the UCNPs.

In 2009, Parak *et al.*<sup>[36]</sup> described a general approach for synthesizing an amphiphilic polymer that could be used for the coating of hydrophobic nanoparticles. The amphiphilic polymer was based on a poly(maleic anhydride) backbone that was modified with hydrophobic side chains and functional organic molecules. Au, CdSe/ZnS QDs and Fe<sub>3</sub>O<sub>4</sub> inorganic colloidal nanoparticles (with the ligand of TOPO, trioctylphosphine or dodecanethiol etc.) were successfully transferred to aqueous solution. The attachment of the polymer to the inorganic nanoparticles was shown to be only mediated by the hydrophobic interaction and did not depend on the particular surface chemistry of the nanoparticles.

However, it is obvious that the polymer encapsulation method has one large disadvantage and that is that it increases the hydrodynamic diameter. The polymers are often not commercially available and functionalized polyethylene glycol (PEG) derivatives are therefore expensive.

### ***1.5.3 Silica coating***

Silica coating is one of the most popular methods for nanoparticle surface modification.<sup>[37]</sup> This coating technique can introduce a silica shell to protect the core nanoparticle from the external environment. It can be used for both hydrophilic and hydrophobic nanoparticles of metals, metal oxides, and quantum dots in a size range of 1-100 nm.

The silica coating method has also several drawbacks. The formation of insoluble silica-nanoparticle aggregates is a common problem due to the sensitive surface chemistry of nanoparticles. Preparation of finer core/shell particles by this method is also difficult as particles with thin silica coatings have a very high tendency to aggregate, leading to multicore formation within the silica shell. Moreover, this method is difficult to apply to

nanoparticles that are insoluble in the alcohol-water media.

#### **1.5.4 Other methods**

Apart from the popular methods to convert the hydrophobic UCNPs into hydrophilic ones such as ligand exchange, polymer encapsulation and silica coating, a few other strategies such as ligand oxidation reaction, cyclodextrin assembly and ligand-free UCNPs have also been developed recently.

Huang *et al.*<sup>[38]</sup> introduced a new strategy to prepare hydrophilic upconversion nanoparticles. The Lemieux-von Rudloff oxidation method was utilized to oxidize selectively a carbon-carbon double bond (R-CH=CH-R') in the oleic acid ligand to provide two carboxylic acids. Owing to the presence of the free carboxylic acid groups on the surface, the oxidized UCNPs could be directly conjugated with some biomolecules. This method, however, still suffers from some limitations such as a relatively long reaction time (> 48 hours) and a low yield.

Li *et al.*<sup>[39]</sup> developed a method for converting hydrophobic UCNPs into amphiphilic UCNPs based on epoxidation of the surface oleic acid ligand and further coupling with polyethylene glycol monomethyl ether (mPEG-OH). No clear changes were observed in the morphologies and luminescent properties of the UCNPs during the epoxidation and functionalization processes. However, this method is still limited to the oleic-capped nanocrystals.

In 2010 Li *et al.*<sup>[40]</sup> developed a simple (only stirring or shaking was needed) and rapid (< 20 s) synthetic method to “draw” hydrophobic UCNPs into water through self-assembly of  $\beta$ -cyclodextrin and adamantaneacetic acid, which was coated on the surface of UCNPs. However, 1-adamantaneacetic sodium is expensive and not a common chemical used in the synthesis of UCNPs. Moreover, conjugation of UCNPs and biomolecules is difficult because of the absence of proper coupling groups outside  $\beta$ -cyclodextrin, which necessitates an additional functionalization process.

In 2011, Capobianco *et al.*<sup>[41]</sup> obtained water-dispersible ligand-free UCNPs by removing the oleate ligand from the surface of oleate-capped UCNPs synthesized via thermal decomposition using a simple acid treatment process. After adding HCl solution, the carboxylate groups of the oleate ligand were protonated to yield oleic acid. The ligand-free nanoparticles could be dispersed in water. Further modification of these UCNPs offers potential bio-applications.

In conclusion, when one chooses a phase transfer method, many parameters need to be considered, such as cost, change of size, or more specific, hydrodynamic diameter, luminescence properties, stability in aqueous solution, type of biofunctional groups on the surface of the particles, mono-dispersibility and cytotoxicity after phase transfer etc. Encapsulating nanoparticles in amphiphilic polymers is a well-established method for generating biocompatible nanoparticles. However, the process is costly and/or nontrivial, and the hydrodynamic diameter of the final water-soluble nanoparticles is significantly

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increased by this method. For some *in-vitro* experiments, or experiments in which small size is not required, amphiphilic polymers encapsulation is not a bad choice. If nanoparticles are intended to be used for loading drugs or some special molecules, silica coating might be more suitable.

Ligand oxidation reaction and cyclodextrin assembly are not mature enough and not used widely. Modification of ligand-free UCNPs might be a novel and facile method to make functionalized and water dispersed nanoparticles.

### **1.6 Upconversion nanoparticles in bio-applications**

Traditional fluorophores such as fluorescent organic dyes and quantum dots (QDs) are mainly based on downconversion fluorescence. These fluorophores have several inherent drawbacks such as photobleaching, high background noise from bio-tissue autofluorescence, and considerable photodamage to biological materials. Upconversion luminescent nanoparticles emit detectable photons of higher energy in the visible and near-infrared range upon irradiation with NIR light, and thus avoid some of the disadvantages of conventional downconversion labels. Upconversion nanoparticles are presently widely regarded as an ideal photonic label/platform for biological applications.

#### ***1.6.1 Fluorescence cell imaging and bioimaging in vivo (multimodal imaging)***

Nowadays, the molecule has become the key to understand at a fundamental level processes that occur in wide variety of sciences ranging from physics to biology to health sciences. In health sciences, for example, one aims to be able to detect disease and injury at a stage that one still can employ less invasive therapies, as well as to develop highly specific therapies. There is thus an increasing need to develop methods that allow one to measure biological pathways and observe cellular events at a molecular level using non-invasive methods. Examples of such bioimaging modalities include X-ray imaging, magnetic resonance imaging (MRI), positron emission tomography (PET), single photon emission coherence tomography (SPECT), ultrasound imaging, optical coherence tomography (OCT), computed (X-ray) tomography and fluorescence imaging.

Fluorochrome imaging is an important method. Conventional fluorescent markers are separated into endogenous and exogenous fluorophores. Endogenous fluorophores are the fluorescent biomolecules naturally present in tissue, exogenous fluorophores are external markers like quantum dots, Cy3, Cy5, GFP, CFP etc. with downconversion fluorescence. There are several problems one has to face when using these fluorophores for imaging: the fluorescence signals from endogenous fluorophores cause background noise (autofluorescence), the exogenous fluorophores need to be excited by UV or visible light. In general, tissue strongly absorbs UV and visible light. As a result, penetration depth is limited.

Traditional organic dyes and fluorescent proteins have been used successfully for *in*

*in vivo* imaging, but suffered from a high bleaching rate in these high-intensity cell imaging studies, thus making long-term experiments unfeasible. More recently, metal and semiconductor nanocrystals have been employed as labels in biological detection.<sup>[42]</sup> The advantages of the fluorescent nanocrystals are high quantum yield, tunable emission wavelength and high stability against photobleaching. Furthermore, in contrast to downconversion fluorescent materials, upconversion nanoparticles show very low background emission and deep penetration depth. Due to their unique luminescence properties they have become a new generation of fluorescent labels.<sup>[43]</sup>

### ***1.6.2 Immunoassay and nucleic acid detection***

Upconversion-based homogeneous assays are commonly based on a Förster resonance energy transfer (FRET) process (**Figure 1.5**) between a donor and an acceptor.<sup>[44]</sup> FRET, also known as fluorescence resonance energy transfer, is in some cases called luminescence resonance energy transfer (LRET). FRET is an interaction that strongly dependent on the distance of the donor and the acceptor. Excitation energy is transferred from the donor to an acceptor molecule without emission of a photon. The efficiency of FRET is inversely proportional to the sixth power of the intermolecular separation.<sup>[45]</sup> The FRET efficiency ( $E$ ) is the quantum yield of the energy transfer transition, i.e., the fraction of energy transfer event occurring per donor excitation event:

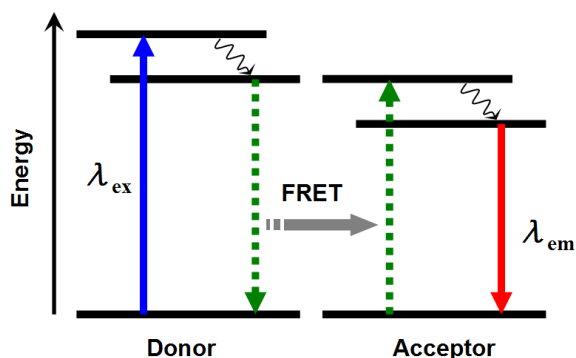
$$E = k_{ET} / (k_f + k_{ET} + \sum k_i)$$

where  $k_{ET}$  is the rate of energy transfer,  $k_f$  the radiative decay rate and the  $k_j$  the rate constant of any other de-excitation pathway. Based on the dipole-dipole coupling mechanism the FRET efficiency can be described as:

$$E = 1 / [1 + (r / R_0)^6]$$

where  $R_0$  is the Förster distance of this pair of donor and acceptor.

FRET is a simple and effective method to investigate the changes of biological phenomena in nanoscale, partly because unbound acceptors in the solution are too far to accept energy nonradiatively due to the sensitivity to the distance between donor and acceptor. Upconversion based FRET typically relies on FRET coupling of UCNPs (donor) and a downconversion fluorophore (acceptor). The fluorophore is chosen such that the emission spectra of the UCNPs overlap with the excitation spectra of the downconversion fluorophore. It is advantageous for FRET to use UCNPs as donor because NIR does not excite the fluorophore acceptor. This results in a good detection sensitivity of the signal from the acceptor. Another advantage is that the narrow and sharp lanthanide emission band is easily distinguishable from the emission wavelength of the acceptor. The photostability, low cytotoxicity, deep penetration depth, and minimum photodamage enable prolonged FRET sensing. Many preliminary applications of using UCNPs for FRET have been published. Some representative ones are listed below.



**Figure 1.5.** Schematic illustration of FRET process between donor and acceptor

In 2005, Li *et al.*<sup>[46]</sup> constructed a FRET system with upconversion luminescent nanoparticles as energy donors and gold nanoparticles ( $\lambda_{abs} \sim 520$  nm) as energy acceptors to detect trace amount of avidin. Both UCNPs and gold nanoparticles were biofunctionalized with biotin. Because the absorption of gold nanoparticles matched well with the UCNPs emission, the luminescence of UCNPs is quenched when UCNPs and gold nanoparticles are in close proximity due to the biotin-avidin interaction. The results indicated the FRET system is a sensitive and simple system for biological analyses.

Xu *et al.*<sup>[47]</sup> utilized a UCNP-gold nanoparticle system to determine the amount of goat antihuman IgG in a sandwich-type bioassay. Rabbit antigoat IgG functionalized  $\text{NaYF}_4:\text{Yb}^{3+}, \text{Er}^{3+}$  nanoparticles served as the donor, human IgG functionalized gold nanoparticles as the acceptor. A short spacing between the donor and acceptor nanoparticles was achieved through sandwich-type immunoreactions. The presence of the goat antihuman IgG as a bridge leads to energy transfer, which enables the determination of the concentration of the goat antihuman IgG in the system.

In 2007, Rantanen *et al.*<sup>[48]</sup> changed this model to a two-step energy transfer homogeneous immunoassay system for which enhanced energy transfer efficiency and a large anti-Stokes shift were observed. In this system, the acceptor was a tandem dye molecule that was constructed using B-phycoerythrin as an absorber and the Alexa Fluor 680 (AF680) dye as emitter. Biotinylated tandem dye molecules can be captured by streptavidin-coated upconversion nanoparticles. The emission of the acceptor is modulated by a binding event. Energy transfer from UCNPs through a FRET process is only possible for acceptors in the proximity. Energy transfer from B-phycoerythrin to AF680 dye leads to 704 nm emission from the AF680 dye. With the tandem dye, it was possible to achieve four times higher signal from a single binding event compared to the conventional AF680 dye on its own. The applications of tandem dyes are currently expanded to fluorescence-based homogeneous assays.

Some immuno-applications of upconversion nanoparticles are not based on FRET

processes.<sup>[49]</sup> In 2001, Hampl *et al.*<sup>[50]</sup> reported upconversion particles used in immunoassays as alternatives to conventional labeling agents. Submicron-sized (400 nm diameter)  $\text{Y}_2\text{O}_3\text{:Yb}^{3+},\text{Er}^{3+}$  particles were shown to enable a detection limit of 10 pg human chorionic gonadotropin in a lateral flow (LF) immunochromatographic assay format. In contrast to conventional labeling agents such as gold nanoparticles or colored latex beads, the upconversion particles exhibit a 10-fold improvement in detection sensitivity.

In 2001, Niedbala *et al.*<sup>[51]</sup> developed an LF-based strip assay for the simultaneous detection of amphetamine, methamphetamine, phencyclidine, and opiates in saliva by using multi-color (550 nm diameter) particles. By analyzing the test strip for each colored phosphor, the drug molecules were successfully detected on the basis of phosphor color and position.

### **1.6.3 DNA detection**

DNA/RNA analysis is of great importance in molecular biology, genetics, and molecular medicine. Many efforts have been made to identify specific variations and mutations in the human genome. DNA hybridization-based detection is a major technique for the diagnosis of genetic disease where clinical symptoms are linked to DNA sequence alterations.<sup>[52]</sup>

In 2006, Li *et al.*<sup>[53]</sup> combined magnetic-field-assisted bioseparation and concentration techniques with magnetic nanoparticles. Magnetic nanoparticles were modified with capture DNA and UCNPs were modified with probe DNA, respectively. Capture DNA was hybridized with target DNA, after which probe DNA was hybridized with the overhanging region of the target sequences. In this way UCNPs were conjugated indirectly to the magnetic nanoparticles. After magnetic separation and luminescence measurement, capture DNA detection was achieved.

In 2009, Rantanen *et al.*<sup>[54]</sup> simultaneously utilized the separate emission bands of the UCNPs donor. Such a method can be used as long as the emission wavelengths of the acceptor fluorophores are sufficiently apart from each other and do not overlap with the other labels in the assay. In a dual-parameter sandwich-hybridization assay, two probe oligonucleotides with sequences complementary to a target sequence of  $\beta$ -actin or HLA-B27 were selectively conjugated to AF546 and AF700. The oligonucleotide-modified dye molecules (AF546 and AF700) and target oligonucleotides were then mixed with UCNPs premodified with capture oligonucleotides. Upon formation of the sandwich complex through hybridization, the upconversion emissions at 540 and 653 nm were quenched by AF546 and AF700, respectively. By measuring the intensities of probe-specific emissions at 600 and 740 nm, two different target-oligonucleotide sequences could be detected simultaneously and quantified with a measurement range from 0.35 to 5.4 nM.

FRET is also an excellent tool used in DNA detection. P. Zhang *et al.*<sup>[55]</sup> and C.H. Huang *et al.*<sup>[56]</sup> designed a highly sensitive and specific single-stranded nucleotide sensor

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based on the upconversion nanoparticles in 2006. A sandwich-type hybridization format was adopted by using two short oligonucleotides with designed sequence to capture the longer target oligonucleotide. One of the short oligonucleotides was covalently bound to the UCNPs, the other was labeled with a TAMRA fluorophore (580 nm emission) with the excitation spectrum overlapping with the emission spectrum of UCNPs. In the presence of target oligonucleotides, the TAMRA was brought close to the UCNPs. Under NIR excitation, the excitation energy was transferred to TAMRA from which the target oligonucleotide could be detected.

In 2009 P. Zhang *et al.*<sup>[57]</sup> designed a more simple DNA biosensor: upconversion nanoparticles as energy donor and an appropriate intercalating dye (SYBR Green I) as energy acceptor. This biosensor is of high sensitivity, specificity and simplicity. The detection limit of this approach was 20 fmol, and targets could be distinguished with an accuracy at the single-nucleotide level.

In 2010, Y. Zhang *et al.*<sup>[58]</sup> constructed a FRET model for RNA detection and studied the intracellular fate of siRNA (small interference RNA) in live cells. In this complex system, BOBO3 (intercalating dye) – stained siRNA was attached to the surface of amino groups modified UCNPs, and energy was transferred from UCNPs to BOBO3 in close proximity under excitation of nanoparticles with NIR laser. The FRET efficiency in the complex increased with the siRNA/nanoparticle ratio. The occurrence of FRET between the UCNPs and BOBO3 provided real-time evidence for the release and biostability of siRNA molecules in a buffer and even in live cells. This system could detect some other biomolecules such as nucleic acid and proteins in both PBS buffer and live cells.

### **1.6.4 Photodynamic therapy**

Photodynamic therapy (PDT) is a novel treatment that involves the use of photosensitizers (so-called PDT drugs), oxygen molecules, and light of appropriate wavelengths that can excite photosensitizers. Under proper light illumination, the photosensitizers act as catalysts that convert oxygen molecules into reactive oxygen species (ROS), amongst which singlet oxygen ( $^1\text{O}_2$ ). Singlet oxygen is very reactive and can cause oxidative damage to biological substrates. It can ultimately lead to nearby cell death, thus displaying antitumor and/or antibacterial properties. PDT is a relatively new treatment compared to chemotherapy and radiation therapy. It is also not as widely practiced. However, most PDT treatments are so far limited to cancers very close to surface, because visible light which is needed for excitation of photosensitizers does not penetrate well through tissues. Light of longer wavelengths, such as far red and infrared light, can penetrate deeper into tissues.

In recent years, the development of upconversion nanoparticles (UCNPs) capable of converting NIR to the visible range under normal conditions has attracted considerable interest in PDT because the excitation at NIR can penetrate deep into bio-tissues. In comparison to traditional fluorescent labels such as organic dyes and quantum dots (QDs),

UCNPs can be excited by IR radiation with an excellent signal-to-noise ratio owing to the absence of auto-fluorescence and reduction of light scattering. NaYF<sub>4</sub>:Yb<sup>3+</sup>,Er<sup>3+</sup> UCNPs are one of the mostly studied upconversion materials.

The first report of using UCNPs for PDT was in 2007 when NaYF<sub>4</sub>:Yb<sup>3+</sup>,Er<sup>3+</sup> nanoparticles were coated with a thin layer of SiO<sub>2</sub> doped with Merocyanine-540 photosensitizer and functionalized with a tumor-targeting antibody on the surface.<sup>[59]</sup> Under 980 nm excitation, the number of cancer cells *in vitro* was decreased by <sup>1</sup>O<sub>2</sub>. This nanosystem could be delivered to cancer cells in a highly specific way, and was capable of targeting different types of cancer cells. The only problem was the low photosensitizer loading.

In 2008 Y. Zhang *et al.* made 50 nm PEI/NaYF<sub>4</sub>:Yb<sup>3+</sup>,Er<sup>3+</sup> nanoparticles modified with a zinc phthalocyanine photosensitizer and targeted to folate receptors on human colon cancer cells.<sup>[60]</sup> Significant cell destruction was observed. But still, loading of the photosensitizer ZnPc was low because it was non-covalently adsorbed to the nanoparticles' surface. After that, Y. Zhang's group coated NaYF<sub>4</sub> UCNPs by a uniform layer of mesoporous silica,<sup>[61]</sup> which has a large surface area of ~ 770 m<sup>2</sup>g<sup>-1</sup> and an average pore size of 2 nm. A high loading of zinc phthalocyanine photosensitizers could be achieved by incorporation into the mesoporous silica. It was claimed that "the photosensitizer encapsulated in mesoporous silica is protected from degradation in the harsh biological environment and was not released out of the silica during the NIR excitation". The nanoparticles are reusable since the photosensitizers encapsulated in the silica can be removed by soaking them in ethanol. However, this approach does not work in deionized water, PBS buffer, or cell culture medium.

## 1.7 Outline of the thesis

In the next chapter (**Chapter 2**), the upconversion photoluminescence properties of NaYF<sub>4</sub>:Yb<sup>3+</sup>,Er<sup>3+</sup> and NaYF<sub>4</sub>:Yb<sup>3+</sup>,Er<sup>3+</sup>@NaYF<sub>4</sub> core/shell structure UCNPs are characterized. In **Chapter 3** we systematically study the effect of the surface-related organic vibrational modes on the spectroscopic properties of rare earth ions in NaYF<sub>4</sub> nanoparticles. In **Chapter 4** a new structured doping strategy, which could efficiently enhanced upconversion luminescence intensity was investigated in detail. In **Chapter 5** we introduce a new design of a dual-functional nanosystem where the bioimaging and photodynamic therapy can be realized simultaneously. In **Chapter 6** core/shell structured UCNPs are introduced to a FRET system for singlet oxygen generation. As energy donor in a FRET process, a proper shell thickness of core/shell UCNPs can facilitate not only strong luminescence but also optimization of energy transfer efficiency compared to pure core UCNPs.

## 1.8 References

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