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Control of Luminescence and Interfacial Properties as Perspective for Upconversion Nanoparticles

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Near-infrared (NIR) light is highly suitable for studying biological systems due to its minimal scattering and lack of background fluorescence excitation, resulting in high signal-to-noise ratios. By combining NIR light with lanthanide-based upconversion nanoparticles (UCNPs), upconversion is used to generate UV or visible light within tissue. This remarkable property has gained significant research interest over the past two decades. Synthesis methods are developed to produce particles of various sizes, shapes, and complex core–shell architectures and new strategies are explored to optimize particle properties for specific bioapplications. The diverse photophysics of lanthanide ions offers extensive possibilities to tailor spectral characteristics by incorporating different ions and manipulating their arrangement within the nanocrystal. However, several challenges remain before UCNPs can be widely applied. Understanding the behavior of particle surfaces when exposed to complex biological environments is crucial. In applications where deep tissue penetration is required, such as photodynamic therapy and optogenetics, UCNPs show great potential as nanolamps. These nanoparticles can combine diagnostics and therapeutics in a minimally invasive, efficient manner, making them ideal upconversion probes. This article provides an overview of recent UCNP design trends, highlights past research achievements, and outlines potential future directions to bring upconversion research to the next level.

1. Introduction

Many biomedical and bioanalytical questions can be attractively answered using optical methods, as demonstrated by the popularity of fluorescence microscopy or the frequent application of fluorescent assays.[1,2] Along with this also improved luminescent nanoprobes are required and here the latest developments in the field of upconverting nanoparticles (UCNPs) display their great potential. Upconversion nanoparticles based on a crystalline host lattice (in most cases hexagonal $NaYF_4$) and doped with different combinations of lanthanide ions diverge from other nanoscale luminescent probes mainly in that they are NIR-excitable and thus have advantages as high tissue penetration, low autofluorescence, and high signal-to-noise ratios. In addition, they are distinguished by exceptional photostability and, unlike other nanomaterials, exhibit continuous emission without blinking, which is particularly advantageous for applications in the field of imaging, for example. Those characteristics allow those nanoparticles to act as small light sources—so-called "nanolamps"—in biological environments. The NIR light is converted into visible and UV light in a nonlinear process by sequential multiphoton absorption of the many long-lived electronic states of lanthanide ions. Compared to the two-photon excitation of dyes, this process increases the probability of reaching higher excited states and therefore allows lower excitation intensities.[3]

Despite this simple explanation of the upconversion principle in lanthanide-doped nanoparticles, the detailed mechanism is complex in nature. This is mainly due to the limitations imposed by Laporte's rule on the occupation of the energy levels and myriad competing nonradiative transitions within the ions.[4] Consequently, their brightness is limited compared to other luminescent probes, which is also reflected in low quantum yields. These are usually <5% for UCNPs, while, for example, quantum or carbon dots tend to achieve quantum yields of <90%.[5] Luckily, the knowledge in nanoparticle synthesis has grown significantly, allowing much more complex particle architectures, and thus leading to smart and creative particle designs that can compensate for the former weak luminescence. By implementing several different lanthanide ions in a host crystal, intentional crosstalk between these ions is induced, leading to processes such as energy migration, cross-relaxation, or energy trapping. Additionally, depending on the chosen lanthanides, the excitation, and emission wavelength can be tuned. The clever choice of doping and its spatial arrangement in the nanocrystal thus enables a precise adjustment of the luminescence regarding spectral properties, intensity, and lifetime.[6]

Before UCNPs can be used in biological environments, they must be equipped with a surface coating that keeps the

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particles colloidally and chemically stable even in the presence of proteins, high salt concentrations, and at different pH values.[7] Exciting progress has also been made in the area of surface engineering, particularly with respect to efficient functionalization with receptors for sensing, light-driven local generation of reactive species, or controlled release of drugs.[8] However, more research is needed, not only to fully control and understand the processes on the particle surfaces but also to protect the nanoparticle itself so that its luminescent properties can be used in chemically complex environments and remain stable over time.

In the following, the most important milestones of the last few years of upconversion research are shown, but also existing research gaps are pointed out and supplemented with ideas on how the research could be continued. The focus is on applications of UCNPs where the lanthanide emissions themselves are detected, such as in biosensing or bioimaging, and in applications where the UCNPs are used more as transducers, triggering other mechanisms such as drug release, photodynamic therapy (PDT), or optogenetics.

2. Strategies in Particle Design

Naturally, the relatively low efficiency of photon upconversion in lanthanide-doped nanoparticles due to the multiphoton processes still provides sufficient motivation for scientists to improve them. Depending on the application, significant successes have been achieved through energy harvesting using antenna dyes and through coupling with plasmonic structures or photonic crystals.[9–12] However, for bioapplications that require, among other things, long stability and low toxicity, strategies that address particle composition and architecture are often more promising. An improved understanding of the synthesis of small, monodisperse nanoparticles has led to remarkable advances in this field. Probably the greatest implications in novel particle architectures are due to a) the development of nanoparticles with a core–shell structure and b) the realization that the concentrations of sensitizer and activator ions can be higher than it has been assumed for many years.[6,13–24] A combination of both approaches opens a wide field of previously unexplored particle systems, with possibilities to adapt the spectral properties of these particles even better to the requirements for medical-diagnostic applications. This can be achieved either by improving the general efficiency of upconversion or by specifically increasing the probabilities of individual transitions in the complex energy term scheme of UCNPs. The beginning of these new trends is marked, among others, by the development of UCNPs that omit sensitizer ions at all. Tm-doped UCNPs, which are excited by photon avalanche (PA) and therefore benefit from a high concentration of Tm^{3+} within the $NaYF_4$ crystal, caused a stir. A high excitation power (in most cases >10 kW cm^{-2}) favors the excited-state absorption (ESA) of Tm^{3+}, followed by cross-relaxations (CR) caused by the high Tm^{3+} concentrations in the crystal. The combination of ESA and CR sets the bulk of Tm ions in intermediate excited states, which then leads to extremely high populations of the higher excited states since ESA now occurs without the further possibility of CR (**Figure 1**a). This upconversion process follows a strongly nonlinear behavior, as the emission intensities increase immensely once a certain excitation power threshold is reached. As a result, these particles surpass all other UCNPs reported to date in brightness, and are therefore of great interest for application in high-resolution microscopy, since the high-power dependence confines the excitation beam to the maximum of the Gaussian laser distribution, enabling it to beat the Abbe diffraction limit.[25–27]

However, apart from high-resolution microscopy, the applicability of such particles in biological systems is limited due to the high excitation power densities, even when trying to choose the excitation wavelength in the optically silent regions within the biological window (800, 1064, or 1450 nm). When it is desired to work with the lowest possible power densities, core–shell architectures are increasingly showing their advantages, especially in multishell systems, where spatial separation of different types of lanthanide ions is achieved. Recently, Liu et al.[28] showed that the separation of sensitizers from activators can lead to an increased luminescence intensity compared to particles in which sensitizers and activators are combined. This study compares three different structures based on $NaLuF_4$ host lattices: Yb,Er@inert, Yb@Er@inert, and Er@Yb@inert (Figure 1b). While the Er@Yb@inert approach showed about a four-fold UCL enhancement compared to the Yb,Er@inert particles, the Yb@Er@inert particles were less bright than the particles with both ions mixed in the core. This clearly shows that not only the separation is important, but also the exact sequence of the lanthanide layers. In general, separation of Yb^{3+} and Er^{3+}-ions minimizes the $Er^{3+} \rightarrow Yb^{3+}$ reverse energy transfer processes, which are known to reduce luminescence intensities. The superiority of Er@Yb@inert particles compared to Yb@Er@inert is mainly attributed to two effects: on the one hand shorter energy migration paths from the outer Yb^{3+} ions to the Er^{3+} in the core and on the other hand to the fact that the Er^{3+}-ions are stronger affected by quenching processes because they are closer to the particle surface.[28] Similar results were obtained by Yao et al.[30], showing that growth of the sensitizer shell around a core particle doped only with activators allows controlled energy migration from the shell to the core and thus upconversion efficiency gets improved.

Even omitting the spatial separation of the sensitizing and activating groups can result in bright UCL, if directional energy migration from the shell to the core is forced via sensitizing Yb-shells protected by inert shells. This has been shown, for example, by Zhou et al.[31] on Yb,Tm@Yb@inert particles. A recent review article gives an excellent overview of different types of multishell approaches, including doping with different sensitizers to shift excitation wavelengths and combining different activators to achieve multicolored emissions.[32] A striking example of the design of complex, efficient multishell particles is provided by Jin et al. by synthesizing particles of Gd,Yb,Tm@Yb@Gd,Yb,Nd@Gd architecture to generate a six-photon energy cascade (Figure 1c). Directed energy migration from sensitizer shells to activator-containing cores brings a decisive advantage. A combination of the two different sensitizers Yb^{3+} and Nd^{3+} in an additional shell enables excitation at 808 nm, thus avoiding sample overheating in aqueous media upon 980 nm excitation. The presence of Gd^{3+} enables energy trapping in highly excited states, which reduces the probability of luminescence quenching, leading to an even brighter upconversion.[29]

These examples clearly illustrate that there is potential in novel core–shell particle architectures and doping ratios that are unusual at first glance. As the developments are only just beginning, a great deal of progress can still be expected. The future

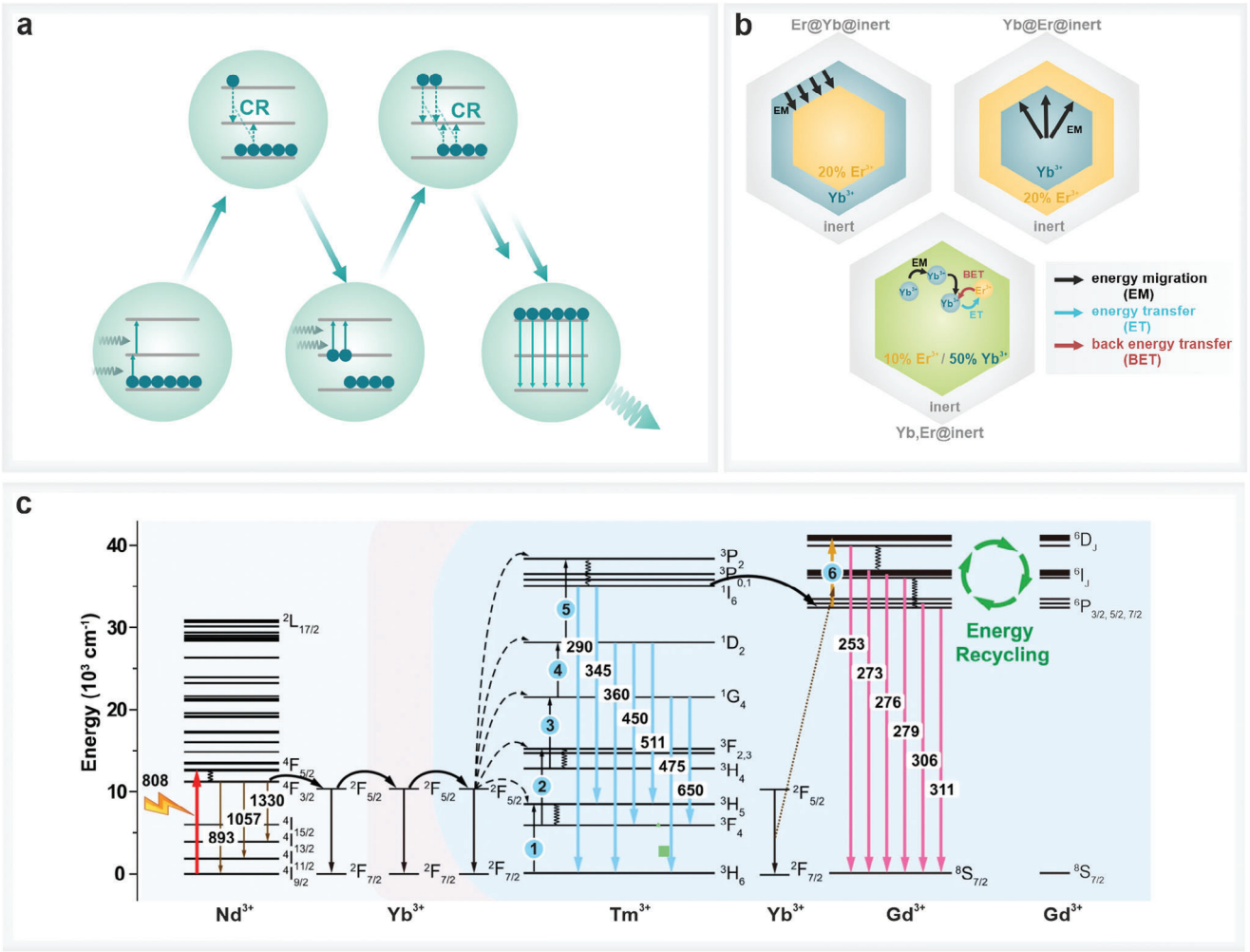

Figure 1. a) Mechanism of cross-relaxation-assisted photon avalanche. b) Energy migration pathways in core-shell approaches w/ or w/o segregation of sensitizer and activator ions. Adapted with permission.[28] Copyright 2022, Springer Nature. c) Energy level diagram of a multishell approach using particles of the type Gd,Yb,Tm@Yb@Gd,Yb,Nd@Gd. Adapted with permission.[29] Copyright 2021, Springer Nature.

certainly lies in complex particle architectures in which different lanthanides are combined but in different compartments of the particles. This can be achieved as described in the examples above, but even more complex structures can be imagined, for example by using anisotropic particles such as rods and platelets. Upconversion nanoparticles with a hexagonal crystal structure possess two different types of crystal facets, the hexagonal (001) and the rectangular (100) facet, which have different affinities for different ligands. In principle, this affinity can be used to achieve anisotropic growth, as Dayong Jin et al.[33] have already shown impressively (**Figure 2**a). This could allow even more complex combinations of lanthanide ions and morphologies. A major goal would be to achieve efficient multicolor emissions by codoping different lanthanides in different particle compartments. As a result, this could lead to different sensitivities of the respective elements to the environment, as exemplified in Figure 2b, where Tm^{3+} in the core provides a reference emission, while the Er^{3+}emission, generated closer to the surface, is sensitive to environmental changes, thus enabling self-referential nanosensors for intracellular applications. By growing Yb^{3+} anisotropically, high absorption and energy migration from outside to inside can be achieved without increasing the distance between Er^{3+} and the surroundings. Such an approach could improve applications that exploit energy transfer processes from the particle surface to ligands or analytes in solution. The additional codoping of Gd^{3+} not only enables energy trapping, but also creates multimodality as such particles also become accessible for magnetic resonance imaging. The second approach (Figure 2c) gives an example of how one could create a particle system that might be able to detect different analytes near the surface since the analytes that prefer the hexagonal face will see a stronger change of Er^{3+} emissions while binding to rectangular areas would alter Tm^{3+} signals.

3. Challenges in Surface Chemistry

The famous quote "God made the bulk; surfaces were invented by the devil",[34] which is attributed to the Nobel Prize winner Wolfgang Pauli, describes quite well in an exaggerated way what also applies to UCNPs: No matter how much you can optimize the luminescent properties of the nanoparticles, in the end, it is

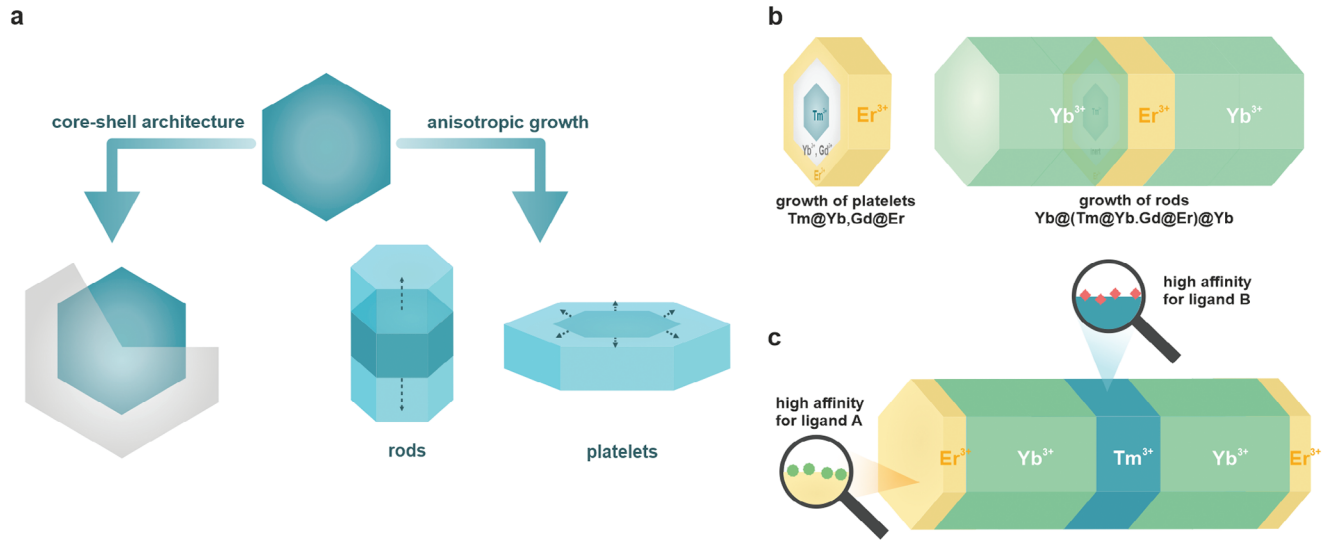

Figure 2. a) Strategies for particle design. b) Platelet growth (Tm@Yb,Gd@Er) followed by rod growth, where the platelets are prolonged by strongly absorbing $NaYbF_4$. The Er^{3+} is exposed to the environment and therefore accessible to analytes, while Tm^{3+} on the inside can be used for reference. c) Design of upconversion rods with Er^{3+} on the outer edges and Tm^{3+} in the center. Different sensitivities of different ligands to the hexagonal or rectangular surface could allow recognition of different ligands by Er^{3+}/Tm^{3+}.

the interfacial phenomena on the particle surface that determine their usefulness. This is precisely why many research groups are united by the question: How can one gain control over the surface and thus stabilize and functionalize the particles at the same time? Nanomaterials have an exceptionally high surface-to-volume ratio, which is great because this high surface area provides an excellent platform for functionalization. At the same time, however, it is also a major disadvantage as it entails a high surface-free energy. This means that literally, anything sticks to the surface of a nanoparticle that can lower that energy, or the nanoparticles stick together and form agglomerates.[35] Attempts are thus being made to stabilize the nanoparticles and prevent aggregation by attaching ligands to the particle surface in a targeted manner via electrostatic forces or steric repulsion.[36] The biggest hurdle in surface design is that, in addition to high stability, functionalization is also desired at the same time (**Figure 3**).

In a biological environment, the phenomena described at the particle surface apply in principle to all types of nanoparticles, and many findings can be transferred from one type to other types of nanoparticles. In the specific case of UCNPs, two phenomena occur that deserve special attention. On the one hand, the upconversion luminescence is strongly quenched by O-H vibrations, and on the other hand, the particles tend to dissolve in high dilution.[37,38] As a result, certain types of surface modification, some of which are very efficient, are less suitable for biological applications.

In particular, the most common, simplest, but also most vulnerable type of surface modification would be the method of ligand exchange. First, the original ligand (usually oleate) is removed from the UCNP surface by addition of $NOBF_4$ or HCl in a two-phase system of organic and aqueous solvent, and then the desired ligand molecule is offered for binding.[39,40] The method

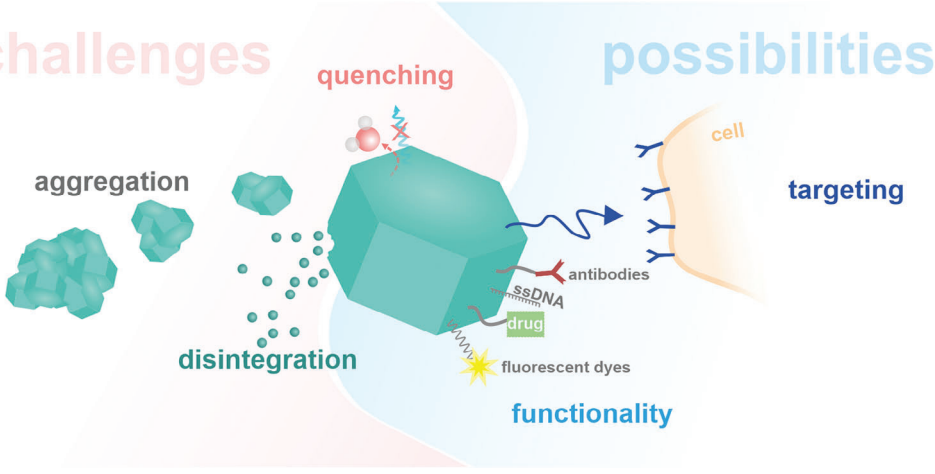

Figure 3. Challenges and possibilities in the surface design of upconversion nanoparticles.

is very popular because it is extremely versatile in terms of the selection of the attached ligands and can be carried out quickly without a large laboratory effort. In addition to the incomplete coverage of the particle surface—often only about 20% of the theoretical maximum coverage density is achieved[41]—the main problem with this method is that the ligands are bound to the nanoparticle surface according to their affinity. In the case of UCNPs, these are functional groups such as phosphate, sulfonate, or carboxylate, which are omnipresent in biological media as well. Consequently, depending on their affinity to the nanoparticle surface, an exchange of these groups is expected, and thus the properties of the particle will change over time.[42] The process of exchange can be slowed down by the addition of polymers instead of monodentate ligands since the probability that all polymer functional groups are exchanged at the same time is low.[43] However, multidentate ligands also harbor the risk of cross-linking individual particles thus promoting agglomeration. In addition, the already mentioned low degree of surface coverage does not represent a diffusion barrier for water molecules, which is why nanoparticles modified by such methods usually suffer greatly from luminescence quenching by water.[44]

In contrast, methods based on silica shell growth have the advantage that binding of the ligands to the silica surface is in most cases of covalent nature, enabled, for example, by the integration of (3-aminopropyl)triethoxysilane into the silica shell, which means that the ligands cannot be easily replaced.[45,46] However, in silica-coated UCNPs the distance between the particle and the ligand is greatly increased, which can be disadvantageous for applications including Förster resonance energy transfer (FRET). Furthermore, it deserves special experimental skills to synthesize silica-coated UCNPs free of aggregates or to have them later in monodisperse distribution through purification steps.

A very promising alternative strategy is the ligand addition method, in which the hydrophobic oleate remains on the particle surface and an amphiphilic ligand is attached to it. The resulting hydrophobic layer prevents hydrophilic molecules from diffusing to the particle surface, thereby reducing the likelihood of exchange, dissolution, and luminescence quenching. Various strategies have been developed, using amphiphilic polymers, phospholipids, or more simply, just oleate molecules to form this bilayer.[47–52]

In addition to colloidal stability, the chemical stability of nanoparticles is also an extremely important issue, which is often somewhat neglected in the excitement of the outstanding properties of the nanoparticle itself. Most inorganic nanoparticles cannot be regarded as inert systems but are in constant equilibrium with their environment and tend to disintegrate. This alters the photophysical properties of the systems on the one hand and makes the toxicity of the individual components relevant on the other. In the case of UCNPs, fluoride carries the risk of toxic effects because it can interact with many proteins, induce oxidative stress and tissue damage, and liberate free radicals.[53] However, rare earth ions also have toxic effects.[54] The formation of lanthanide phosphates is strongly thermodynamically favored, which is why the lanthanide ions extract phosphate from the cells, for example, from lipid membranes, leading to organelle damage, or inflammatory processes.[55] Since toxicity is always highly dependent on the concentration of the specific agent, it is of utmost importance to know about the chemical stability of the used nanomaterials before using them for biological applications. For UCNPs, long-term studies revealed that partial release of the ions occurs most rapidly within the first three days, with a fluoride ion release rate of about 0.12 mol% h^{-1} in phosphate buffered saline (pH7.4).[38] This rate can even be accelerated by increasing the temperature or decreasing the particle size. For applications in living organisms, it would be desirable to prevent the dissolution of the nanoparticles to minimize the toxic effects of their components. Lisjak et al.[56] have shown that an amphiphilic surface coating drastically reduces the disintegration rate by a factor of eight. This again emphasizes the importance of carefully designing the nanoparticle surface and, if possible, incorporating hydrophobic barriers to increase the colloidal and chemical stability of the nanoparticles.

Although surface modification is crucial for any application of nanomaterials, what actually happens at the surface of a nanoparticle still is largely an enigma. This is mainly due to the difficulty of molecularly characterizing the interfaces of nanoscale objects with high local precision and at the same time across an entire particle ensemble. Light scattering experiments or nanoparticle tracking analysis can demonstrate whether monodispersity can be maintained, while zeta potential measurements are commonly used to determine surface charge changes; but in most cases, the characterization of the particle surface ends here. There are, of course, countless methods available to determine the exact composition of ligands on the surface. Methods such as thermal gravimetric analysis or quantitative nuclear magnetic resonance can determine the total amount of bound ligands, while colorimetric assays are used, for example, to quantify the available functional groups.[57–61] However, these methods are not very widely used for the characterization of nanoparticles, which is probably due to the large variety of ligands that differ in the method of quantification and the lack of standardized and validated protocols and techniques.[61] There is definitely an urgent need for further development in this field of analytical chemistry. Critics of this thesis may object that it is debatable whether it is negligent not to look in detail at the composition of the surface, or whether one must assume that the composition at the particle interface changes significantly as soon as particles enter an organism and one can therefore save oneself the trouble. As proof of this, it can be provided that in the first 30 s after a nanoparticle enters an organism, it is surrounded by a biomolecular corona.[62] This phenomenon is extremely frustrating for nanoparticle researchers as it reduces control over the chemical processes on the particle surface. Imagining an ideal nanoparticle for bioapplications, one would likely create a multifunctional nanoplatform that not only has therapeutic functions enabling, for example, PDT or optogenetics, but also contains a targeting entity.[63–67] A protein corona significantly limits functionality, especially with respect to targeting. This is confirmed by a comparative study of the targeting efficiencies achieved to date. It was discovered with disillusionment that only about 0.7% of the nanoparticles reach the target tissue.[68] In general, the response of an organism to nanoparticles is strongly influenced by the formation of the protein corona, since the corona is first recognized by the cells, and hence the inherent particle plays a rather minor role from this point on.[69] Therefore, the protein corona is crucial for parameters such as blood circulation time, cytotoxicity, or biodistribution. On the one hand, the production

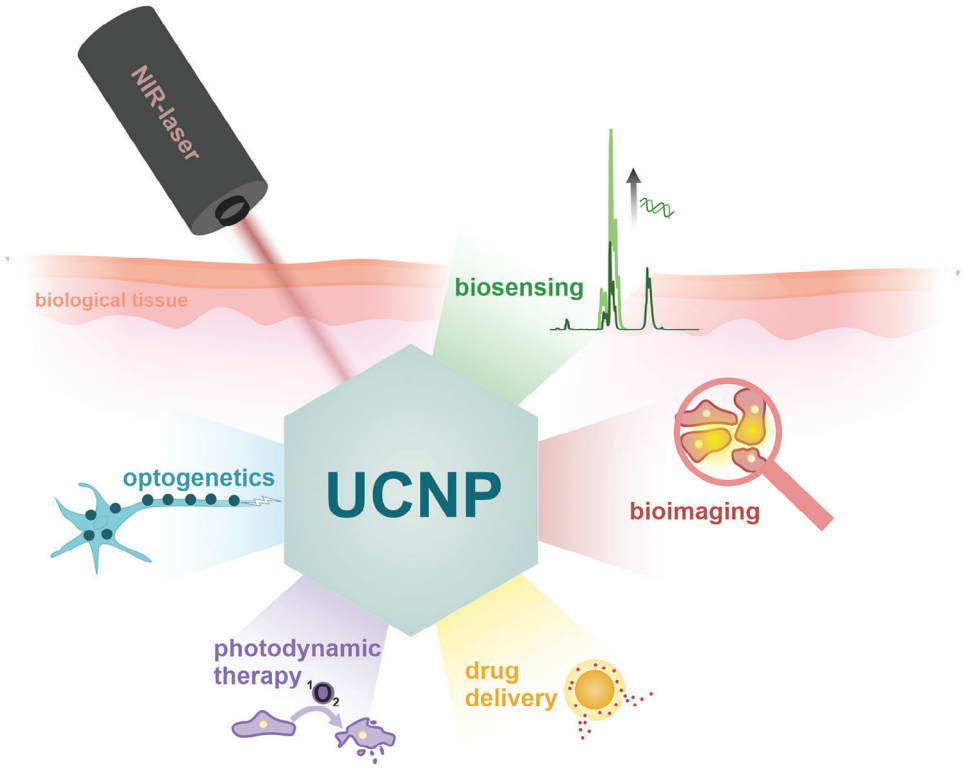

Figure 4. Possible applications for UCNPs in bioanalytical and biomedical sciences.

of reactive oxygen species (ROS) and thus the cytotoxicity can be reduced, on the other hand, the blood flow time is influenced by the absorption of macrophage cells, and thus the removal from the body can be increased, or reduced depending on the adsorbed proteins.[70]

Although the formation of the protein corona appears to be an undesirable phenomenon at first glance, it can be of great benefit. In addition to reduced cytotoxicity, a protein corona can also help internalize nanoparticles into cells.[62,70,71] In addition, the dissolution of nanoparticles is slowed down when the particle is protected by proteins.[72] However, it was found that the formation of the protein corona is highly dependent on the ligands used. A high surface charge density favors the attachment of proteins to the particle surface,[73] while long-chain polymers such as polyethylene glycol (PEG) lead to reduced protein corona formation due to the formation of a hydrophilic protective layer.[74,75] Since this, in turn, leads to lower cell uptake, it is a difficult task to find the optimal balance between the desired and undesired consequences of a protein corona.[62,70,76] It is clear, that the characterization of the surface of UCNPs before and after entering complex biological processes in terms of their functionality, colloidal stability, and media toxicity should become more of a focus in the coming years of research. This will also help to make the processes inside an organism more predictable and further improve the design of the particle surface.

4. Potential in Bioapplications

For many biomedical or bioanalytical questions that are based on optical probes, UCNPs represent an attractive way to solve them. Due to the NIR-excitation, they are particularly impressive where autofluorescence and low penetration depths of the excitation light have a limiting effect (**Figure 4**).

In sensor applications, NIR-excitation benefits above all from the high signal-to-noise ratio. The recent reviews by Chen et al.[77] and de Camargo et al.[78] summarize the wide variety of possible sensor applications based on UCNPs, ranging from parameters like temperature or pH to the detection of inorganic ions or biomolecules (nucleic acids, proteins, etc.). Such sensors usually use the change in emission band intensities of the respective upconversion emission bands. Considering another advantageous feature, the several spectrally well-separated emission bands of UCNPs, another advantageous feature, one clearly might envision ratiometric sensing applications. This is especially important when intracellular sensing is desired where calibration is not possible and self-referencing is the solution. When designing such a sensor particle, it is important to understand in detail the energetic processes between the lanthanide ions. Considering Er^{3+}-activated UCNPs, the green (≈525/545 nm) and the red emission (≈650 nm) are usually used for sensing purposes. However, due to the multiphoton process, the intensities of the individual bands depend on the power density of the excitation light. This represents a major challenge, especially in tissue, where light scattering is practically unavoidable and hence the excitation power density becomes an unknown quantity, which can fluctuate locally. The preferred area of use for sensing applications is therefore more in cell culture rather than in vivo. Furthermore, considering that within one kind of lanthanide ion, the energy levels are related to each other to a certain extent, it becomes clear that sensor applications of UCNPs pose

several challenges. Considering Er^{3+}-doped systems, two excitation paths of the red-emitting level are based on the green-emitting levels. In specific terms, this means that the red emission would also lose intensity if the green-emitting energy level gets depopulated by an energy transfer.[79] The more complex core–multishell-particles with compartmentation of different lanthanide ions as presented earlier in this article offer a possible way out of the dilemma. The benefit of having two different types of activator ions, such as Er^{3+} and Tm^{3+}, which do not interact when they are spatially separated was recently demonstrated by Zhou et al.[80] Here, the intensity ratio of 450 nm (Tm^{3+})/540 nm (Er^{3+}) of multishell nanoparticles of the type Yb,Er,Ce@inert@Yb,Tm could be used for thermometry with a sensitivity of almost 10% signal change in the physiological range. This impressive result was achieved for particles dried to powder. Thus, to apply this method under biologically relevant conditions, a precise surface design is required. However, the idea of compartmentalization is obviously beneficial, as is the introduction of anisotropic structures, as exemplified in Figure 2a, where perhaps even different ligand affinities could be achieved on a particle.

If the scattering and the absorption of the excitation light lead to problems, then of course this also applies to the emitted light, which cannot cross the tissue undisturbed either. Since this becomes a particular issue for shorter wavelength emissions, this could also lead to changes in peak ratios and therefore be an issue for ratiometric sensing. This does not mean that UCNPs are unsuitable for in vivo applications, as one way to circumvent this disadvantage is to look at luminescence lifetimes instead of luminescence intensities. Zhang et al. developed a FRET-based tumor detection sensor that provides information about changes in the lifetime of Nd^{3+}-doped UCNPs via the marker molecule peroxynitrite, which affects the spectral properties of a FRET-acceptor dye bound to the particle surface. This method impresses with its extremely stable lifetime signals, which are unaffected by scattering effects or light absorption.[81]

Similar considerations apply to imaging, where photoluminescence lifetime imaging has proven to be an efficient tool, especially for multiplexed approaches that typically use different emission wavelengths to label different regions. Instead, using nanoparticles with the same wavelength but different luminescence lifetimes enables bioimaging with high sensitivity and accuracy.[82,83] In addition to upconversion, bioimaging also offers the charm of exploiting the Stokes-shifted NIR emissions. The so-called NIR-II range (1000–1700 nm) enables almost background-free imaging due to the greatly reduced scattering and autofluorescence in this range.[84] It has also been shown several times that imaging in this range has clear advantages compared to imaging visible emissions from lanthanides.[85] Probably the most commonly used system for NIR-II imaging are nanoparticles doped with Yb^{3+}/Nd^{3+} sensitizers and Er^{3+} activators, sometimes additionally codoped with Ce^{3+}, as reported by Dai et al.[86] An approximately nine-fold enhancement of the 1550 nm emission was obtained when 2% Ce^{3+} was doped into the nanocrystal. A disadvantage of the Er^{3+} emission, although it is one of the brightest, is its quenching by O-H vibrations of water.[86] Recently, Kong et al. proposed a completely new concept for NIR-II imaging using $LiTmF_4$(0.2%Er)@$LiYF_4$ nanoparticles that can be excited at 800 or 1208 nm and show emissions between 1600 and 1900 nm. These can be traced back to the $^3F_4 \rightarrow {}^3F_6$ transitions and have never been reported for imaging purposes before. This example illustrates the potential of a carefully tailored particle design with exceptional doping. According to Kong et al.[87] a high Tm^{3+} content in the core particle, in combination with thick inert shells (5 nm), leads to bright emissions with a quantum yield of 14% for about 25 nm sized particles, which can even be increased to 16% by adding small amounts of Er^{3+} (0.2%) in the core.

Such examples demonstrate the potential of the great variety of UCNPs. While the upconversion part of the spectrum can be used for sensors or actuators, the Stokes-emission in the NIR-II range seems to be very promising for imaging processes due to the high penetration depth. Upconversion is difficult to work with in tissues, at least when the goal is to quantify the emitted upconversion signals. However, detecting the upconversion signals is not always the goal. Perhaps the greatest not fully utilized potential of upconversion in bioapplications so far is to use them as signal converters in organisms, locally converting low-energy light into high-energy light. The high-energy visible or UV light can be used for therapeutic purposes by initiating certain desired photochemical reactions. The major classes of such therapeutic applications are drug delivery, PDT, and optogenetics.

As an example of the use of UCNPs for drug delivery, see the work of Wu et al. who succeeded in delivering drugs locally with UCNPs which are coated with mesoporous silica. The anticancer drug doxorubicin has been incorporated into the mesoporous silica, while a blue-light-sensitive ruthenium complex acts as a molecular valve. Under NIR-irradiation, the Tm^{3+} ions in the UCNP excite the ruthenium complex and release the anticancer drug.[88] Another smart approach encapsulates UCNPs and doxorubicin together in a light-sensitive disulfide-linked polymersome, which gets dissolved by the NIR-triggered upconversion luminescence and thus releases the active ingredient.[89]

PDT uses a related but different concept to treat diseases such as cancer. The original principle is based on the local excitation of a photosensitizer (PS) into the excited singlet state, from which, in addition to internal conversion and fluorescence, intersystem crossing into a triplet state also takes place. This triplet state can interact with a substrate and generate radicals and oxygenated products (Type I) or react directly with oxygen and generate $^1O_2$ (Type II). These highly reactive compounds can kill the surrounding cells, which is why the principle is very attractive for tumor therapy. A major problem with this type of light therapy is that the PS used can be excited in the UV and visible range, which in turn means that noninvasive application is hardly possible due to the low penetration depth of the light.[90] For this reason, the combination of UCNPs with PS is extremely attractive, since the principle functionality (**Figure 5**a) has already been proven in several in vitro and in vivo studies.[91] An example of such an approach is the recent study presented by Kim et al.[92], in which two different types of PS were attached to the PEG-coated surface of a nanoparticle together with the tumor-targeting agent folic acid. (Figure 5b). This approach impressively demonstrates the importance of surface design for such applications, as the challenge is to combine ligands that ensure colloidal stability, ligands that enable therapeutic activity, and ligands for targeting. A major limitation of PDT is that tumor tissue is often hypoxic, which limits the

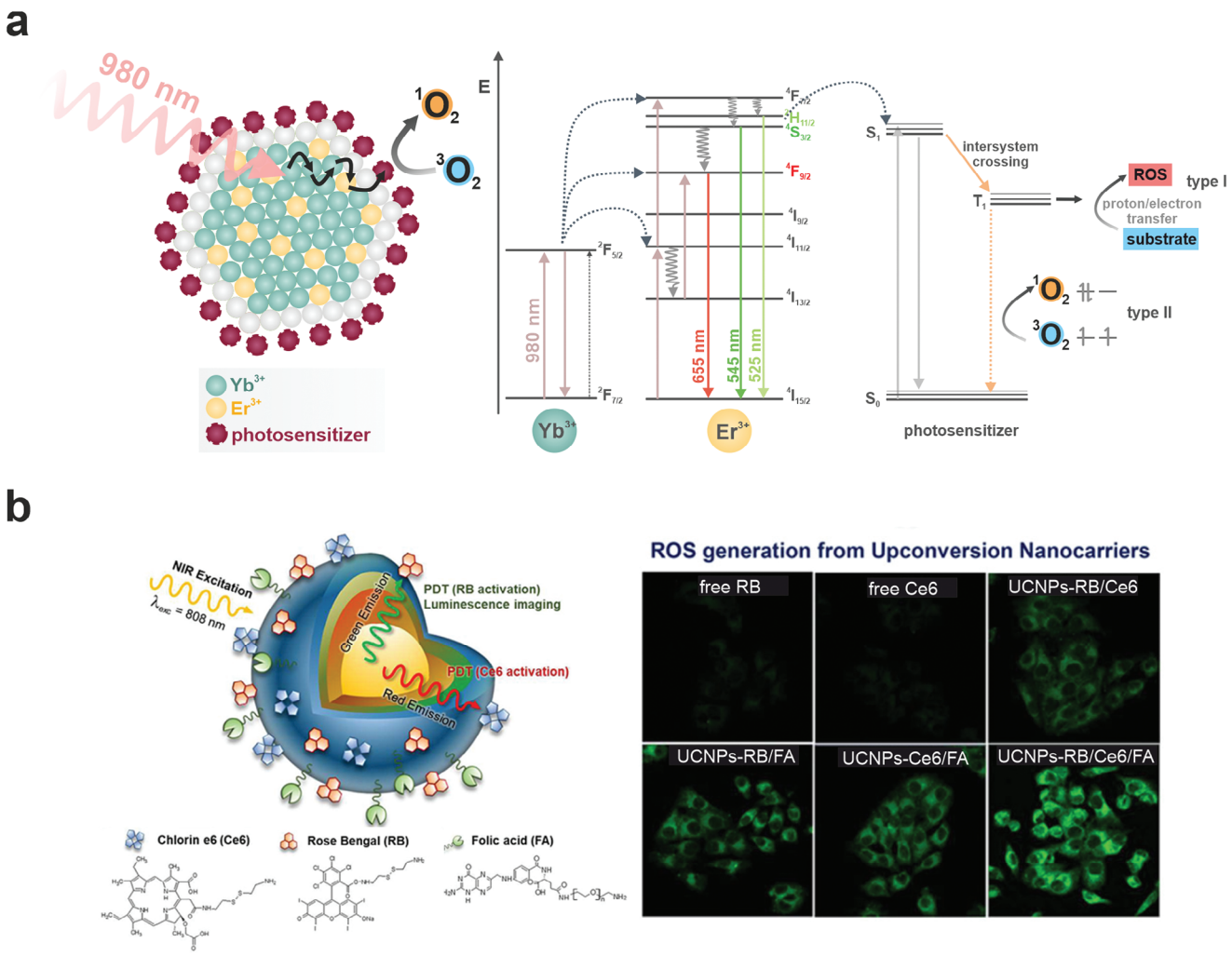

Figure 5. a) Mechanism of photodynamic therapy involving UCNPs shown schematically (left) and in a simplified energy level diagram (right). The mechanism is shown for Yb^{3+}/Er^{3+}-doped UCNPs and a PS absorbing in the green range. b) PDT approach by Kim et al.,[92] with two kinds of PS attached to a multishell UCNP. Schematic drawing of the particle (left) and ROS generation visualized with the ROS indicator DCFH-DA in KB cells incubated with the free photosensitizers, UCNPs with one or two PS attached and UCNPs w/ and w/o attached folic acid, which acts as tumor targeting agent. Adapted with permission.[92] Copyright 2023, Elsevier.

production of ROS and thus the efficiency of the therapeutic approach. To solve this problem, several ideas have already been proposed, such as by Shi et al., who coupled cyanobacteria to UCNPs that produce $^3O_2$ in situ, which is directly converted to $^1O_2$ by an upconversion-PS combination.[93] Another recent approach attaches the PS Chlorin-e6 together with CeO_2 to the surface of UCNPs. The CeO_2 can decompose H_2O_2 generated from a tumor to $^3O_2$, which is converted to $^1O_2$ by the PS.[94]

A major opportunity to improve the therapeutic effect of upconversion systems is to focus on developing multimodal approaches. To give an example, one could imagine an upconversion nanocrystal doped with two activator ions, Er^{3+} and Tm^{3+}, and two surface functionalities, a drug-release system triggered by Tm^{3+} emissions and a PS that is activated by Er^{3+} emissions combined with two different types of sensitizers (Yb^{3+} and Nd^{3+}). Such a PS could be Rose Bengal (green absorbing) or Chlorin-e6 (red absorbing), or ideally a combination of them to exploit both Er^{3+} emissions. With a well-designed core–multishell structure, which separates both sensitizer-activator pairs by an inert layer, it should then be possible to switch between PDT and drug delivery by changing the excitation wavelength.

The final application of UCNPs for biomedical purposes that shall be discussed here is the use of UCNPs for optogenetics. In optogenetics, light is used to control neurons or other cell types. This is done by introducing foreign genes into the target cell, resulting in the expression of light-sensitive ion channels. The channels can be opened or closed under irradiation with light, generating specific signals (**Figure 6**a). Since the activation in most cases works with visible light, which has low penetration depths, optical fibers are typically inserted into the brain. Therefore, the idea came up to use UCNPs for this method, allowing non-invasive excitation via NIR-light.[95]

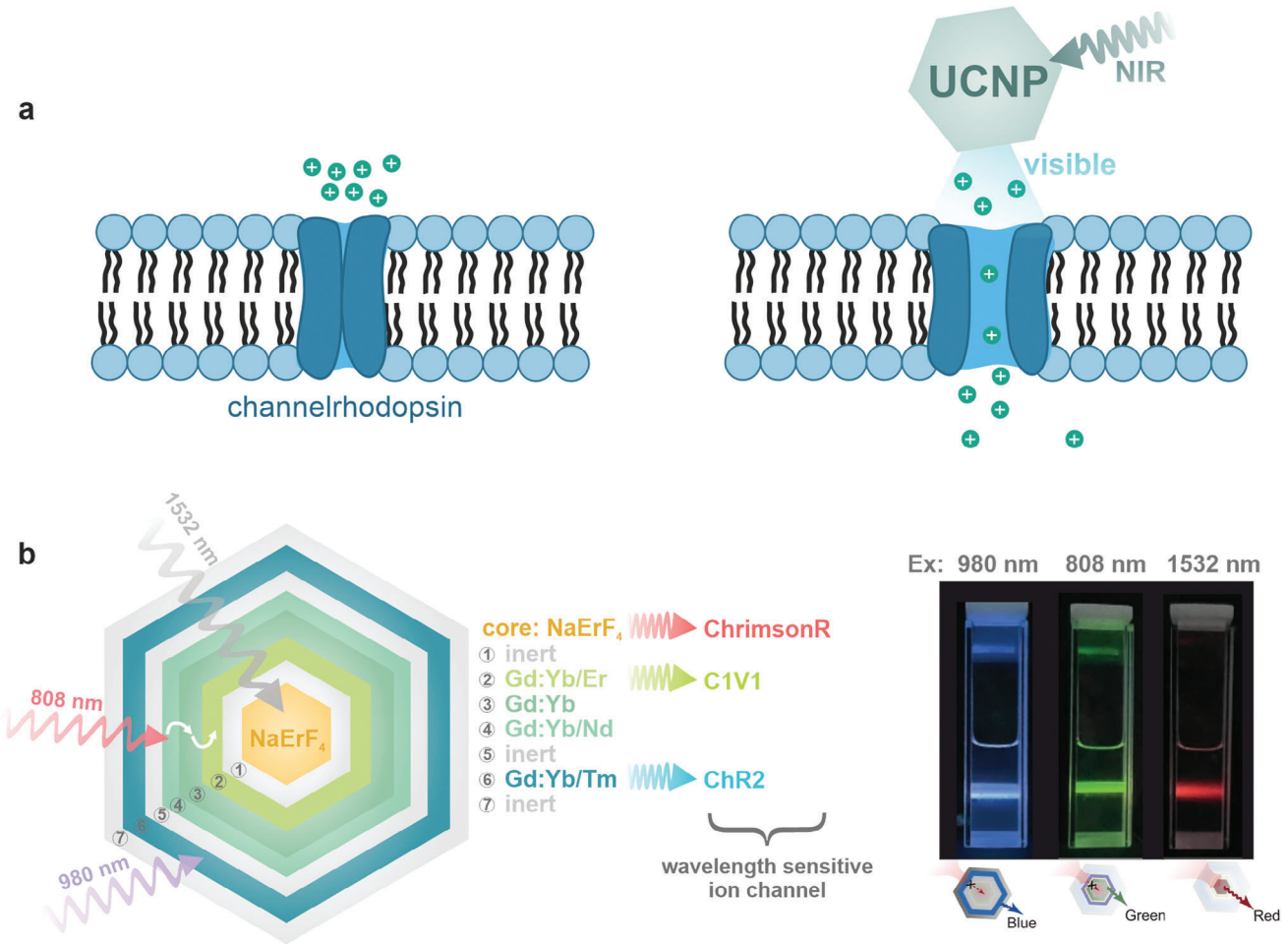

Figure 6. a) Principle of UCNPs in optogenetics. Light sensitive ion channels (channelrhodopsins) open upon NIR-irradiation due to the upconversion of the NIR-light into visible light. b) Particle design of a trichromatic upconversion nanoparticle suitable for optogenetic applications. Depending on the irradiation wavelength blue, green or red light is created which interacts with different kind of channelrhodopsins (ChR2, C1V1, ChrimsonR). Adapted with permission.[96] Copyright 2021, Springer Nature.

With optogenetic approaches using UCNPs, it was already possible to achieve locomotion control in C. elegans, which changed their direction under 980 nm irradiation[97] or manipulation of the food intake behavior of mice, as recently demonstrated by Zhong et al.[98]

Zhang et al. presented a strategy that is elegant in both, the particle architecture as well as their application to neural manipulations (Figure 6b). They fabricated UCNPs with a total of seven shells, resulting in particles that are excitable at three different wavelengths (1532/808/980 nm) and exhibit trichromatic green, red, and blue emissions. Combined with the expression of three different light-sensitive channel proteins (ChR2, ChrimsonR, and C1V1) that perfectly match the emission wavelengths, they were able to precisely control different types of ion channels. Manipulation of the locomotion behavior of mice was achieved since the average running distance of the mice was increased under irradiation with 808 or 1532 nm, while it was decreased under irradiation with 980 nm.[96]

Investigating the influences of ion channel manipulation on various behaviors will help neuroscientists gain a deeper understanding of the detailed mechanisms in the brain and will contribute to a better understanding of neuronal diseases. With optogenetics, it may also be possible to switch regions in the brain on or off, correcting aberrant neuronal signals that cause Parkinson's disease or epilepsy, for example. To achieve therapeutic relevance for humans, the use of UCNPs in optogenetics still must go through a long road of optimization. In mice, whose brains are only a small fraction of the size of the human brain, the NIR light can reach all parts and stimulate the neurons. In the human brain, the light would have to cover far greater distances, while at the same time, the excitation power densities of the laser light are limited since the heating would cause tissue damage. Therefore, further optimization is required, for example, by implementing the multishell approaches described above. This could lead to improved upconversion efficiencies, together with a shift in excitation wavelengths to ranges—ideally above 1000 nm—where water absorption does not occur and scattering is reduced even more in comparison to the widely used 980 nm excitation. One should also pay special attention to surface engineering in optogenetics applications since an additional challenge appears here: To

get close to the neurons, the blood-brain barrier, which protects the brain from pathogens and toxins, must be conquered. Surface design is important for the process of transcytosis since, for example, positive ligands or peptides, which can bind to specific receptors on the membrane, can help to cross the barrier.[99,100] This once again emphasizes how important the entire interplay of particle architecture and surface design is and that neither of the two areas should be neglected. However, it also becomes clear that one has different specific requirements for each application, to which one must precisely adapt the design to achieve optimal results in the end.

5. Outlook

The recently developed strategies together with the latest examples in the development of UCNPs demonstrate the outstanding potential of such probes in bioapplications. The bottom lines for further research are identified as follows:

(a) Design and synthesis of complex particle architecture: The spatial confinement of different doping within one particle gives rise to surprising features and better efficiencies, caused by energy migration and energy trapping zones. The possibilities in multishell architectures in combination with the large variety of lanthanide ions are not fully explored yet, especially when thinking of anisotropic particle shapes such as rods or platelets. One challenge besides small and efficient particle design is still to characterize and maintain the stability of the individual compartments during multishell growth at high temperatures.

(b) Thorough characterization of the particles' surface and controlled surface engineering: The progress, which was made in designing the surface coating of nanoparticles is enormous. However, the knowledge regarding the exact events happening on the particle surface is relatively low, especially as soon as the particle enters complex matrices. To better estimate whether and to what extent the function of the particle is preserved and how great the stability and toxicity of the particle are, precise surface characterization methods regarding composition, surface coverage, and density need to be established. With this, the next level can be entered by controlling a complex particle surface consisting of targeting moieties, reporter molecules, building blocks providing colloidal stability, or reservoirs capable of hosting drugs.

(c) Better linkage of the individual disciplines in UCNP development: So far, standard particle compositions and architectures have often been used for smart applications without particular attention to surface chemistry, while outstanding particle designs are often characterized only outside of a biological system. The highly complex field of UCNP research, which demands expertise in so many disciplines, could be much more prospective with better collaboration among materials chemists, photophysicists, and medical scientists.

(d) Establishment of standards: To speed up the progress in particle development, especially when one thinks of the almost countless possibilities of multishell approaches it would be very desirable to be able to easily compare the findings of individual researchers. A great benefit would be to have a minimal set of standards when reporting on luminescence features, surface composition, colloidal stability, or toxicity.

It is expected that this fascinating field of UCNP research will continue to grow and that topics like multimodality by generating hybrid composite particles will get into focus, with lots of further room for experimentation and improvement.

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Conflict of Interest

The authors declare no conflict of interest.

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