Upconversion Nanoparticle-Mediated Optogenetics

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Abstract

Upconversion nanoparticle-mediated optogenetics enables remote delivery of upconverted visible light from a near-infrared light source to targeted neurons or areas, with the precision of a pulse of laser light in vivo for effective deep-tissue neuromodulation. Compared to conventional optogenetic tools, upconversion nanoparticle-based optogenetic techniques are less invasive and cause reduced inflammation with minimal levels of tissue damage. In addition to the optical stimulation, this design offers simultaneously temperature recording in proximity to the stimulated area. This chapter strives to provide life science researchers with an introduction to upconversion optogenetics, starting from the fundamental concept of photon upconversion and nanoparticle fabrication to the current state-of-the-art of surface engineering and device integration for minimally invasive neuromodulation.

Keywords

Upconversion nanoparticle · Near-infrared · Remote control · Opsin · Optogenetics · Neuromodulation

Abbreviations

Ca^{2+}	Calcium
Dy^{3+}	Dysprosium
Er^{3+}	Erbium
Eu^{3+}	Europium
Gd^{3+}	Gadolinium
Ho^{3+}	Holmium
Lu^{3+}	Lutetium
Mn^{2+}	Manganese
NIR	Near-infrared
Sm^{3+}	Samarium
Tb^{3+}	Terbium
Tm^{3+}	Thulium
Y^{3+}	Yttrium
Yb^{3+}	Ytterbium

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44.1 Introduction

Optogenetics is an advanced optical approach for targeted, spatiotemporal modulation of specific neural functions through dynamic manipulation of light-sensitive opsin proteins in the plasma membrane of neurons. In contrast to conventionally pharmacological, genetic, electrical, and magnetic strategies (Kramer et al. 2013; Miller 1989; Zamponi 2016; Kringelbach et al. 2007), optogenetic tools take advantages of light modulation with fast "on–off" kinetics, tunable excitation and emission wavelengths, and precise delivery of stimulatory light to targeted regions. This approach has made it possible to enable spontaneous modulation of depolarization and hyperpolarization of neurons at an even single-cell level. In this context, light-sensitive opsin proteins play a core role in mediating cellular response through external light excitation. When triggered by light at a specific wavelength, such proteins undergo conformational changes that affect an influx or efflux of cations or anions through the ion channels of the cell membrane. The current generated by the cation flux depolarizes the membrane of the neuron, resulting in the activation of cellular signaling pathways. The neuron fires an action potential that propagates to adjacent neurons. Optogenetics provides a powerful toolbox to investigate cellular biology, neuroscience, and behavior correlation at a wide range of spatiotemporal scales (Fenno et al. 2011; Deisseroth 2011).

The evolving field of optogenetics is mainly attributed to the discovery of various opsin variants (e.g., channelrhodopsins and halorhodopsins) combined with the implementation of different light sources (e.g., lasers and light-emitting diodes). Action potential stimulation was first demonstrated in Aplysia ganglia neurons using a blue laser (Fork 1971). A milestone in optogenetics was the manipulation of mammalian neurons, which was achieved with the use of Channelrhodopsin-2 (ChR2) (Boyden et al. 2005; Ishizuka et al. 2006). Despite numerous successful efforts in vitro, effective stimulation of targeted neurons in vivo remains a formidable challenge because current opsins only respond to visible light usually at 430–630 nm, a spectral range where living tissues show strong absorption and scattering. It is difficult to directly deliver visible light through layers of tissues to achieve efficient, targeted neuromodulation. To address this issue, current optogenetic approaches generally adopt optical fiber insertion or implantation of microchips with light-emitting diodes to deliver visible light into targeted areas in deep tissues and organs (Cardin et al. 2010; McAlinden et al. 2015). However, these invasive procedures inevitably induce side effects and damages such as inflammation and limit free movement of the animal under study, thereby hindering their practical use in long-term and behavioral studies.

Two possible strategies could be carried out to overcome the limitations imposed by conventional techniques. One is to develop new types of opsin proteins that can respond to near-infrared (NIR) light capable of deep-tissue penetration. Although channelrhodopsins with red-shifted wavelength responses have been exploited, their stimulating wavelengths are still confined within the visible range (Zhang et al. 2008; Urmann et al. 2017). The other possible strategy is to deliver NIR light to the targeted area and simultaneously convert to visible light in situ utilizing certain converters. In 2010, Deisseroth et al. put forth an innovative concept that realizes NIR-to-visible conversion in vivo with the mediation of upconverting nanoparticles (Deisseroth and Anikeeva 2016). From then onward, luminescent nanomaterials, especially upconversion nanoparticle-mediated optogenetics has witnessed tremendous research efforts and progress (All et al. 2019; Wang et al. 2019; Yu et al. 2019).

Upconversion nanoparticles can serve as powerful nano-antenna absorbing NIR light and emitting visible light ranging from ultraviolet to NIR regions, bridging the spectral gap between established opsin proteins and NIR light source excitation. Importantly, upconversion nanoparticle-mediated optogenetics holds the unique advantages of wireless control, minimal invasion, and negligible cytotoxicity in vivo

(Wang et al. 2017a, b; Chen et al. 2018; Chen 2019). Therefore, this could become a general trend in the customization of upconversion nanoparticles for specific research purposes in neuromodulation and optogenetics. Mastering basic knowledge of optical mechanisms, chemical engineering, and properties optimization is necessary to accommodate the expansion of the upconversion nanoparticle-based optogenetic tool. In this regard, we will focus on introducing basic mechanisms, synthesis methods, surface modifications, and excitation/emission optimization strategies of upconversion nanoprobes, as well as presenting recent progress in upconversion nanoparticle-mediated optogenetics.

44.2 Background Information on Upconversion Nanomaterials

44.2.1 Upconversion Mechanism

The concept of upconversion was established in the 1950s by Nicolas Bloembergen (Bloembergen 1959). In recent decades, upconversion nanomaterials have been theoretically and experimentally investigated because of their potential applications in optics, display, security, storage, nanomedicine, and neuroscience. In brief, upconversion nanomaterials consist of a host crystal and lanthanide dopants capable of absorbing two or more low-energy photons and converting them into one photon with higher energy. As shown in Fig. 44.1, upconverting processes can be classically categorized into five types: (a) excited-state absorption, (b) energy transfer upconversion, (c) photon avalanche, (d) cooperative sensitization, and (e) energy migration-mediated upconversion (Auzel 2004; Wang et al. 2011a, b).

Energy transfer upconversion, as the most efficient and adopted upconversion process, requires a sensitizer for photon absorption and an activator for photon emission. This requirement can be met with specific lanthanide pairs such as ytterbium/erbium, ytterbium/thulium, and ytterbium/holmium (Yb^{3+}/Er^{3+}, Yb^{3+}/Tm^{3+}, and Yb^{3+}/Ho^{3+}, Wang and Liu 2009). Various emission colors can be easily achieved by tuning the types of lanthanide dopants and their concentrations in the host lattice. Energy transfer efficiency is sensitive to doping concentration. The tradeoff between energy loss through cross-relaxation effects and the probability of energy transfer determines the critical doping concentration for maximum energy transfer efficiency. Energy migration-mediated upconversion is an attractive approach to realize photon upconversion through other types of lanthanide activators (dysprosium Dy^{3+}, terbium Tb^{3+}, europium Eu^{3+}, samarium Sm^{3+}, etc.) and even transition metal activators (manganese Mn^{2+}). Through the use of a multiple core-shell nanostructure design, the energy migrators (typically gadolinium Gd^{3+} ions) can be controlled to absorb photons from Yb^{3+}/Tm^{3+} couples and then migrate the excitation energy to activators over a large distance (Wang et al. 2011a, b; Han et al. 2016; Liu et al. 2017). The energy migration strategy dramatically improves the upconversion efficiency of these activators with ladder-like intermediate energy states through spatial confinement of lanthanide ions.

The characteristics of photon upconversion nanoparticles include large anti-Stokes shift, narrow emission peaks, full-spectral tunable emission, stable physicochemical and optical properties, good biocompatibility, and programmable surface functionality. Upconversion nanoparticles are considered as alternative probes to organic dyes and quantum dots and hold promise for applications in biosensing, deep-tissue imaging, disease diagnosis, and therapy as well as in neuroscience.

44.2.2 Synthesis Methods

Size, morphology, and surface functional group of nanoparticles affect their physicochemical properties such as dispersity and stability in physiological conditions and their in vivo behaviors such as interactions with cells, circulation time, biodistribution, and biocompatibility (Wang et al. 2012; Gnach et al. 2015). Therefore, precise

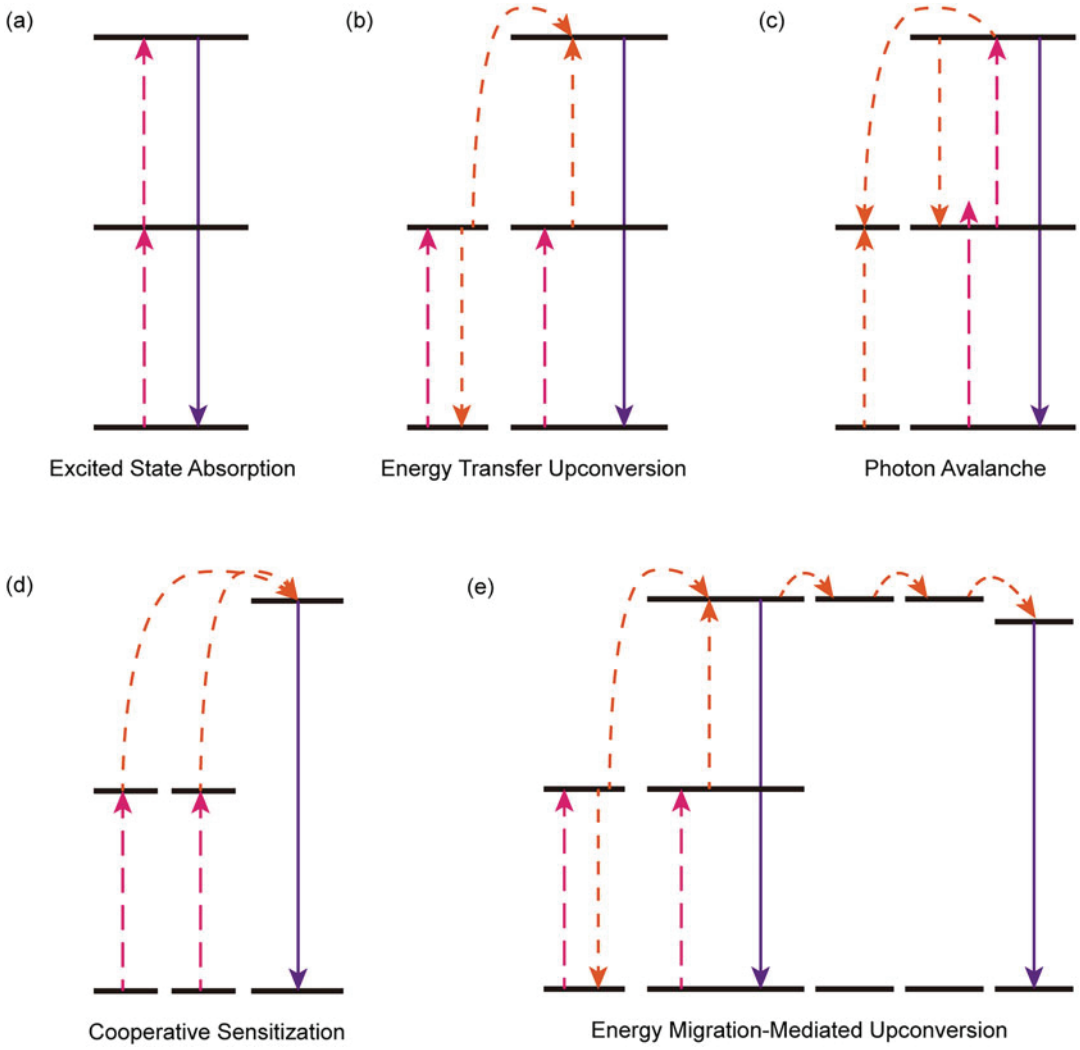

Fig. 44.1 Schematic illustration of typical upconversion processes, where dashed arrows in the red stand for direct excitation processes, dashed arrows in orange represent energy transfer processes and full arrows in purple show radiative emission processes

control of these factors in fabrication and post-treatment of upconversion nanoparticles is crucial to their further bio-applications, particularly in optogenetics. It is worth mentioning that current chemical synthesis methods mainly contain thermal decomposition, coprecipitation, and hydrothermal reactions (Fig. 44.2).

Thermal decomposition is the most effective method to fabricate ultrasmall upconversion nanocrystals, even sub-10 nm in diameter. This method takes advantage of an oxygen-free reaction where organic precursors are decomposed in boiling organic solvents at high temperatures with the assistance of surfactants. The first demonstration of this method was the synthesis of high-quality LaF_3 nanocrystals (Zhang et al. 2005). In the work, one-step, mass production of single-crystalline and monodisperse LaF_3 triangular nanoplates (2.0 nm in thickness) was realized via thermolysis of lanthanum trifluoroacetate in a hot oleic acid/octadecene solution. In 2007, Yi and Chow applied hot

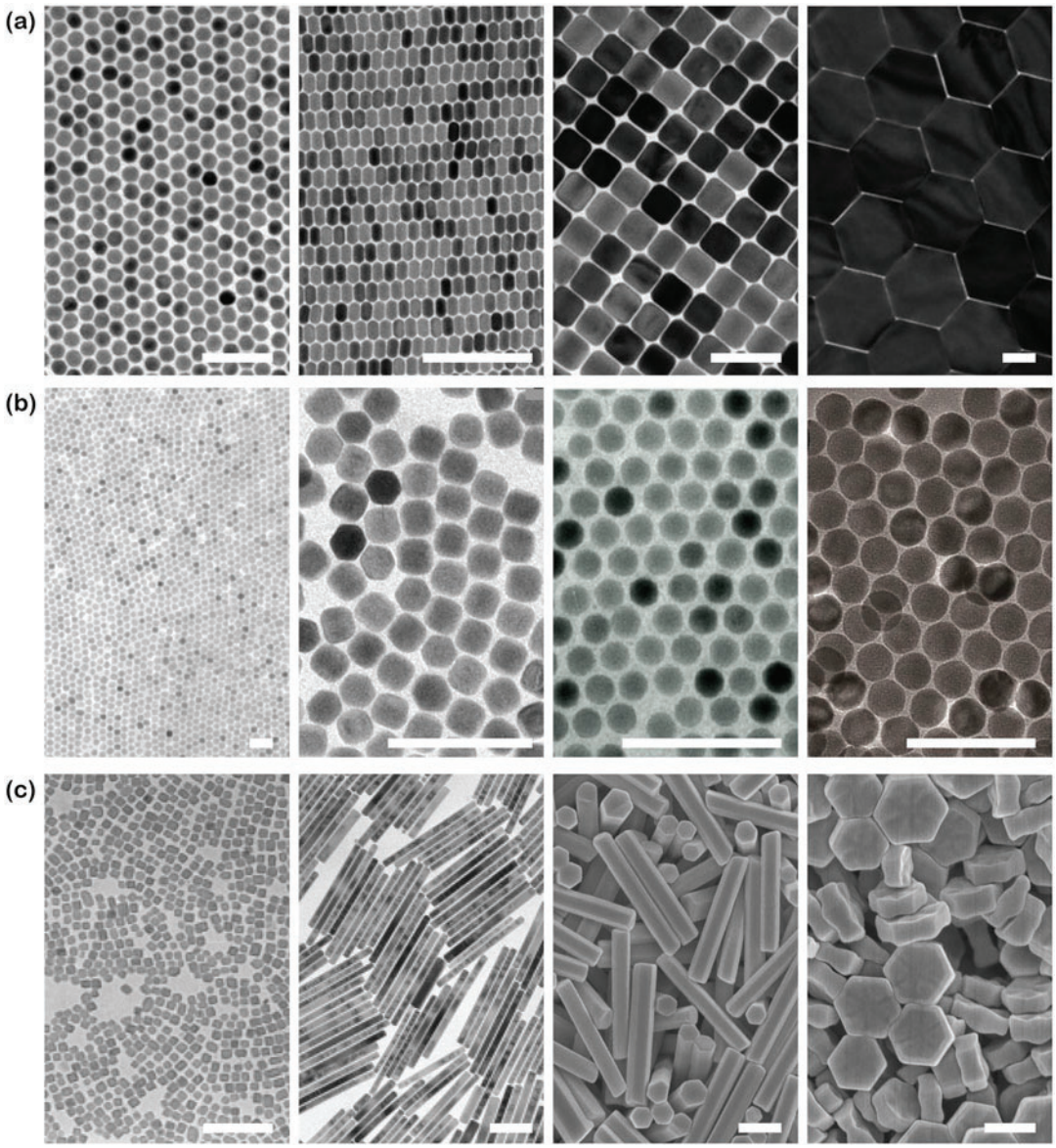

Fig. 44.2 Electron micrographs of typical sodium lanthanide fluoride-based upconversion materials. (**a**) $NaYF_4$ nanoparticles, nanorods, nanocubes, and nanodisks synthesized by thermal decomposition method (Ye et al. 2010). (**b**) $NaYF_4$, $NaGdF_4$, $NaYbF_4$, $NaLuF_4$ nanoparticles synthesized by coprecipitation method (Li and Zhang 2008; Wang et al. 2014, 2015; Chen et al. 2019). (**c**) $NaYF_4$ nanoparticles and nanorods, $NaYF_4$ micro-rods, and $NaLuF_4$ micro-disks synthesized by hydrothermal method (Wang and Li 2007; Zhang et al. 2016). Scale bar: 500 nm for the last two graphs and 100 nm for the rest. Note that only host materials other than dopants are denoted here

injection techniques with the thermal decomposition method and successfully obtained core-shell structured nanoparticles with an average diameter of ~12 nm (Yi and Chow 2007). Ye et al. fabricated monodispersed, homogeneous upconversion nanoparticles with a controllable size ranging from several to hundreds of nanometers using sodium and lanthanide

trifluoroacetate as precursors and a molten salt bath as a heat source (Ye et al. 2010).

Coprecipitation is one of the most popular methods to fabricate upconversion nanoparticles, which involves nucleation at room temperature and subsequent epitaxial growth at high temperatures. Li and Zhang first developed a coprecipitation method to synthesize hydrophobic hexagonal $NaYF_4$:Yb/Er/Tm nanoparticles by adding a methanol solution of sodium hydroxide and ammonium fluoride into a uniform solution containing lanthanide chlorides, oleic acid, and 1-octadecene to realize room-temperature nucleation and then elevating the temperature to 300 °C to perform Ostwald ripening growth of nanoparticles (Li and Zhang 2008). With this method, homogeneous nanoparticles with different shapes and sizes from several to a hundred nanometers can be fabricated through changing reaction parameters such as reaction time, temperature, and dosage of reagents. Liu's group and van Veggel's group further improved the coprecipitation method by replacing lanthanide chlorides with lanthanide acetates as starting materials to synthesize core-shell nanostructures (Wang et al. 2011a, b; Abel et al. 2009).

Another versatile approach to synthesize highly crystallized upconversion nanoparticles is the hydrothermal method, where a homogenous aqueous solution containing lanthanide ions and anions (typically fluoride) is subjected to thermal treatment under high pressure and temperature provided by a sealed, Teflon-lined autoclave. Li group first proposed a general hydrothermal approach to synthesize nanocrystals through a liquid–solid-solution phase transfer and separation process, where they fabricated $NaYF_4$ nanocrystals of 10 nm in diameter (Wang et al. 2005; Wang and Li 2007). Liu group reported a modified hydrothermal method to simultaneously tune the phase, size, and shape of $NaYF_4$ nanoparticles through lanthanide doping (Wang et al. 2010). In their work, lanthanide dopants with large ionic radii such as Gd^{3+} played a significant role in stabilizing the hexagonal-phase structure and decreasing crystal size. Liu group further developed an epitaxial growth strategy based on a hydrothermal method for controllable fabrication of upconversion micro-disks and micro-rods that can emit multiple colors at single-particle levels under NIR excitation (Zhang et al. 2016). The advantages of hydrothermal methods are that the crystallographic phase, size, shape, and luminescent properties can be easily tuned by controlling experimental parameters, such as hydrothermal temperature, reaction time, solvents, surfactants, pH value, concentration, the ratio of dopants, and so on. Distinctive from thermal decomposition and coprecipitation, the hydrothermal method starts with inexpensive reagents and can produce nano−/micro-sized crystals with hydrophobic or hydrophilic surfaces through a one-pot or multistep synthesis. Nevertheless, the homogeneity and monodispersity of hydrothermally synthesized nanoparticles are inferior to those obtained from decomposition and coprecipitation. Although several other methodologies have been established, the three strategies mentioned earlier are the most widely used to realize efficient upconversion emission, especially in fluoride host materials and core-shell nanostructure design.

44.2.3 Surface Functional Modification

Reliable biocompatibility and efficient optical output of upconversion nanoparticles are the prerequisites to their practical use in in vivo optogenetics and deep brain modulation. Although hydrophilic nanoparticles can be directly prepared by a hydrothermal reaction in aqueous solutions, they often have a broad size distribution, unsatisfied water solubility, and insufficient optical output. In contrast, commonly adopted approaches in organic solvents can produce nanoparticles with uniform morphology and high-efficiency photon conversion. But these nanoparticles are indispersible in aqueous solutions because of hydrophobic ligand coating. To address this problem, the surface functional modification should be implemented (Fig. 44.3).

A straightforward approach to improve the aqueous dissolution of upconversion nanoparticles is to eliminate hydrophobic ligands

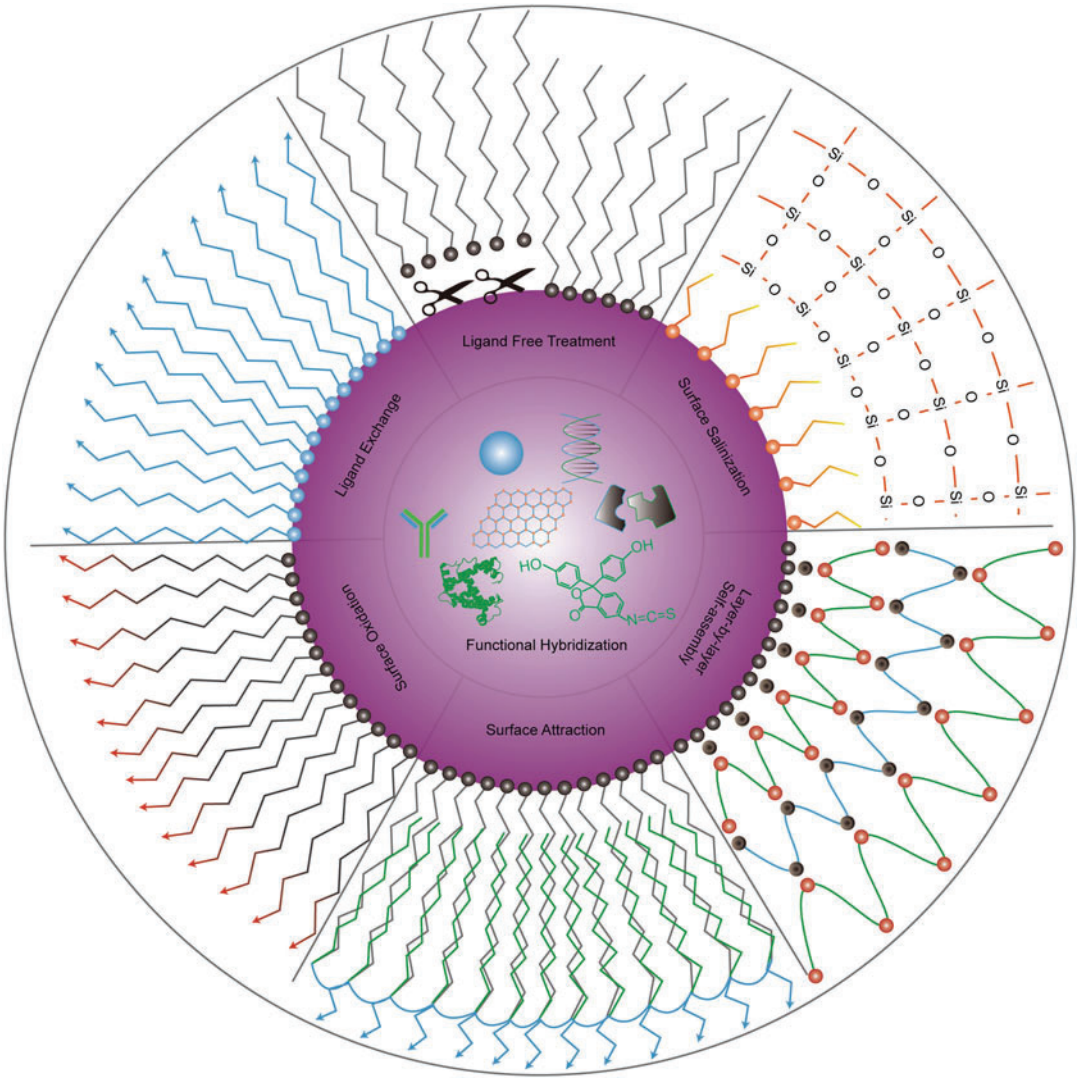

Fig. 44.3 Common strategies for surface modification of upconversion nanoparticles, including ligand-free treatment, ligand exchange, surface oxidation, surface attraction, layer-by-layer self-assembly, surface salinization, and functional hybridization

on the surface through an acid treatment (Bogdan et al. 2011; Deng et al. 2011). However, the exposure of "naked" nanoparticles to physiological conditions may lead to unexpected aggregation and cytotoxicity. Attractive strategies include posttreatment with hydrophilic segments through ligand exchange, ligand oxidation, polymer attraction, and surface salinization, as well as layer-by-layer self-assembly using polyelectrolytes (Wang and Liu 2009; Sedlmeier and Gorris 2014). Ligand exchange method utilizes hydrophilic organic ligands with a highly coordinative capability to replace those hydrophobic and poorly coordinative ligands. For instance, long carbon chains from oleic acid can be easily exchanged by polyacrylic acid or polyethylene glycol (Xiong et al. 2010; Zeng et al. 2012). The carbon–carbon double bond of surface ligands can be oxidized by Lemieux-von Rudloff reagent, thus improving the nanoparticles' water

solubility. But this method only works for a limited number of ligands. Surface salinization is the most widely used method to make nanoparticles biocompatible. A solid or mesoporous silica layer can be directly coated onto hydrophobic particles (Li and Zhang 2006; Liu et al. 2012).

Wrapping upconversion nanoparticles with other functional nanomaterials or biomolecules as a hybrid nanoplatform opens the opportunity to advanced optogenetics with specific purposes and multiplex neuromodulation. For example, Liu group reported the integration of manganese dioxide nanosheet on the surface of upconversion nanoparticles, which was demonstrated for the detection of intracellular glutathione through optical sensing (Deng et al. 2011). Surface attachment of ultrasmall gold nanoparticles can suppress upconversion emission at short wavelengths and enhance the photothermal conversion of upconversion nanoparticles (Han et al. 2017). Antibody-conjugated upconversion nanoparticles have been applied to in situ molecular mappings of different cancer biomarkers (Zhou et al. 2015a, b). Recently, cell membranes have been cloaked onto nanoparticle's surface to achieve immune escaping and homologous targeting of cancer (Rao et al. 2016; Yi et al. 2019). Upconversion nanoparticles have been combined with a variety of functional materials or molecules, including quantum dot, graphene, perovskite, organic dye, enzyme, protein, and DNA.

44.3 Optical Optimization of Upconversion Nanoparticles

44.3.1 Spectral Overlapping

To perform upconversion nanoparticle-mediated optogenetics, the emission wavelength of nanoparticles must be tuned to match the absorption bands of targeted opsins for efficient neural modulation (Yi and Chow 2006; Suyver et al. 2006). Visible emission modulation can be readily achieved through co-doping of Yb^{3+} with Tm^{3+}, Ho^{3+}, Er^{3+} in nanocrystals (Fig. 44.4). Although blue band (ChR2) and green band (VChR1 and C1V1) absorption can be well addressed by Tm^{3+} and Er^{3+} doping, the yellow band from 550 to 650 nm for controlling Cl^--pumping halorhodopsins remains a challenge. This deficiency can be overcome by utilizing Eu^{3+} emission at 590 and 616 nm with energy-migration or cooperative-sensitization processes (Wang et al. 2011a, b; Zhou et al. 2015a, b).

Lanthanide activators usually emit at multiple wavelengths under NIR excitation. For optogenetics, the impurity of stimulating light may cause unwanted effects in some cases, meaning that these redundant emission peaks need to be removed. For example, Er^{3+} ions exhibit four characteristic upconversion emission peaks at 409, 525, 546, and 659 nm, of which 409 and 659 nm emissions are not used for stimulating VChR1 and C1V1. There are several smart approaches to address this issue. For instance, inhibition of red emission at 659 nm can be achieved by decreasing the dopant concentration of Yb^{3+} in the matrix while enhancing green emission at 546 nm (Wang and Liu 2008). Additionally, pure red color with single-band emission can be achieved through an energy back-transfer process involving codoping of Mn^{2+} ions in host lattices (Tian et al. 2012; Wang et al. 2011a, b). Apart from compositional modulation, nano-sized color filters are also effective for realizing monochromatic upconversion emission. In 2015, a general method was developed to achieve single band upconversion by using organic dyes as a filter to absorb unwanted emissions (Zhou et al. 2015a, b).

44.3.2 Irradiation Wavelength Broadening

Examples of upconversion-mediated optogenetics are currently focused on Yb^{3+}-sensitized nanoparticles, in which a 980 nm laser was usually applied as the excitation source. Considerable absorption by water in this spectral region may result in tissue overheating, a major concern especially for in vivo deep brain stimulation. The currently adopted solution is to minimize the overheating effect through modulation

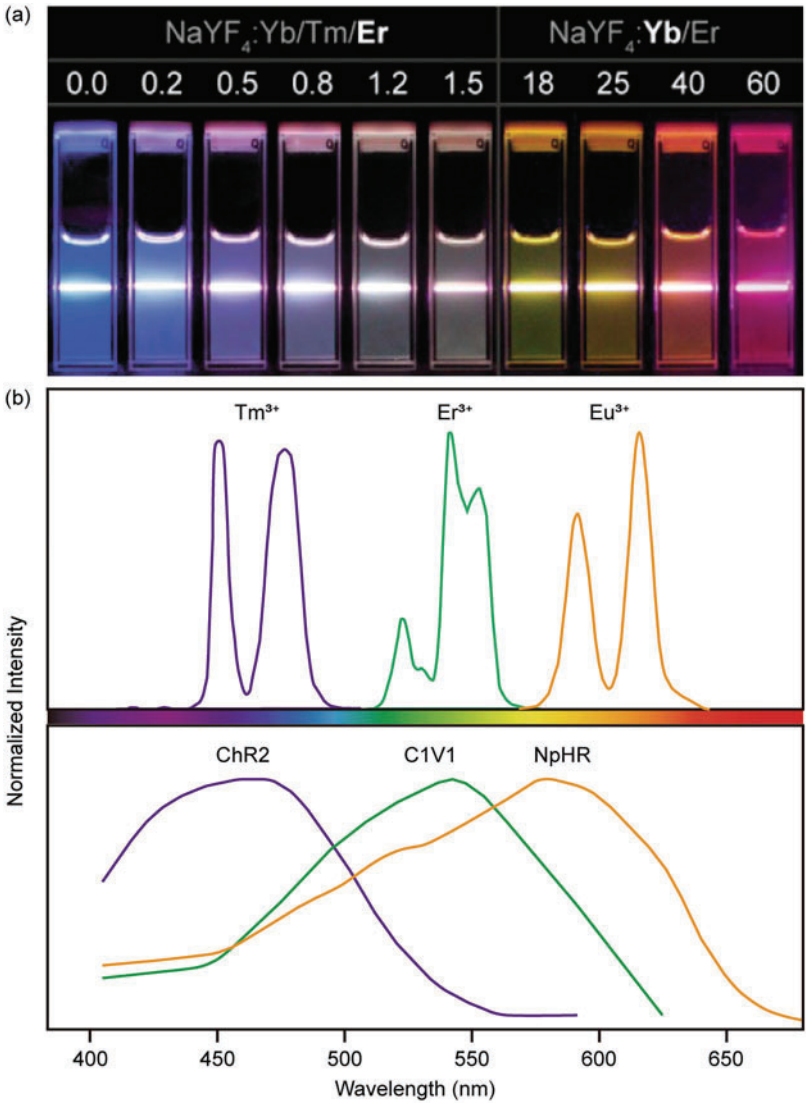

Fig. 44.4 (**a**) Photographs of upconversion nanoparticles under NIR excitation showing multicolor emission tuning in $NaYF_4$ host with varying concentrations of Er^{3+} and Yb^{3+} (Wang and Liu 2008). The upper labels represent the corresponding concentration of highlighted dopant ions. (**b**) Representative emission peaks from typical lanthanide activators and absorption bands from commonly used opsins

of peak intensity, pulse frequency, and duration time of the laser input, as well as reserving sufficient time for heat dissipation (Wang et al. 2017a, b; Chen et al. 2018; Miyazaki et al. 2019). Another interesting strategy is to vary the irradiation wavelength at a spectral region where absorption from water and bio-tissue is relatively low. Recently, co-doping of Nd^{3+}/Yb^{3+} as sensitizers has allowed effective excitation at 808 nm, where the coefficient of water absorption is almost one-tenth of that at 980 nm (Shen et al. 2013; Xie et al. 2013; Liang et al. 2016). Liu group also reported an Nd^{3+}-free approach by which intense visible emissions can be obtained through self-sensitizing Er^{3+} at multiple wavelengths such as 808, 980, and 1532 nm (Chen et al. 2017). Additionally, the excitation wavelength for photon upconversion can be broadened by utilizing organic dyes as sensitizers (Wu et al. 2016; Liang et al. 2019a, b). The variation in excitation responses of different sensitizers renders upconversion nanoparticles with orthogonal emissions under irradiation at different wavelengths (Zheng et al. 2018), which

can be utilized for the design of multiplex neuromodulation.

44.3.3 Output Brightness Enhancement

As with spectral overlapping, the intensity of upconversion emission is also essential for in vivo optogenetics. A strong upconverted emission guarantees sufficient stimulation of opsins so that light-induced depolarization can surpass the threshold potential and generate an action potential in targeted neurons. Enhancing upconversion emission intensity can be achieved through external or internal factors. In principle, a higher excitation power density (below the saturation intensity) produces a higher emission intensity according to the classical theory of nonlinear optics (Pollnau et al. 2000). However, increasing the input may not be the best choice since it may aggravate living neurons considering the risk of overheating. Instead, pulsed excitation input with high peak intensity but relatively low average power density is commonly adopted for effective delivery of light with minimal tissue heating and dynamic modulation of neurons in vivo (Wang et al. 2017a, b; Chen et al. 2018; Miyazaki et al. 2019).

Luminescence enhancement could boost energy conversion efficiency (Fig. 44.5). Lanthanide ions intrinsically exhibit low light-absorption coefficients because of parity-forbidden f–f transitions. For example, Nd^{3+} ion possesses a relatively higher photon-absorption coefficient at 808 nm compared to the conventional sensitizer Yb^{3+} at 980 nm. Co-doping of Nd^{3+} with Yb^{3+} in the host lattice would be an effective way to enhance upconversion emission (Shen et al. 2013). Organic dyes have also proven effective in boosting photon absorption in upconversion nanocrystals as their light-absorption coefficients are orders of magnitude higher than those of lanthanide ions (Zou et al. 2012; Chen et al. 2015a, b; Wang et al. 2017a, b).

To promote conversion efficacy in upconversion nanoparticles, the main challenge is to reduce nonradiative energy loss. Over the past few decades, several strategies have been developed, including the host–lattice modulation, crystal composition variation, nanostructure design, and nanoplatform engineering. Heer et al. first identified $NaYF_4$ as a matrix material with relatively low phonon energy (~350 cm^{-1}) to achieve multicolor emission (Heer et al. 2004). An effective method to boost upconversion emission involves the modulation of host lattice symmetry by host–dopant ion substitution (Chen et al. 2008). Other practices have dedicated to regulating dopant types and concentrations for alleviated quenching effects (Zhao et al. 2013; Gargas et al. 2014). One commonly adopted approach is surface passivation of nanoparticles through a core-shell design. Suitable shell coating can restrain defect-induced radiative quenching (Wen et al. 2018). In view of composition and functionality, a shell layer can be categorized as an optically inert shell (e.g., CaF_2, SiO_2, and dopant-free host crystal) and an optically active shell (e.g., sensitizer or activator ions doping) (Chen et al. 2012; Li and Zhang 2006; Ren et al. 2012). In a recent study, tetherless deep brain stimulation was reported by Lin et al., who carried out a core–shell–shell design and demonstrated a considerable emission enhancement by optimizing the Yb^{3+} concentration of the middle shell (Lin et al. 2018). Nanoplatform engineering mainly involves surface plasmon coupling, photonic crystal engineering, and microlens amplification. Recently, Liu group reported a new method to amplify upconversion emission through dielectric superlensing modulation (Liang et al. 2019a, b). The utilization of dielectric microbeads can modulate the wavefront of both excitation and emission fields, resulting in emission enhancement up to five orders of magnitude. Another extraordinary work by the same group has realized ultrafast radiative decay of activators with remarkable emission enhancement by taking advantage of surface plasmon coupling effects (Wu et al. 2019).

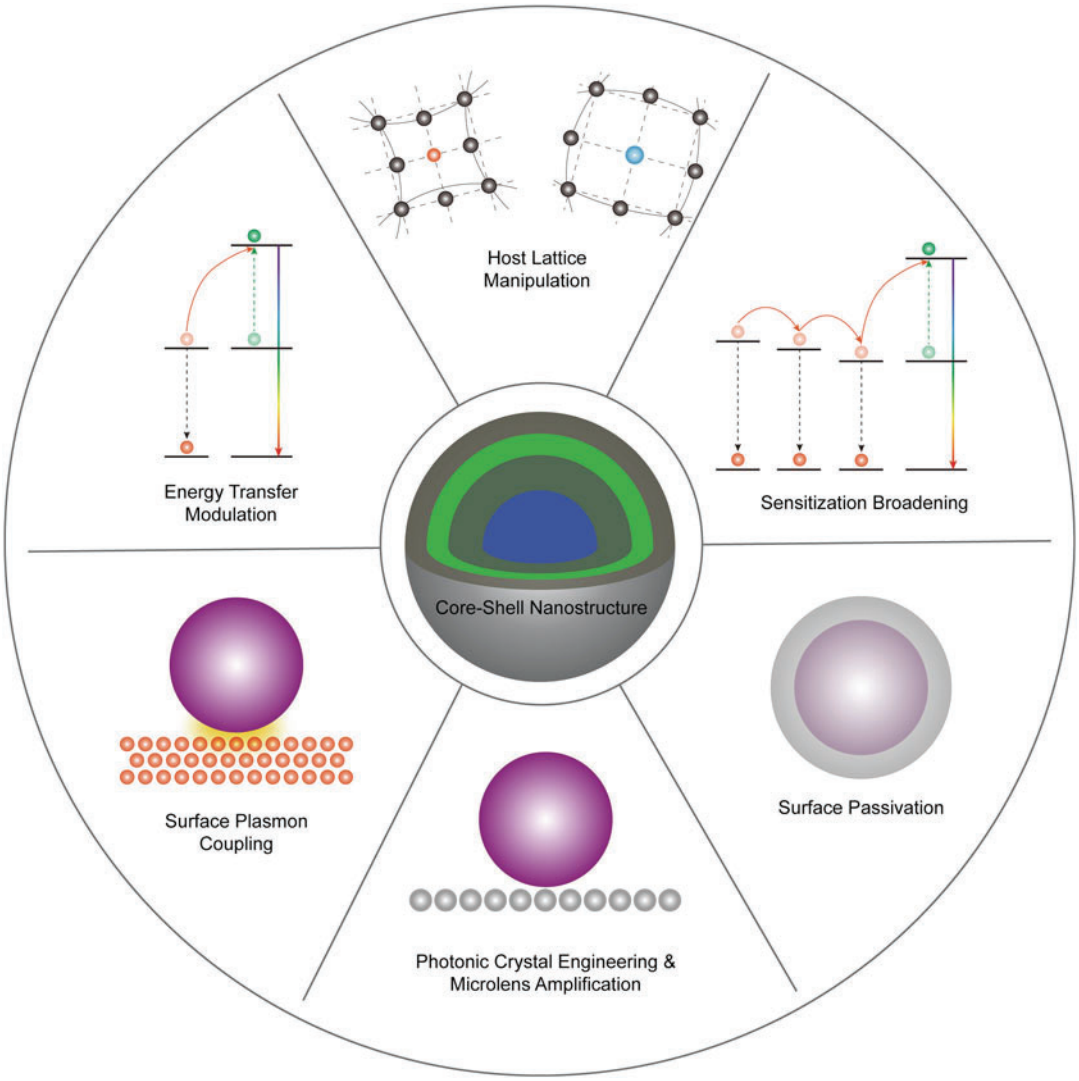

Fig. 44.5 Generic strategies for emission enhancement of upconversion nanoparticles, including host lattice manipulation, energy transfer modulation, surface plasmon coupling, photonic crystal engineering, microlens amplification, surface passivation, sensitization broadening, and core-shell nanostructure design

44.4 Recent Progress in Upconversion Nanoparticle-Mediated Optogenetics

NIR optogenetic systems overcome the limitation of traditional optogenetics, such as insufficient delivery of visible light to the targeted area and inevitable tissue damage induced by invasive insertion of optical fiber and light source implantation. Over the past few years, upconversion nanoparticle-mediated optogenetics has been applied in in vitro cultured neurons and in vivo animal models such as *Caenorhabditis elegans*, zebra fish, and rodents (Fig. 44.6).

In 2015, in vitro neuron activity induced by NIR light was performed using a well-designed neuron culture system in which core-shell structured upconversion nanoparticles were

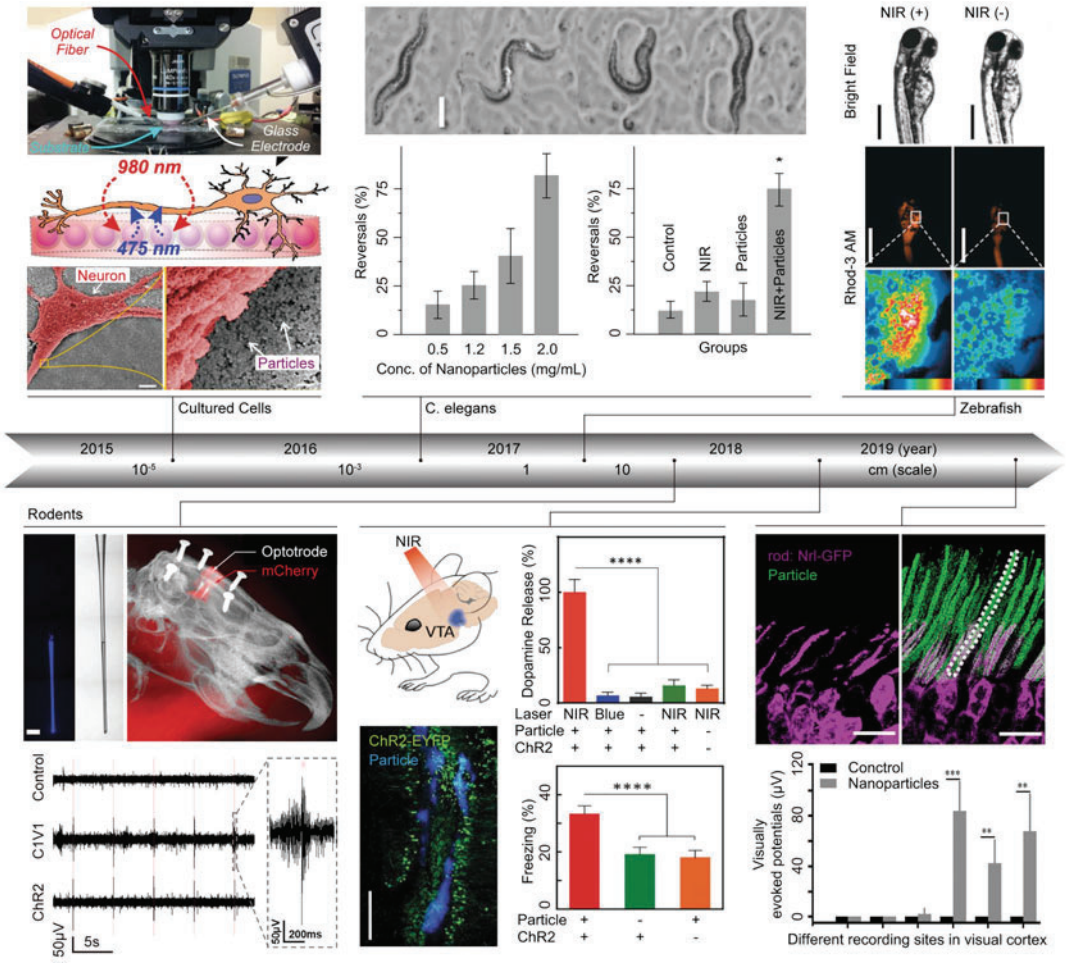

Fig. 44.6 Representative breakthroughs in upconversion nanoparticle-mediated optogenetics. Panel for cultured cells: a photograph of the whole-cell patch-clamp system for in vitro NIR-enabled electrophysiological measurements (top), a schematic illustration of neurons cultured on the nanoparticle-containing substrate (middle), and the corresponding electron microscopy images (bottom) (Shah et al. 2015). Scale bar: 5 μm for the bottom left and 200 nm for the bottom right. Panel for *C. elegans*: microscopic images showing reversal behaviors of a ChR2-expressing worm under 980-nm laser irradiation, the effect of nanoparticle concentrations on the percentage of worms showing a reversal response (bottom left), and the reversal response percentage of different experimental groups (bottom right) (Bansal et al. 2016). Panel for zebra fish: bright-field and luminescence images of zebra fish incubated without/with upconversion nanoparticles (Ai et al. 2017). Scale bar:10 μm. Panels for rodents. Left panel: bright-field and fluorescent photographs of the nanoparticle-embedded micro-optrodes (top left), X-ray and luminescent merged image showing the co-localization of implanted micro-optrodes in ChR2-expressing region (top right), and electrophysiological records of NIR-driven spiking traces in rat expressing ChR2 or C1V1 (bottom) (Wang et al. 2017a, b). Scale bar: 500 μm. Middle panel: schematic illustration showing transcranial NIR stimulation of VTA dopamine neurons in vivo (top left) and dopamine release percentage in different experimental groups (top right), a confocal image showing the overlap between nanoparticles and ChR2 expression (bottom left), and freezing level of different groups (bottom right) (Chen et al. 2018). Scale bar: 200 μm. Right Panel: merged fluorescent images of the retina without/with nanoparticle injection (top) and visually evoked potentials triggered by 980-nm light at different sites in the visual cortex (bottom) (Ma et al. 2019). Scale bar: 10 μm

embedded into the cell culture substrate to in situ convert remotely delivered NIR light to the visible light (Shah et al. 2015). Under pulsed NIR excitation, high-temporal resolution neuron responses were recorded, and repetitive action potentials were observed at frequencies up to 10 Hz. In the same year, Yawo group also demonstrated the feasibility of NIR-induced neurostimulation using a whole-cell patch-clamp technique at the cellular level (Hososhima et al. 2015). In this study, C1V1 and *Platymonas subcordifonnis* (PsChR) expressing ND7/23 cells were cultured on collagen films containing upconversion nanocrystals, and whole-cell patch-clamp studies were adopted to record electrical signals on the cell membrane. Upon excitation of upconversion nanocrystals with a NIR laser, the researchers observed activated expressions of C1V1 or PsChR and neuronal responses induced by the upconverted visible light.

Early demonstrations of NIR-enabled in vivo optogenetics were carried out in *C. elegans* and zebra fish. *C. elegans* is a micron-sized, optically transparent worm with a nervous system of 302 neurons. *C. elegans* can be regarded as a simple in vivo model for genetic tractability and known connectome (Shipley et al. 2014). Bansal et al. successfully operated upconversion-mediated in vivo optogenetics in *C. elegans* (Bansal et al. 2016). In their study, nanoparticle-containing *C. elegans* with ChR2-expressing mechanosensory neurons exhibited altered movements in the presence or absence of NIR irradiation. Overheating and particle-induced toxicity were suppressed by utilizing a quasi-continuous wave laser with a low average power density and by optimizing the dosage of nanoparticles. Additionally, zebra fish is also a popular model in neurobehavioral studies because of its physiological and genetic homology with mammals (Best and Alderton 2008). In 2017, Ai et al. performed in vivo optogenetic manipulation on Ca^{2+}-mediated biological functions of zebra fish through activation of ChR2 ion channels by 808 nm-excitable and blue-emitting nanoparticles (Ai et al. 2017). The substitution of 980 nm with 808 nm excitation can effectively eliminate tissue overheating due to water absorption at 980 nm.

Despite the implementation of successful practices in cultured cells and transparent in vivo models, it is still challenging to apply NIR light-enabled optogenetics in mammals for transcranial deep brain modulation. The most effective way is to design better nanoparticle systems with the adequate capability of photon conversion under "remote" delivery of NIR light. Additionally, direct injection of nanoparticles to targeted areas is likely to maintain a desirable density of nanoparticles in situ in living animals. Suitable modifications on the surface of nanoparticles may address the concerns of possible particle-induced cytotoxicity, diffusion, and metabolism.

Mice is a commonly used animal model in genetic and medical research for the physiological resemblance of humans. Shi and Wang's groups managed to manipulate the motor behaviors of mice through activation and inhibition of neurons with implantable micro-scaled fibers embedded with upconversion nanoparticles (Wang et al. 2017a, b; Lin et al. 2017, 2018). Recently, Yamanaka's group also remotely controlled the free movement of mice by activating and inhibiting neurons where upconversion microparticles were directly injected at 2 mm below the brain surface (Miyazaki et al. 2019).

In 2018, Chen et al. demonstrated that precise control of multiplex deep brain modulation in rodents is viable through transcranial NIR excitations (Chen et al. 2018). In their work, ChR2-expressed neurons activated fear memory recollection through dopamine regulation, where core-shell structured nanoparticles with a silica coating at the outer layer were directly injected into the hippocampus of transgenic mice. Under NIR irradiation, the freely movable mice exhibited fear-induced freezing behavior even at 2 weeks after the injection. This approach was further expanded for inhibition of neural circuitry and provoking hippocampal theta oscillations. Nanoparticles were observed in the targeted area even 1 month after injection with low diffusion and long-term stability. Meanwhile, no obvious tissue damage, inflammation, or apoptosis was

found, implying low particle- or photo-induced cytotoxicity. This investigation elucidated the potential of luminescent nanomaterial-mediated optogenetics in mammals, especially on deep brain stimulation and neurological disorder therapies.

Upconversion-mediated optogenetics can also be a tool for breaking physiological visual limits. In 2019, Ma et al. first realized NIR image vision in rodent models through subretinal injection of photoreceptor-binding upconversion nanoparticles (Ma et al. 2019). After ocular injection, the mice could not only perceive NIR light but also see NIR light patterns with native daylight vision unaffected. This technique may present a new opportunity for security, ophthalmic therapy, and other medical use.

In addition to lanthanides-doped nanomaterials, triplet–triplet annihilation (TTA) molecules also present photon upconverting capability which may be used for NIR-mediated optogenetics. A recent study demonstrated the application of a TTA molecule-embedded hydrogel in NIR optogenetic genome editing where the morphology of hippocampal neurons can be regulated (Sasaki et al. 2019). However, TTA based optogenetics technology is still in its infancy since most of these molecules suffer common drawbacks from insolubility, structural and optical stability in physiological conditions, massive optical quenching effects, and their resultant upconversion efficiency limitation.

Other types of nanomaterials with photothermal or magnetothermal conversion capability can be used for neuromodulation. Commonly used photothermal nanotransducers include small molecules such as indocyanine green dye, organic polymers, carbon-based materials, noble metals, and semiconductors. For example, semiconducting polymer nanobioconjugates bound to TRPV1 (transient receptor potential cation channel subfamily V member 1) channels can act as energy transducers to convert NIR light to heat for the activation of intracellular Ca^{2+} influx (Lyu et al. 2016). Similarly, magnetothermal conversion regulated by ferroferric oxide nanoparticles under alternating magnetic field was also identified for triggering widespread and reversible firing of $TRPV1^{+}$ neurons (Chen et al. 2015a, b). With further advances in nanoscience and nanotechnology, nanomaterials with energy-conversion capability can be instrumental to uncover the complex neural activities that would not be otherwise discoverable using conventional probes.

44.5 Outlook and Perspectives

Recent decades have witnessed significant progress in NIR light-enabled optogenetics. In this chapter, we have illustrated how upconversion nanomaterials can be used to manipulate cation flux in membrane vesicles with minimal invasiveness, deep-tissue penetration capability, and precise spatiotemporal perturbation. As with any other technique, upconversion nanoparticle-based optogenetics is not free of limitations. In particular, upconversion efficiency and biocompatibility of the nanoparticles need to be improved. Surface quenching effects may dominate in small-sized upconversion nanoparticles and cause complications for optogenetic manipulation. This could be overcome by rational design of dopant composition and core-shell structure. Additional surface modification of nanoparticles can boost biocompatibility and impart added functions such as bio-labeling, targeting, and multiplex control of neurons. The advances in NIR-mediated optogenetics will also rely on new populations of opsin variants as well as innovative instrumentation. Exploiting new classes of opsins will enable us to maximize the potential of upconversion emission and optimize the effect of the optical response. Next-generation NIR-mediated light sources and opsin-mediated neural modulation in combination with novel recording instruments will certainly improve outcomes of in vivo optogenetic techniques.

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